Environmental conditions at the Last Interglacial (Eemian) site Neumark-Nord 2, Germany inferred from stable isotope analysis of freshwater mollusc opercula

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Mollusc biogenic carbonates are valuable records of past environmental conditions. In particular, carbonate oxygen ($\delta^{18}$O) and carbon ($\delta^{13}$C) stable isotopes can be used to reconstruct different physical and chemical parameters, according to the different genera used (marine, freshwater or terrestrial). The Last Interglacial (early Eemian) palaeolake of Neumark-Nord 2 (NN2), Germany provides an excellent example of a Neanderthal archaeological site with abundant freshwater carbonate remains. As in other European contexts, one of the most abundant species is *Bithynia tentaculata*. In order to provide a robust regional baseline for the interpretation of the archaeological data, this study includes a calibration phase on modern *B. tentaculata* opercula. The results indicate that these calcitic structures are likely to be subjected to a growth slowdown/cessation during summer, which influences their geochemistry, reflecting mainly the water properties of the rest of the year. This modern calibration, together with the existing palaeoenvironmental reconstructions developed for NN2 (e.g. pollen data), represents a valuable opportunity to establish *B. tentaculata* opercula as reliable environmental proxies applicable to several other freshwater contexts. The isotope data of the NN2 opercula, in agreement with the pollen record, indicate that the major archaeological horizon was formed during a rather wet period.
and potentially in a semi-forested environment. However, human occupation occurred also during
drier phases at the site and within a wide temperature range, indicating the absence of restricted
environmental preferences by the local Neanderthal groups.

**Keywords**: Neanderthals; Last Interglacial; Middle Palaeolithic; Oxygen and carbon stable
isotopes; Bithynia tentaculata; Modern calibration

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Remains of freshwater molluscs, and in particular *B. tentaculata*, are abundant and well preserved throughout the Quaternary sedimentary record across Europe (Preece 2001). Recent advances in biomolecular techniques and biomineral understanding have resulted in the application of amino acid racemization analyses of these biominerals for dating (Penkman et al. 2008, 2011). However, only few studies have previously used these materials as palaeoenvironmental records (Filippi et al. 1997; Hammarlund et al. 1999; Anadón et al. 2006).

Given that the *B. tentaculata* habitat range includes closed and slow-running water bodies, shells and opercula are often found in interglacial palaeolake infills (Anadón et al. 2006; Waghorne et al. 2012; Milano & Szymanek 2019). Previous studies suggested that *B. tentaculata* opercula constitute suitable environmental records and that their oxygen and carbon stable isotope compositions are useful proxies for water temperatures and moisture regimes, respectively (Milano & Szymanek 2019). For instance, oxygen incorporation by freshwater molluscs is influenced mainly by the water oxygen δ¹⁸O and temperature (Anadón et al. 2006; Waghorne et al. 2012). Furthermore, the carbonate δ¹³C is influenced by the water DIC (dissolved inorganic carbon), which, in turn, is controlled by water inflow and evaporation related to the moisture extent of the environment (Leng & Marshall 2004 and references therein).

High-resolution climatic reconstructions of interglacial periods, and especially of the Last Interglacial, are valuable tools for the palaeoecological interpretation of Pleistocene human-environment interactions. Evidence of human activity during the Last Interglacial in central Europe has been attested to by the discovery of several sites in northern and central Germany such as Gröbern, Grabschütz, Weimar, Neumark-Nord, Rabutz, Lehringen and Stuttgart-Untertürkheim
Roebroeks et al. 1992; Gaudzinki-Windheuser & Roebroeks 2011). Among them, Neumark-Nord 2 (NN2) stands out: the record includes not only abundant evidence for hominin activity (Gaudzinski et al. 2014; Pop 2014), but it also contains several organic and inorganic materials useful for palaeoenvironmental reconstructions, including pollen (Sier et al. 2011; Bakels 2014; Wansa et al. 2014), sediment (Mücher 2014) and mammalian biominerals (Britton et al. 2019). The aim of this study is to produce additional evidence for palaeoenvironmental conditions at NN2.

The abundance of B. tentaculata opercula throughout the Hauptprofil 7 (HP7) sequence, together with the existence of numerous other environmental proxies, represents a unique opportunity to achieve such objective. In addition, the present study includes a modern calibration of B. tentaculata $\delta^{18}O$ and $\delta^{13}C$ based on a year-long collection and environmental monitoring at Hundewasser (near Leipzig, Germany). This part of the study was designed to further refine our understanding of the freshwater biogenic carbonates as environmental records.

