ECOLOGICAL STASIS IN SPINICAUDATA (CRUSTACEA, BRANCHIOPODA)? EARLY CRETACEOUS CLAM SHRIMP OF THE YIXIAN FORMATION OF NORTH-EAST CHINA OCCUPIED A BROADER REALIZED ECOLOGICAL NICHE THAN EXTANT MEMBERS OF THE GROUP

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Abstract: The palaeoecology of Spinicaudata, the dominant group of benthic invertebrates in many pre-Cenozoic freshwater environments, remains poorly understood. In analogy with extant taxa, it has been oversimplified and often reduced to shallow, temporary environments characterized by few trophic levels, implying ecological stasis from the Devonian to the Recent. We excavated 43 horizons of the Lower Cretaceous Yixian Formation (Anjiagou and Hengdaozi beds) to evaluate whether spinicaudatan ecology can be simplified to such an extent. Sedimentological evidence suggests general perennial conditions during the excavated lake interval. Based on 33 226 specimen counts, we identified three arthropod-dominated macrobenthic associations and two assemblages. Response curves indicate that the spinicaudatan *Eosestheria middendorfii* was exceptionally tolerant to environmental gradients, followed, in decreasing order, by mayfly larvae, water boatmen and oligochaetes. Many spinicaudatan-yielding layers represent death after reproductive senescence rather than mass-mortality events. Spinicaudatan size varies significantly according to faunal association. A forward stepwise regression model suggests that growth responded to population density and diversity: lower densities and higher diversities triggered fast growth, and vice versa. The proposed strong density effect on carapace size has been corroborated by rearing experiments: as for *E. middendorfii*, natural log regression provided the best fit for the two extant species *Eulimnadia texana* and *Eocyzicus argillaquus*. Hence, *E. middendorfii* was a tolerant and morphologically variable species adapted to perennial waters and the frequent presence of higher trophic levels. Although there are rare records of extant taxa co-occurring with fish, the described Early Cretaceous environment is commonly not inhabited by extant Spinicaudata.

Key words: ecology, *Eosestheria middendorfii*, fossil Lagerstätte, Jianshangou Member, Spinicaudata, Yixian Formation.

THE niche breadth of fossil Spinicaudata, one of the three groups formerly combined into the paraphyletic taxon 'Conchostraca' (clam shrimp) and the dominant group of benthic invertebrates in many pre-Cenozoic lake environments, has traditionally been interpreted as being restricted to ephemeral ponds, implying ecological stasis from the Devonian to the Recent (e.g. Gueriau *et al.* 2016). Interpretations of fossil clam-shrimp population ecology are often based on an analogy with extant 'shallow and temporary' habitats (e.g. Frank 1988; Todd 1991; Wang 1999; Vannier *et al.* 2003; Fürsich *et al.* 2007; Stigall *et al.* 2017) which has been critically challenged by

Orr & Briggs (1999) and Olsen (2016). The vast majority of extant clam-shrimp species live in fresh to subsaline waters (Timms & Richter 2002) and the group is generally a good indicator of freshwater conditions from the Late Carboniferous to the Recent (Petzold & Lane 1988; Vannier *et al*. 2003). However, the 'shallow and temporary' interpretation of ancient water bodies inhabited by clam shrimp often contradict associated sedimentological features (Hethke *et al*. 2013a; Barth *et al*. 2014; Olsen 2016), which led Olsen to coin the phrase 'the paradox of clam shrimp paleoecology'.

This oversimplification of fossil (and extant) clam-shrimp ecology has hampered the development of palaeoenvironmental interpretations, exemplified by the volcanically influenced fossil Lagerstätte of the Barremian to Aptian Yixian Formation of western Liaoning, China, which forms the archive of the so-called Lake Sihetun (Jiang *et al*. 2012). Clam shrimp of this fossil Lagerstätte have been proposed to have lived in quiet and shallow waters of 2–50 cm to a maximum of 2 m depth near the lake coast (Wang 1999; Fürsich *et al*. 2007). In the present study, we re-investigate the factors that determined the general high abundance and distribution pattern of the Early Cretaceous clam shrimp *Eosestheria middendorfii* (Jones 1862; Hethke *et al*. 2018a).

Sedimentological and taphonomic analyses subdivide the Jianshangou Member of the Yixian Formation into four beds, which correspond to four phases of lake evolution (Jiang *et al*. 2012; Hethke *et al*. 2013a; Wang *et al*. 2016). Most of the excellently preserved biota, such as feathered dinosaurs or early angiosperms (Xu *et al*. 1999a, b, 2001; Sun *et al*. 2002) are found in beds 2 and 3. This study quantitatively documents the community relicts of 43 horizons excavated near Jianshangou in the Sihetun area (excavation area 25 m^2). While previous works (Fürsich *et al*. 2007; Pan *et al*. 2012a) focused on a single lake phase (Phase 2), this work represents the first account on Phase 3 lake communities and investigates the community development across the marked sedimentological transition between beds 2 and 3 (Fig. 1).

