Scientific drilling projects in ancient lakes: Integrating geological and biological histories

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1.	Introduction
2.	Data and methods
2.1.	<i>Geological data and methods</i>
2.1.1.	<i>Site selection and drilling strategies</i>
2.1.2.	<i>Coring techniques and data recording</i>
2.1.3.	<i>Borehole logging</i>
2.1.4.	<i>Sediment-core data</i>
2.1.5.	<i>Age-depth models</i>
2.1.6.	<i>Stable isotopes</i>
2.2.	<i>Biological and paleontological data and methods</i>
2.2.1.	<i>Macrofossils</i>
2.2.2.	<i>Microfossils</i>
2.2.3.	<i>Subsurface biosphere</i>
2.2.4.	<i>Biomarkers</i>
2.2.5.	<i>Ancient amino acids</i>
2.2.6.	<i>Ancient DNA</i>
2.3.	<i>Data accessibility and storage</i>
3.	Integrating geological and biological data
3.1.	<i>Paleolimnological reconstruction</i>
3.3.	<i>Molecular dating</i>
3.4.	<i>Tracing character evolution</i>
3.2.	<i>Consequences of environmental change: A biological diversification perspective</i>
4.	Conclusions
	Glossary
	Acknowledgements
	References

ABSTRACT

Sedimentary sequences in ancient or long-lived lakes can reach several thousands of meters in thickness and often provide an unrivalled perspective of the lake's regional climatic, environmental, and biological history. Over the last few years, deep drilling projects in ancient lakes became increasingly multi- and interdisciplinary, as, among others, seismological, sedimentological, biogeochemical, climatic, environmental, paleontological, and evolutionary information can be obtained from sediment cores. However, these multi- and interdisciplinary projects pose several challenges. The scientists involved typically approach problems from different scientific perspectives and backgrounds, and setting up the program requires clear communication and the alignment of interests. One of the most challenging tasks, besides the actual drilling operation, is to link diverse datasets with varying resolution, data quality, and age uncertainties to answer interdisciplinary questions synthetically and coherently. These problems are especially relevant when secondary data, i.e., datasets obtained independently of the drilling operation, are incorporated in analyses. Nonetheless, the inclusion of secondary information, such as isotopic data from fossils found in outcrops or genetic data from extant species, may help to achieve synthetic answers. Recent technological and methodological advances in paleolimnology are likely to increase the possibilities of integrating secondary information, e.g., through molecular dating of molecular phylogenies. Some of the new approaches have started to revolutionize scientific drilling in ancient lakes, but at the same time, they also add a new layer of complexity to the generation and analysis of sediment core data. The enhanced opportunities presented by new scientific approaches to study the paleolimnological history of these lakes, therefore, come at the expense of higher logistic, communication, and analytical efforts. Here we review types of data that can be obtained in ancient lake drilling projects and the analytical approaches that can be applied to empirically and statistically link diverse datasets for creating an integrative perspective on geological and biological data. In doing so, we highlight strengths and potential weaknesses of new methods and analyses, and provide recommendations for future interdisciplinary deep drilling projects.

1. Introduction

The vast majority of the world's lakes has existed or will exist for up to a few ten thousand years (e.g., Brooks, 1950). Primarily due to sediment infill, they become progressively shallower and subsequently vanish. Ancient or long-lived lakes, on the contrary, exist for over 100,000 years (100 ky), sometimes millions of years (My) (Brooks, 1950; Gorthner, 1994; Martens, 1997). They typically occur in settings where sedimentation rates are low or balanced by subsidence (Cohen, 2012). Accordingly, most of today's ancient lakes are oligotrophic and situated in active tectonic graben settings or impact craters with low sediment supply from the catchment.

Because of the long-term availability of accommodation space (Jervy, 1998), sediment sequences in ancient lakes can reach several hundreds to thousands of meters in thickness (e.g., Scholz et al., 1993, 2011; Lindhorst et al., 2015). Lake deposits contain material that mostly derives from the lake proper and the catchment area and, hence, provide an unparalleled perspective of the lake's history through time (O'Sullivan, 2004). Combining the paleolimnological records from different lakes permits to reconstruct continental and global environmental, and climatological histories. It is this potential, captured in the often continuous lacustrine sedimentary archives, that has inspired several deep drilling projects in ancient lakes (reviewed in Cohen, 2012; Fig. 1).

However, over the past decades, drilling operations became increasingly multidisciplinary, as data bearing on physical, chemical, biochemical, and biological research questions can also be obtained from sediment cores. Because of a wealth of new information, scientists from different fields, such as sedimentology, climatology, geochemistry, paleolimnology, paleontology, biochemistry, microbiology, evolutionary biology, physics, and modeling, currently aim to use ancient lakes as paradigms to interactively look into natural phenomena from various angles, emphasizing the need for truly interdisciplinary collaborations (*sensu* O'Sullivan, 2004; Birks and Birks, 2006).

Fig. 1. Map showing location of ancient lakes with a presumed age of > 1 My at which deep drilling has been done (Cohen, 2012; Russell and Bijaksana, 2012).

Multidisciplinary and interdisciplinary studies enable a more holistic approach to scientific problems, provide excellent opportunities for hypothesis-driven research, and are likely to have greater success in generating a widespread interest in the broader scientific community. However, these projects pose several challenges for the diverse science teams. The interests of the various groups involved need to be aligned; participants may lack the required knowledge of other disciplines; traditions and common practices may differ widely between disciplines. Finally, larger teams increase the challenge to communicate and coordinate efforts effectively. The various goals of individual teams call for compromises on several levels, such as drill site selection, subsampling strategies, and choice of analyses (see section 2.1.1.). Life-scientists are typically not familiar with drilling operations and often lack basic geological knowledge whereas earth-scientists may not be acquainted with biochemical or biological procedures. More practically, the difficulty arises that life-scientists do not know exactly how to retrieve the archives they hope to study, and that earth-scientists cannot evaluate applicability and performance of biological methods. Similar problems persist on smaller scales, and given the rapid advancement of many of the individual fields, specialists may even struggle with

methodological innovations in their field over the often year-long duration of deep drilling projects, involving the planning, the actual drilling campaign, and the interpretation of the final datasets. These issues are also relevant for core storage, which may affect geological and biological properties differently. Sedimentologists are typically acquainted with long-term changes in sediments after core retrieval, but others may draw erroneous conclusions when linking biological and geological data without accounting for potential contamination, drilling artifacts, decay processes, and other complications (see section 2.1.2.). In general, greater logistic, communicative, and administrative efforts are required with increasing complexity of interdisciplinary projects, and drilling methods may have to be optimized to guarantee the required data quality.

Perhaps the most challenging task, however, is to integrate the diverse datasets various teams collect from drilling cores. These datasets typically have differences in resolution, data quality, and dating uncertainty, but combining them is required to answer interdisciplinary questions. Whereas the physical linkage of information directly obtained from sediment cores is, in most cases, relatively straightforward due to the chronological constraints on the data, the challenge grows when primary data, i.e., data generated from sediment cores or in boreholes, are to be linked with external (secondary) data, i.e., data obtained independently of the drilling operation. Examples of secondary data sources include stable isotope information from fossils found in outcrops (see section 2.1.6.) or genetic information from extant species (see section 3.3.).

Here we review the types of geological and biological data that can be obtained from ancient lake drilling projects (section 2.) and the methods that can be used to analyze these data against the backdrop of the abovementioned practical and analytical challenges (section 3.). Acknowledging the increasing number of approaches and analyses that can be applied to drilling data, we narrow our focus on data and methods that have a high potential towards integrating geological and biological data and for hypothesis-testing related to interdisciplinary questions. We also provide a retrospect on how the actual drilling operation and conditions of sediment-core storage can affect data and subsequent multi- and interdisciplinary analyses. Although this review focusses on extant ancient lakes, some of the information given might also be applicable to lakes from the past and even young lakes.

Our aim is to provide scientists from various disciplines with a background to strengthen interdisciplinary approaches to ancient lake drilling projects. We thus explain data acquisition and analyses in broad terms and provide information as to the underlying fundamental principles that may be equally useful for earth and life scientists. Given this scope, we refrain from detailed discussions that are constrained to a specific field, nor do we provide a historic overview of drilling operations for which other reviews exist (Cohen, 2012).

As such, this review intends to encourage scientists from diverse disciplines to join scientific deep drilling projects, and to utilize these unique records of global change during the earth's history for understanding current and future changes on a planetary scale.

2. Data and methods

2.1. Geological data and methods

2.1.1. Site selection and drilling strategies

Careful consideration of the drill site(s) and the drilling strategy are a prerequisite to optimize the chances that the goals of a deep drilling project can be reached. Scientific objectives are the foremost criteria for the selection of drill sites and strategies, but financial and time constraints also have an distinct impact. The extensive infrastructure needed and the shipping of highly specialized gear are important cost factors of deep drilling (Fig. 2).

Fig. 2. Deep Lake Drilling System of DOSECC (USA) in operation at Lake Ohrid. In this case, the equipment had to be shipped from Salt Lake City (USA) to this inland lake on the Balkans (photo credits: T. Wilke).

Given a certain budget, the costs for logistics, including the transport of the drilling equipment to and from the lake and daily operational expenses (e.g., labor costs, fuel consumption, daily shuttle of drill team and cores), define the number of drilling days. Technical failures, weather delays, or lithologies that are challenging to drill are difficult to predict and may further reduce available drilling time. In contrast, unexpected drilling progress may provide the opportunity to drill deeper than anticipated or to add additional sites, addressing for example 2nd or 3rd order scientific objectives. For these reasons, a well-calculated budget, which includes sufficient contingency to cover unforeseen costs and/or delays, may significantly increase the chances for success. Finally, site selection may also be influenced by the time required for daily commutes of the drill teams to and from the drill site. Experience has shown that the one-way commute time should not exceed 1.5 h when working in 12 h shifts.

Most of the scientific criteria for site selection and drilling strategies are reviewed in Cohen (2012). Catchment characteristics, such as the location of inlets, providing terrigenous, clastic, and organic sediments to the basin, the bathymetry and surrounding topography, and the presence of undisturbed successions may affect site selection. However, bathymetry, catchment topography, and the location of inlets may have changed over time, especially in tectonic basins. Most commonly, the site selection is therefore based on data from reflection-seismic surveys, providing information about bathymetry and the

three-dimensional sub-bottom sediment structure, as well as the stability of sediments in target areas. For most scientific questions, areas with undisturbed sedimentation are preferred, but for others, such as the reconstruction of earthquake history, a focus on disturbed successions may be beneficial (e.g., Takemura et al., 2013). Moreover, most drilling campaigns focus on the depocenter of a lake. If biological questions are of interest, such a drilling strategy will almost certainly omit valuable information of the nearshore benthic communities where most of the biodiversity is concentrated (see also section 2.2.2). However, during initial lake phases or massive drops in lake level, shallow water species may have lived nearby the depocenter and thus be preserved in the old sediments just overlying the basement rock (e.g., Wagner et al., 2014a). Coring in shallower or littoral regions is facing other problems, such as obtaining discontinuous or incomplete records of biotic evolution, hydrological characteristics, and catchment processes. In these environments, sediments from the initial lake phase or low-level periods will be missing.

The drilling strategy also includes criteria, such as the number of drill sites, their priorities, or the number of holes per site needed for a composite core. As a general rule, the site with the highest scientific priority should be drilled first, as unforeseeable technical or weather issues may delay the coring progress. However, it may be advisable to start with a site of secondary priority, for example, if the sedimentary characteristics are poorly known or if the main drill site requires high logistic efforts. In many campaigns, the initially retrieved sediment cores have a poorer quality compared to cores drilled at later stages of the operation, because drill and science teams need to develop communication and drilling strategies (e.g., the choice of drill tools) suitable for the encountered lithologies. As core recovery in a single borehole can be as low as 10% (see also section 2.1.2.), parallel coring is needed to fill the gaps. Sometimes 3–4 holes are required to obtain 100% or close to 100% recovery at one site (resulting into a so-called composite core).

In some cases, the drilling strategy may need to be adapted to effectively address specific scientific questions or deviating lithological intervals. For example, in studies focusing on subsurface microbial activity, contamination by drilling fluids needs to be minimized and samples may need to be taken directly upon recovery of the cores (see section 2.2.3.). If the exact depth of the recovered sediments is not of highest priority, the hole of the first core drilled for subsurface biosphere studies can be used for

borehole logging (section 2.1.3.) and to obtain initial information about lithological characteristics at depth. This facilitates adaptation of drilling strategies for the subsequent holes and sites to be drilled.

In summary, several criteria affect site selection and drilling strategies. Because of the specifics of each lake, these criteria need to be balanced carefully based on the exact setting encountered. Meticulous preparation, including the collection of site-specific information, such as data on sediment structure from seismic surveys and lithological characteristics from pilot coring surveys may serve to construct a drilling strategy that can then be evaluated against scientific objectives, budget, logistic requirements, and the available time.

2.1.2. Coring techniques and data recording

The main lake coring tool is a piston-type corer, which allows penetration under hydraulic power into generally cohesive sediments of various aggregation up to a pre-determined length. This tool permits collection of the least 'contaminated' or 'disturbed' type of core as it is pushed into the formation ahead of any disturbance caused by the rotational drill bit and sediments are collected before they get into contact with drilling fluids. Before the actual drilling, the piston corer is sealed with a piston to avoid contamination of the drill string with surface lake water and cuttings or cavings, which may be in the borehole before and during the core collection, to enter the coring tube.

In soft to firm clays, sample recovery can be 100%. The slight under-pressure caused by the piston avoids shearing of sediment packages in front of the corer during penetration, but it leads to expansion of the core, particularly at its top and may promote degassing. In more granular sediments, compression rather than expansion is common due to grain packing. Moreover, as the drill string is frequently advanced to the next firing point by the length of recovery rather than the length of the core barrel, there can be overlap of 'strata' between two core runs, with the top of a subsequent sample consisting of cavings from the previous sample. It is not easy to completely clean the borehole in this type of sediment and the coarser and less cohesive it is, the more likely it will be that full recovery of the section will not fill the core barrel due to packing and dewatering occurring. Again, recalibration of any subsample depth with regard to the composite borehole log and marker horizons will ensure that correct depths for correlation with other scientific data results are achieved. Although the core length may not reflect the

full sampled length, the stratigraphic coverage typically will be complete and essentially free from other types of mechanical disturbance and fluid mixing. Therefore, piston cores allow for the best stratigraphic resolution of all drill-sampling method.

Other frequently used lake drilling tools are the extended nose corer and the alien corer. Both of those tools require a rotation while coring. The core is cut and collected into the core barrel in close proximity to the drill bit while it is operating and, hence, while fluid discharge is required to keep the drill bit cool and clear of cuttings. This makes it difficult to allow the core an undisturbed passage into the core barrel, and results in artifacts by the drilling and the use of drilling fluid. These artifacts depend also on the lithology of drilled sediments, but there may be mixing of drilling and formation fluids, or selected portions of the core may be washed away, and disturbance to the structure and stratigraphy of the core may also occur.

The extended nose corer is used when the material in the stratigraphic section is non-cohesive or friable but becomes too coarse or compacted to be able to operate a piston corer for any reasonable length into the subsurface. To use this corer, the sediment needs to be still soft or granular enough to allow extending a thin diamond bit nose ahead of the main bit and main fluid flush when progressing the hole. Ideally, the core will enter the extended bit section without too much interference or influence from the rotation and drilling fluid. If there is any movement of the drill string due to heave of the platform while drilling, then discs of core and cuttings may also occur and show up as bands in the core. Recovery is unlikely to reach 100% even under ideal conditions.

The alien or rotary core barrel is essentially a method of collecting cores from hard, cohesive sediments. It may selectively wash away material that is too soft or non-cohesive before it can be properly cut to enter the core barrel. It may also induce drilling fractures in otherwise good quality rock. There is always an interaction between the drilling and formation fluids as the core is being cut and collected. Recovery will range from poor (<10%) to excellent (100%), depending on lithology, weather conditions, and type of drill bit. However, good recovery does not equate to quality core for laboratory analyses given the earlier highlighted artifacts. The length of the recovered core may not be a good reflection of the length of the stratigraphic section cored. Harder material is most likely to be recovered while soft material can be crushed or washed away, thus reducing its occurrence in the recovered

stratigraphy. In these cases, composite borehole log interpretation, utilizing borehole geophysical and Multisensor Core Logger (MSCL) data, is important to indicate what sections are recovered and how they are to be placed in the actual stratigraphy. Stratigraphic resolution with this type of corer will be very good in hard materials but will quickly diminish where uncemented, friable, or non-cohesive sediments occur in the section. Subsamples of such cores, hence, need to be carefully extracted.

In general, core portions used for bulk analyses should be disassembled and cleaned of foreign materials before use. If, in later years, bulk samples are made available for analyses by scientists that were no part of the drilling team, it is important that the core's full history is known, so that anomalous or unexpected results can be interpreted in the light of possible artifacts of the drilling, storage or preparation of the material. Detailed drilling and core interval logs should be held with the composite geological core log and identify, as a minimum, the type of drilling and coring tools used, and details of any drilling mud/fluid utilized. The latter information is important to eliminate geochemical anomalies in scientific results. For example, contact of long-deposited sediments with current lake water may leave a signature in the stored core. Although the drilling mud will not directly affect fossil material (sections 2.2.1., 2.2.2.), unless there are secondary reactions during storage, it may affect microbial activity, metabolites, and decay products as some types of drilling mud contain guar, which is a food source. For microbiological studies (see section 2.2.3.), tracers used to detect 'contamination' need to be documented. Moreover, if microspheres were utilized, they may still be present in some samples.

Subsamples of sediment cores from scientific drilling projects are increasingly being used for multidisciplinary studies in a much wider scientific aspect than that of the original project and thus particular emphasis should be placed on understanding the circumstances in which the original data were collected and records were archived (for a review see Cohen, 2012). Any subsample from a core section needs to have the depth of the core section as an unmistakable criterion, as correlation depths derived from the correlation of cores from parallel holes (so-called core composites) may change with results from ongoing measurements (e.g., high resolution logging). Ideally, these core composites are recalibrated to borehole logging data prepared from a number of boreholes at the same site. However, significant marker horizons (e.g., tephra deposits), being used as the correlation basis for the composite borehole log are not always available.

Storage in controlled and cool temperatures will minimize moisture loss, bacterial activity (see section 2.2.3.) and ancient DNA decay (see section 2.2.6.), and allow physical property measurements to be extended by a few months. Cooler temperatures will also slow down any secondary chemical reactions or existing core alteration.

Drilling operation and coring methodology define initial core quality and determine the degree of physical or chemical 'contamination' that may be anticipated. Archiving and storage imprints, further characteristics on the core, and accurate subsampling interpretation require a full tracking record of the core from collection to interpretation with subsample positions clearly archived in relation to the final composite borehole log. These logs should also show where subsamples have been taken from and regularly updated as new information is generated.