Study site: Neumark-Nord 2 (NN2)

Neumark-Nord is located ~ 35 km west of Leipzig in Saxony-Anhalt, Germany (51°19′28″ N, 11°53′56″ E). The first excavations uncovered the Neumark-Nord 1 basin and subsequently, less than 200 meters northeast, a second basin, Neumark-Nord 2, was discovered in 1996 (Fig. 1; Gaudzinski-Windheuser et al. 2014). The NN2 basin infill revealed archaeological deposits related to the Last Interglacial period, with the lake estimated to have started forming in the last part of Saalian and to have lasted throughout the Eemian (Sier et al. 2011). In July 2007, the NN2 profile HP7, located in the deep part of the infill, was sampled at 5-cm intervals with the deepest sample at 595 cm from the top of the section. The lithostratigraphic succession was recorded by Mücher.
The pollen record was documented along the entire HP7 profile and allowed a reconstruction of the (local) vegetational change of the Eemian Interglacial (Sier et al. 2011; Bakels 2014; Wansa et al. 2014). Numerous archaeological finds, including faunal and lithic assemblages, were excavated from the margin of the basin in different units that are well correlated to the HP7 profile. Based on multidisciplinary analyses of the finds, previous studies suggested that the area around NN2 was used primarily for food processing and flint tool production (Kindler et al. 2014; Pop 2014). Ongoing studies are focused on correlating the fine-grained archaeological data from NN2 to the record obtained during rescue excavations of the neighbouring, much larger, lake of Neumark-Nord 1 (e.g. Gaudzinski-Windheuser et al. 2018).

Along the NN2 HP7 stratigraphic succession freshwater molluscs dominated the mollusc fauna (Strahl et al. 2010; Kuijper 2014). Especially abundant were the species of B. tentaculata (used in this study), Valvata piscinalis, Radix ovata, Anisus spirorbis, Gyraulus acronicus and Gyraulus crista. The mollusc fauna indicated calm, shallow freshwater conditions (Kuijper 2014).

Material and methods

*Bithynia tentaculata* and modern sampling

*B. tentaculata* (Linnaeus, 1758) is a prosobranch gastropod living in European slow-running rivers and lakes (Schäfer 1953; Frömming 1956). The snail has a short lifespan of about 1-3 years, with sexual maturity reached within the first 12 months of growth (Tashiro & Colman 1982; Richter 2001). It is characterized by a small shell (up to 13 mm) with five whorls and an upside-pointing operculum (Clarke 1981; Jokinen 1992; Welter-Schultes 2012). As for all molluscs,
biomineralized structures are deposited throughout the animal’s life. The shell is composed of aragonite while the operculum is made of calcite (Filippi et al. 1997; Anadón et al. 2006).

For the modern component of this study, collection of live specimens was performed on a monthly basis for a period of one year from November 2017 to November 2018. Molluscs were collected from the small river Hundewasser in Lützschena near Leipzig, Germany (51°22ʹ37.3ʺN, 12°16ʹ50.7ʺE) ca. 30 km east of NN2 (Fig. 1). The molluscs (N = 39) were collected using small fishing nets at a depth of 50-100 cm. Juveniles and small specimens were avoided. On average, the opercula collected were 4.6 ± 0.2 mm long and 3.2 ± 0.1 mm wide.

The river is about 8 m wide and 1 m deep and it is characterized by extremely low current levels. Its banks are vegetated with abundant aquatic macrophytes. Water samples were collected and stored in 40-mL glass bottles with no headspace at ~ 4 °C until measurement. Air and water temperatures were measured during collection using a Voltcraft DT-300 thermometer.

Sample preparation and analysis

The soft tissues of the modern molluscs were discarded immediately after collection. In order to remove foreign material, the carbonates were immersed in 3 vol % NaOCl for 2 hours and ultrasonically washed using deionized water for circa 3-5 minutes. After being air-dried for 24 hours, the opercula (N = 39) were individually crushed using an agate mortar and pestle.