As Lake Sihetun existed at a palaeolatitude of 41.9°N (Enkin *et al*. 1992; Zhou *et al*. 2003) with mean air temperatures of about 10°C (Amiot *et al*. 2011) it was probably influenced by seasonal fluctuations, expressed in summer hypoxia (stagnation) and winter re-oxygenation of the bottom waters (Fürsich *et al*. 2007). Seasonal community successions are exemplified by the rhythmic presence of chrysophycean cysts within the sediments of Phase 2 (Hethke *et al*. 2013a).

In addition to seasonal successions, pronounced environmental stress due to major disturbances of the lake's ecosystem potentially affected population dynamics and long-term temporal patterns in community composition. The proposed main disturbances of Phase 2 are connected to volcanic activity (Fig. 1; Jiang *et al*. 2011, 2012; Hethke *et al*. 2013a) and recurrent anoxia (Fürsich *et al*. 2007; Pan *et al*. 2012a; Hethke *et al*. 2013b). During this phase, bottom waters were governed by dysoxia with sporadic anoxia, and the lake assumed holomictic, eutrophic conditions that episodically alternated with meromictic interludes. Spatial variations in bottom-water redox state were widespread (Hethke *et al*. 2013b; Wang *et al*. 2016; Zhou *et al*. 2016) creating sub-environments within the lake. Clam-shrimp occurrences have been frequently interpreted as mass mortality events (Fürsich *et al*. 2007; Pan *et al*. 2012a) but life-cycle based explanations such as death from senescence should also be considered. During Phase 3, bottom waters were fully oxygenated and possible mass mortality events must have been caused by factors other than oxygen depletion. Here, we distinguish between true mass mortalities and life-cycle events.

In the present study, we: (1) identify long-term community successions to understand ecological disturbances to the ecosystem of Lake Sihetun; and (2) characterize the population ecology and life history patterns of *E. middendorfii* to reveal similarities and differences between this Early Cretaceous setting and modern clam-shrimp habitats.

GEOLOGICAL SETTING

The Lower Cretaceous Yixian Formation was deposited within an interval of about 7 myr (129.7 ± 0.5 Ma and 122.1 ± 0.3 Ma; ^{40}Ar/^{39}Ar; Chang *et al*. 2009) and its depositional environment was governed by repeated volcano eruptions, manifested in lava flows, magma intrusions and ash layers (Jiang *et al*. 2012; Hethke *et al*. 2013a). In the Sihetun area, the Yixian Formation comprises three members (Jiang & Sha 2007; Wang *et al*. 2016): the fluvial and alluvial volcaniclastic Lujiatun Member, the extrusive-basaltic Xiatulaigou Member and the lacustrine Jianshangou Member. The so-called Upper Lava Unit, previously interpreted as a unit overlying the Jianshangou Member (Jiang & Sha 2007), has recently been reinterpreted as intrusive igneous rock (Wang *et al*. 2016, see fig. 1 for an updated geological map of the area).

The sediments of the so-called Lake Sihetun are represented by the Jianshangou Member (125.7 ± 2.6 Ma to 124.2 ± 2.5 Ma; ^{40}Ar/^{39}Ar; Zhu *et al*. 2007) which can be subdivided into four depositional regimes (beds 1–4; Jiang *et al*. 2012) that generally correspond to four phases of lake evolution. Phase 1 was initiated by a caldera collapse (Jiang *et al*. 2011) and the subsequent rising of water levels. Phase 2 yielded very fine, mainly suspension-derived deposits, while slightly coarser hyperpycnal flows governed Phase 3. A prograding fan delta led to the eventual siltation of the lake during Phase 4. Not all of the four beds of the