2.1.3. Borehole logging

Borehole logging is the process of measuring physical, chemical, and structural properties of penetrated geological formations via tools that are lowered into a borehole on a wireline cable. It provides *in situ* information about the physical properties of the rock or sediment strata and groundwater. Borehole logs deliver a continuous record that provides information on the lithological changes with a precision of decimeters to a few centimeters. Since it allows depicting actual depth and petrophysical characteristics, information from borehole logging is often used in combination with seismic reflection data to construct geological models. In addition, the combination of downhole logging data and petrophysical datasets from several drill holes and cores from the same site are essential to construct a composite lithological log. More recent applications include the derivation of paleoclimatic indicators and cyclostratigraphic analyses.

The main components of logging equipment are a surface unit, winch, cable, and logging tools equipped with variable detectors and/or sensors. The surface unit is used to control the measurements, including the movement of the tool in the borehole. It also provides the energy supply to the tool and records, displays, and stores the data generated in the borehole. The depth of the measurement in the borehole is independently recorded by a gauge on the winch. To allow depth correlation between all logging runs, each tool is equipped with a gamma ray sensor, which records the formation's natural

gamma radioactivity caused by its occurring contents of uranium (U), thorium (Th), and potassium (K).

A number of tools have been developed over the recent decades that maximize the number of physical parameters that can be measured in slim boreholes. The equipment, field application, and analytical methods have been described by, e.g., Ellis and Singer (2007) and Rider and Kennedy (2011). The most important tools/physical aspects of borehole logging in a lake drilling project are spectral gamma ray (natural gamma ray plus contents of U, Th, and K), magnetic susceptibility, resistivity, acoustic velocity, vertical seismic profiling (VSP), dipmeter, and caliper (borehole diameter and orientation). Furthermore, tools that register the density, neutron porosity, and the content of a selection of geochemical elements of the drilled formations are available. These tools emit ionizing radiation and contain either radioactive sources or neutron accelerators. The regulations for the import and export of these tools are complex and differ from country to country, which regularly limits their use in ancient lake drilling projects. Tools based on optical methods like video cameras or optical televiewers exist as well, but they cannot usually be operated in lake drillings because the drilling fluids are not translucent.

The various downhole logging methods together with a sensor configuration adapted to the measurement conditions is used to obtain data from a limited, irregular rock volume. The vertical and radial extent of this volume is influenced by the borehole diameter, the physical properties of the content of the borehole, the ratio of the borehole diameter to the diameter of the tool, the position of the tool in the borehole, and the design of the tool (detector size, electrode spacing, transmitter-receiver spacing, radioactive source-detector spacing). Thus, each tool has a characteristic depth resolution and an average radial depth of investigation under the given conditions.

The logistical effort involved with transport and installing logging equipment at the drill site can be high, particularly when the gear has to be transported to a floating drilling barge. These barges are typically not equipped with heave compensation, so that logging operations need to be conducted during appropriate weather conditions. Final decisions about holes to be logged during or at the end of drilling operations can change quickly and are dependent on the overall progress of the drilling operation.

Downhole logging measurements in ancient lake drilling projects are typically made in unconsolidated sediments. Due to the specific sensor requirements, most physical parameters have to be measured in an open hole. To reduce the impact of potential borehole wall collapses, logging is

performed in borehole sections. Their number and individual length (down to 30 m) need to be defined in close cooperation with the drilling supervisor and leads to a significant prolongation of the logging time. The logging speed depends on the tools/sensors used, and has a large influence on the quality of the data. Typical logging speeds are between 60 m h⁻¹ and 600 m h⁻¹. The sampling interval also influences data quality. It is typically 5–10 cm, with a vertical resolution of about 20 cm.

The quality of the borehole logs are dependent on the measurement conditions and the depth resolution of the tool. The conditions of logging are determined primarily by the borehole geometry, the physical properties of the drilling fluid (density, electrical resistivity, pH, neutron braking, and absorption properties), and the properties and size of the flushed and invaded zone. Borehole logging is thus an important and versatile tool in ancient lake drilling projects and applications include:

i) Lithological classification of the strata penetrated by a borehole.

Characteristic physical rock parameters, especially when combined, can be used to determine or confirm the lithology of the rocks or sediment successions. The most useful parameters for this are gamma radiation, magnetic susceptibility, resistivity, acoustic velocity, and caliper.

ii) Site-to-site/hole-to-hole correlation of lithological units.

Correlation of lithological units are crucial for obtaining core-composites and for linking stratigraphic positions across boreholes (see section 2.1.2.). Best suitable are gamma-radiation and magnetic susceptibility. Furthermore, magnetic susceptibility data has the potential for identifying tephra layers that are important chronostratigraphic marker horizons in sediment successions.

iii) Paleoenvironmental/paleoclimatic reconstructions.

Paleoclimatic indicators were derived from physical properties of the sediments from lakes El'gygytgyn (Nowaczyk et al., 2013), Van (Baumgarten and Wonik, 2014), and Ohrid (Baumgarten et al., 2015). Sediment records from these lakes display strong shifts in physical and chemical properties with depth that are tied to different environmental/climatic states.

iv) Cyclostratigraphic analyses.

Contrasting physical properties and therefore changes in the sediment characteristics can trigger cyclic changes in the logging data. Cyclostratigraphic analyses in lakes based on physical parameters from cores and/or boreholes were conducted by Nowaczyk et al. (2013), Baumgarten and Wonik

(2014), and Baumgarten et al. (2015). Under favorable sedimentation conditions, results from cyclostratigraphic analyses based on downhole logging data even allow estimates of varying sedimentation rates and establishment of reliable age-depth relationships (see section 2.1.4.).

v) Time-depth conversions.

Measurements of acoustic velocities in boreholes are useful for the interpretation of shipborne seismic reflection surveys. Therefore, for the geological modeling based on seismic data, downhole logs (acoustic velocity and VSP) can provide essential information necessary to develop time-depth conversions for seismic datasets.

vi) Inference of structural/tectonic features.

Important information needed for tectonic and structural geology investigations include the dip angle, strike direction, and angle of formations. These parameters can be inferred in the borehole using a dipmeter tool. Layers with thicknesses as small as 1–2 cm can be detected.

vii) Hydrogeological and geotechnical parameters (e.g., clay content, porosity).

Data registered from the acoustic velocity logging tool can be used to determine porosity.

Assumptions about the lithology and fluid properties based on local knowledge or other measurements have to be made to estimate porosity. Spectral gamma ray logs may help to distinguish between different clayey sediments based on their U, Th, and K content. Acoustic velocity logs via the compression modulus provide indirect information about the storage coefficient of the rock or sediment successions.

Due to technical difficulties, micro-resistivity imagers and nuclear magnetic resonance (NMR) equipment have not been applied in slim boreholes so far (for details of the methods see, e.g., Kenyon, 1997; Coates et al., 1999; Dunn et al., 2002). With the former method, detailed, high-resolution spatial data providing structural and textural information can be obtained from the measurement of resistivity with a large number of electrodes (Lovell and Parkinson, 2002). Using a NMR tool, a real time permeability log may be possible. Also, the application of interpretative methods for hydrocarbon exploration and basement rock data (e.g., multivariate statistics) to ancient lake drilling projects can provide further possibilities to combine geophysical data from boreholes with other geophysical and geochemical datasets towards more holistic analyses in the future.

2.1.4. Sediment-core data

Standard measurements on sediment cores can be separated into those conducted on whole core sections, on split core surfaces, and on discrete samples (Fig. 3).

Fig. 3. Generalized scheme of core processing for basic sedimentological analyses. Individual steps and analytical methods may vary across projects.

Whole core measurements are typically performed just after drilling on site and/or shortly before core opening in the laboratory. For these purposes, so-called multi-sensor core loggers (MSCL), which can be described as a logging bench unit, are commonly used. MSCL systems allow stepwise measurements of geophysical parameters at centimeter resolution and are commonly equipped with variable sensors/detection systems for magnetic susceptibility, gamma ray density (GRAPE), P-wave velocity, natural gamma spectroscopy, and resistivity (e.g., Weber et al., 1997). These datasets provide a first, rough overview about changes in sediment composition (density, detrital/magnetic mineral content, U-, Th-, K-concentrations) before the cores are opened and sediments are exposed to the atmosphere. They

are useful to establish hole-to-hole correlations, to identify gaps in the core recovery, and to assist adjusting the drilling strategy. P-wave velocity data are needed to establish time/depth conversions for acoustic and seismic reflection datasets, thus allowing for more sophisticated interpretations of seismic units and their correspondence to changes in lithology. Furthermore, obtaining these whole core measurements is a prerequisite for geomicrobiological sampling (see also section 2.2.4.), which needs to be undertaken in the field shortly after core recovery and consumes entire core sections. Hence, if geomicrobiological datasets need to be tied to a stratigraphy/chronology or compared to other analyses obtained on overlapping intervals later on, this can only be achieved using whole core MSCL datasets. MSCL datasets can be biased by cracks in the sediments due to gas expansion or by incompletely filled liners, such as is common when using rotational drilling tools (see section 2.1.2.).

Macroscopic lithological core descriptions on split core surfaces, supported by microscopic smear slide analyses of major biogenic and minerogenic sediment components, are used to define major lithologic (i.e., pelagic sediments, mass wasting deposits, tephra layers) and sedimentary/stratigraphic units. This information is not only important to determine changes in the depositional environment but also allows more targeted lithotype-specific subsampling strategies to be developed. Detailed and spatially highly resolved (down to 0.2 mm) geochemical analyses on split core surfaces can be performed using X-Ray Fluorescence (XRF) core-scanners. Newer systems allow the analysis of major and minor elements heavier than Al at high precision and permit, besides inference of relative changes in concentration, also quantification upon calibration (e.g., Russell et al., 2014). Relative changes in intensities as well as ratios of specific elements are useful indicators for changes in sediment composition (e.g., Melles et al., 2012), sediment provenance (e.g., Kylander et al., 2013), redox conditions (e.g., Naeher et al., 2013; Costa et al., 2015), diagenetic overprinting, grain size/depositional processes (e.g., Bloemsma et al., 2012; Francke et al., 2016), and to identify the occurrence of tephra or cryptotephra layers (e.g., Vogel et al., 2010a). The quality of the data depends on the analytical time and on several sedimentological characteristics, such as surface smoothness, water content, or grain size distribution. XRF scanning of marine and lacustrine core sequences has become a standard method to obtain a rapid, spatially highly resolved overview of changes in element composition, but the method is time consuming at high resolutions. Requiring an analysis time of up to several hours per meter sediment

core, the scanning of several sediment sequences from one site with a total length of hundreds of meters may take several weeks. Moreover, due to the high scanning resolution, the amount of data produced is immense and often requires smoothing or filtering in order to facilitate the identification of major trends of element variation in sediment cores and to reduce background noise. Therefore, careful selection of the resolution based on other data, e.g., reduction of resolution if a section of sediments appears homogeneous, for example, due to bioturbation or mass movement processes, can significantly reduce measurement time and facilitate data handling.

Analytical methods on discrete samples comprise a broad range of granulometric, geochemical, and biological methods. Much variation exists in the amount of preparation and time that are required to apply individual methods, e.g., sample preparation or identification of micro- and macrofossils can be very time consuming. Significant differences also exist in the required amount of sediment needed to perform each analysis. Some geochemical methods require only a few milligrams of powdered material, but others, such as paleontological studies may need several grams of sediment or more. Both labor-intensity and availability of material affect the sampling resolution, with the separation between subsequent samples usually varying from one centimeter to several decimeters.

Granulometric analyses are used to obtain information on sediment transport history and energy (fluvial, aeolian, pelagic, gravity driven). The measurement of grain-size distributions can be done relatively fast with a laser particle size analyzer for sand- to clay-sized fractions, as are common in lacustrine sediments. However, sample preparation is necessary to extract information on transport and depositional processes unbiased by autochthonously derived sediment components. Sample preparation can be labor-intensive if removal of organic matter, carbonates (authigenic, biogenic), biogenic silica from diatoms, phytoliths, or sponge spicules is required. Standard geochemical analyses often comprise the measurement of total organic carbon (TOC), total inorganic carbon (TIC), total nitrogen (TN), total sulphur (TS), and require only relatively low amounts of powdered material. TOC and TN concentrations are useful indicators that provide estimates on changes in intralacustrine productivity (e.g., Wagner et al., 2009; Vogel et al., 2010b) and/or changes in the supply of terrestrial organic matter (e.g., Meyers, 2003). However, TOC and TN concentrations in the sediment are not only influenced by their initial fluxes but also by the degree of post-burial remineralization, which in most settings is

controlled by lake-mixis and the availability of oxygen in the hypolimnion and top sediments (e.g., Melles et al., 2007). TIC concentrations are a measure of the amount of carbonate, which can be present in biogenic, endogenic, detrital, and/or authigenic form in lacustrine sediments. Calcite and aragonite (both CaCO_3) are the predominant carbonate phases in most freshwater settings and changes in their concentrations are usually driven by temperature, productivity, and hydrological variations (e.g., Kelts and Talbot, 1990; Wick et al., 2003; Wagner et al., 2009; Vogel et al., 2010b). However, other carbonate phases, such as siderite (FeCO_3), dolomite ($\text{CaMg}(\text{CO}_3)_2$), and/or ankerite ($\text{Ca}(\text{MgFeMn})(\text{CO}_3)_2$), can also be present in certain settings (e.g., De Decker and Last, 1988; Stevens et al., 2012; Lacey et al., 2015). Moreover, the measurement of isotopes, such as carbon or oxygen isotopes (see section 2.1.6.), require relatively small quantities of sediment if the concentration of biomineralized carbon or oxygen is sufficient.

A new analytical method that has been applied in recent deep drilling projects for the analysis of discrete samples is Fourier Transformed Infrared Spectroscopy (FTIRS). FTIRS is a relatively fast and cost efficient method, which requires small sample amounts and can be used to infer absolute concentrations of biogenic silica (bSi), carbonate (TIC), and organic matter (TOC) with a single measurement (Vogel et al., 2008; Meyer-Jacob et al., 2014a). The low processing time might be important for the continuous analysis of long drilling sequences (e.g., Meyer-Jacob et al., 2014b) and for high-resolution studies of specific time slices in these sediment records (e.g., Cunningham et al., 2013). Further applications of FTIRS involve the determination of relative changes in the abundance of different carbonate phases (Lacey et al., 2016).

Overall, the combination of information from individual proxies and future progress in the development of analytical methods as well as scanning and logging techniques will significantly help to improve the study of environmental changes from sedimentary records in ancient lakes.

2.1.5. Age-depth models

Creating a reliable and robust chronological framework is fundamental for drilling studies across disciplines and, hence, also for the synthesized interpretation of paleoenvironmental, climatological, and biological data. Age-depth relationships in non-marine records are commonly established by combining

absolute chronological information from radiometric and magnetic dating methods, and from varve counting with chronostratigraphic information derived from comparisons of a proxy response to a reference record (e.g., Nowaczyk et al., 2013; Stockhecke et al., 2014; Francke et al., 2016). Similar to dendrochronology (tree ring counting), varve counting provides robust age-depth control points as varves consist of thin (millimeter scale), characteristic seasonal summer and winter deposits (so-called laminae). However, the preservation of varves in lacustrine sediments may depend on several factors, such as the absence of sediment-dwelling organisms or the presence of anoxic bottom water conditions.

Obtaining absolute ages from radiometric dating techniques (Table 1) or techniques that utilize radiometrically induced lattice effects in certain mineral phases requires the presence of suitable/datable materials in the studied sediment sequence (reviewed in Bradley, 2014). In addition, the different dating methods cover different time ranges. Consequently, various radiometric dating techniques are typically used in combination and choices depend on the age range covered in a core, the anticipated/estimated age of a specific dating point, and the presence of suitable materials. Whereas the relative error of some of these techniques is small, absolute uncertainties increase in the deeper sediment record and obtained dates can be biased by a variety of physical and chemical effects. Nevertheless, dates from radiometric methods provide the most precise chronological tie points, and are therefore introduced as 1st order constraints in age-depth calculations.

Table 1

Selected absolute dating techniques for sediment cores. ESR = Electron Spin Resonance; OSL = Optically Stimulated Luminescence. ¹⁰Be refers to burial dating.

	¹⁴ C	Ar/Ar	U/Th	U/Pb	OSL	ESR	¹⁰ Be
Material	Organic matter/ carbonate fossils	Volcanic glass	Endogenic/ authigenic carbonates	Endogenic/ authigenic carbonates	Quartz/ feldspar	Carbonates	Quartz
Age range (My)	0–0.05	~0.01–4,600	0–0.5	1–4,600	0.001–0.2	0.001–0.2	~0.01–4
Precision (%)	1–5	5	1	0.1–1	~10	~10	10–20

Additional 1st order constraints can be obtained by correlating the chemical composition of tephra

layers to the volcanic eruption of which the age is known or to tephra layers with identical composition found in other, well-dated sediment sequences (e.g., Wulf et al., 2008; Sulpizio et al., 2010; Vogel et al., 2010a; Leicher et al., 2015). However, an unequivocal correlation of tephra layers to known eruptions based on their chemical composition is, due to the often encountered similarity in glass shard chemistry of ejectas from different eruptions, not always straightforward. In recent studies, these limitations have been partly overcome by additional measurement of the trace element and isotope chemistry of glass shards (e.g., Sulpizio et al., 2013; Insinga et al., 2014; Albert et al., 2015; Tomlinson et al., 2015).

Further independent age control can be provided by paleomagnetic data. Polarity reversals and excursions of the earth's magnetic field are common in the geological record (Merrill et al., 2006). Moreover, the intensity and vector (secular variation) of the Earth's Magnetic Field varies within magnetic chrons. As magnetic particles will be aligned during and after settling on the lake floor, their orientation in the sediment core depends on the direction and strength of the ambient magnetic field during or shortly after deposition (depositional remnant magnetization, DRM). Alignment of particles during deposition is, however, not always an instantaneous process and different factors, such as the lock-in depth of DRM (i.e., the sediment depth where magnetic particles are aligned to the ambient magnetic field, important for sedimentation rates of $<10 \text{ cm ky}^{-1}$; Roberts and Winklhofer, 2004) and early diagenetic formation of ferrimagnetic minerals (important in low oxygen environments) should be considered (see Tauxe, 1993; Roberts et al., 2013 for more details). Age information from well-dated, paleomagnetic reference records can be transferred to the analyzed sediment sequence, if similar magnetic features were identified (Peck et al., 1996; Frank et al., 2002; Nowaczyk et al., 2013). A number of magnetic reversals and excursions have been dated using absolute radiometric dating methods (Laj and Channel, 2009 and references therein). This is, however, not yet the case for the entirety of these events in the geological history. In some cases, the chronology of the paleomagnetic reference records is based on synchronization of marine benthic oxygen isotope data of either individual or stacked records with orbital parameters, standard oxygen isotope stacks (SPECMAP and LR04), or ice-core records (GISP2). Where absolute age control of paleomagnetic events based on radiometric methods is absent, ages derived from magnetostratigraphy should not be regarded as absolute age control points.