The opercula from NN2 were bleached, washed and crushed following the same protocol as the modern specimens. A total of 107 opercula were analysed from the NN2 collection. On average, three opercula were selected from 41 5-cm mollusc-rich intervals in the HP7 stratigraphic succession. The analysed opercula came from units (and archaeological levels): 5, 6 (NN2/3), 7, 8 (NN2/2b), 9 (NN2/2a), 10, 11 (NN2/1c), 17 (NN2/1a) and 18.
The stable isotope analyses of the carbonates were performed at two laboratories, the Iso-Analytical laboratory in Crewe, United Kingdom and the Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology in Leipzig (henceforth MPI-EVA), Germany. At the Iso-Analytical laboratory, carbonate powder samples were digested in He-flushed borosilicate exetainers using a water-free phosphoric acid. The released CO$_2$ gas was then measured in continuous flow mode with a Europa Scientific 20-20 gas source isotope ratio mass spectrometer (CF-IRMS). At MPI-EVA, samples were digested at 70 °C and the CO$_2$ gas was then measured with a ThermoFisher MAT 253 Plus IRMS linked to a Kiel IV automated carbonate preparation device. At Iso-Analytical, stable isotope ratios were calibrated against an IA-R022 calibrated NBS-19 ($\delta^{13}C = +1.95\%$; $\delta^{18}O = −2.20\%$), whereas at MPI-EVA, data were calibrated against an IAEA-603 calibrated Carrara marble ($\delta^{18}O = −1.64\%; \delta^{13}C = +1.87\%$). Results are reported in per mil ($\%$) relative to the Vienna Pee-Dee Belemnite (VPDB) standard. At Iso-Analytical, the average precision error ($1\sigma$; computed from eight injections per sample) was better than 0.05$\%$ for both $\delta^{18}O$ and $\delta^{13}C$. At MPI-EVA, the average precision error ($1\sigma$; computed from eight measurements per sample) was better than 0.05$\%$ for $\delta^{18}O$ and 0.03$\%$ for $\delta^{13}C$, and the long-term accuracy based on IAEA-603 samples measured blind ($n = 268$) was better than 0.05$\%$ for $\delta^{18}O$ and 0.03$\%$ for $\delta^{13}C$.

The oxygen stable isotope analyses of the water collected on monthly basis from Hundewasser were performed at the Iso-Analytical laboratory in Crewe, United Kingdom. The samples were measured by continuous flow isotope ratio mass spectrometry using a Europa Scientific ANCA-GSL and 20-20 IRMS. The analyses were performed in duplicate by the equilibration technique. The samples were measured against three reference standards. The first standard being IA-R063 with $\delta^{18}O_{V-SMOW} = −0.41\%$, the second IA-R065 with $\delta^{18}O_{V-SMOW} = −33.57\%$ and the third IA-
R064 with $\delta^{18}$O _V-SMOW = $-12.34\%$. All three standards are traceable to the primary reference standards V-SMOW2 (Vienna-Standard Mean Ocean Water) and V-SLAP2 (Vienna-Standard Light Antarctic Precipitation) distributed by the IAEA. All the modern isotope and environmental data can be found in the Supplementary Information (Table S1).

In order to test the water isotopic equilibrium deposition of _B. tentaculata_ opercula, the measured $\delta^{18}$O values were compared to the expected $\delta^{18}$O calculated using the following fractionation equation:

$$T = 15.70 - 4.36 (\delta^{18}O_c - \delta^{18}O_w) + 0.12 (\delta^{18}O_c - \delta^{18}O_w)^2$$  (1)

(Hays & Grossman 1991) where $T =$ water temperature; $\delta^{18}O_c =$ calcite $\delta^{18}$O; $\delta^{18}O_w =$ water $\delta^{18}$O.

For the fossil material from NN2, to standardize the dataset and to enhance long-term isotope trends, departures (“deviations” or “anomalies”) in the values of the single specimens from the $\delta^{18}$O and $\delta^{13}$C overall averages of the sequence were calculated. The $\delta^{18}$O and $\delta^{13}$C 2-sigma standard deviations within the monthly modern datasets (0.7‰ for both isotope ratios) were used as significance thresholds in the interpretation of the archaeological results. The isotope data from NN2 can be found in the Supplementary Information (Table S2).

**Results**

*Modern water and carbonate data*

At Hundewasser, the seasonal water temperatures fluctuated between 0.6 and 21.7 °C, with a similar variation for the recorded air temperatures (from −4.2 to 21.2 °C; Fig. 2A,B). In association with these variations, the water $\delta^{18}$O ($\delta^{18}O_w$) showed a variance range of $1.6\%$, with more negative values in winter and more positive ones during summer (Fig. 2B). The opercula showed relatively
narrow seasonal variances in their isotope values of 0.7‰ for δ¹⁸O and 0.9‰ for δ¹³C (Fig. 2C).

Significant offsets occur between measured and expected δ¹⁸O monthly values (calculated using Eq. (1)), with rather flat data distributions compared to the expected value distributions (Fig. 3A). The largest offsets occurred during the summer months of June, July and August, suggesting a growth slowdown/cessation during this time of the year (Fig. 3A). The expected average δ¹⁸O value (−5.9±1.4‰) using the annual dataset resulted lower than the measured value (−5.4±0.2‰; Fig. 3B). This offset decreased (from 0.5‰ to 0.3‰) when the period of slow growth was excluded from the average calculations, with an expected δ¹⁸O value of −5.2±1.0‰ and a measured δ¹⁸O value of −5.5±0.3‰ (Fig. 3B). Likewise, the average reconstructed water temperature using the whole annual dataset was calculated to be 7.7±1.8 °C, about 2 °C colder than the measured one (9.8±7.4 °C; Fig. 3C). By excluding the period of slow growth, the reconstructed water temperature (7.4±1.9 °C) and the measured one (6.7±5.1 °C) showed an offset of 0.7 °C.