FIG. 1. Litholog of excavation JSG, which can be separated into two main beds belonging to the Anjiagou and Hengdaozi beds, respectively, whose boundary is indicated by a dotted line; 16 segments have been recognized and briefly characterized in Hethke *et al.* (2018*b*, S1). Bed 2 is marked by μm-thick, clay–silt couplets and intercalated tuff layers. The uppermost layers of Bed 2 are associated with 1–2 cm thick layers of plant remains. Bed 3 is characterized by consecutive units of normal-graded silty fine sandstones that are marked by pervasive meiofaunal bioturbation and overlain by biofilms and background sediments. There is a general coarsening and thickening-upwards trend throughout Bed 3. Thicknesses of normal-graded units in Bed 3 are exaggerated in the litholog.
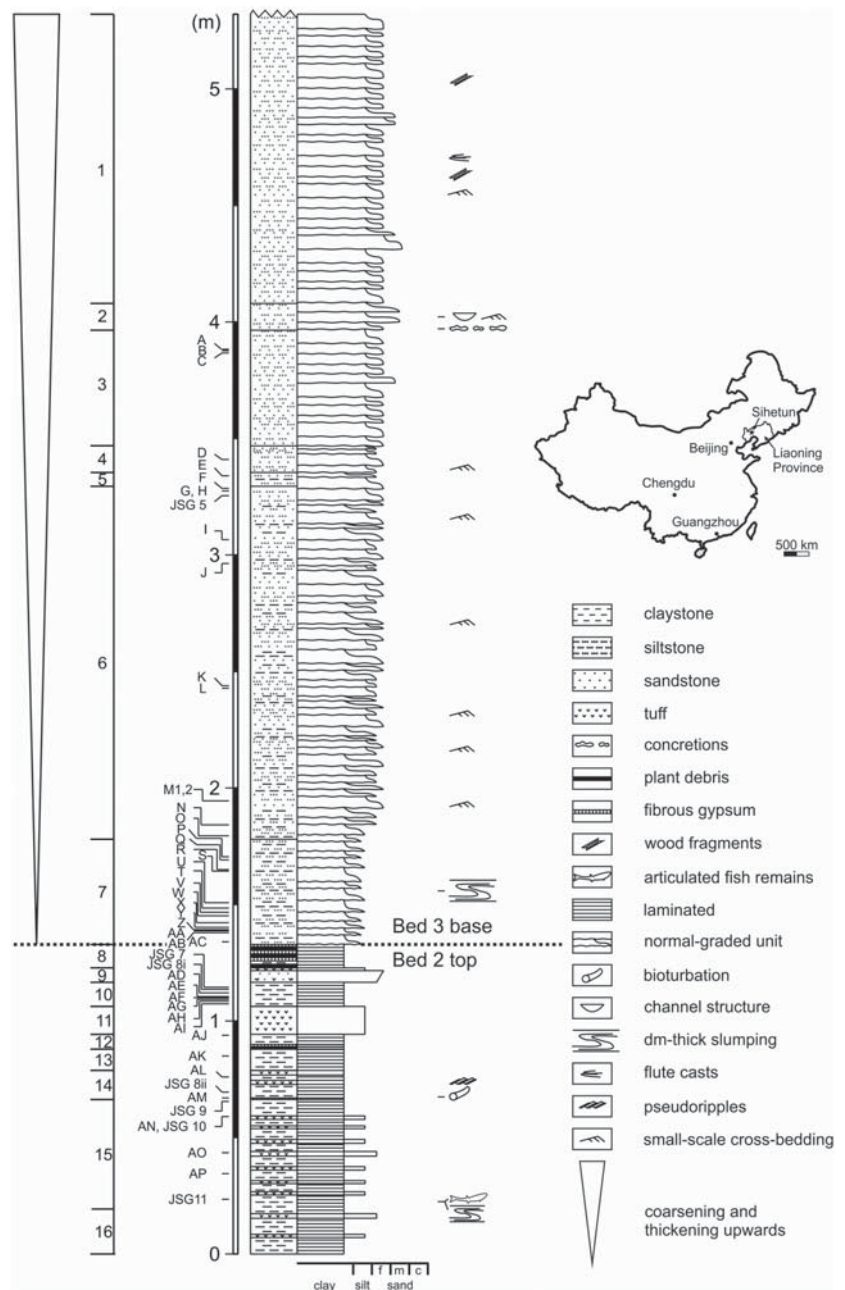
Jianshangou Member are present throughout the Sihetun area. Locality Erdaogou (LXBE; see Hethke *et al.* 2013*b*, fig. 1) for example, is lacking the delta deposits of Phase 4. Facies changes within the lake resulted from local relief. Recently, Wang *et al.* (2016) defined four beds: Dajianshanzi Bed (shallow water), Anjiagou Bed (microlaminated mudstones, meromictic lake), Hengdaozi Bed (alternating dysoxic and oxygenated bottom waters) and Huangbanjigou Bed (alternation of mudstones and volcaniclastic sandstones), which partly correspond to the four beds recognized by Jiang *et al.* (2012). This case study concentrates on beds 2 and 3 (= Anjiagou and Hengdaozi beds) from

which the majority of the excellently preserved fossils known from the Sihetun area originates.

MATERIAL AND METHOD

The present study focuses on palaeoenvironmental changes recorded by the excavation near Jianshangou (JSG, 2008; locality indicated in Hethke *et al.* 2013*b*, fig. 1) across the sedimentological transition from Bed 2 into Bed 3. Bed 3 was not sampled by the two previous excavations near Erdaogou and Zhangjiagou (LXBE and ZJG;

Fürsich *et al.* 2007; Pan *et al.* 2012*a*). Thus, this study marks the first examination of palaeocommunity changes across the transition between phases 2 and 3. The sedimentological data of excavation JSG are summarized in Hethke *et al.* (2018*b*, S1). Fossil communities were quantitatively documented for 30 horizons of Bed 3 and 13 horizons of Bed 2. The abundance data employed in this study consist of 33 226 quantitative specimen counts of nine benthic invertebrates (Hethke *et al.* 2018*b*, S2). Horizons JSG A and C had to be recounted under a lab microscope, due to mass occurrences of very small spinicaudatans (about 1 mm) that were overlooked during fieldwork. Mayfly-larva counts from the excavation were standardized accordingly, resulting in two mayfly counts below 1 (Hethke *et al.* 2018*b*, S2). The collection received the number SNSB-BSPG 2018 XIV (Bavarian State Collection of Palaeontology and Geology Munich, Germany) but will be housed in the Freie Universität Berlin, Germany, until 2020.

Biodiversity and sample standardization to area

Example fossil horizons and aquatic benthic organisms are illustrated in Figures 2–5, and biodiversity indices are listed in Hethke *et al.* (2018*b*, S2). Richness has been rarefied to the same number of individuals to identify whether differences in species richness (*S*) between horizons are influenced by sample size. Rarefaction is performed using `rarefy` function in `vegan` on the original counts (Oksanen *et al.* 2017). A third biodiversity estimate is based on the Shannon–Wiener index (H'), which considers the number of species and the relative abundance of taxa. H' will be 0 for monospecific samples ($H' = -\Sigma\ p_i \ln p_i$; p_i = proportion of a species).