Downhole magnetostratigraphic measurements can be performed even with logging-while-drilling

tools, such as the geological high-resolution magnetic tool (GHMT), which is based on high-precision measurements of the total magnetic field and the susceptibility field with a magnetometer. Combining these two measurements with the Earth's total magnetic field at the surface close to the borehole allows deriving *in situ* remnant magnetizations of the drilled sedimentary units (Luthi, 2001; Paulissen et al., 2011).

Correlation of a proxy response measured in a sediment record to local insolation or to the global benthic foraminifera oxygen isotope stack LR04 (Lisiecki and Raymo, 2005), which primarily displays ice-volume/sea-level changes during the Quaternary, is a chronostratigraphic method that is often used to refine age-models when independent age control points are sparse. At Lake Ohrid, for example, variations in the length of local insolation during summer and winter trigger changes in the primary productivity and mixing in the lake (Francke et al., 2016). These variations affect the TOC content of Lake Ohrid's sediments, which allow tuning the total organic carbon concentrations with the Earth's orbital parameters (i.e., local summer insolation and winter season length). At Lake El'gygytyn, various stratigraphic parameters, which are related to the redox conditions at the lake floor and to the climatic conditions in the surroundings of the lake, show variation synchronous with the northern hemisphere insolation and the global benthic isotope stack LR04 (Nowaczyk et al., 2013).

Tuning of a proxy response against reference records requires a thorough understanding of the processes that shape the proxy response to interpret possible leads and lags compared to the reference dataset (Prokopenko et al., 2006). This is particularly important in lake studies as proxy/forcing relationships are strongly dependent on site characteristics. Ideally, leads and lags between proxy responses and reference records should be tested on horizons for which absolute time markers are available in both the studied and reference records. Zanchetta et al. (2015) showed that tuning against an absolutely-dated reference record from the vicinity of the study site can improve the understanding of a proxy response compared to insolation and ice-volume reference records. Furthermore, this approach may enable the identification of the synchronicity or asynchronicity of climate events compared to the reference records and other, absolutely-dated regional records. The feasibility of such endeavors, however, depends on the availability of suitable reference records in the region.

Chronological constraints adopted from tuning against a reference record comprise two potential

errors: Uncertainties introduced by the tuning and the uncertainty of the age model of the reference record. Therefore, these tie points are not independent and introduced as 2nd order constraints (e.g., Nowaczyk et al., 2013; Francke et al., 2016). For example, the chronology of the global benthic isotope stack LR04, which is frequently used as reference record (see above), comprises uncertainties in a range of ± 4 –40 ky for a timeframe of 0–5.3 My, as the age model of LR04 is based on tuning the benthic isotope data to the 21 June insolation at 65°N (Lisiecki and Raymo, 2005). For records younger than 1 My (a relevant range for most lake sediment studies), the error of LR04 is estimated close to ± 4 ky.

Fig. 4. Flow chart illustrating the required steps to establish an age-depth model.

Once a sufficient number of data points have been obtained via the outlined methods, a quantitative age-depth model can be calculated by interpolation between the individual age control points (Fig. 4). For this purpose, various interpolation methods, such as linear interpolation, linear regression, polymodal interpolation, or smooth spline interpolation, can be used. Considering the sedimentological characteristics, expected sedimentation rates, and other stratigraphic information, including the position of event layers (mass wasting deposits, tephra layers) and hiatuses forms the basis for deciding which interpolation method is most appropriate. Linear interpolation implies abrupt changes in the sedimentation rate at each age control point (cf. Blaauw, 2010; Blaauw and Christen, 2011), which, in most cases, is not supported by the lithological characteristics and stratigraphic information from the studied sediment succession. Polymodal or smooth spline interpolation methods calculate more gradual changes of sedimentation rates and are often more suitable for age-depth modeling. Non-linear changes in sedimentation rates between two age points are incorporated in new age-depth modeling software, e.g. Bacon v2.2 (Blaauw and Christen, 2011), which has been applied only recently for age-depth modeling

on long lacustrine sediment sequences (e.g., Shanahan et al., 2013; Francke et al., 2016). It uses Bayesian statistics and Markov Chain Monte Carlo iterations to infer the accumulation history based on a priori assumptions about the sedimentation rate and its variability over geological time (Fig. 5). Another advantage of this approach is that uncertainties of tuning and independent age points can directly be included into the age-depth modeling (Fig. 5).

Fig. 5. Age model of the DEEP site sediment sequence from Lake Ohrid (Macedonia, Albania) down to 247.8 meter composite depth (mcd) corresponding to an age of 637 ky. Modified from Francke et al. (2016).

In summary, age-depth models, which reveal the relationship between increasing sediment depth and age (Fig. 5), are a prerequisite to integrate and interpret biological and geological data. However, as each dating technique has its specific limitations, their respective uncertainties should be considered during age-depth calculations (Blaauw and Heegaard, 2012).

2.1.6. Stable isotopes

The analysis of stable isotope ratios in mineralized components from lake sediments (Leng and Marshall, 2004), are well established in paleoclimatology, paleolimnology, and limnogeology since the early work of McCrea (1950) and Urey et al. (1951). Stable isotopes (in particular $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$; Leng

and Marshall, 2004) are incorporated into a number of different components that precipitate or grow in lake waters and subsequently get deposited within the sediments, for example shelly materials, fine grained calcium carbonate crystals (a chemical precipitate called endogenic carbonate), and diatoms (siliceous algae, see also section 2.2.2.). Paleoclimate studies commonly use changes in the isotope compositions throughout the sediment succession to infer changes in either temperature ($\delta^{18}\text{O}$) or the isotopic composition of lake water ($\delta^{18}\text{O}/\delta^{13}\text{C}$). With $\delta^{18}\text{O}$, the latter could be a function of changes in/or at the source of water to the lake (changes in moisture source) or the precipitation/evaporation balance. By looking at changes in $\delta^{18}\text{O}$ through time, and depending on the characteristics of the lake in question, it is possible to reconstruct an aspect of paleoclimate for a particular location. With $\delta^{13}\text{C}$, changes are usually ascribed to the source of the dissolved carbon ion or changes in productivity of the aquatic plants and phytoplankton, which preferentially utilize ^{12}C (Leng and Marshall, 2004).

In ancient lakes, where the isotope composition of the lake water has been demonstrated to reflect mean annual precipitation, it has been shown that a signal of climate change can be determined from the analysis of biogenic calcite from the shells of benthic ostracods, which live below the thermocline and are thus not affected by seasonal shifts in temperature or evaporation. For endorheic lakes, the isotopic composition of the carbonate has been shown to reflect lake-level, most significantly between glacial-interglacial cycles. In Lake Ohrid, for example, these cycles suggest that the lake has been subject to hydroclimate fluctuations on orbital and millennial timescales (Lacey et al., 2016).

Despite an increase in the application of stable isotope techniques in paleolimnology, interpreting stable isotope results can be challenging. These challenges may be general or specific to a core, lake, or basin. The vast majority of studies focus on oxygen isotopes because their interpretation can be linked directly or indirectly to climate change. Factors that have an influence on the oxygen isotope composition of the lake water ($\delta^{18}\text{O}_{\text{lakewater}}$) are given in Fig. 6. The oxygen isotope composition of water in hydrologically open lakes ($\delta^{18}\text{O}_{\text{water}}$) will dominantly reflect the isotopic composition of the precipitation received by the lake ($\delta^{18}\text{O}_{\text{precipitation}}$ or δp). Many studies have shown that the $\delta^{18}\text{O}$ signature of mean annual precipitation varies globally between regions and covariation in δp (and δD) defines a global meteoric water line (Craig, 1961). Outside the tropics, where ‘amount’ effects (relating to intense

precipitation events) are common, and δp varies systematically with mean annual temperature (Clark and Fritz, 1997; <http://isohis.iaea.org/GNIP>), δp thus broadly correlates with latitude and altitude of a site (Bowen and Wilkinson, 2002 and references therein). The global relationship between changes in δp with temperature is referred to as the ‘Dansgaard relationship’ (after Dansgaard, 1964).

Fig. 6. Overview of factors that can influence the isotope composition of a lacustrine carbonate or biogenic silica ($\delta^{18}\text{O}$). For equilibrium mineral precipitation, the oxygen isotope composition of the mineral is controlled only by the temperature and by the isotope composition of the lake water from which the mineral precipitated. If a mineral is precipitated in isotope equilibrium, paleotemperature equations can be used to estimate past temperatures and their changes. Other factors, such as pH, mineral speciation, and rates of mineral precipitation, may affect the fractionation relationship. (From Leng and Marshall, 2004).

In many lacustrine environments it cannot be assumed that either the modern or past $\delta^{18}\text{O}_{\text{lakewater}}$ reflect that of mean annual precipitation. The residence time of water in the lake and modification of water compositions by catchment and lake processes are particularly important to consider as evaporation will affect the water composition. The size of a lake in comparison to its catchment is important because the isotope composition of rain and snowfall are very variable on short time scales: A lake therefore needs to be big enough and well enough mixed for its isotope composition to ‘average out’ the short-term variation and reflect mean annual precipitation. The greatest degree of variation in the isotope composition of rainfall occurs on the time scale of hours to days. Seasonal variation in

precipitation is likely to be much more significant in small, short residence time lakes as these tend to have $\delta^{18}\text{O}_{\text{water}}$ values that are regularly displaced by later precipitation. However, if the inference of intra-seasonal fluctuations is of interest, growth increments analyses of the isotope composition of molluscan shells will often provide intraseasonal information (Kaandorp et al., 2005; Taft et al., 2012; Leng and Lewis, 2014).

At temperate latitudes, monthly mean rainfall $\delta^{18}\text{O}$ typically have an overall range of 2–8‰. The range increases with continentality of the site. At such sites, winter rainfall has significantly more negative $\delta^{18}\text{O}$ than its summer equivalent. If a lake is very small in relation to its catchment (with residence times of < 1 year), winter rainfall will be physically displaced by summer rainfall and thus $\delta^{18}\text{O}_{\text{water}}$ will be influenced by seasonal variation. Therefore, deep ancient lakes with their long water residence time are particularly useful for isotope studies. The precise isotope composition of lake water at any time will depend on the amount of rain in the different seasons and the degree of mixing of winter and summer rainfall. Even in lakes with relatively long overall residence times, surface waters may have isotope compositions that reflect summer rainfall rather than mean annual precipitation if the waters become stratified.

The $\delta^{18}\text{O}$ of components within lake sediments contain information on temperature, and there are many empirically derived 'paleotemperature equations'. During equilibrium precipitation, carbonates for example, have $\delta^{18}\text{O}$ compositions that decrease by about 0.24‰ for each 1°C increase in temperature (Craig, 1965). There is a number of paleotemperature equations for the equilibrium precipitation. For example, Kim and O'Neil (1997) proposed an equilibrium fractionation relationship, which was reordered by Leng and Marshall (2004). Assuming that equilibrium precipitation has occurred, the interpretation of oxygen isotope data in terms of paleotemperatures requires an understanding of two processes that have opposing effects on the composition of a carbonate or silicate precipitate. At intermediate and high latitudes, the oxygen isotope composition of mean annual precipitation correlates directly with change in temperature with a gradient of approximately +0.6‰ °C⁻¹ (Dansgaard, 1964). Opposing this effect from being transferred directly into the sediment record, the equilibrium isotope fractionation between carbonate and water has a gradient of around –0.24‰ °C⁻¹. For many lake records,

the carbonate response to temperature will be dominated by the change in the isotope composition of precipitation and effectively ‘damped’ by the opposing effect of mineral-water fractionation. In this case, the measured carbonate values will covary with temperature – with an increase of $\sim 0.36\text{‰ }^{\circ}\text{C}^{-1}$ (Eicher and Siegenthaler, 1976). This is reasonable for the paleoclimatic interpretation of many lakes but it implicitly assumes that $\delta p/dT$ always changes according to the Dansgaard relationship.

Evaporation has a major influence on the oxygen isotope composition of any standing water body. For closed (terminal) lakes, where water loss is mainly through evaporation, lake waters tend to have high $\delta^{18}\text{O}$ (Fig. 6). Measured $\delta^{18}\text{O}$ (and δD) values are always higher than those of average precipitation as the lighter isotopes of ^{16}O and (^1H) are preferentially lost to evaporation. Isotope records from such lakes show large swings in composition as the ratio of the amount of precipitation to evaporation (P/E) change with climate. Any interpretation of the isotope records from a lake must take into account the hydrology of the lake and likely changes in hydrology that may have occurred in the past.

Endogenic carbonates are still the most commonly utilized materials for stable isotope analysis. Endogenic carbonates are formed by photosynthetic utilization of CO_2 and resultant calcium carbonate supersaturation (often mistakenly referred to as authigenic carbonate). In most temperate and high-latitude regions, endogenic carbonates are precipitated mainly in the summer months during periods of maximum phytoplankton productivity (Leng et al., 1999b, Teranes and McKenzie, 2001). In mid-latitude and tropical lakes, phytoplankton growth may occur throughout the year although other mechanisms, such as supersaturation, may also cause continuous carbonate precipitation (e.g., the Dead Sea aragonite; Niemi et al., 1997). More commonly in the tropics, carbonate precipitation is related to phytoplankton blooms associated with annual lake-water mixing and nutrient availability (*sensu* Lamb et al., 2002). The advantage of using endogenic carbonate in stable isotope studies is that it provides an integrated climate signal for the whole sample, which may be time-averaged over several years, depending on sedimentation rate. However, there are some potential problems. It can be difficult to distinguish between authigenic (diagenetic carbonate formed within the sediment) and allogenic (detritus derived from the terrestrial environment) carbonates, especially in karstic regions, so there is always the possibility of contamination of the isotope signal from a washed-in component (cf. Hammarlund and Buchardt, 1996). Some carbonates do not precipitate in equilibrium with their environments.

Disequilibrium effects (often called ‘vital effects’ in biogenic materials) have been attributed to rates of precipitation, pH effects, incorporation of metabolic fluids, and growth in microenvironments not typical of the water body as a whole (Leng and Marshall, 2004). Also, there are several endogenic carbonate minerals that could precipitate out in a lake and each mineral has its own mineral-water fractionation (see above). In freshwater systems, calcite (CaCO_3) usually forms. However, with increasing evaporation, other forms can occur, such as aragonite (CaCO_3) and dolomite ($\text{CaMg}(\text{CO}_3)_2$) (e.g., Lake Bosumtwi; Talbot and Kelts, 1986). Physically separating different carbonate minerals is not easy, although respective protocols are available (e.g., Dean et al., 2015). Diatom silica $\delta^{18}\text{O}$ is being increasingly utilized in paleolimnology, and many of the issues are the same as with carbonates. However, the main consideration is that almost pure diatom samples are required as extraction techniques will liberate oxygen from all the components in the sediment (Leng and Barker, 2006).

Overall, isotope geochemistry is an essential part of paleoclimatological and (paleo-) limnological research. Over the last few decades, the analysis of isotopes in carbonate materials has contributed significantly to our understanding of a broad array of environmental change research. Nevertheless, advances are still to be made. These include better preparation, analysis, and interpretation of some of the less routine materials (other than carbonate), such as biogenic silica and chironomid chitin (Leng and Henderson, 2013). There is also the up and coming field of determining the excess of ^{13}C – ^{18}O bonds in clumped isotopes (Leng and Henderson, 2013). Bulk carbon and nitrogen isotope ratios within organic materials are also frequently analyzed but do not provide specific information and, therefore, are not further discussed here. Moreover, paleoenvironmental studies targeting paleohydrology and biome dynamics increasingly use carbon and hydrogen isotope ratios of specific organic sources and compounds, such as leaf waxes and algal lipids (for details see chapter 2.2.4).

2.2. *Biological and paleontological data and methods*

2.2.1. *Macrofossils*

Macrofossils include all remains of organisms from the remote past large enough to be visible without a microscope. Although fossils are widely reported from lake sediment cores, obtaining

macrofossils is often a hope, though rarely an objective of drilling campaigns. The larger individual fossils are, the smaller the chance that such fossils will be picked up in a sediment core. The diameter of drill cores (typically 48–85 mm) sets a strong constraint on the possible recovery of macrofossils and their size. Even fossils with a maximum linear size of 2–4 cm unlikely fall within the categories of exquisite preservation and complete recovery. An additional difficulty for the recovery of macrofossils is that cores are typically cut and observed perpendicular to bedding planes, so that macrofossils may easily be overlooked. Recovery of macrofossils from a drilling core may also require disturbing the sediments more than is desirable at the early stages of core documentation (see section 2.1.2.). To overcome this issue, CT- or radiographic images can be obtained of core sections during the core documentation procedure just after opening a core.

Macrofossils may be obtained from sediment cores as complete body fossils, such as mollusks, plant leaves, or isolated biomineralized parts, such as bones, teeth, charophyte oogones, seeds, plant cuticles, and wood fragments (Taviani et al., 2000; Reinthal et al., 2011; Cohen, 2012; Pepe et al., 2013; Mazzini et al., 2015). Lake sediments regularly yield fossils with exceptional preservation (e.g., Richter and Wedmann, 2005; Smith, 2012), and sometimes the temporal resolution is extraordinary, i.e., centennial to even annual scales (Bell et al., 2006; Hunt et al., 2008; Van Bocxlaer and Hunt, 2013). The choice of drilling sites affects the chances to recover macrofossils as well as fossil preservation (reviewed in Cohen, 2012). For example, drilling in the deeper waters of a meromictic lake is unlikely to yield many macrofossils because the benthic habitats at these sites can usually be expected to have been inhabitable for benthic life for most of the lake's history (see section 2.1.1.), although remains of pelagic organisms, such as lake sardines (Cyprinidae) may end up in such cores (Reinthal et al., 2011). Hence, macrofossils from nearshore habitats generally can be found only in low abundances in offshore cores. Drill sites relatively close to the shores, in general, provide greater potential to recover macrofossils (Harzhauser et al., 2013). However, such sites usually contain large proportions of sand and gravel (indicative for greater water energy and poorer preservation potential), and are subject to sediment redeposition, which makes them undesirable targets for paleoclimate studies and difficult to drill (see also section 2.1.1.). Mid-lake topographic highs are often selected as disturbance can be anticipated to have been limited, but such sites are unlikely the most suitable target of drilling from the perspective of macrofossil recovery. If

recovered, macrofossils may serve several research goals, and because of the practical limitations to find macrofossils, we discern here between goals that can be reached with a low number of specimens and those that require the sampling of larger numbers of macrofossils.