Oxygen isotopes of B. tentaculata opercula from NN2

Compared to the average opercula δ¹⁸O values of modern specimens, the archaeological δ¹⁸O values were from 2.5‰ to 6.5‰ higher. Along the HP7 profile, the geochemical composition of B. tentaculata opercula showed a certain degree of variation. The average δ¹⁸O value varied among the different stratigraphic units (Fig. 4A), with the most negative values recorded in units 6, 10 (respectively −1.7±1.1‰ and −2.3±1.1‰) and 18 (with only two specimens; −2.9±2.0‰) and the most positive values recorded in units 5 and 17 (resp. 0.8±0.6‰ and 0.9±1.9‰). Data in units 6, 7, 8 and 17 recorded the largest δ¹⁸O variances due to the large spread among the opercula (Fig. 4A). In unit 6, the high variance was due to the particularly low δ¹⁸O values of the opercula at 545-550 cm and to the particularly high values of the specimens at 550-555 cm (Fig. 5A). In unit 7,
spread of the data was likely affected by the discontinuous sampling of opercula for the unit.

Within unit 8, high δ\(^{18}\)O values occurred in the lower part but they decreased toward lower values in the central part of the unit and then they showed a tendency towards high values in the final parts of the unit (Fig. 5A). Furthermore, unit 17 showed a clear tendency toward lower values in the upper part (Fig. 5A).

When considering the deviations from the profile δ\(^{18}\)O average, some data resulted to be smaller than the threshold of 0.7‰ (based on the 2σ standard deviations within the monthly modern dataset). Only deviations above this threshold were considered significant for the palaeoenvironmental reconstructions and therefore further discussed (Fig. 6A). Warm (positive) anomalies were observed at the end of unit 5, at the beginning of unit 7 and 8 (NN2/2b) and, to a lesser degree within unit 11 (NN2/1c). Exceptionally positive anomalies were observed at the beginning of unit 17 (NN2/1a). Cold (negative) anomalies were observed at the end of unit 6 (NN2/3), 7, 8 (NN2/2b) and, to a lesser degree in unit 9, at the end of unit 10 and in the central part of unit 11 (NN2/1c). A rather large negative anomaly was also observed at the beginning of unit 18 (Fig. 6A).

**Carbon isotopes of B. tentaculata opercula from NN2**

As with oxygen isotope composition, the archaeological δ\(^{13}\)C values were from 5.6‰ to 6.8‰ higher than the values of modern specimens. The average δ\(^{13}\)C values varied among the different units (Fig. 5B), with the most negative values recorded in units 8 and 10 (resp. −6.1±0.9‰ and −5.4±1.4‰) and the most positive values recorded in units 7, 11 and 17 (resp. −4.5±1.0‰, −4.1±1.2‰ and −4.5±1.94‰). Likewise for δ\(^{18}\)O data, large δ\(^{13}\)C variances were recorded in units 8 and 17 (Fig. 5B). As for unit 8, the variance was primarily associated with exceptionally high δ\(^{13}\)C
values at depth 430-435 cm, contrasting with the rest of the unit characterized by lower values. In
unit 17, the δ¹³C showed a tendency toward lower values in the uppermost parts. The same trend
was visible within unit 9 (Fig. 5B).

When considering the significant deviations (>0.7‰) from the profile δ¹³C average (Fig. 6B),
dry (positive) anomalies were observed at the end of unit 5, at the beginning of units 6 (NN2/3), 7
and 17 (NN2/1a), in unit 11 (NN2/1c). Wet (negative) anomalies were observed at the beginning
of units 5, 6 (NN2/3) and 7, through most of unit 8 (NN2/2b) and at the end of unit 17 (NN2/1a).

Discussion

Modern calibration of B. tentaculata

The results of modern B. tentaculata specimens demonstrated the potential of the opercula δ¹⁸O
values to be proxies for water temperature, with an error of less than 1 °C. Due to the small size of
the opercula, the sampling technique used did not ensure the right spatial resolution to resolve
seasonal isotopic fluctuations and therefore sub-annual temperature reconstructions. Other
analytical techniques, such as SIMS (Secondary Ion Mass Spectrometry) and SHRIMP (Sensitive
High Resolution Ion Microprobe), would be needed to achieve a higher temporal resolution of the
data. Although the δ¹⁸O seasonal variability in the opercula was limited, it was possible to identify
a period of potential growth slowdown/cessation during the warmest months of summer (Fig. 3A).