S is generally regarded as being dependent on sample size (e.g. Colwell *et al.* 2004). In case of the present study, sample size equals area. For example, 2205 of 2207 counted individuals of sample JSG B are clam shrimp (Hethke *et al.* 2018*b*, S2). One might think that the high number of clam-shrimp individuals renders the occurrences of one mayfly larva and one water boatman unimportant. However, this is not the case when looking at specimen counts in a given surface area. Standardized to a surface area of 10 m^2, JSG B exhibits an even higher abundance of the mayfly larva *Ephemeropsis trisetalis* (~39 individuals; column O in Hethke *et al.* 2018*b*, S2) than horizons JSG Q (~31 individuals) or JSG Y (~25 individuals), which are marked by considerably higher relative abundances than JSG B. Clam shrimp of horizon JSG B (Fig. 4B, C) are small and marked by a high population density, while those of JSG Y are all large and scattered (Fig. 6). JSG Q exhibits exclusively water boatmen and mayfly larvae.

This raises the question of how reliable diversity indices are in the case of a mass occurrence of one species, which undermines the environmental information of co-occurring taxa. As individual counts have always been conducted on a defined area during our excavation (Figs 2D, 4F) taxon occurrences may be regarded not only in relation to the entire community but also to excavated area. Therefore, individuals have been standardized to excavated area (Hethke *et al.* 2018*b*, S3) to enable the comparison of abundances per unit area among horizons.

Orientation patterns of Ephemeropsis *larvae*

To check for possible post-mortem transportation of faunal remains, we collected *in situ* biostratinomic data during our excavation. Directional patterns of mayfly larvae were obtained by defining 12 directions, each of which spanning 30° (Fig. 3D; *n* = 43, 11, 83, 49, 14 for horizons JSG H, J, AA, AB, AP). These directional data were analysed using Rayleigh's test as described by Davis (1986, eqn 5.42, 5.43) and as implemented by the PAST software (https://folk.uio.no/ohammer/past/; Hammer *et al.* 2001). The non-parametric Rao's *U* seems inappropriate for such interval-based data. Each directional measurement can be thought of as a unit vector. The resultant length *R* gives the average direction of a set of vectors. Dividing *R* by the number of observations gives a range between 0 (vectors are randomly dispersed) and 1 (all vectors are pointing towards the same direction). The resultant quantity, 'mean resultant length' ($\bar{R}$) can be understood as a measure of dispersion, with larger values indicating orientation in the data and smaller values indicating a wide dispersal of vectors around a circle.

The data collected in the field give the 360° orientation of the larval head as plotted in Figure 3D, where one dot represents one individual. However, the vector length of the resultant vector will be 0 for two individuals of *E. trisetalis* whose heads point towards opposite directions, even though both individuals exhibit the same orientation. Hence, instead of signalling a clearly oriented sample, $\bar{R}$ would be falsely low. Therefore, categories 210°–0° have been mirrored (e.g. 210° = 30°, 240° = 60°) in order to perform Rayleigh's test with only six remaining categories ('Orientations' option in PAST). The probability model used is the circular equivalent of the normal distribution (von Mises; Davis 1986). The test for randomness is based on the assumption that the data are drawn from a population with the von Mises distribution.

H_0: *Ephemeropsis* larvae are randomly oriented.