Single or scarce macrofossil finds may provide valuable information for the study of paleoenvironments or organismal evolution. They can also facilitate dating analyses, either directly if the fossil provides datable substances (e.g., charcoal, shells, seeds of terrestrial plants, etc.), via the study of ancient amino acids (see section 2.2.5.), or if the macrofossil can be placed into a biostratigraphic framework for the studied basin. It can also be used indirectly if the fossil can be inserted with reasonable constraint into a time-calibrated phylogeny of extant taxa (see section 2.2.9.). Moreover, macrofossils may provide unique opportunities for paleolimnological reconstructions, either via isotope studies (see section 2.2.8.) or via reconstructions directly based on the fossils' properties or habitat characteristics (see section 3.1.). Beyond calibrating the molecular clock (see section 3.2.), macrofossils may give a good insight into organismal evolution including information on how long the taxon is present in the basin, on ancestral character states (see section 3.4.), and on morphological adaptation by comparing the morphology of the fossil to that of modern populations. In the most fortunate case, the fossil may be exceptionally preserved so that attempts to isolate ancient amino acids or DNA can be undertaken (see sections 2.2.5., 2.2.6.).

However, most biological goals relate to community changes over time or morphological evolution, and thus require sampling multiple taxa or individuals per interval (also see section 3.1.). As indicated above, chances to recover macrofossils in substantial numbers relate first to the size of such fossils, but furthermore also to preservation potential (e.g., CaCO_3 -bearing fossils will corrode when deposited below the local carbon compensation depth), general abundance, and lifestyle (e.g., benthic vs. pelagic). Therefore, most of the macrofossils that can be used for goals requiring abundant finds are only marginally larger than microfossils (e.g., fish teeth, or small mollusks; see section 2.2.2.). Continuity of the recovery of such fossils throughout a core relates primarily to the preservation potential under changing chemical conditions (dissolution, corrosion, and abrasion diminish preservation potential) and the patchiness of a taxon's occurrence. Patchy taxa can be expected to occur with great numbers at few intervals, whereas more evenly distributed taxa would occur in smaller numbers throughout more

intervals within a sediment core. The abundance of the target taxon per interval of sediment is the main determinant of the resolution at which the taxon's macrofossils can be studied. For example, if an interval of 2 cm of sediment yields on average 40 seeds, but only 2 fish teeth and 4 mollusks, then the core can be analyzed in 2 cm intervals for plant remains, but perhaps only in 10+ cm intervals for the study of fish teeth and mollusks, which affects the power to discover trends (see the simulation in Fig. 7).

Fig. 7. The feasibility to detect ecological or evolutionary signals from drilling data depends on the length of a time series (here expressed as relative time). Simulated time series of a morphological trait (scaled in within-sample standard deviation units; sdu) are represented in A) and B). The trait indicated in blue was simulated with a model of directional change, the red trait with a model of morphological stasis. Parameters were identical for each model in A and B. Only over an extended period of time (B), morphological stasis and directional change can be distinguished (blue area = 2 sdu). Modeling was performed in R 3.3.1 (R Development Core Team, 2015) with the paleoTS package (Hunt, 2006, 2012).

Upon detecting macrofossil remains in lacustrine sediment cores, the specimens are usually picked out, identified and/or counted prior to further analyses, such as dating or the study of biomarkers, isotope and element geochemistry. The advantage of using macrofossils for the latter studies is that analyses may be performed on a single fossil and may yield data on, e.g., seasonal fluctuations. This procedure allows obtaining a more accurate signal than when individuals are pooled or a sample of bulk organic remains is analyzed, because these latter samples provide averaged signals.

Identifications of macrofossils may be challenging because taphonomic processes, such as time

averaging, sorting, and post-mortem pooling (e.g., deposition of two taxa with fine-scaled habitat differences in the same assemblage), might considerably alter the amount of variation observed in a fossil assemblage in comparison to that in a modern population (e.g., Bell et al., 1987; Bush et al., 2002). Furthermore, chronospecies or taxa with character states intermediate to those of two or more modern species remain a problem. They result in the poor applicability of identification keys and potentially in doubtful identifications—much of these aspects are inherent to fossils and hence, apply for microfossils as well. Initial exploratory analyses may be conducted to examine occurrence data (counts) belonging to different groups of taxa (e.g., endemics vs. non-endemics), or to calculate biodiversity or community estimators/indices to compare a number of target assemblages (e.g., faunal and floral compositions before, during, and after a climatic or geological event). If qualitative observations suggest potential morphological changes, measurements may be taken to accurately document these changes through time. Such measurements can range from traditional caliper-measurements to studies of size and shape with fractal dimensions or geometric morphometrics. The choice for a particular method typically depends on the complexity of the signal, the time required to document/measure a single specimen, and the total number of specimens to be studied. ‘Targeted macrofossils’ may also be studied with more time-consuming 3D scanning methods and/or biogeochemical analyses (e.g., to document chemical composition, to study diagenetic processes, or to get information on an environmental proxy).

After exploratory analyses, more in-depth statistical and time-series studies can be undertaken. Fossils (mainly microfossils) encountered in sediment cores are regularly used for analyses of community composition, often in relation to environmental change (e.g., Cohen et al., 2007; Kröpelin et al., 2008; Harzhauser et al., 2013; also see sections 2.2.2., 2.2.3.) or to document morphological change and evolution in the fossil record (e.g., Pearson and Ezard, 2014 and references therein). For studying shape/community changes related to environmental change or organismal evolution, statistical and time-series analyses provide a useful framework to explore and test relationships between predictor variables and organismal change, or to fit models of morphological evolution. Major determinants of the power of such time-series approaches to discover ecological patterns will be the strength of the association between the predictor variables and organismal/community change, the range of values of the predictor variable observed throughout the core, and the variation in the dependent variable. To reliably document

patterns of morphological evolution in a fossil lineage, a major determinant of analytical power will be the ratio of variation within individual samples to the changes between consecutive samples. For example, fewer specimens per interval will be required to document a strong morphological trend in a time series with limited within-sample variation than for a time-series with the same trend and great within-sample variation. Additionally, as mentioned, the number of sampled intervals throughout the core for which all required data are available and, hence, the length of the time-series is a determining factor of analytical power (Fig. 7). To assess the feasibility of time-series analyses with drill core data, detailed analyses of the anticipated patterns of change and the various components that contribute to the variation in fossil assemblages may be required. Explorations examining variation in homologous or analogous modern faunas and floras may be required to understand how different sources of variation contribute to the total variation observed in fossil assemblages. Dieleman et al. (2015) presented such an exploration for the study of fossil cichlid teeth from the African crater Lake Challa.

Offering suggestions on how future lake drilling campaigns could be designed optimally for the study of macrofossils is not easy. First, strategies depend on the group of macrofossils that is specifically targeted. Second, each ancient lake has unique ecosystems that differ from those of other lakes and the design of the program needs to be adjusted to the specific target lake. Third, the multidisciplinary nature of many drilling operations may regularly weaken the feasibility of obtaining a continuous fossil record due to compromises in site selection (Cohen, 2012; also see section 2.1.).

Nevertheless, promising subprojects based on macrofossils can be constructed, and the following aspects can augment the potential of such programs. Drilling campaigns usually aim to retrieve multiple cores and one (or some) of the drilling locations may be selected where abundant macrofossils can be expected (e.g., Wagner et al., 2014a). Moreover, fossil-bearing outcrops may be present in the basin, and these too may contain a rich archive of faunal and environmental history. An outstanding example is the mollusk fauna from the Turkana Basin (Williamson, 1981, 1985; Van Bocxlaer et al., 2008). However, this example also highlights that interpretations of outcrop data are highly dependent on the available stratigraphic control. Integrating data from lake drilling and the study of fossiliferous outcrops would be a powerful approach to eliminate the weaknesses of individual strategies, and hence to solidify the documentation of the lake's natural history. Specific hypotheses that were constructed based on the study

of the modern fauna and that are testable with macrofossils from sediment cores can provide good objectives for a drilling strategy that maximizes the potential to recover macrofossils.

Several future trends in data generation and analyses specifically geared to macrofossils are conceivable. An important starting point is the diameter of the core. Bigger diameters would increase the potential to obtain macrofossils, however, they would also increase the chance of technical difficulties. Core scanning (i.e., CT-scanning) in a way that allows the non-invasive detection of various macrofossils on bedding planes based on biomineral concentrations, density contrasts, or shape would be invaluable to select targeted parts of the cores for detailed examination, with minimal disturbance to other parts. Furthermore, more automated methods in fossil retrieval and visualization would help and speed up the development of goals based on large numbers of fossils. Automated identification, measurement, and digitization would greatly facilitate the study of macrofossils, but the desirability of such developments ranges beyond the drilling communities at large (e.g., Houle et al., 2003).

2.2.2. *Microfossils*

Lake sediments are also valuable archives of microfossils, i.e., remains or traces of organisms from the past that require a microscope for study. Typical microfossils in sediment cores include plant pollen from seed plants, single-celled protists with biomineralized shells (e.g., calcareous dinoflagellate cysts and siliceous diatoms), as well as small shelled invertebrates, such as ostracods and micromollusks. As the latter share many characteristics with macrofossils (see section 2.2.1.), this review focuses mainly on the discussion of pollen and diatom microfossils, which have great potential for environmental studies based on lake-cores.

Diatoms

Diatoms (phylum Bacillariophyta) are photosynthetic eukaryotes. Their silica shell ('frustule') preserves exceptionally well, making diatoms one of the most abundant microfossil taxa in lacustrine systems (Gross, 2012). Moreover, the frustules often display species-specific ornamentation, providing important information for identification and classifications (Round et al., 1990).

Ancient lakes regularly display an exceptional diatom biodiversity (Cocquyt, 1998; Levkov et al.,

2007; Mackay et al., 2010; Kulikovskiy et al., 2012; Wagner et al., 2014a) with the total number of morphotypes often exceeding 1,000 (Levkov and Williams, 2012). Most diatom species are benthic; only few species are euplanktonic and spend their entire life cycle in the water column (Stoermer and Edlund, 1999; Khursevich and Prokopenko, 2009; Snyder et al., 2013; Recasens et al., 2015).

Current diatom research in ancient lakes strongly focuses on the reconstruction of paleoenvironments and past environmental fluctuations. One of the most frequently used approaches is the chronological assessment of changes in species composition and relative abundances of the dominant species at various temporal scales (orbital, millennial, centennial, or pluriannual). These community studies are typically undertaken at low taxonomic resolutions (i.e., species from one or several genera are combined) and with a relatively large number of valves to be counted per sampled sediment interval (e.g., 400–600). As habitat characteristics and ecological preferences of many diatom species are well characterized, the collective diatom community found in a sediment sample then becomes an important source for analysis, e.g., with paleoecological transfer functions (see section 3.1.).

However, given that diatom communities in ancient lakes are often dominated by endemic species, approaches based on transfer functions may be problematic due to the lack of analogues in the existing calibration sets. In fact, given the old age of many diatom fossils in ancient lakes, they may not even have extant analogues in the lake (= supralimital evolution; Wesselingh, 2007; Mackay et al., 2010). Nonetheless, some attempts have been made to establish modern diatom calibration sets for ancient lakes (e.g., Lake Baikal; Mackay et al., 2003). These attempts try to overcome the limitation of missing modern analogues by examining modern species compositions along environmental/spatial gradients within the lake. Moreover, some studies suggest that species with similar size and comparable ultrastructural features have similar ecological preferences (e.g., Winder et al., 2009), and this assumption may also be used in establishing calibration sets. In combination with other proxies (e.g., biogeochemical data derived from the same sediment record), these new approaches enable a more reliable paleoenvironmental interpretation of diatom communities (Bradbury, 1999).

Another focus of research on diatoms obtained from ancient lake sediment cores is the assessment of a lake's primary productivity (*sensu* Battarbee, 1986). As diatoms are important primary producers, their concentration is a good indicator for productivity and epilimnetic nutrient availability (Zhang et al.,

2016). Productivity data have been used, for example, to identify successive glacial and interglacial stages (Rioual and Mackay, 2005; Melles et al., 2012; Meyer-Jacob et al., 2014b). This approach is especially promising if environmental fluctuations are not anticipated to have caused significant changes in diatom species composition (e.g., Cvetkoska et al., 2015).

However, diatom concentrations in drill cores may be affected by taphonomic processes, such as differential dissolution (Ryves et al., 2006). Parameters, such as low temperature, high pH, high detrital mineral content, and grazing, may (selectively) decrease valve preservation, and can lead to the loss of specific taxa, morphological details on individual frustules, or even the entire assemblage (Mackay, 2007; Reed et al., 2010). Moreover, biases in diatom concentrations may result from inwash of specimens from rivers, or from the loss of specimens via the lake's outflow (O'Sullivan, 2004). In addition, diatom concentrations do not account for size differences in taxa or changes in sediment accumulation rates. Therefore, the biovolume accumulation rate, i.e., diatom concentration divided by sediment accumulation rate and corrected for size differences between taxa (O'Sullivan, 2004), could be a more powerful proxy for a lake's productivity (Rioual and Mackay, 2005).

Several other aspects complicate interpretations of diatom records from ancient lakes (Bradbury, 1999). Besides the taxonomical and ecological uncertainties mentioned above, only a fraction of the actual (endemic) biodiversity is known. Moreover, recent changes in diatom species and genus concepts have led to an introduction of more than 5,000 new names (Fourtanier and Kociolek, 2011). Even specialists with several years of experience have difficulties keeping up with these changes and, more importantly, to unify their taxonomic opinions with those of colleagues. Finally, diatom analyses are time consuming, thus limiting the number of samples that can be analyzed. Therefore, samples from sediment cores are often subdivided and analyzed in parallel by several researchers, creating a potential bias in observations. Moreover, species within common planktonic genera, such as *Cyclotella* and *Stephanodiscus*, differ in morphological features that are difficult to assess with a light microscope, potentially increasing determination errors. The problem of taxonomic accuracy is further increased by the cryptic nature of some closely related diatom species, on the one hand, and by a partially high phenotypic plasticity, potentially driven by environmental factors, on the other hand (e.g., Cvetkoska et al., 2012; García et al., 2012). These problems are progressively addressed via molecular analyses and by

cultivation experiments, which have shown that diatoms can change morphological features during cultivation (e.g., Abarca et al., 2014; Rose and Cox, 2014). Nonetheless, diatom studies can provide very valuable insights into the primary productivity of ancient lakes, and into the interpretation of past environmental changes (see section 3.1.).

Pollen

Plant pollen produced by terrestrial seed plants is frequently deposited in lacustrine systems by wind ('pollen rain'), riverine inflow, and animals (e.g., O'Sullivan, 2004). Similar to diatoms, pollen is often well-preserved in long sediment cores. The high resistance to degradation under anoxic/hypoxic conditions is due to the polymer sporopollenin, which is a component of the outer wall (= exine) of pollen grains (Brooks and Shaw, 1978).

The pollen deposited in sediment cores from ancient lakes is an important archive of past environmental and climatic changes. Pollen is typically present throughout the sediment core, thus enabling continuous analyses over long time scales. Moreover, their deposition is usually only affected to limited extent by hydrological and chemical changes within the lake, and, hence, pollen records typically provide accurate reflections of terrestrial events near the studied lake (O'Sullivan, 2004). Finally, pollen deposition in sediment cores may permit the reconstruction of regional changes as pollen rain is often dispersed over long distances. Thus, that the record may contain the signal of both local and regional drivers of environmental change (e.g., Wagner et al., 2014a).

As the composition and provenance of pollen in lake cores are often complex due to varying pollen productivity and dispersal rates (Faegri et al., 1989), significant efforts have been made to understand the extent to which these records represent past vegetation covers (e.g., Davis et al., 2013; Schüller et al., 2014; Trondman et al., 2015; see also section 3.1.) and, hence, how such records should be interpreted. Pollen is generally identified to the genus level, because the pollen of many closely related species cannot be distinguished using transmission light microscopy. Subsequent paleoreconstructions are generally done using pollen diagrams, which utilize information on pollen composition, concentration, and influx values (Berglund and Ralska-Jasiewiczowa, 1986). Past vegetation covers and floral compositions can then be related to the underlying climatic drivers. Tzedakis et al. (1997), for example,

observed a close correlation between herbaceous vegetation and ice volume at a global scale during glacial intervals, whereas forest physiognomy and development appeared closely related to changes in temperature and humidity during interglacials. This study and following work (Tzedakis et al., 2001) reinforced evidence for a broad correspondence between climate signals provided by pollen data in long lacustrine records and oxygen isotopes from marine cores. Other pollen records have allowed to link short-term vegetation oscillations and centennial-scale climatic events on various smaller scales (e.g., Dansgaard et al., 1993; Broecker, 1994; Bond and Lotti, 1995). Pollen records from cores of ancient lakes that have a well-established chronology can thus provide a better understanding of climate forcing from local to global scales over several glacial/interglacial cycles (Litt et al., 2014; Sadori et al., 2016).

Other objectives of pollen analyses in long and continuous sediment records are the reconstruction of species dynamics, dating of extinction events (see also section 3.2.), and the inference of possible refuge areas. Bertini (2010), for example, could show that extinction events following climate changes did not occur synchronously across ecosystems. However, geographically-related records, in general, may show somewhat different vegetation dynamics. Part of the problem is that many previous sediment records have not been studied with a high temporal resolution and/or lack a precise chronological control.

Given the challenges and limitations outlined above for diatom and pollen microfossil analyses from sediment cores, the following recommendations for future deep drilling projects are suggested:

- i) Microfossil studies should be hierarchically structured. As analyses from long records are very laborious and time-consuming, the first target should be to produce low/medium resolution data (e.g., skeleton pollen diagrams with key pollen curves, Sadori et al., 2016 or stratigraphic diatom diagrams, Cvetkoska et al., 2015). This enables a preliminary chronological alignment with major environmental and climatic fluctuations. Only then, high-resolution diatom and pollen studies should be conducted.
- ii) The temporal sampling design should adhere to the main question and time-scale of the respective project. However, in case of uncertainties about the temporal resolution required, subsampling should be set up in a way that samples for higher resolution studies are available even if a first

analysis is to be conducted in lower temporal resolution. This is because resampling of sediment cores that are already in long-term storage may be difficult and/or costly.

- iii) Sediment subsampling should be coordinated (see section 2.1.4.). In order to be able to combine microfossil data sets and link them to other biotic and abiotic data generated in multidisciplinary deep drilling projects, samples should, whenever possible, be taken from the same sample depth.
- iv) Protocols for sample preparation and microfossil identification to be used by all biologists involved in the project have to be implemented. A 'taxonomy working group' should be established that elaborate and share the taxon list with all investigators, defines diagnostic characters for problematic taxa, solves taxonomic disputes, and implements quality control procedures (e.g., Munro et al., 1990).
- v) Microfossil studies should involve specialists in climate reconstruction and empirical modeling at an early stage to improve data quality and the power of subsequent analyses.

We expect to see considerable advances in microfossil biodiversity research in the future, partly driven by ongoing deep drilling projects in ancient lakes. Comparative molecular investigations of recent taxa, particular for diatom analyses, may help clarifying systematic problems, mainly in respect to cryptic species and species with high phenotypic plasticity (Kermarrec et al., 2013). Robust species-level phylogenies could then be used to statistically identify those morphological character states or combinations thereof that have a high diagnostic power. These characters could possibly also be applied to paleo-morphotypes.