Generally, in bivalves, the main shell growth coincides with the warm season (Richardson 2001).
However, several studies previously reported cases of summer growth cessation (Ramón 2003;
Milano et al. 2017). Waghorne et al. (2012) investigated the growth of B. tentaculata opercula
from southern Great Britain and they estimated the growing season to be between May and
October. The different growing season recorded here is likely related to the animal’s exposure to different environmental factors such as food availability, temperature and water conditions (Witbaard et al. 1997). Furthermore, *B. tentaculata* is known to spawn between May and July (Richter 2001). The observed slow-down in opercula formation can be a result of energy reallocation from growth to reproduction, which was previously observed in several other mollusc species (Sato 1995; Purroy et al. 2018). Our results indicated that *B. tentaculata* δ18O values reflected well the water temperature throughout the year with the exception of about three months during summer. When applied to palaeoenvironmental studies, this temporal window has to be taken into account.

Although the modern data are rather promising, the application of the opercula δ18O as quantitative palaeothermometer for fossil remains has to be considered with caution. The superficial δ18O value of freshwater bodies is known to reflect the rainfall δ18O value, which varies primarily with latitude and altitude (Craig 1961; Bowen & Wilkinson, 2002). At a specific location, the rainfall δ18O is mainly controlled by air temperature and, in the tropical regions, by the amount of precipitation (Dansgaard 1964). However, the size and type of water body significantly affects the relationship between the water δ18O and rainfall δ18O (Darling et al. 2003). In fossil contexts, in which the water δ18O value is not known, a quantitative temperature reconstruction is not advisable, especially when applied to lacustrine environments. Open and large lakes have different hydrological properties compared to small and closed ones, whose δ18O water can be significantly affected by evaporative processes (Darling et al. 2003). For this reason, only qualitative temperature reconstructions are recommended (Waghorne et al. 2012). Our results from NN2 confirmed that hydrological characteristics significantly influence water and carbonate stable isotope signature. The δ18O difference observed between modern and archaeological
The stable isotope composition of the NN2 lake was rather different than the modern Hundewasser. A previous rainfall δ¹⁸O value estimation based on the oxygen isotope analysis of Equus tooth enamel from NN2 showed similar values to today’s average, with δ¹⁸O precipitation values calculated from main find level NN2/2b being -9.1±1.1 ‰, for example (Britton et al. 2019). This indicates that the differences observed in the opercula was likely related to fractionation processes in the lake water itself. Considering the small size of the lake, NN2 might have been affected by evaporative processes inducing the lake water and therefore the carbonates to have higher δ¹⁸O values. The discrepancy between the two types of environments (NN2 and Hundewasser) could have been reduced by collecting modern specimens from a water body similar to NN2. However, several field inspections and personal communications with local malacologists, revealed that the modern distribution of B. tentaculata in Saxony is largely confined in small rivers and channels. Therefore, a modern collection from lakes proved unfeasible.

As for the δ¹⁸O values, the δ¹³C modern variability was low (Fig. 2). Biogenic carbonate δ¹³C values in lacustrine environments are known to be mainly related to the δ¹³C values of the water DIC and therefore water inflow and evaporation processes (Milano & Szymanek 2019 and references therein). In particular, when evaporation exceeds water inflow, the water DIC δ¹³C is more enriched, with consequent higher values in the biogenic carbonate δ¹³C (Leng & Marshall 2004 and references therein). Previous works on B. tentaculata suggest that the δ¹³C of this species can be used as an evaporation/inflow proxy (Hammarlund et al. 2003; Anadón et al. 2006; Milano & Szymanek 2019). Hundewasser is a flowing water body with percolation of groundwater recharge. This, together with the small opercula size and consequent isotope signal averaging, could explain the limited variability of the opercula δ¹³C values throughout the year. On the other
hand, closed hydrological systems, especially when small, are significantly more sensitive to evaporative processes. As for oxygen, $\delta^{13}C$ values of specimens from NN2 were significantly higher than the Hundewasser opercula, supporting the hypothesis that the local water isotope composition would have been significantly different than modern running systems. However, records from evaporation-sensitive settings such as NN2 might offer important insights into the humidity variations at a local scale. For the $\delta^{18}O$ values, the larger sensitivity to evaporation may bias the interpretation of the isotopic signal purely based on water temperature. Therefore, the temperature cannot be reconstructed in a quantitative way and the results have to be considered with caution.

**Palaeoenvironmental reconstruction of NN2**

*B. tentaculata* opercula excavated from NN2 (HP7) indicated that, within the Eemian Interglacial, variations in the local environmental conditions occurred. Two important issues have to be considered when interpreting the opercula NN2 isotopic record: (i) the opercula, as all biogenic minerals, offer snapshots of past environmental conditions, and (ii) the environmental signal refers to the average annual conditions, summer excluded.