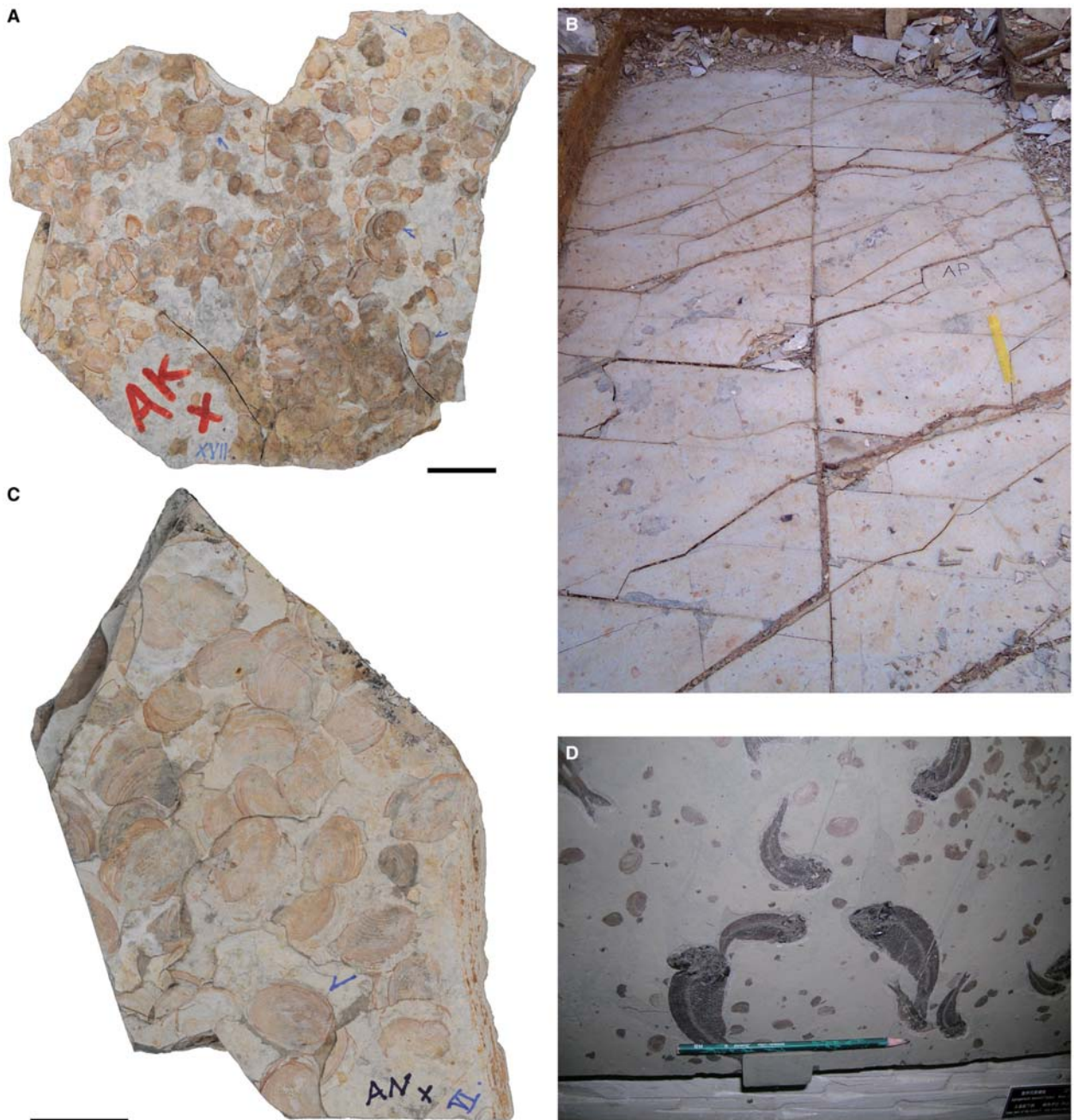H_1: There is a preferred orientation among *Ephemeropsis* larvae.

FIG. 2. Overview of Bed 2. A, JSG AK (SNSB-BSPG 2018 XIV_AK17); concentration of *Eosestheria middendorfii* individuals of various sizes in multiple laminae. B, excavated bedding plane of JSG AP. C, JSG AN (SNSB-BSPG 2018 XIV_AN6); concentration of large individuals in multiple laminae. D, rock slab from the Jingangshan area, Yixian Formation; photo taken in the Sihetun Fossil Museum; although spinicaudatans and fish are preserved on different laminae in this example, it indicates that spinicaudatans frequented perennial lakes yielding fish during the Early Cretaceous. Scale bars represent 2 cm (A, C). Colour online.