As to future pollen fresearch, a better link to studies of plant macrofossils could be established (Birks and Birks, 2000; see also section 2.2.1.). Macrofossils can often be identified with more taxonomic precision than pollen, and in the context of lake drilling, they may provide supplementary information, particularly on water plant communities (Birks and Birks, 2000; Sadori et al., 2010), and taxa that produce very little or no pollen. Integrated paleobotanical analyses may allow creating a more comprehensive picture of paleoenvironmental changes in ancient lakes and their watersheds.

Another promising development is the 3D reconstruction of microfossils. Whereas scanning electron microscopic 3D approaches are still costly and time consuming (e.g., Mansilla et al., 2015), light

microscopic 3D solutions are meanwhile offered by several companies.

Bioinformatic advances in image analyses have also led to improved automatic identifications of diatoms (e.g., Jalba et al., 2005; Mosleh et al., 2012; Kloster et al., 2014) and pollen (Guru et al., 2013; Holt and Bennett, 2014; Marcos et al., 2015), potentially reducing processing time and identification biases. Future improvements may further increase the performance of these approaches beyond their current application as preliminary sorting tools.

2.2.3. *Subsurface biosphere*

Over the past decades, marine and continental drilling projects have shown a growing interest in understanding the role of microbes in the complex chemical reactions occurring in the sediments and or the sediment/water interface (Konhauser, 2007). Microbial activity in the water column of modern lakes and oceans is well known in comparison to effects of microbial life in sediments, but despite controversy on specifics, scientists generally agree that the impact of the deep marine biosphere on global biochemical cycles is massive (Whitman et al., 1998; Kallmeyer et al., 2012). As a result, there has been a substantial increase in investigations dealing with microbial activity in freshly retrieved sediments (D'Hondt et al., 2002; Inagaki, 2010). This hidden microbial mass as well as the study of the interactions between microbes and sediments in the marine environment is known as 'deep biosphere research'. Analogously, the study of active microbes in lake sediments through scientific drilling have been labeled as 'subsurface biosphere research'. Many microbes in this subsurface biosphere grow extraordinarily slowly and under extreme conditions (Røy et al., 2012). Their study is critical to understand the physiological abilities and biogeochemical impact of subsurface life within the sedimentary column.

Subsurface biosphere studies have only recently been implemented in continental deep drilling projects (Vuillemin et al., 2010; 2013a, b; 2014a; b; Glombitza et al., 2013; Thomas et al., 2014, 2015; also see Ariztegui et al., 2015 for a detailed description of the different sites).

The systematic study of the subsurface biosphere in lacustrine sediments will allow geologists and biologists to identify the magnitude and impact of microbes during early diagenesis on both sediments and biological remains. Geomicrobiological studies permit the identification of signatures of former microbial activity recorded in the sediments as well as investigating their impact in biogeochemical

cycles. Some open questions are:

- i) What are the source, composition, and global significance of subsurface communities in lacustrine basins?
- ii) What is the impact of environmental change on subsurface biodiversity? Do changes in environmental conditions generate changes in microbiomes population diversity and density?
- iii) How does the diversity and activity of microbial life vary with depth, geochemistry, sediment composition, and age?
- iv) How does the diversity of active and non-active microbes relate spatially across the lacustrine basin at given time slices?
- v) How do microbes resolve the paucity of nutrients and energy as well as the limits of life in the subsurface of lakes under contrasting physicochemical conditions?
- vi) What is the influence of subsurface communities on paleoenvironmental and paleoclimatic proxies, minerals, and hydrocarbon reservoirs? How do they alter sediments and hence the data and interpretations of other workers on deep drilling cores?

Cell counts in recent continental deep drilling in mesosaline Lake Potrok Aike (Patagonia, Argentina) and alkaline Lake Van (Turkey) indicate variable microbial activity at sediment depths reaching down to 100 mcd (Vuillemin et al., 2010; Kallmeyer et al., 2015). Additionally, investigations in hypersaline Dead Sea sediments (Levantine region) have shown a dominant Archaea population down to 200 mcd (Thomas et al., 2014, 2015). These results indicate that further research is critical to obtain more detailed information about the fine-scale mechanisms controlling microbial life in the deep subsurface of ancient lake sediments while answering several of the aforementioned questions.

Compared to their marine counterparts, the study of lacustrine sediments is often logistically easier. However, the implementation of subsurface biosphere studies in lake systems has required new sampling techniques that provide the needed aseptic conditions to avoid contamination. Whereas most ocean drilling vessels have a dedicated laboratory for geomicrobiological sampling on board, the size and configuration of lake drilling platforms prevent setting up a comparable sampling laboratory with conditions of asepsis. Therefore, special on-shore facilities are required to solve this problem. Core sections are transported to this laboratory as frequently as possible, depending on the logistics of the

drilling (e.g., distance to the shore, frequency of drilling, and crew rotation; also see section 2.1.).

Moreover, *in situ* sampling procedures allow recovering aseptic samples as well as determining the presence of active microbes (Fig. 8; for details see Vuillemin et al., 2010).

Fig. 8. Subsurface biosphere sampling from sediment cores obtained from Lake Potrok Aike (Patagonia, Argentina). A) Sampling window cut in the core under aseptic conditions. B) Methane sampling. C) DNA sampling (photo credits: A. Vuillemin and D. Ariztegui).

Ideally, a geomicrobiological study should be planned well ahead of the start of a drilling operation. It is preferable to have a dedicated core for microbiological and geochemical studies. This will secure the best possible sampling conditions but can cause problems of hole-to-hole correlation because the microbiological results have to be tied with other sedimentological, biological, and geochemical parameters as well as core chronologies. To overcome this issue of missing stratigraphic information, petrophysical properties (whole core MSCL; see section 2.1.4.) of cores dedicated for geomicrobiological research can be measured in the field, or borehole logging can be used (see section 2.1.3). These petrophysical or logging datasets can then be used to generate at least a rough hole/core-to-hole/core correlation and help to embed the geomicrobiological datasets into the common stratigraphic framework and other continuously generated datasets from other holes/cores of the same site.

In some cases it may, for logistical and/or budgetary reasons, not be possible to dedicate an entire drill core to microbiological investigations. If this is the case, there is the possibility to sample the core catchers for microbiological studies. When working with core catcher materials, special care should be

taken to avoid contamination. A recent study that utilized core catcher samples for microbiological analyses from the Dead Sea ICDP sediment cores and followed appropriate sampling protocols, has proven to produce reliable results (Thomas et al., 2014, 2015).

The significance and validity of the results of subsurface biosphere studies is largely dependent on the quality, speed, and prevailing conditions of the initial sampling. Hence, it is critical to attain a methodological standardization for all lake and ocean deep drilling sites in order to be able to compare results between different campaigns and environments. A generalized protocol would allow reducing the impact of contamination issues, determining the best method to accomplish on-site cell counting, choosing the appropriated sampling methods for further molecular characterization, and designing a proper strategy for sample archiving. Due to the different nature of each drilling project, a protocol for standardized biological sampling, processing, and analysis would be a significant accomplishment. Recently, the development of genomics and the emergence of high-throughput DNA sequencing technologies have been opening up new possibilities including the expansion of databases, which contain crucial information to define the metabolic pathways of different microbes (also see section 2.2.5.). The latter combined with laboratory culture experiments will be critical to constrain the impact of active microbes on, for example, the carbon cycle and diagenetic processes in the sediments.

As in ocean research, a main challenge for the development of subsurface biosphere studies in lacustrine settings is to communicate the potential of these investigations to the broader scientific community participating in deep drilling projects. It is also important to involve geomicrobiologists in discussions about the effects of an active biosphere on the subsurface environment and sediment composition. Paleoclimatological reconstructions largely depend on the use of a variety of petrophysical, geochemical, and biological proxies. Proxy responses are typically interpreted to reflect the environmental/climatic conditions during the time of deposition. However, organic compounds (ancient DNA, see section 2.2.5; biomarkers, see section 3.3.) as well as element and isotope compositions (see section 2.2.8.) may be altered by microbially induced processes long after deposition, thus biasing and complicating the interpretation of proxies. A precise understanding of the influence of different microbial communities on compositional changes after burial is of vital importance for a better understanding of proxy responses and their interpretation.

2.2.4. *Biomarkers*

Biomarkers are source-specific organic molecules, i.e., they are synthesized by living organisms in aquatic or terrestrial settings for specific organic tissue types, e.g., cell membranes or protective and supporting tissue, or to fulfill specific functions, e.g., energy storage or pigmentation. The presence of such compounds in lacustrine sedimentary records is a testimony that, in the past, certain groups of organisms occurred in aquatic and terrestrial habitats of the catchment of the studied lake basin. In ancient lakes, occurrences of organisms may even be modified by geological processes, such as tectonic subsidence or uplift, changing catchment topography, and material fluxes within the watershed. This, in turn, can be detected by biomarkers. Very few individual biomarkers are species-specific, and there is considerable overlap in biomarker profiles of large groups of organisms. Still, many biomarkers can be chemotaxonomically assigned to groups of organisms that characterize a specific habitat. Furthermore, organic matter pools of living and decaying biomass (aquatic/terrestrial vegetation, sinking particles/plant litter, soil organic matter) produce equally specific combinations of biomarkers, i.e., an organic geochemical fingerprint that can be interpreted to represent an ecosystem (e.g., Holtvoeth et al., 2016). Thus, ‘source-specific’ may refer to a specific organic matter pool, a group of organisms, or to individual species. The most fundamental distinction as to the sources of organic matter in sedimentary records that biomarkers can provide is between aquatic and terrestrial plant biomass, based on the fact that vascular plants (higher land plants) require structurally supportive and protective tissues that are not present in non-vascular, aquatic plants. Some other biomarkers indicate highly specific adaptations of their source organisms to environmental conditions. For example, pigments of anaerobic phototrophic bacteria indicate past anoxia in the photic zone of the water column when found in lacustrine sediments (Hanisch et al., 2003; Castañeda and Schouten, 2011).

The association of biomarkers to specific ecological functions or conditions highlights a fundamental principle behind many biomarker applications in paleo-environmental research. Organic matter inventories over time may document fluxes in various biomarkers in response to largely climatically controlled environmental parameters, e.g., temperature and the supply of moisture. Factors, such as catchment topography and lake bathymetry, also determine organic matter pools through, for example,

soil thickness and stability, weathering and erosion rates, run-off modes, the extent of the littoral, all of which also affect organic matter degradation during storage, transport, and deposition. Thus, the two main approaches in biomarker studies are to infer i) sources of organic matter and ii) environmental parameters (Table 2.)

Table 2

Biomarker-based approaches that determine sources of organic matter and environmental parameters, with examples of relevant literature.

Parameters	Molecular evidence	References
Organic matter source		
Aquatic biomass (phytoplankton/algae, zooplankton, macrophytes, bacteria, archaea)	- chemotaxonomic compounds, incl. membrane lipids, storage lipids, pigments - compound-specific stable isotopes ($\delta^{13}\text{C}$, $\delta^2\text{H}$, $\delta^{15}\text{N}$)	Volkman et al. (1998), Meyers (2003), Castañeda and Schouten (2011)
Terrestrial vegetation (woody/non-woody vegetation, angiosperms/gymnosperms, C3/C4 plants)	- chemotaxonomic compounds and compound distributions, incl. membrane lipids, leaf waxes, lignin phenols - compound-specific stable isotopes ($\delta^{13}\text{C}$, $\delta^2\text{H}$, $\delta^{15}\text{N}$)	Meyers (2003), Castañeda and Schouten (2011)
Soil organic matter (belowground biomass, incl. degraded plant debris, roots, bacteria, archaea, fungi)	- biomarkers from soil microbial organisms (membrane lipids) - biomarkers from root material (suberin monomers)	
Burned biomass	- pyrogenic compounds	Denis et al., 2012
Environmental parameters		
Lake surface temperature (LST)	- alkenone unsaturation index (Uk_{37} , from C_{37} alkenones of haptophyte algae) - tetraether index (TEX_{86} , from glycerol dialkyl glycerol tetraethers/GDGTs of aquatic archaea)	Castañeda and Schouten (2011); Schouten et al. (2013)
Mean annual air temperature (MAT)	- methylation and cyclisation of branched archaeal tetraethers (MBT, CBT)	
Moisture source (hydrology)	- compound-specific hydrogen isotopes ($\delta^2\text{H}$)	Huang et al. (2002, 2004), Sachse et al. (2004)
Soil pH	- MBT, CBT	Weijers et al. (2007), De Jonge et al. (2014)

Both approaches are frequently applied in paleoenvironmental studies as ecosystem functions are adjusted to environmental parameters. In tropical and subtropical settings, for example, information on moisture supply and evapotranspiration, which can be detected by plant wax $\delta^2\text{H}$, can be combined with carbon isotope data of the same plant wax $\delta^{13}\text{C}$ (e.g., Berke et al., 2012) to trace the abundance of C4 vegetation through time. Carbon and hydrogen isotope records indicating shifts in climatically controlled hydrology and types of organic matter can then be used for climate modeling (Aichner et al., 2015).

Lipid biomarkers provide highly specific proxy data that support and validate data from other organic sediment components, in particular, palynological and bulk organic matter data (see section 2.2.2.). Relatively fast and cost-efficient bulk geochemical proxies, such as carbon to nitrogen ratios ($\text{C}_{\text{org}}/\text{N}_{\text{tot}}$), bulk organic carbon isotopes ($\delta^{13}\text{C}_{\text{org}}$) or hydrogen and oxygen indices (HI, OI) from Rock-Eval pyrolysis, are useful tools to explore aspects of environmental variability. Although bulk proxies provide limited environmental information, they do have the potential to indicate major changes in sources and/or fluxes of organic matter (see Meyers, 2003 for a review). Furthermore, bulk analyses facilitate the formulation of detailed hypotheses and the design of targeted, high-resolution biomarker studies. A popular strategy for paleoenvironmental analyses therefore is to compare biomarkers with proxy data from palynology and bulk organic geochemistry. Examples include studies of East African lakes, combining pollen data with compound-specific carbon and hydrogen isotope data, lignin composition, and lake surface temperature data (Tierney et al., 2010; Berke et al., 2012).

A basic problem is that biomarkers, as any organic substance left behind by a deceased organism, are affected by microbial and physicochemical degradation, which can occur before or after deposition in the sediments. Nitrogen- and oxygen-containing compounds, such as carbohydrates or amino acids, are prone to microbial degradation (see also section 2.2.3.), whereas molecules based on hydrocarbons, such as lipids, may preserve source-specific information over geological timescales, i.e., over hundreds of millions of years (e.g., Marynowski et al., 2011; Izart et al., 2012; Rohrsen et al., 2013). The oldest deep-time context from which biomarkers have been interpreted dates back ~1.6 billion years (Pawlowska et al., 2013). Microbial degradation may selectively alter the relative amounts of lipid biomarkers of different recalcitrance or that are bio-accessible, e.g., in a clay mineral matrix. In order to

minimize diagenetic bias, biomarker proxies are generally based on ratios or distributions of compounds of the same compound class that also likely derive from similar source tissue types.

In the following, we discuss some practicalities of biomarker approaches, focusing on the recovery of paleoenvironmental records from ancient lakes, and including strategic considerations in order to gain maximum and reliable information from biomarker studies.

Lipid biomarkers are principally extracted from sediment samples by organic solvents through the application of a range of extraction techniques, in particular sonication, accelerated solvent extraction (ASE), and microwave-assisted solvent extraction (MAE), all of which are fast methods for high sample throughput (Camel, 2000; Kornilova and Rosell-Melé, 2003). Although time consuming and using greater quantities of solvent, Soxhlet extractions are a suitable option for the study of sediments with very low organic matter content. Once extracted, the lipids can be identified and quantified by gas chromatography-mass spectrometry (GC-MS). In cases where differences in ionization during GC-MS analyses may lead to quantitative bias, a standard gas chromatograph fitted with a flame ionization detector (GC-FID) is used for quantification instead. A typical application is the quantification of haptophyte-derived alkenones, which can provide information on paleo-surface water temperatures (Uk₃₇ LST proxy, Table 3). Larger molecules, such as bacterial bacteriohopanepolyols (BHPs) or archaeal glycerol dialkyl glycerol tetraethers (GDGTs), which also provide temperature proxies for lake surface waters as well as for soils, are analyzed by high-performance liquid chromatography-mass spectrometry (HPLC-MS). Lignin phenols used for vegetation reconstructions or terrestrial input can be analyzed efficiently by pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS; e.g., Ishiwatari et al., 2006). For compound-specific stable isotope analyses ($\delta^{13}\text{C}$, $\delta^2\text{H}$, $\delta^{15}\text{N}$), compound classes or individual compounds can be isolated either through standard flash chromatography or automated preparative methods (prepGC, prepHPLC).

However, a single lab is rarely able to produce the complete suite of biomarker proxy data. One possibility to increase material- and cost-efficiency of multiproxy biomarker studies is to design a coordinated approach and to distribute splits of the total lipid extracts (TLEs) for various applications rather than bulk sediments samples.

The inter- and intra-habitat heterogeneity of biomarkers leads to the fundamental problem that biomarker-based proxies often are not interchangeable between investigated lake basins. Therefore, it is highly recommended to include a survey of the modern biomarker sources within the catchment of an environmental archive (biogeochemical fingerprinting) when planning biomarker-based paleoenvironmental research. In this context, it is crucial to correctly identify the major sources of sedimentary organic matter. For example, rather than the living vegetation itself, plant litter is a major source of terrigenous organic matter. This is important as the biomarker composition of plant litter is already altered compared to living biomass due to degradation processes that begin as soon as a plant dies. Soils are another major organic matter pool within many lacustrine basins. Furthermore, as they accumulate over time, soils integrate the biogeochemical signature of the changing vegetation cover and its degradation products and of belowground biomass (root material plus fungal and bacterial biomass). Thus, biogeochemical fingerprinting of the major organic matter pools facilitates the correct identification of the sources of the organic matter in lake sediments and helps assessing potential input of pre-aged material, which can lead to considerable bias in high-resolution records (Douglas et al., 2014). A large uncertainty also affects the interpretation of stable isotope data, in particular, for hydrogen isotopes (Sachse et al., 2012). Determination of the carbon and hydrogen isotope composition of biomarkers from the major organic matter pools in the modern environment of a lacustrine catchment is needed in order to improve the understanding of the impact of hydrological changes on compound-specific isotope compositions (see Wilkie et al., 2013 for the Lake El'gygytgyn drilling project). For paleotemperature reconstructions, a surface sediment-surface water calibration is highly recommended due to the many factors that can bias biomarker-based temperature proxies in lacustrine settings.

Unless sediment cores are stored in a freezer, microbial breakdown processes continue to alter organic matter in the sediments. Samples for biomarker studies should therefore be taken from the cores at the earliest possible occasion and then be frozen (ideally at $-80\text{ }^{\circ}\text{C}$) or freeze-dried as soon as possible to prevent further microbial degradation. Even after freeze-drying, cold storage is advisable in order to preserve labile biomarkers, such as mono- and poly-unsaturated fatty acids.