In the final phase of unit 5 (corresponding to the Pinus-Betula pollen assemblage zone PAZ II) an increase in temperature and a tendency toward more arid conditions occurred (Fig. 6). Micromorphological analysis of this laminated sedimentary unit indicated the presence of non-turbulent water during its colluvial deposition (Mücher 2014). These features, in agreement with our results, may relate to periods with less intense rainwash.

At the end of PAZ II, the abundance of *Pinus* increased and *Corylus* started to appear (Bakels 2014). In unit 6 (PAZ III), *Pinus* declined and *Quercus* started to dominate the vegetation. In
addition, Ilex and various deciduous trees appeared, suggesting the occurrence of a maritime temperate climate (Bakels 2014). However, the high percentage of herb pollen indicated that, despite the oceanic climatic influence, the environment was still rather open (Fig. 6C; Pop et al. 2015). The B. tentaculata record agreed well with the pollen, indicating a wet climate with periods of more arid conditions (Fig. 6B). Temperatures showed a decrease toward the end unit 6 (Fig. 6A).

The few B. tentaculata data of unit 7 were not sufficient to achieve a robust environmental interpretation during this phase. According to the pollen record, this part of the succession (end of PAZ III and start of PAZ IV) began with a steady rise in Corylus and its subsequent dominance. In general, PAZ IV was characterized by semi-open landscapes with rather scattered forests and some steppe elements (Bakels 2014). During PAZ IVa1 Ulmus reached its maximum abundance and algae of the genus Spyrogyra overgrew in the lake waters. Furthermore, the percentage of AP (arboreal pollen) versus NAP (non-arboreal pollen) increased (Bakels 2014). This indicated that the climate became more humid, as supported by the decreasing trend in the opercula δ¹³C values throughout unit 7 into unit 8 (Fig. 6B). A similar trend was visible for the δ¹⁸O values, suggesting a decrease in temperature (Fig. 6A).

In the first part of unit 8, arboreal species were still dominant (Fig. 6C) and B. tentaculata recorded rather high temperatures and humid conditions. In the second part of unit 8, temperatures dropped and a dry event was recorded (only visible at depth 430-435 cm; Fig. 6 A,B). Simultaneously, Corylus decreased and non-arboreal vegetation became more abundant (Bakels 2014). It is possible that the temperature switch toward cooler conditions may have triggered the decline of the arboreal species. At the beginning of unit 9, the temperatures increased slightly and the humidity level decreased (Fig. 6A,B). From this unit onwards, laminated deposits were
replaced by massive deposits with occasional soil formation (Pop et al 2015). In this phase, the abundance of Corylus remained low (Bakels 2014). Very few mollusc remains were retrieved from PAZ IVb1 (second half of unit 9 and unit 10), suggesting a lake dry up event. Likewise, the shells from unit 11 (PAZ IVb2) indicated that substantial aridity occurred during this period (Fig. 6B), coupled with a rather variable temperature regime (Fig. 6A). Simultaneously, a drop in arboreal vegetation (mainly Corylus) was identified by the pollen analyses, together with an increase of steppe-like vegetation (Bakels 2014). Compared to units without aquatic shell remains (e.g. units 12 to 16), unit 11 (and units with an aridity signal) indicates that the climate was rather dry but it still allowed the basin to contain water. This hypothesis was also supported by the presence of water plants, Lemna pollen and Spirogyra (Pop et al. 2015, Kuiper 2014). Unit 17 was characterized by the highest δ¹⁸O values of the whole sequence, indicating that the temperatures were significantly higher than before, especially in the lower portions corresponding to the last part of PAZ V. With the start of PAZ VI/VII (end of unit 17), the δ¹⁸O values showed a significant decrease, indicating the occurrence of a cooling trend. A similar tendency was observed in the δ¹³C values, going from less humid to more humid conditions at the end of unit 17 and beginning of unit 18. The observed elevated temperatures during PAZ V agreed with the reconstruction based on pollen by Külh & Litt (2003) and the bulk carbonate δ¹⁸O record from Gröbern (an Eemian lake ~ 70 km north-east to NN2) by Litt et al. (1996). Indeed, these studies suggested that the temperatures reached a maximum during PAZ IVb and they remained rather stable during PAZ V. Interestingly, the drier conditions observed in the Bitynia record from unit 11 (PAZ IVb2) do not seem to be reflected in the pollen sequence of NN1 (Neumark-Nord 1), the larger lake located about one hundred meters away from NN2 (Seifert 1990). Previous studies hypothesized that the absence of such environmental change at NN1 might have been related to either a very small-scale
local change or to a bias introduced by the small size of the NN2 lake (Bakels 2014). Indeed, small lakes may catch pollen from a restricted area around the lake itself (Tauber 1967). Furthermore, droppings from animals coming to the lake might have altered the composition of the pollen, enriching the consumed plant species (Bakels 2014). However, the aridity signal from the mollusc opercula confirms that the pollen data were robust and the animal droppings did not significantly affect the vegetation reconstruction. The absence of the Corylus drop in NN1 might be related to the different sizes of the two basins. Being a much smaller lake than NN1, NN2 may possibly have been more sensitive to changes in temperature and humidity levels. Therefore, the interpretation of pollen and opercula data from HP7 at NN2 might describe fairly localized environmental conditions and its application to a broader regional level might be challenging.