Faunal associations and ordinations

In this study, we define the term 'association' as recurrent (par-)autochthonous community relics that include only part or none of the original soft-bodied fauna, whereas (par-)autochthonous relics that lack data on recurrence are defined as 'assemblages' (modified from Fürsich 1984). The original definition of assemblage also includes 'transported, mixed, or diagenetically distorted community relics' (Fürsich 1984, p. 315). So, assemblages can be understood as possible associations or as mixed remains of several former communities from various environments.
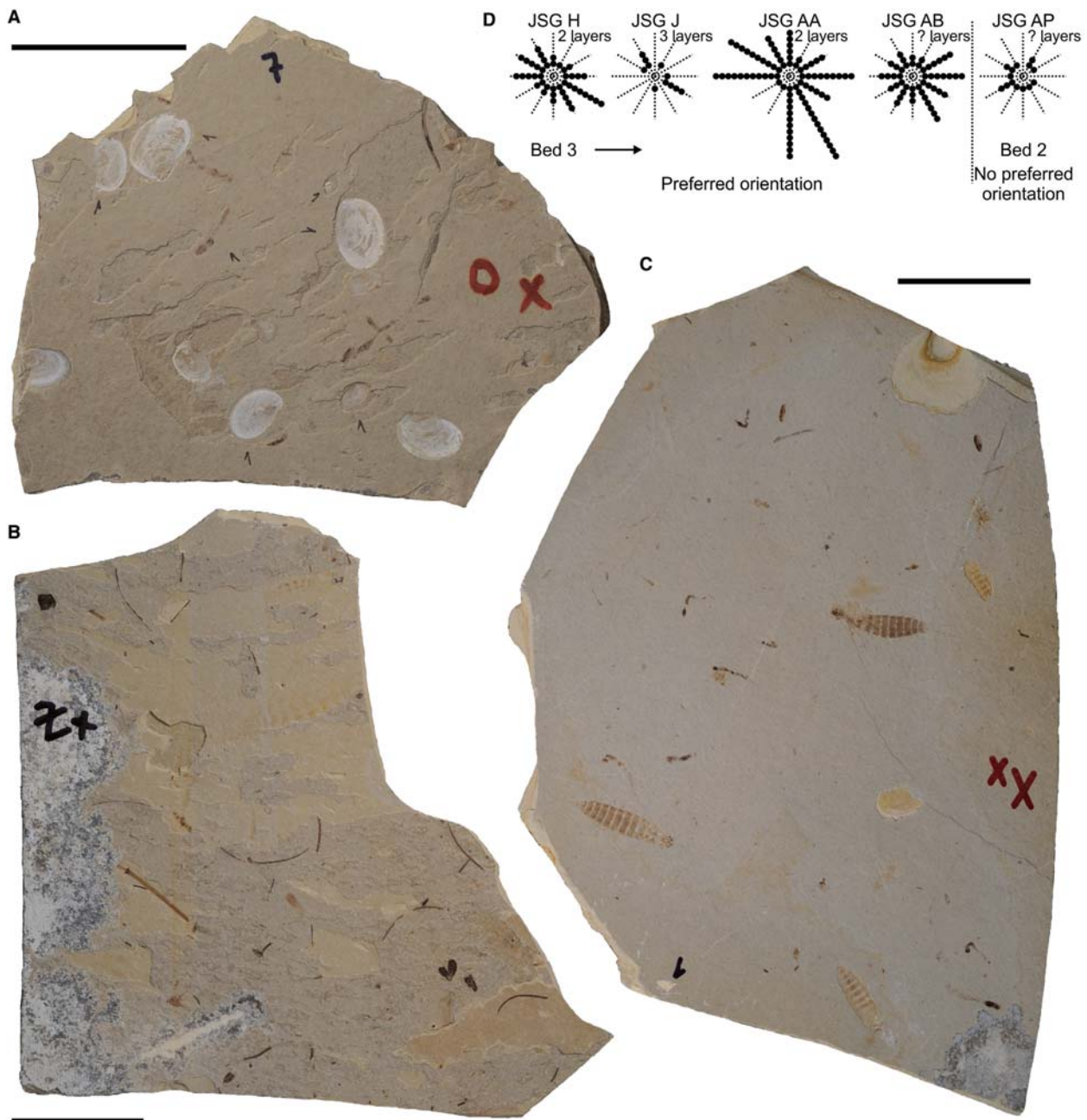
FIG. 3. Overview of Bed 3, Transitional Fauna (Association 3 and assemblages Z and Q). A, JSG O (SNSB-BSPG 2018 XIV_O7). B, JSG Z (SNSB-BSPG 2018 XIV_Z1). C, JSG X (SNSB-BSPG 2018 XIV_X1). Bedding planes are well defined. Nevertheless, the exact number of layers within a horizon has been counted to get an estimate of time averaging. The fauna of JSG X is, for example, distributed over two closely adjacent horizons. D, the orientation of *Ephemeropsis* larvae within the outcrop is visualized with a dot plot (12 categories); each dot represents the direction the head of one individual points to; the column height, therefore, corresponds to the absolute number of observations within a directional category; 360°–180° corresponds to 070-250 (ENE-WSW) and 90°–270° to 160–340 (SSE-NNW) in outcrop position. Scale bars represent 5 cm (A–C). Colour online.

Prior to statistical analyses, the area-standardized (10 m^2) abundance data of 43 horizons (Hethke *et al.* 2018*b*, S2) was double-square-root transformed to reduce the strong influence of *Eosestheria middendorfii* on the pattern. Faunal associations were then determined by means of group-average sorting of compositional dissimilarities based on the Bray–Curtis measure `vegdist` {`vegan`} (Oksanen *et al.* 2017) which has been interpreted to have a robust relationship with ecological distance (Faith *et al.* 1987). The resultant set of

dissimilarities has subsequently been hierarchically clustered using the function `hclust {stats}` (R Core Team 2017).

We represent community relationships using correspondence analysis (CA) and non-metric multidimensional scaling (NMDS; Kruskal 1964). For CA, we used the function `cca {vegan}` based on the algorithm in Legendre & Legendre (2012) which involves the chi-square statistic and weighted samples and species. Each of the nine identified species received a score close to the scores of the samples it was common in (Braak 1985), so species and samples correspond within the plot. CA axes are generally interpreted to relate to underlying environmental variables, and species are expected to exhibit unimodal responses to the environmental variable by becoming less common at higher and lower values of that variable (Braak 1985).

NMDS was performed based on the Bray–Curtis measure as implemented by the PAST3 software (https://folk.uio.no/ohammer/past/; Hammer *et al.* 2001). Stress values of <0.05 are considered to provide excellent representation, 0.05–0.1 good, 0.1–0.2 useable, and values of >0.2 indicate unreliable data (Clarke 1993).

Relay plot. To visualize the species that are commonly associated, we plot schematic unimodal response curves of the six most abundant species along the environmental gradient underlying CA1. The position of horizons in the relay is indicated by the relay index, which derived from the horizon-coordinates on CA1 (Hennebert & Lees 1991).

Carapace size and clam-shrimp population density

During our excavation we noticed that clam-shrimp size and population density were highly variable and we suspected a relationship between these two variables. To identify possible factors influencing the size of *Eosestheria middendorfii*, we: (1) visualize size distributions and population densities for each excavated horizon; (2) investigate diversity effects on size and population densities; and (3) examine population–density effects on size which we corroborate with rearing experiments. Other factors such as temperature will be discussed.