During sampling and storage, it is crucial to avoid contamination with organic compounds derived from petroleum products such lubricants and plastics (also see section 2.1.2.). Polyethylene (PE), which

is commonly used for soft plastics, such as sampling bags or the lids of sample containers, releases a series of compounds, in particular branched alkenes with quaternary carbon atoms (BAQCs), which can contaminate even freeze-dried sediment during prolonged storage (Brooks et al., 2008; Holtvoeth, unpublished data). Samples should be transferred into furnaceed glass jars or vials, or into containers lined with combusted foil (aluminum foil heated to 450°C for at least 4 h).

The study of biomarkers preserved in lacustrine sediments has developed strongly ever since their potential for paleo-environmental reconstructions was recognized and developed from the late 1960s onwards (e.g., Cranwell, 1973; Brooks et al., 1976). This is largely due to the rapid advance of analytical technology and the increase in analytical capacity. Promising analytical methods are established using advanced detectors for mass spectrometry, such as a quadrupole time-of-flight mass detector (GC/Q-TOF) or orbitraps, increasing resolution and precision in the detection of molecular fragments. Moreover, high-temperature GC-MS applications extend the range of GC-amendable compounds towards high-boiling biomarkers (e.g., wax esters, triacylglycerides, GDGTs; Sutton and Rowland, 2012). A dynamic field with great potential for biogeochemical fingerprinting of archaeal and bacterial organic matter sources and reconstructions of microbial ecology is the analysis of compounds derived from bacteriohopanepolyols (BHPs; Talbot et al., 2003; Zarzycki and Portka, 2015) and of intact polar lipids (IPLs; Rethemeyer et al., 2010; Tierney et al., 2012; Buckles et al., 2014). A recent and entirely different approach to the use of biomarkers as indicators of ecosystem change is the application of advanced statistical programs integrated in the analytical software for identifying the key variables in biomarker screening data that may include hundreds of compounds in an environmental sample (lipidomics, environmental metabolomics; Bundy et al., 2009). Finally, genetic research targeting enzymatically controlled molecular adaptations of organisms to certain environmental conditions is fundamentally changing the conventional interpretation of biomarker data. Rather than linking a biomarker to the presence of a certain organism or groups of organisms, it can instead be assigned to a specific ecological niche (e.g., Welander et al., 2012). This change in view is important for the understanding of the geochemical fingerprint that past ecosystems left behind and opens up new possibilities to interpret assemblages of biomarkers that, individually, had been regarded as unspecific.

2.2.5. Ancient amino acids

Towards the end of the 19th century, the first identification of the likely remains of proteins in fossils paved the way for their use in the earth sciences. Amino acids, the building blocks of proteins, are found in all living tissues and can be preserved in subfossil biominerals, such as shells, as well as in sediment. Analysis of these ancient amino acids has proved important for three main avenues of research: dating (via amino acid geochronology), species identification (via paleoproteomics), and to assess the integrity of the organic matter for other biomolecular studies (e.g., aDNA).

Amino acids can be used for dating purposes because of amino acid racemization (AAR), i.e., the time-dependent breakdown of proteins (and their constituent amino acids) in fossils. Spanning an age range from 10 years ago up to as long ago as a few million years, the method is applicable to the whole of the Quaternary Period (see Lowe and Walker, 2015 for a recent review). Advances in chromatography, preparation methods, and the choice of material for dating have greatly improved the accuracy of the methods, and demonstrate the technique's potential for developing regional Quaternary chronologies around the world (e.g., Penkman et al., 2011; Wehmiller, 2012). Identification of endogenous amino acids in Cretaceous and Paleogene samples (Miller and Hare, 1980; Penkman et al., 2013) opens up the opportunity to use other protein degradation reactions to date material over much longer timescales.

The 20 naturally-occurring amino acids all have a central carbon atom (the α -C) with four attached groups: an amino group (NH_3), a carboxylic acid group (COOH), hydrogen (H), and a side chain (R) that defines the type of amino acid. In glycine, the side chain is H , but for all other amino acids, the α -C has four different groups (Fig. 9). The four distinct groups connected by single bonds make the α -C a chiral center, meaning that it can exist as two stereoisomers: the *laevo* (L-form) and *dextro* (D-form), named after the optical activity of glyceraldehyde. In living organisms, proteins are almost exclusively made from the L-form. However, this dominance of one form is thermodynamically unstable, so after death, a spontaneous reaction occurs to balance the abundance of both forms. The extent of AAR is analyzed by gas or liquid chromatography and recorded as a D/L value. AAR continues until a dynamic equilibrium is reached (usually $\text{D/L}=1$). First applied to fossil shells (Hare and Abelson, 1968), AAR geochronology

measures the extent of this degradation in fossils as an index of relative age (an aminostratigraphy), which can provide calibrated ages in combination with known-age samples or detailed temperature records (Fig. 10). This then may allow correlation of deposits with the marine oxygen isotope stage (MIS) record (for lacustrine deposits see McCoy, 1987; Bowen et al., 1989; Magee et al., 1995; Oviatt et al., 1999; Kaufman, 2003a; Ortiz et al., 2004; Penkman et al., 2011), to a sub-MIS level for at least the Late Pleistocene.

Fig. 9. Most amino acids have no plane of symmetry, just like hands, so their mirror images are non-superimposable and therefore distinct from each other. The breakdown of left-handed molecules to the right-handed form over time provides a mechanism for estimating age of fossil material.

Fig. 10. The increase in racemization in the opercula of the snail species *Bithynia tentaculata* with age for the free amino acid (FAA) aspartic acid (Asx; red symbols) and the total hydrolysable amino acids (THAA) valine (Val;

blue symbols) and alanine (Ala; green symbols) from British deposits with independent geochronology. Asx racemizes rapidly and is therefore most valuable for separating sites younger than MIS 7 in these temperate deposits. Val, in contrast, racemizes more slowly and is able to differentiate between sites back to the Pliocene, but provides poorer resolution for young sites. Utilizing multiple amino acids with different rates of degradation therefore enables greater time depth and age resolution. Image modified from Penkman et al. (2011).

Protein degradation consists of a series of chemical reactions that are dependent on time, but also on environmental factors (e.g., pH, availability of water, temperature), which can confound the time signal. These difficulties in AAR's early applications have led to a focus on analyzing 'closed-system' protein from fossil samples (Towe, 1980), where the fraction of protein analyzed is physically or chemically shielded from the environment. The chemically-isolated 'intra-crystalline' fraction found in mollusk and egg shells forms such a closed system, meaning that the AAR within this fraction is solely dependent on time and temperature, and therefore predictable (Brooks et al., 1990; Sykes et al., 1995; Penkman et al., 2008). AAR has been particularly successful in dating carbonate fossils (shells, eggshells, foraminifera, ostracods) and in long-lived biominerals (e.g., corals), providing age information *within* an individual sample (Hendy et al., 2012). In subfossil samples, the different proteins break down at different rates, so analyses are undertaken on monospecific samples (usually individual mollusk shells, a few mg in weight). Labs performing AAR have developed dating frameworks for a large number of commonly occurring species, but tests can be undertaken on additional species to examine whether they would be suitable for AAR dating. The crystal phase of calcite (e.g., opercula, eggshell, ostracods) are more stable over longer timescales and are therefore preferred for material of Early and Middle Pleistocene age (Penkman et al., 2011).

The rate of breakdown towards D/L equilibrium in the intra-crystalline fraction is still affected by temperature, so comparative frameworks need to be applied from regions with a broadly similar temperature history. However where age control is available, the extent of racemization can then be used to estimate the effective diagenetic temperatures (Kaufman, 2003b). Published amino acid data are now being archived by NOAA and are freely available at <http://www.ncdc.noaa.gov/paleo/aar.html>.

The advent of soft-ionization mass spectrometers made protein sequence identification more routine, and this was soon applied to fossil material (Ostrom et al., 2000). The ordering of the amino acids in a

peptide chain (its sequence) can be diagnostic of the species from which it came, and therefore identification of specific ancient proteins informs on the past biota (Buckley and Wadsworth, 2014). While the evolutionary picture from proteins is not as detailed as that from aDNA (as changes in the peptide sequences are significantly slower), proteins are significantly more stable than DNA (see section 2.2.6.), allowing identification of peptide sequences (and hence phylogenetic information) from material where aDNA is not recoverable (Cappellini et al., 2011). Focusing initially on Pleistocene bones and shell, this technique has primarily been applied to terrestrial deposits, but the excellent preservation of organic material in lake sediments (often due to anoxic conditions) lends itself to the expansion of paleoproteomics to lacustrine material.

For all biomolecular analyses, it is critical to identify and exclude contamination. One advantage of protein analysis is that (unlike aDNA) proteins do not have to be amplified for detection, while the predictable nature of protein degradation enables identification of modern contamination (e.g., Buckley et al., 2008). Amino acid analysis of sedimentary material provided a useful tool for identifying the presence of original biomolecules, and hence helped authenticate the oldest DNA sequences yet recovered (Willerslev et al., 2007).

A non-specialist can collect material and/or sediment samples in the field, and samples should be stored at or below room temperature. Biominerals for AAR dating are typically obtained from wet-sieved residues of sediment samples. Development of better preparative and analytical methodologies is allowing analysis of smaller and more degraded samples, while protein databases to match recovered sequences against are ever-growing, enabling more accurate species identifications. Amino acids and proteins are present in geological samples, and our abilities to use the geochronological and biological information they hold are advancing rapidly.

2.2.6. *Ancient DNA*

The analysis of ancient DNA (aDNA), i.e., DNA of long-dead specimens (Krause, 2010), has become an emerging field in evolutionary biology and paleoecology during the last decades (e.g., Hofreiter et al., 2001; Pääbo et al., 2004; Willerslev et al., 2014; Hagelberg et al., 2015; Birks and Birks, 2016). Ancient DNA provides a unique opportunity to assess paleo-biodiversity, and to unravel past evolutionary and

environmental processes by comparing genetic information of fossil and extant organisms. Moreover, as the nucleotide sequence of DNA fragments ('DNA sequences') can be digitally encoded in a standardized way and stored in public databases, newly generated DNA sequences are directly comparable to previously generated data.

Ancient DNA may be isolated from (parts of) specimens preserved in sediment cores (incl. mummified tissues, bones, teeth, or other biomineralized material) or, more commonly, as 'sedimentary aDNA', i.e., organismal DNA that has been released into the water or directly into the sediment, often in the absence of visible fossils (Parducci et al., 2013, 2015; Pansu et al., 2015). However, aDNA is often of poor quality compared to DNA from samples of extant organisms as DNA molecules exponentially degrade in smaller fragments after the death of an organism (Hofreiter et al., 2001; Allentoft et al., 2012). In addition, microorganisms may digest aDNA and even introduce mutations (Hofreiter et al., 2001; Krause, 2010 and Allentoft et al., 2012). These microbial effects are of particular concern for sedimentary aDNA analyses, as the respective DNA molecules are largely unprotected against the environment. Other factors determining degradation are, among others, time, environmental temperature, oxygen content, pH, sediment type and composition, and presence of free water (Allentoft et al., 2012; Hagelberg et al., 2015). Once a critical sequence length of approximately 15–25 nucleotides is reached, these DNA fragments lose their unique fingerprint and can no longer be used for comparative studies.

The two main methods to decode aDNA are PCR-directed sequencing and direct sequencing. For PCR-directed sequencing, short oligonucleotides ('primers') have to be designed that selectively bind to a complementary aDNA target region. The advantage of this method is that a specific region (e.g., a particular gene or part of a gene) can be targeted, that the number of read errors is relatively low, and that contamination (e.g., through microbial DNA) is limited by the use of taxon-specific primers. Disadvantages are that the aDNA fragments targeted have to be relatively long (typically several hundreds of nucleotides) and the need to design specific primers, which is often difficult in the absence of reference data. In contrast, direct sequencing does not require specific primers and thousands or millions of short aDNA fragments can be directly encoded using 'next generation sequencing' platforms (e.g., Metzker, 2010; Mardis, 2011). These high-throughput methods work well with highly degraded aDNA fragments (i.e., with fragments as short as 25 nucleotides; Storvall et al., 2013), but the numerous

individual aDNA reads have to be assembled using reference databases, which so far cover mainly ‘model’ organisms. Moreover, as no taxon-specific primers are used, contamination with exogenous DNA may constitute a major problem even though parts of these contaminations can be recognized *a posteriori* using bioinformatic tools (e.g., Schmieder and Edwards, 2011).

Despite numerous methodological advances, aDNA analyses from lake-core materials remain problematic. Reasons are the small amount of available material, the highly degraded nature of aDNA from sediment cores, read errors, contamination, and incomplete reference databases (*sensu* Krause, 2010; Pedersen et al., 2013; Birks and Birks, 2016). Therefore, aDNA from lake sediments is currently mainly used for biodiversity assessments of Late Pleistocene and Holocene communities (Bissett et al., 2005; Anderson-Carpenter et al., 2011; Boessenkool et al., 2014; Pansu et al., 2015), particularly as complementary information to fossil data (*sensu* Jørgensen et al., 2012; Parducci et al., 2013).

Though respective analyses from ancient lake cores are still lagging, we expect to see more paleo-biodiversity assessments based on aDNA analyses in future deep drilling projects. Given that shallow areas in ancient lakes are typically more biodiverse than deep sites, we suggest to retrieve aDNA samples from near-shore sediment cores (although temperature and O₂ content in these areas might be higher, thus accelerating degradation; see also sections 2.1.1. and 2.2.1. about the disadvantages of near-shore sites and Cohen, 2012 for issues related to geological conditions in general). A principle problem is contamination with exogenous DNA. Therefore, cores have to be sampled under aseptic conditions and sedimentary aDNA sampling is ideally done directly in the field. However, previous studies have shown that aDNA can also be isolated from lake sediments after long-term refrigeration at 4°C (Bissett et al., 2005). Respective procedures of field sampling are similar to those used in deep biosphere studies (Ariztegui et al., 2015; also see section 2.2.4.). For this reason, coupling aDNA and subsurface biosphere studies in ancient lake drilling projects is advisable.

Future studies will probably use direct high-throughput sequencing, perhaps extending the time frame of analyses to the Middle Pleistocene. However, the usefulness of high-throughput approaches may also increase once more extensive reference DNA datasets are established that include genetic information on both fossil and extant species. We therefore recommend to couple future aDNA studies in ancient lake drilling projects with genetic and genomic studies on extant species (see also section 3.3.).

2.3. *Data accessibility and storage*

Long-term core and sample storage as well as data accessibility and curation are of fundamental importance in light of the immense financial, logistic, labor, and intellectual efforts associated with scientific drilling projects. Over the last decades, core repositories (e.g., Bremen Core Repository (BCR), Bremen, Germany; Kochi Core Center (KCC), Kochi, Japan; National Lacustrine Core Facility (LacCore), Minneapolis, USA), equipped for core processing and particularly designed for the long-term storage of cores, samples, and data were established in order to service the scientific drilling community and to guarantee the accessibility of samples, relevant drilling metadata, and initial core descriptions (ICD) for extended periods. Project parties of scientific drilling projects are conventionally obliged to generate ICD-data, which typically encompasses whole core and split core MSCL data, split core surface imaging, visual core descriptions, smear slide analysis, and core correlation points (Fig. 3). ICD data is crucial for subsequent sample selection by those directly involved, or others that intend to study samples after the moratorium has ended (commonly two years after drilling).

All relevant metadata and initial datasets generated on-site during drilling and laboratory-based core processing should, by default, be uploaded into dedicated, specific databases such as the ICDP Drilling Information System (DIS) for archival in professionally managed server facilities. In order to assure globally unique identifiers (Sample ID's) for long-term traceability between samples and data, International Geo Sample Numbers (IGSNs; <http://www.geosamples.org/aboutigsn>) for each type of sample generated should be registered and assigned appropriately through the System for Earth Sample Registration (SESAR, <http://www.geosamples.org/>) by each project. Furthermore, fundamental datasets along with datasets published in scientific publications by scientific drilling project parties have to be made available through online databases such as PANGAEA (<http://www.pangaea.de/>) and/or NOAA National Geophysical Data Center Index to Marine and Lacustrine Geological Samples (<http://www.ngdc.noaa.gov/mgg/curator/curator.html>) in accordance with ICDP and national funding agency rules.

However, most of the data storage and data sharing policies outlined above only apply to primary data generated from sediment cores. In contrast, there are still no generally accepted rules for storing and sharing secondary data and materials obtained during drilling campaigns. As a minimum, geological and biological voucher materials should be deposited in a freely accessible and internationally recognized scientific collection. Moreover, data should be made available through major public databases such as NCBI's GenBank (<http://www.ncbi.nlm.nih.gov/genbank>), the Paleobiology Database (<https://www.paleobiodb.org>), or the citable Dryad Digital Repository (<https://datadryad.org>).

3. Integrating geological and biological data

In section 2, we have shown that many different types of data, both geological and biological, can be obtained from drilling campaigns. Despite this rich variety of data types, lake drilling long remained the domain of earth scientists. As a result, many geological and paleolimnological analyses are well established and have been reviewed abundantly before (e.g., Cohen, 2003; O'Sullivan, 2004). In comparison, the use of organismal approaches to sediment-core data for questions related to environmental and evolutionary biology are newer, and therefore we focus here on data analysis methods that fall within this scope. We start with paleolimnological reconstructions as a nexus of environmental inquiry between strictly geological and biological approaches, then discuss the consequences of environmental change for biological diversification processes, and finally deal with aspects of the natural history of biota, i.e., timing evolutionary events and tracing character evolution.

3.1. Paleolimnological reconstruction

A major goal of scientific drilling projects in ancient lakes is the reconstruction of their paleolimnology and paleoclimate (Cohen, 2012). Sediment records revealed, for example, regional climatic and environmental expressions of Milankovitch/glacial-interglacial cycles (e.g., Hooghiemstra, 1989; Kashiwaya et al., 2001; Wagner et al., 2014a) and their extremes in polar and tropical realms (e.g., Cohen et al., 2007; Melles et al., 2012).

Traditionally, paleolimnological reconstructions are primarily based on micro- and macrofossils, both from sediment cores (primary data) and outcrops (secondary data) (see sections 2.2.1., 2.2.2.). However, modern reconstructions also integrate sedimentological, geochemical, and isotope information, thus enabling a more comprehensive assessment of paleoconditions in a given lake and its watershed. These conditions include, for example, past lake-level fluctuations (Filippov and Riedel, 2009) or changes in water depth (Lyons et al., 2016; Magyar et al., 2006), oxygen conditions (Costa et al., 2015), salinity (Mischke et al., 2010), water temperature (Goodwin et al., 2003; Castañeda and Schouten, 2011; Recasens et al., 2015), or primary productivity (Langlet et al., 2007; Recasens et al., 2015). Paleolimnological reconstructions often provide a well-informed picture about past regional (e.g., Bergner and Trauth, 2004; Stager et al., 2009) or local environmental conditions (e.g., Mourguiart et al., 1998). Although the resolution and the indicative power of paleolimnological reconstructions largely depends on the availability and quality of the respective fossil record, preserved biological recorders sometimes reflect past climate and environmental changes even on the scale of decades or years (e.g., Ghinassi et al., 2015).