**Implications for Neanderthal presence at NN2**

Traces of human presence at NN2 were found from unit 6 to unit 17 (Fig. 6; Table 1; Gaudzinski-Windheuser et al. 2014; Pop 2014). Our reconstruction indicated that the local environmental conditions changed within these phases, suggesting that Neanderthal groups were present at NN2 under varying temperature and humidity regimes. This correlates with the results from the oxygen isotope analysis of horse tooth enamel from the main archaeological find levels in a recent study (Britton et al. 2019). The major archaeological assemblage, with the highest concentration of finds, was NN2/2b (unit 8; PAZ IVa2). The reconstructions based on B. tentaculata opercula indicated NN2/2b was the most humid phase of the whole succession (Fig. 6B). High humidity potentially supported the expansion of forested areas, suggesting that semi-closed environments may have occurred during this period. In support of our data, the percentage of arboreal pollen, (especially
in the first half of unit 8), was particularly high compared to non-arboreal pollen (Bakels 2014). This also correlates with evidence for niche partitioning between horses and bovids in this phase, which suggested a mosaic and semi-forested environment (Britton et al. 2012). Besides NN2/2b, less dense archaeological assemblages were found at various different stratigraphic depths (Fig. 6; Table 1). The corresponding temperature estimations ranged from very warm (NN2/1a), cold (NN2/3), average (NN2/2a) and variable (NN2/1c; Fig. 6A; Table 1). Similarly, the humidity range was rather broad with very dry (NN2/1c), average (NN2/2a) or with variable conditions (NN2/3, NN2/1a; Fig. 6B; Table 1). Virtually no B. tentaculata opercula were recovered from levels NN2/1b and NN2/2c. Therefore, their respective palaeoenvironmental reconstructions are missing.

Our results suggested that Neanderthals were present both during the occurrence of relatively dense forests, semi-open environments, as well as more open steppe-like landscapes around the basin. Our data did not indicate any evident environmental preference that might have affected the presence of Neanderthals during the Eemian Interglacial here, which is in agreement with similar recent studies at Neumark (Britton et al. 2019). The character of the presence of Neanderthal groups in central Europe during the Last Interglacial has been the topic of several previous studies (Gamble 1987; Roebroeks et al. 1992; Gaudzinski 2002; Richter 2006; Wenzel 2007; Gaudzinski-Windheuser & Roebroeks 2011). In the past, some researchers argued that Neanderthals might have had limited adaptive abilities to survive successfully in fully forested interglacial conditions (Roebroeks & Tuffreau 1999). However, in Central Europe, Neanderthals show a high flexibility in subsistence strategies in a variety of habitats (Gaudzinski-Windheuser & Roebroeks 2011 and references therein; Gaudzinski-Windheuser et al. 2018), indicating that they were highly responsive to climatic and environmental shifts (Gaudzinski 2002, 2004; see also Antoine et al.
2006, and Sier et al. 2015 for comparable evidence from north western France). These inferences are in line with the results obtained by this new proxy for palaeoenvironmental reconstructions.

Conclusions

The present study presents the reconstruction of palaeoenvironmental conditions occurring during the Last Interglacial at Neumark-Nord 2 (NN2), Germany. Oxygen and carbon stable isotopes of B. tentaculata opercula were used as environmental proxies. Modern specimens were analysed for calibration purposes and the results suggest that the opercula are generally formed throughout the year, with a minor slowdown during summertime. Therefore, the water properties (i.e. temperature and moisture regimes), encoded in the opercula bulk isotope signal in form of $\delta^{13}$C and $\delta^{18}$O values, encompasses annual averages with potential exclusion of the warmest period. The isotope data from the NN2 opercula indicate the local conditions during the Last Interglacial were characterized by alternations between warm and cold conditions as well as successions of phases with different humidity levels, although indicate that the absolute values measured were influenced by local hydrological conditions in the lake. The major evidence of human activity, related to Neanderthals, coincides with a humid phase and potentially with a semi-closed type of vegetation. However, human presence was detected in six additional levels within NN2 which were characterized by rather variable environmental conditions. In the light of the results presented in this study, and other recent analyses, we conclude that the local Neanderthal groups in NN2 area did not manifest a clear environmental preference, and instead were active across a wide range of temperature and humidity conditions.
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Author contributions. — S. M., K. P. and K. B. conceived the study and were in charge of overall direction and planning. E. P., W. K. and W. R. gave access to the samples analysed in the project. S. M. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Figure and table captions
Fig. 1 Location of the modern *B. tentaculata* collection site (Hundewasser: 51°22′37.3″ N, 12°16′50.7″ E) and the archaeological site of Neumark-Nord 2 (NN2: 51°19′28″ N, 11°53′56″ E).