Clam-shrimp axes (Hethke *et al.* 2018a) have been measured using a calliper with a precision of ±0.1 mm. Most of these measurements were taken in the field in Liaoning, where it was impossible to distinguish between closely adjacent, sub-millimetric sedimentary layers, whose number has been subsequently counted in the lab. The data are displayed in beanplots. Each bean consists of individual observations in the form of small lines and a density trace. The implementation in *R* as provided by Kampstra (2008) was employed.

Distributions of mean carapace size and population density in monospecific and higher-diversity associations. We examined the relationship between the categorical variable 'monospecific' (Y/N) and: (1) population density of *Eosestheria middendorfii* in clam-shrimp yielding associations (individuals/100 cm²); and (2) mean carapace size, respectively, using the JMP Pro 13 software package (https://www.jmp.com). Horizon JSG C was excluded from the analysis, as only large carapace axes were measured in the field, while density reflects both mm and cm-sized individuals within two separate layers of the same horizon. A lack of size data in clam-shrimp yielding horizons JSG V, W and AC limited the second analysis to 37 data points.

Stepwise regression analysis. To identify possible variables influencing carapace size, we built a regression model based on forward stepwise regression as implemented by the JMP Pro 13 software package, with size (mean axis measurements) as the dependent variable. Our 10 potential model factors were diversity (Shannon) and the population densities of the nine benthic taxa identified in our excavation. Specimen counts were standardized to an area of 100 cm^2 (Hethke *et al.* 2018b, S3), and horizons JSG C, Q, V, W, Z and AC were excluded from the analysis due to lack of size data. Factors with the strongest statistical significance were added to the model in a stepwise process, and we used the minimum Bayesian Information Criterion as a stopping rule for adding terms. After non-zero terms in the linear regression model were predicted, we estimated the parameters using least squares regression.

Aquarium experiments. We hypothesize that the pronounced variations in carapace size and population densities observed in clam-shrimp yielding horizons of our excavation are related. To test the effect of density on size, MH and SCW set up two rearing experiments using two extant species of different families. *Eulimnadia texana* individuals were reared from soil collected by SCW in Arizona (USA; 'Wallace' pool) in 2003. Soil containing *Eocyzicus argillaquus* eggs was collected in Western Australia in 2007 (Cullimbin Pool5). The rearing tanks and cups were placed in a room kept at 24°C and all tanks remained constantly lit under sunlight-simulating fluorescent bulbs (Duro-Test Corp.) throughout the duration of the experiments.

Eulimnadia texana was hydrated in a 10-gallon tank on 18 September 2017. Three days after hydration of the resting eggs, we transferred 15, 10, 5 and 1 juveniles into plastic cups of 400 ml each, which were filled with wet-sieved (74 μm mesh size) aquarium water from an earlier (6-day old) branchiopod population. We replicated the experiment five times for single-individual
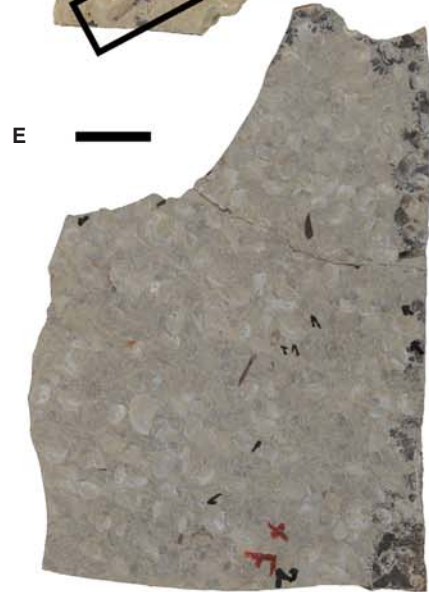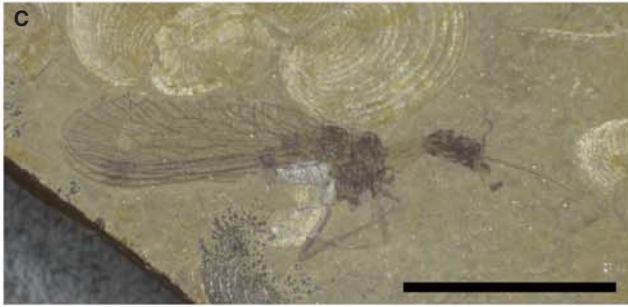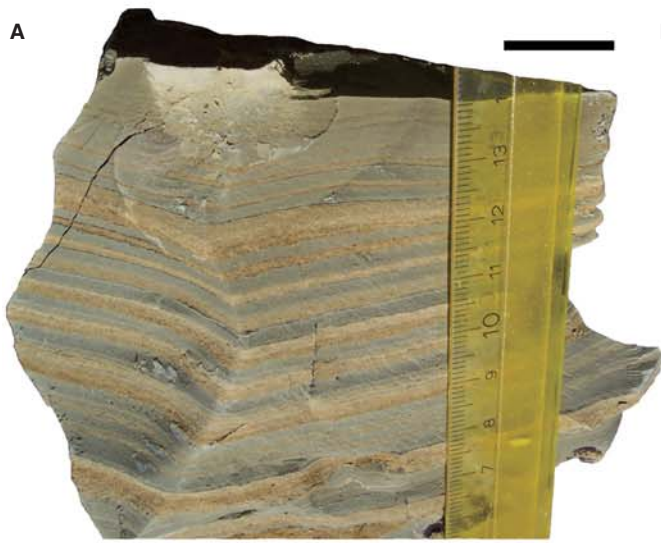
containers, and twice for five and ten-individual containers, respectively. Growth (size) of all 50 individuals was recorded on the day of transferring them into the 400 ml cups and 14 days after hydration, using an Olympus microscope equipped with a COHU High Performance CCD Camera and the Scion Image software package.