Analytical approaches for paleolimnological reconstructions range from qualitative to semi-quantitative and quantitative analyses. Qualitative inference is based on linking ecological and environmental information using indicator species, whereas semi-quantitative analyses utilize estimated changes in fossils proportions and involve a link between environmental and biotic information (Cohen, 2003; Birks et al., 2012; also see section 2.2.). These approaches usually employ models ('paleoecological transfer functions', Fig. 11) that correlate modern species assemblages (i.e., composition or abundances) and their environmental characteristics ('calibration'; Cohen, 2003; Juggins and Birks, 2012). Subsequently, fossils are used to infer paleoenvironmental conditions based on the revealed assemblage-environment relationship. Transfer functions require a reference data set (= 'training set'), i.e., a data matrix that contains information on the distribution and abundance of recent species, either found in the water column or in surface sediments, together with information on the environmental variables that drive these distributional patterns. Ideally, a training dataset should be produced under controlled laboratory conditions, subjecting an organism or community (e.g., phytoplankton) to a range of environmental conditions for establishing a causal link between the

environmental (independent) variable and a set of response (dependent) variables (Saros et al., 2012). However, because of the often complex relationship between environmental and distributional data, these models are typically built with data from a range of modern lakes.

For ancient lakes, the training of transfer functions might be restricted to the lake itself due to the lag of analogues for endemic taxa (see also section 2.2.2.; Mackay et al., 2003). However, care is required, because the non-independency of the training and testing datasets may cause statistical problems (for details see Cohen, 2003).

Fig. 11. Flow of transfer function development for paleolimnological reconstructions. Species abundances (Arab numerals) and environmental variables (letters) are measured in extant assemblages. These data are used to model species-specific curves of environmental preferences, which then can be utilized to develop and refine predictive functions of environment-abundance relationships using fossil assemblages from time-resolved sediment-core samples in the lake of interest (modified from Fritz et al., 1999).

Whereas it is typically straightforward to produce environmental and biological datasets from extant lakes, it is naturally more difficult to obtain the necessary fossil record from sediment cores. Of concern are site selection (i.e., drilling shallow or deep sites; see also section 2.1.1.), continuity of records, as well as data quality and resolution. The latter aspects are particularly important because they strongly affect the applicability of transfer functions. It is also important to account for diagenetic and taphonomic

processes that might mask or even mislead interpretations (see sections 2.2.1., 2.2.2.). Environmental reconstruction based on transfer functions and sediment proxies can give conflicting information for the interpretation of the same paleoenvironment (Cohen, 2003). Therefore, it is necessary to assure that there is, indeed, a causal link between the physico-chemical variable and the bioindicators' response (Juggins, 2013). Strategies have to be developed to implement uncertainty in paleolimnological reconstructions. Another challenge is the need to disentangle individual and joined effects of multiple causal factors in the species-environment relationships (Juggins, 2013). Moreover, the species-environment relationship needs to be constant over time (i.e., no niche shift; for a critical assessment of transfer functions see also Juggins, 2013). These environmental variables should cover the range of environmental variation in the particular time frame of interest.

Systematic knowledge and taxonomic concepts, in general, are major sources of misinterpretation of communities, meta-communities, and related ecological measures. For example, the biological relevance of morphological characters for species delimitation in the fossil record (e.g., diatoms, ostracods, mollusks) should be studied in extant analogs ideally using an integrative taxonomic approach based on phylogenetic relationships and character evolution analyses (see section 3.4.). New methods to be developed should also identify and estimate the range of effects of diagenetic and taphonomic processes on the records used for paleolimnological reconstructions (Birks et al., 2012).

Undoubtedly, we will see several technical and methodological advances in the future, including improved dating of sediment records (e.g., Shanahan et al., 2013; Zanchetta et al., 2015; see also section 2.1.5.), high-resolution datasets (Lacey et al., 2015), and high-throughput technologies (Tolu et al., 2015), allowing a more efficient production of long-term datasets that can be used for paleolimnological reconstructions (also see Seddon et al., 2014).

The application of new proxies for paleolimnological reconstructions, such as biomarkers, is rapidly increasing as the relative costs for analytical instrumentation are coming down (section 2.2.4.). By now, a broad range of proxies has been established for lacustrine sediment records. These markers allow tracing temperatures, both surface water and mean annual air temperature, and moisture supply as key climatic factors in habitat dynamics (reviewed in Castañeda and Schouten, 2011). Paleothermometry based on estimations of racemization rates of different amino acids is another direction of analytical

developments (Collins and Demarchi, 2014; see also section 2.2.6.). Further aspects of ecosystem change that can be targeted through biomarker proxy applications are photic zone anoxia or the dynamics of the terrestrial surroundings, including vegetation change, biomass burning, and soil erosion (see section 2.2.4), all of which affect the trophic state of a lacustrine ecosystem.

Despite the exiting results derived from integrating geological and biological data for paleolimnological reconstructions, there are many questions remaining how past ecosystem dynamics have shaped extant structures, resilience, and dynamics of ancient lakes. Future drilling projects might also aim at identifying early ecosystem warning signals for catastrophic regime shifts, and they may attempt to identify common environmental signals in multiple records spanning different spatial and temporal scales. Methods should be implemented that address problems of time lead and lag, conflicts between datasets derived from the various proxies used, data uncertainties, and spatial autocorrelation.

3.2. *Consequences of environmental change: A biological diversification perspective*

The often extraordinary endemic species richness and high morphological disparity of ancient lake taxa have inspired evolutionary biologists to unravel the underlying mode, tempo, and drivers of biological diversification. Increasingly, non-biologists, such as geologists and climatologists, are also interested in the balance between speciation and extinction events – the two sides of biological diversification. Their reasoning is straightforward: linking environmental and evolutionary data in space and time may help inferring and possibly even quantifying the consequences of past geological and/or climatic change. Moreover, information on past speciation and extinction events can be used to assess if and how an ecosystem can buffer such perturbations, i.e., its ecosystem resilience. Finally, an understanding of how abiotic factors have driven diversification processes in the past might help to predict the impact of future environmental changes on the biotic world under various global change scenarios (Condamine et al., 2013; Lawing and Matzke, 2014).

Indeed, integrating chronologically constrained primary geological and biological datasets from sediment cores has become a quasi-standard in interdisciplinary drilling projects. Increasingly, these data are supplemented with secondary data such as fossil or stable isotope information from outcrops (see

sections 2.1.6., 2.2.1., 2.2.2.) or with genetic information from extant species (section 3.3.). Three main, non-exclusive objectives are of potential interest:

- i) Inferring the drivers of diversification; of concern are, for example, potential effects of changes in temperature, lake-level fluctuations, and tephra depositions on speciation and/or extinction events (e.g., Schultheiß et al., 2009; Brown et al., 2010; Wagner et al., 2014c; Jovanovska et al., 2016).
- ii) Inferring the tempo of speciation; of relevance are whether, for example, environmental perturbations affect changes in diversification rates over time and triggered radiation events (e.g., Day et al., 2008; Sherbakov, 1999; Schön and Martens, 2011; Wysocka et al., 2014).
- iii) Inferring the mode of speciation; of interest are, for example, whether intralacustrine barriers or lake-level associated vicariance events have promoted allopatric speciation (Trajanovski et al., 2010; Kobl Müller et al., 2011; Schreiber et al., 2012) or whether environmental changes opened new ecological niches, thus promoting adaptive radiation (e.g., Schön and Martens, 2004; Herder et al., 2008; Young et al., 2009; Muschick et al., 2012, 2014).

Previous studies, for example, have shown that lake-level changes in ancient lakes had very different effects on their endemic taxa. Depending on the magnitude of lake-level changes, the ecosystem resilience of the respective lake, and the resistance of its biota, these changes may have caused massive extinction events across higher taxa (e.g., Schultheiß et al., 2009, 2011), selective extinction in some but speciation in other taxa (e.g., Kroll et al., 2012; Takahashi and Moreno, 2015; Weiss et al., 2015), or no visible effect on patterns of diversification (e.g., Föller et al., 2015).

However, linking geological and biological data from drilling projects, particularly in the context of hypothesis testing, is not always straightforward (see also section 3.4.). This issue is particularly true when secondary data are used, which are not *per se* chronologically constrained with the primary sediment core data. For example, linking a sequence of diversification events inferred from DNA phylogenies to a series of lake-phases (e.g., Kroll et al., 2012) is challenging because of uncertainties in the timing of both datasets. Furthermore, even when a correlation can be demonstrated, it does not imply causality. In addition, drilling projects largely focus on the generation of abiotic data, which may not affect tempo and mode of diversification directly. Instead, abiotic changes possibly act indirectly through biotic drivers such as changes in character states (Hansen, 2014; Cantalapiedra et al., 2014; Salzburger et

al., 2014; see also section 3.4.) or community structures (Hauffe et al., 2015). Moreover, to unravel the consequences of environmental change (*sensu* Condamine et al., 2013; Lawing and Matzke, 2014), it is not only important to understand if abiotic drivers are involved, but also to what extent. These problems, however, are not unique to scientific deep drilling projects (Rabosky and McCune, 2010).

In the following, we therefore exemplify recent developments of statistical approaches that allow testing specific hypotheses about drivers of diversification. Depending on the type of dataset to be linked in drilling projects, three categories of analyses can be distinguished.

The first set of methods (secondary + secondary datasets) is based on dated molecular phylogenies and tests for deviation from a constant diversification rate (see also section 3.3. and Morlon, 2014). In particular, the methods of Stadler (2011a, 2011b), Morlon et al. (2011), and Rabosky (2014) allow detecting distinct shifts and a non-linear time-dependence of diversification rates. These molecular data can be complemented by another set of secondary data, i.e., biotic factors hypothesized to drive diversification such as habitat use or changes in morphological character states (see also section 3.4. and the review of Ng and Smith, 2014).

The second set of methods (secondary + primary datasets) uses dated molecular phylogenies in combination with abiotic factors that can be directly obtained from the sediment core and/or the borehole. Though the idea of abiotic control on diversification ('Court Jester hypothesis'; Barnosky, 2001) is conclusive, to the best of our knowledge, only two approaches exist for testing the influence of abiotic factors on speciation and extinction events (Condamine et al., 2013; Cantalapiedra et al., 2014). The method of Cantalapiedra et al. (2014), for example, enables the identification of the relative importance of abiotic versus biotic drivers, their consequences on diversification rates, and the change of rates over time. Previous studies have indicated an interplay of present-day abiotic factors, such as area and depth of the African Great Lakes, and biotic characteristics, such as mating behavior, in determining the probability of cichlid lineages to form intralacustrine species flocks (Wagner et al., 2012).

The third set of methods (primary + primary datasets) for inferring drivers of biological diversification uses dated origination and extinction events obtained from fossils, their biotic features, and abiotic conditions. The estimation of diversification rates based on fossil occurrence including taphonomic uncertainties is constantly improving (Foote, 2000), and the influence of abiotic drivers on

these rates can be assessed using standard regression techniques (e.g., Neubauer et al., 2015). However, an alternative framework suggested by Silvestro et al. (2014) enables a simultaneous inference of diversification rates and identifies how these rates are shaped over time by biotic or abiotic factors. This allows including various factors such as morphological changes, standing species richness for diversity-dependent diversification, and environmental changes.

Whereas these three sets of methods have largely improved our ability to test evolutionary hypotheses based on data derived from interdisciplinary deep drilling projects, they all share the same major limitations and pitfalls:

- i) Typically only the influence of a single abiotic or biotic driver of diversification can be estimated due to the current lack of tests for multiple drivers (Rabosky and McCune, 2010; Morlon, 2014).
- ii) Most analyses require a large set of species for correctly identifying the effect of a driver (Davis et al., 2013). Though many ancient lake species flocks are relatively species rich, the statistical power for smaller flocks might not be sufficient. For such cases, simulations have been suggested (Rabosky and Goldberg, 2015). Another option to increase the power is to test for a common signal across species groups by combining the phylogenetic information from several species flocks (i.e., phylogenetic meta-analyses; Adams, 2008).
- iii) Constraining primary and secondary datasets from drilling projects is often challenging because they are independently dated. Moreover, the dating uncertainties of primary datasets from sediment cores (see section 2.1.5.) may be lower by orders of magnitudes than those of some secondary datasets (Wilke et al., 2009; see also section 3.3.).
- iv) Environmentally-triggered diversification events frequently occur with a time lag (Stadler, 2011b; Dynesius and Jansson, 2014), making a direct link of geological and biological datasets difficult. A solution for this problem could potentially come from the field of epidemiology where the spatial extent and duration of disease outbreaks and their causes are analyzed (e.g., Liang et al., 2010). In interdisciplinary drilling projects, similar approaches (e.g., marked point pattern analyses; Ripley, 1976) could be used to identify abiotic drivers and the temporal extent of their influences on species diversification.
- v) The ability to estimate extinction rates based on phylogenies remains difficult (Rabosky, 2010) and

the incorporation of the fossil record has been advocated (Quental and Marshall, 2010). However, as macrofossils are rare in sediment cores (section 2.2.1), statistical approaches would need to be applied that enable inferring extinction rates based on phylogenies alone (e.g., Morlon et al., 2011).

vi) Another problem involves the suitability of the model taxa studied. Many ancient lakes harbor a high number of endemic species, often with diverse niches and different life styles (e.g., benthic vs. planktonic and generalist vs. specialist). However, baseline studies are necessary to verify that the candidate taxa, indeed, represent suitable model systems for the questions of interest in terms of, for example, monophyly and species richness (for details of sampling requirements and potential sampling biases see section 3.3.).

In general, the candidate abiotic and biotic drivers to be studied in interdisciplinary deep drilling projects have to be selected based on the specific scientific objectives. Apart from ‘standard’ abiotic factors such as lake-level changes, desiccation, and salinization events, parameters such as temperature and productivity (Condamine et al., 2013) have been proposed to influence diversification events and rates. Biotic drivers might be even more diverse. The underlying mechanisms and causality of some biotic drivers are well understood (e.g., the pharyngeal jaw of cichlids, Salzburger et al., 2014; depth preferences, Stelbrink et al., 2015), whereas the influence of species richness and composition on diversification is controversially discussed (e.g., Day et al., 2008; Harmon and Harrison, 2015; Hubert et al., 2015; Rabosky and Hurlbert, 2015). Also, the timeframe of evolution has to be considered when linking geological and biological datasets. Some ancient lakes such as lakes Baikal (e.g., Müller et al., 2001) and Ohrid (e.g., Albrecht and Wilke, 2008) are considered to harbor many old species or groups of species. Other lakes, though being old, experienced a series of major environmental perturbations, and the respective endemic species are often comparably young. Examples are Lake Malawi (e.g., Genner et al., 2007), the Caspian Sea (e.g., Dumont, 1998), and Lake Titicaca (e.g., Benavides, 2005; Kroll et al., 2012). Due to the young age of many taxa and problems of incomplete lineage sorting (for details see section 3.3.), phylogenetic relationships may be difficult to ascertain. In such cases, the use of primary data, such as fossils from the sediment core, is recommended for the study of old and/or long-term diversification processes, though sufficient data are rarely available.

3.3. Molecular dating

The ability to reconstruct older evolutionary events based on aDNA (see section 2.2.6.) remains limited. However, the lack of aDNA may be compensated for using DNA information from extant specimens. During organismal evolution, mutations become fixed over time (= substitutions) in the genome. Comparing these substitutions using DNA sequences of individuals that share a common ancestor may allow to reconstruct past evolutionary events, potentially driven by ecological, environmental, and/or geological processes (*sensu* Avise, 2000). A large ancestral population of lacustrine animals may, for example, become separated into two geographically separated subpopulations due to a severe lake-level lowstand. These resulting subpopulations might then evolve independently. After time, the two subpopulations thus become genetically distinct and potentially even new species. By comparing the substitutional patterns of the extant subpopulations/species using statistical approaches, the genetic structure of the common ancestor of these populations/species at the time of population subdivision (= ‘most recent common ancestor’) can be reconstructed. Under certain conditions, these divergence events might be time-calibrated using a methodology called molecular dating. This approach is based on the molecular clock hypothesis (Zuckerkandl and Pauling, 1965), which relates nucleotide or amino acid substitutions to time. In other words, knowing the number of substitutional differences between extant populations/species may allow for calculating divergence time and thus the age of the underlying event that potentially drove the separation of the ancestral population.

Today, molecular dating is widely used in evolutionary biology to infer such past evolutionary events. However, the accuracy and even the applicability of molecular dating have long been subject to controversy (e.g., Takahata, 2007; Wilke et al., 2009; Ho, 2014; Ho and Duchêne, 2014). Substitution rates may, for example, vary among taxa, with life history traits, and/or over time, making a precise dating of evolutionary events challenging. They are gene-specific and highly sensitive to problems such as incomplete lineage sorting and substitutional saturation. Moreover, results from molecular dating analyses can easily be misinterpreted if, for example, the sampling design of extant species is insufficient (e.g., Wilke, 2004; Wilke et al., 2009; Friedman et al., 2013). For most of these problems, test statistics and mathematical solutions are available, which have made molecular dating statistically sound.

However, a problem that continues to exist is that the molecular clock has to be calibrated in order to be able to calculate absolute times. This can be done using calibration points or bounds from externally derived dates such as ages of fossil occurrences and biogeographical events (e.g., vicariance events resulting from the closure of the Isthmus of Panama or the Mediterranean Salinity Crisis; Ho et al., 2015). Alternatively, calibration can be achieved via external clock rates that are gene- and often also taxon- or trait-specific (reviewed in Wilke et al., 2009). All of these different calibration methods have in common that uncertainties introduced by the calibration process often far outcompete mathematical problems with the clock approach.

The two main approaches that are currently used for molecular dating are molecular clock and coalescence analyses. The former typically calculates divergence times between species or groups of species by estimating the number of substitutions that occurred along the respective branches of a phylogenetic tree (Fig. 12). The latter models the timing of demographic and spatial expansion events of populations (e.g., mismatch analyses; Rogers and Harpending, 1992), or past changes in population sizes (e.g., Bayesian Skyline Plots; Ho and Shapiro, 2011).

Fig. 12. Methods for calibrating molecular clock trees. Substitutions along the branches of a phylogenetic tree are shown as black rectangles. A) Calibration with point(s) from externally derived dates. In this example, the known age of a divergence event (blue circle and blue dashes line) is used to estimate the timing of three other divergence events in the tree (red dashed lines). B) Calibration with bounds from externally derived dates. In this example, the known minimum and maximum ages of two divergence events (upward and downward pointing blue arrows, respectively, and blue dashes lines) are used to estimate the timing of two other divergence events in the tree (red dashed lines). C) Calibration with a known external molecular clock rate. In this example, a fixed external clock rate of 1 substitution per My and derived depth of nodes in the tree (blue dashed lines) are used to calculate absolute divergence ages (red dashed lines). For reasons of clarity, error bars of calibration uncertainties are not shown.