Fig. 2 A. Air temperatures (open circles) measured at Hundewasser and modelled monthly rainfall δ¹⁸O values (grey triangles and secondary y-axis) obtained from OIPC. B. Water temperature (black circles) and δ¹⁸O values (grey triangles and secondary y-axis) measured throughout the
annual collection in Hundewasser. C. Modern *B. tentaculata* opercula (N = 37) $\delta^{18}$O (open circles) and $\delta^{13}$C values (black circles and secondary y-axis). Error bars indicate standard deviation.
**Fig. 3** Modern *B. tentaculata* δ¹⁸O data. A. Monthly opercula δ¹⁸O values measured throughout the annual collection in Hundewasser (open triangles) and the respective expected values (circles) calculated using Eq. (1). The highlighted area represents the potential period of cessation/slowdown of the opercula growth. B. Average measured and predicted values of the opercula δ¹⁸O calculated using the inverse function of Eq. (1). For this purpose, the calculations were carried out using both the whole annual dataset (i.e. 13 measured water δ¹⁸O values and temperatures) and the partial dataset excluding the period of slow growth (i.e. using 10 water and temperature datapoints except the data from June, July and August). C. Average measured and predicted (Eq. (1)) values of the water temperature using the whole annual dataset and the partial dataset excluding the period of slow growth. Error bars indicate standard deviation.

**Fig. 4** Overviews of the stable oxygen (A) and carbon isotopes (B) data obtained on *B. tentaculata* opercula from NN2, grouped by stratigraphic units (18 to 5).
Fig. 5 Raw stable oxygen (A) and carbon isotope (B) data obtained on *B. tentaculata* opercula throughout the NN2 stratigraphic sequence. The bars above the two graphs represent the subdivision of the succession in 14 units (18 to 5). The units in grey contain archaeological findings.
Fig. 6 Stable oxygen (A) and carbon isotope (B) anomalies throughout the different stratigraphic units (18 to 5) and PAZ (Pollen Assemblage Zones; VII to II). Grey areas mark the significance level of 0.7‰ representing the annual isotopic variability. Data outside these envelopes were
considered significant for the palaeoenvironmental interpretation. C. Pollen diagram from Pop & Bakels (2015) showing the percentages of arboreal pollen (AP; using the three key taxa of Pinus, Corylus and Carpinus) versus non-arboreal pollen (NAP; including grasses and sedges). $Q-U-C = Quercus-Ulmus-Corylus$.

Table 1. Find levels at NN2 with corresponding palaeoenvironmental conditions reconstructed from B. tentaculata geochemistry record.

<table>
<thead>
<tr>
<th>Find level</th>
<th>Corresponding unit</th>
<th>Finds m$^2$</th>
<th>Total finds</th>
<th>Palaeoenvironmental reconstruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN2/3</td>
<td>6</td>
<td>68</td>
<td>271</td>
<td>cold variable</td>
</tr>
<tr>
<td>NN2/2c</td>
<td>7</td>
<td>11</td>
<td>2296</td>
<td>n/a n/a</td>
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<tr>
<td>NN2/2b</td>
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<td>125,322</td>
<td>warm than cold very humid</td>
</tr>
<tr>
<td>NN2/2a</td>
<td>9</td>
<td>3</td>
<td>1501</td>
<td>average average</td>
</tr>
<tr>
<td>NN2/1c</td>
<td>11</td>
<td>37</td>
<td>8811</td>
<td>variable very dry</td>
</tr>
<tr>
<td>NN2/1b</td>
<td>13</td>
<td>17</td>
<td>1250</td>
<td>n/a n/a</td>
</tr>
<tr>
<td>NN2/1a</td>
<td>17</td>
<td>1</td>
<td>4</td>
<td>very warm dry than wet</td>
</tr>
</tbody>
</table>

Table S1. Modern environmental data and B. tentaculata isotope values during the annual collection conducted at Hundewasser. SD = standard deviation.

Table S2. B. tentaculata opercula isotope values throughout the HP7 profile in NN2. SD = standard deviation.