Both molecular clock and coalescence analyses have been used extensively in ancient lake studies including lakes Baikal (e.g., Sherbakov, 1999; Koskinen et al., 2002; Fazalova et al., 2010), Tanganyika (e.g., Nevado et al., 2013; Koblmüller et al., 2015), Malawi (e.g., Genner et al., 2010; Schultheiß et al., 2011), and Titicaca (e.g., Kroll et al., 2012). However, despite the high potential of molecular clock approaches, as yet there are only few examples for the application of molecular dating in ancient lake drilling projects. Wagner et al. (2014b), for example, found a temporal correspondence between a lake-level low stand in Lake Prespa on the Balkan as inferred from sediment-core data, the Late Pleistocene Toba eruption, and a spatial expansion in the lake's most abundant mussel species. Moreover, Föller et al. (2015) used lineages-through-time plots (i.e., a visualization of the number of accumulated evolutionary lineages over time inferred from molecular clock analyses; Harvey et al., 1994) and diversification-rate analyses in an attempt to link major environmental events inferred from the deep drilling project in Lake Ohrid to changes in diversification rates of endemic species.

Certainly, molecular clock analyses will gain more importance in future deep drilling projects. However, given the 'vagaries' (Ayala, 1997) of the molecular clock, we recommend a careful planning of molecular dating approaches. This concerns the choice of methods, molecular markers, calibration means, and taxa. The latter requires especially thorough planning because sampling in ancient lakes can be expensive and time consuming, and incomplete sampling may bias molecular dating results. As a rule of thumb, molecular clock analyses in ancient lakes work best with large, monophyletic groups of endemic species – so-called species flocks (e.g., Schön and Martens, 2004). They typically evolved within the lake and thus are likely to reflect its environmental, ecological, and geological history.

Research on the behavior of the clock, calibration means, and data basis is continuing at high pace. Of interest for drilling projects in ancient lakes are, for example, newly developed models that enable improved fossil calibrations (e.g., Heath et al., 2014; Gavryushkina et al., 2015). However, notwithstanding the progress that will be made in the future, confidence intervals of molecular dating results will likely remain wide. It is therefore important to consider these uncertainties in all conclusions made based on molecular clock or coalescence approaches in order to avoid misinterpretations (reviewed in Hipsley and Müller, 2014; Warnock et al., 2015).

3.4. *Tracing character evolution*

Understanding character (= ‘trait’) evolution of a species’ individual characters over time, such as changes in morphological/anatomical traits, ecological niches, functional roles in ecosystems, reproductive modes, or changes in geographic distributions, is of great relevance for linking geological and biological histories in ancient lakes. Tracing character evolution ideally involves groups of species that originated within the lake (species flocks) and which are comparatively old, thus allowing for inferring character evolution along the lake’s entire geological history.

Studies of character evolution can be done utilizing two main approaches. The first involves the direct observation of character-state change using chronological fossil information, often supplemented with other primary information obtained from the sediment cores. Examples include stratigraphic series of gastropods (Williamson, 1981; Van Bocxlaer and Hunt, 2013) and diatoms (e.g. Khursevich, 2006). However, continuous, high quality fossil information is typically not available (see section 2.2.1.) or only for selected groups of microfossils (section 2.2.2.). In the latter case, secondary data have to be used, i.e., information on characters states of extant species together with a hypothesis about their evolutionary relationships (typically a phylogenetic tree).

There are two particular interests in tracing character evolution along a phylogenetic tree. The first involves ‘ancestral state reconstruction’, i.e., the reconstruction of either categorical or continuous character states back in time (sometimes also referred to as ‘character mapping’ or ‘character optimization’; see Fig. 13).

Fig. 13. Tracing character (‘trait’) evolution along a phylogeny. Either categorical (pie charts) or continuous (grey-step gradient) character states can be estimated along the branches of a phylogeny solely based on trait information of extant species. Uncertainties of character tracing are here exemplified for the categorical states by the proportions of the pie charts, but uncertainty can also be estimated for continuous traits.

The second interest concerns modeling tempo (i.e., trait divergence per time) and mode (e.g., adaptive, non-adaptive, divergence, convergence) of mainly continuous character evolution. The underlying assumption is that these two parameters might not be constant over time but are potentially driven by extrinsic factors (*sensu* Schluter, 2000; Coyne and Orr, 2004). This assumption offers the possibility to test the effects of geological, climatic, and/or environmental changes in ancient lakes inferred from sediment cores on species traits over time using specific models. Thereby, a major goal of interdisciplinary deep-drilling projects can be addressed – understanding the consequences of environmental change for the biotic evolution of ancient lake taxa as well as the capacity of an ancient lake to buffer such perturbations.

Three testable models are particularly useful for ancient lake studies (Fig. 14). The simplest model (‘Brownian motion’; Edwards and Cavalli-Sforza, 1964), assumes a constant trait mean of species over time, which would indicate that extrinsic factors have no effect on character evolution (Fig. 14A). Because of its neutral characteristic, it is often used as null model to compare to empirical data.

The ‘early burst model’ assumes that character variance decreases exponentially with time (Blomberg et al., 2003; Harmon et al., 2010; Fig. 14B). It can, for example, be used to test whether the formation of an ancient lake triggered the onset of adaptive radiations (*sensu* Schluter, 2000). The third, the ‘Hansen model’, allows species to be influenced by past environmental events but assumes that the trait mean adapts to an optimum over time (Fig. 14C; Hansen, 1997). This model focusses on the relationship between environment and the pull towards an optimal character state (‘selection’; see Hansen, 2014).

Potential changes over time in tempo and mode of character evolution can be visualized by disparity-through-time plots (Harmon et al., 2003; see Muschick et al., 2012 for an example from Lake Tanganyika), displaying mean pairwise differences of traits among all species of one clade that is present at a particular moment in time (Fig. 14).

Fig. 14. Simulation of three modes (A–C) of trait evolution (grey circles) showing their signatures in the respective disparity-through-time plots (trait divergence $\sigma^2 = 0.3$). Observed traits at the tips of the phylogeny are used to estimate the 95% confidence interval over time (grey area). Deviation of the observed disparity (solid black line) from this expectancy may indicate periods of environmental influence on evolution or changes in selection strength. A) Brownian motion, which is a model of phylogeny-wide stasis in trait mean and the null expectancy in evolutionary studies. B) Early burst of trait evolution with decreasing trait variance over time. C) Hansen model of trait evolution with trait mean approaching an optimum.

However, tracing character evolution is subject to several pitfalls and challenges. Phylogeny-based analyses of character evolution through time can be biased as morphological exuberant species may be more prone to extinction (Huang et al., 2015). The effects of missing extinct species on phylogeny-based character analysis has not been studied comprehensively yet (but see Albert et al., 2009; Slater et al., 2012). However, fossil species may provide complementary insights into character evolution (e.g., Van Bocxlaer and Hunt, 2013; Benson et al., 2014) and could be used to fine-tune or even verify models of character evolution (Betancur-R et al., 2015). Another challenge is that information on species traits and detailed information on morphology, ecological attributes, and distribution of the taxa of concern is often sparse. Quality issues include sampling completeness, taxonomic coverage, the presence of cryptic species, and DNA marker choice, all affecting the quality of phylogenetic trees (node support, resolution) and subsequent state reconstructions (ambiguous states).

Examples of tracing character evolution in ancient lakes include studies on the effect of lake origin on changes in morphological traits. Gonzalez-Voyer and Kolm (2011), for example, showed two periods of increased morphological disparity in Lake Tanganyika, the older was related to the initial colonization

after lake origination but the younger one could not be explained by limnological history. Tracing character evolution was also used to infer trophic specialization and coloration patterns of endemic invertebrates of the ancient Malili Lakes of Sulawesi, Indonesia (von Rintelen et al., 2004, 2010) and to uncover convergent evolution in ancient lakes (e.g., Meixner et al., 2007; Young et al., 2009). The latter phenomenon has been puzzling evolutionary biologists for years (e.g., Mahler et al., 2013) and might be of eminent importance for the interpretation of fossil records from sediment cores. Other applications include reconstructing the colonization history of ancient lakes over time (e.g., Van Bocxlaer et al., 2015; Daniels et al., 2015), habitat or niche occupation of ancient lake species such as the colonization of rocky habitats in Lake Tanganyika by cichlids (Koblmüller et al., 2004), or the bathymetric range evolution of limpet gastropods in lakes Baikal (Stelbrink et al., 2015) and Ohrid (Albrecht et al., 2006).

From a methodological point of view, there are three trends in tracing character evolution that might be of importance for future deep drilling projects. First, phylogenetic uncertainties (Sorenson et al., 2014; Shi and Rabosky, 2015) and character variance caused by measurement errors or intraspecific variation (Revell, 2012; Clavel et al., 2015) need to be considered. Second, shifts in the tempo (i.e., different rates of trait divergence; Eastman et al., 2011; Thomas and Freckleton, 2012) or mode (e.g., from neutral divergence to adaptation; Clavel et al., 2015) of character evolution over time are to be identified, which may help mitigating erroneous ancestral state reconstruction (King and Lee, 2015). Third, characters may not evolve independently of each other but co-vary or even constrain one another. Therefore, multivariate evolutionary models that simultaneously use a set of characters with several states each are being designed (Freckleton, 2012; Mahler et al., 2013; Adams and Collyer, 2015; Clavel et al., 2015).

Whereas most earlier attempts did not integrate geological and evolutionary data from ancient lakes at once, such integration is becoming increasingly important in analyses of character changes. Integrated approaches allow to test whether geological or environmental changes previously inferred from deep drilling campaigns had an effect on patterns of character change in extant species (e.g., Danley et al., 2012; Van Bocxlaer and Hunt, 2013; Lyons et al., 2015; Stelbrink et al., 2015). Potential drivers of previously inferred changes in species traits can also be studied with data that are subsequently generated from deep drilling campaigns (e.g., von Rintelen et al., 2010 for Lake Towuti and Trajanovski et al., 2010 for Lake Ohrid).

For future deep drilling campaigns, we expect to see more explicit attempts to link geological and biological histories. As more phylogenetic data become available, future comparative analyses across taxa will help shed light on general evolutionary processes in ancient lakes that affect entire communities (e.g., Salzburger et al., 2014). If common temporal signals in phylogenies can be detected (e.g., simultaneous character or rate changes across taxa; O'Meara et al., 2006), a more straightforward link between environmental changes and evolutionary patterns may be established. These signals could also help to understand the biological consequences of environmental change in ancient lakes, even when fossil information is absent.

4. Conclusions

- 1) Over the past years, scientific drilling projects in ancient lakes became increasingly interdisciplinary and have intensified the use of secondary data, i.e., data obtained independently of the drilling operation. Comprehensive interdisciplinary projects enable a more holistic view on scientific problems and provide excellent opportunities for hypothesis-driven research.
- 2) One of the most challenging tasks for answering novel research questions in deep drilling projects is to link diverse datasets with different resolutions, different data qualities, and potentially different age uncertainties to solve complex problems.
- 3) Careful consideration of drill sites and drilling strategies are a prerequisite to optimize the chances that the goals of a deep drilling project can be reached. Meticulous preparation, including the collection of site-specific information from pilot studies may serve to construct a strategy for collecting primary and secondary data that can then be evaluated against scientific objectives, budget, logistic requirements, and the available time.
- 4) Accurate subsampling and data interpretation requires a full tracking record of the core. Moreover, sediment subsampling should be coordinated and samples should, whenever possible, be taken from the same sample depth to aid data integration.
- 5) Data analyses should be hierarchically structured. As studies from long records are very laborious and time-consuming, the first target should be to produce low/medium resolution data. Only then,

high-resolution analyses are to be conducted.

- 6) Though many new methods and analyses (e.g., analysis of isotopes in organic materials, high throughput image analyses and high throughput DNA sequencing, low-concentration biomarker analyses, studies of ancient amino acids and ancient DNA, molecular dating) are of high potential for integrating diverse datasets, their weaknesses in terms of resolution, quality of data, and practicability have to be considered.
- 7) Whereas the physical linkage of primary information obtained from sediment cores is, in most cases, straightforward due to the chronological constraints on the data, integrating secondary data and/or interpretations into a consistent representation of the natural history of ancient lakes remains challenging.
- 8) Moreover, in most previous deep drilling projects, geological and biological data were linked empirically. However, recent statistical developments enable a better mathematical integration of diverse types of datasets and the testing of hypotheses based on specific null models.
- 9) For future deep drilling campaigns we expect to see more explicit attempts to statistically link geological and biological histories aided by methodological advances in data generation (e.g., automated methods of fossil retrieval, visualization and identification) and data analyses (e.g., a better integration of uncertainties in age-depth calculations).
- 10) Interdisciplinary projects should integrate earth and life scientists, statisticians, and modelers in the planning phase, to establish clear communication strategies, to align interests, and to discuss data requirements.
- 11) Finally, the interdisciplinary character of modern deep drilling projects not only requires a constant adaptation to methodological innovations, but also targeted scientific training components. Therefore, thematic workshops, network training events and/or field schools, particularly for early stage researchers, should be an integrative part of interdisciplinary deep drilling campaigns.

Glossary

Accommodation space: Available space for accumulation of sediments.

Adaptive radiation: Rapid diversification of species accompanied by adaptation into various niches. The term is used both to describe an evolutionary process as well as the result of this process.

Age-depth model: Synthetic model that explains the relationship between sediment depth and sediment age in depositional environments.

Allopatric speciation (= *geographical speciation*): Speciation due to the evolution of (geographical) reproductive barriers in populations that prevent or interfere with gene flow.

Amino acid racemization: Spontaneous reaction describing the interconversion between the chiral forms of an amino acid.

Aminostratigraphy: Relative dating framework based on the extent of amino acid racemization in subfossil biominerals.

Anagenesis: Directional evolutionary change from an ancestor species to a descendant species without lineage splitting (also see *chronospecies*).

Ancestral polymorphism: The amount of heterogeneity that is present in an ancestral population prior to the separation of the descending species.

Ancestral state reconstruction: Reconstruction of ancestral phenotypic or genetic states of species along a phylogenetic tree based on information of extant taxa.

Ancient lake: A lake that has continuously existed for > 100 ky or even > 1 My. The meaning of the term is not universally accepted. Some authors use this term synonymously with ‘long-lived lake’. Others use the term ancient lake only for extant long-lived lakes.

Ancient lake species flock: Species rich, monophyletic group of endemic taxa that typically evolved within the lake (i.e., intralacustrine).

Bioindicator: Extant species that are used to infer the present ecological conditions of an ecosystem.

Biomarker, sedimentary: Source-specific organic molecules (‘molecular fossils’).

Biovolume accumulation rate: Diatom concentration divided by sediment accumulation rate and corrected for size differences between taxa.

Borehole logging: Process of measuring physical, chemical, and structural properties of penetrated geological formations using logging tools that are lowered into a borehole on a wireline cable.

Bottleneck, genetic: Sudden decrease in population size, which potentially reduces the genetic variation within a population.

Coalescence analyses: Population genetic analyses that relate patterns of genetic diversity in an extant population to its demographic history.

Chronospecies: Arbitrary divisions of a single evolutionary lineage, defined on the basis of morphological change within the lineage (also see anagenesis).

Cladogenesis: Evolutionary branching of an ancestor species into two or more descendant species.

Composite core: Layer-to-layer correlation of core segments from multiple boreholes drilled at the same drill site, i.e., best-case scenario of a continuous, undisturbed sediment profile.

Convergence: Similarities that have arisen independently in two or more organisms that do not share a common ancestry.

Cyclostratigraphy: Study of stratigraphic records of astronomically forced climate cycles.

Depocenter: Location of the thickest deposit in a sedimentary basin.

Divergence time: Time since separation of descendent taxa from a most recent common ancestor.

Endemism: Characteristic of a taxon that is restricted to a geographic location (such as an ancient lake).

Evolution, biological: Change in heritable traits of populations from generation to generations.

Evolution, biotic: Gradual change in the structure, composition, or dynamics of biological objects or systems.

Evolution, geological: Gradual change in the structure, composition, or dynamics of geological objects or systems.

Global benthic isotope stack: Stack of 57 benthic marine $\delta^{18}\text{O}$ records reflecting global ice volume and deep ocean temperature for the past 5.3 My, often used as stratigraphic reference record.

Hiatus: Discontinuity ('break') in the stratigraphic succession.

Incomplete lineage sorting: Phenomenon that not all genetic lineages are segregated at the time of species splitting.

Interdisciplinarity: Integration of two or more scientific disciplines.

Intralacustrine: Within a lake.

Lineages-through-time plot: Plot showing the accumulation of lineages through time in a time-calibrated phylogeny.

Lake proper: Lake body excluding peripheral water bodies or effluents/affluents.

Long-lived lake: A lake that has continuously existed for > 100 ky or even > 1 My. Some authors use this term synonymously with the term ‘ancient lake’.

Macrofossils: Remains of organisms from the remote past large enough to be visible without a microscope.

Magnetic susceptibility: A measure of the degree of magnetization of a material after the application of a magnetic field.

Microbiome: It refers to the entire microbial population within a specific environmental niche.

Microfossils: Microscopically small remains of organisms from the remote past.

Molecular clock: A concept that correlates number of nucleotide or amino acid substitutions (i.e., mutations fixed in the genome) to time.

Multidisciplinarity: Concurrent combination of two or more scientific disciplines.

Phylogenetic tree: Graphical representation of evolutionary (genealogical) relationships of several species or other units, which are assumed to have a common ancestor.

Pollen rain: The cloud of airborne pollen produced by plants.

Proxy: Measured variable used to model or generate the value of a variable that is typically more difficult to obtain.

Radiation, evolutionary: Event of rapid cladogeneses.

Relaxed clock: A dating approach that relaxes the assumption of a single substitution rate within a phylogeny and allows rates to vary across the branches.

Resilience, ecosystem: Ability of an ecosystem to resist disturbances.

Speciation: Evolutionary process leading to new species.

Species flock: In ancient lakes, monophyletic group of endemic species that evolved intralacustrine.

Stable isotope: An isotope of an element that does not tend to decay over time.

Substitution rate: Here used in terms of number of fixed mutations per site and time unit.

Subsurface biosphere: Term used to designate the active microbial life in lacustrine sediments in analogy to deep biosphere that refers to the marine environment.

Tephra: Pyroclastic material ejected from a volcano including fragmented rocks and smaller particles.

Trait: An inherited morphological, molecular, or ecological characteristic of a species.

Trait-specific clock: A single molecular clock rate of a specific gene that can be assigned to a range of taxa that share similar biological and life history characteristics that are supposedly affecting rate heterogeneity.

Transfer functions, paleoecological: Models that correlate modern species assemblages and their environmental characteristics to fossil assemblages for reconstructing past environmental conditions.

Watershed: Catchment area of a drainage basin.

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