Eocyzicus argillaquus was hydrated on 25 September 2017 in a 5 l tank. Larvae hatched a day later and juveniles were transferred into 400 ml plastic cups on 30 September 2017. Single-individual cups were replicated four times, while 3, 5, 7, 10 and 15-individual cups were each replicated twice. Carapace size was measured 16 days after hydration, when mortality rate seemed to increase substantially. In both experiments, all individuals were raised under the same conditions prior to their separation into cups of various population densities.

RESULTS

Sedimentary geology

The lithological analysis of excavation JSG yielded 16 segments (Hethke *et al.* 2018b, S1) and the prominent sedimentological change that is highlighted by a dotted line in the litholog of Figure 1 marks the transition between beds 2 and 3. The microfacies analysis of Hethke *et al.* (2013a) yielded five microfacies for Bed 2, of which very thin (~20–30 μm on average) allochthonous siliciclastic laminae (Microfacies 1; Hethke *et al.* 2013a, fig. 5) form the bulk of the sediments deposited. Tuff horizons of varying thicknesses (several μm to 17 cm) are abundant. Varves, indicated by chrysophycean-cyst accumulations, are present but only rarely preserved, and they contain several silt–clay couplets per annual layer. This shows that time is not well constrained within these deposits and, contrary to previous interpretations (Wang *et al.* 2016), that a varying number of silt–clay couplets represent an interval of 1 year (Hethke *et al.* 2013a, fig. 6). The sedimentological transition between Bed 2 and Bed 3 is marked by several tuff layers that are topped by an anomalously large amount of plant debris in association with fibrous gypsum layers (Fig. 1; Hethke *et al.* 2018b, S1, Segment 8).

Single units of Bed 3 (Hethke *et al.* 2013a, fig. 11d–g) are made up of horizontally stratified, normal-graded silty fine-grained sandstones that are overlain by microbially bound argillaceous siltstone layers, which partly form mound-like structures. The normal-graded part is marked by pervasive meiofaunal bioturbation, indicated by a circular arrangement of silt and fine-sand grains and with burrow diameters ranging between 114 and 228 μm. Several layers are cross-bedded (Hethke *et al.* 2018b, S4). The sequence is coarsening and thickening-upwards within the examined section, with sub-millimetric thicknesses in the lower part of Bed 3 to several mm-thick units near horizon JSG K (Fig. 4A). Single units in the upper part of the excavation are of mainly cm-scale and often marked by mm-long mud clasts indicative of reworking (segments 1 and 2; Hethke *et al.* 2018b, S1).

Biostratinomic observations. The orientation of *Ephemeropsis trisetalis* larvae has been measured as a proxy for currents or wave action (Fig. 3D; Table 1). Preferred directions can be inferred for JSG H, J and AA, but according to Rayleigh's *R*, the null hypothesis of a random distribution cannot be rejected for JSG AB and JSG AP. Importantly, the studied horizons consist of several sub-millimetric laminae, which introduces an aspect of time averaging. However, JSG H exhibits an unambiguous pattern with an average orientation of 10–190 (NNE–SSW). In turn, the distribution overlap of two or more sub-millimetric consecutive layers suggests two directions for JSG AA (Bed 3). The main direction can be summarized as NE–SW.

Community composition of beds 2 and 3

Overview plates of benthic organisms and their respective horizons are presented in Figures 2–5. Figure 2 gives an impression of representative Bed 2 horizons. Clam-shrimp density is variable, ranging from pavement-like accumulations (Fig. 2A, C) to widely scattered occurrences (Fig. 2B). An overview of the so-called Transitional Fauna is given in Figure 3. It is characteristic of Bed 3 sediments that immediately overlie the major sedimentological transition between beds 2 and 3.

FIG. 4. Overview of Bed 3, shallower facies. A, example of normal-graded units, which become progressively coarser throughout Bed 3. B–D, JSG B (SNSB-BSPG 2018 XIV_B1); well defined horizon with excellently preserved insect fossils, such as the snakefly *Alloraphidia* (C) or the water boatman *Karataviella* cf. *pontoforma* (D). E, JSG F (SNSB-BSPG 2018 XIV_F2); densely distributed clam shrimp of small to medium size. F, JSG P; horizon with scattered larger clam shrimp. G, JSG G (SNSB-BSPG 2018 XIV_G7); bimodal size distribution with larger individuals restricted to the lower layer and smaller individuals occurring within the upper layer; the latter is an example of an environmentally induced mass mortality event. Scale bars represent: 2 cm (A, E, G); 5 cm (B); 1 cm (C); 2 mm (D); 10 cm (F). Colour online.
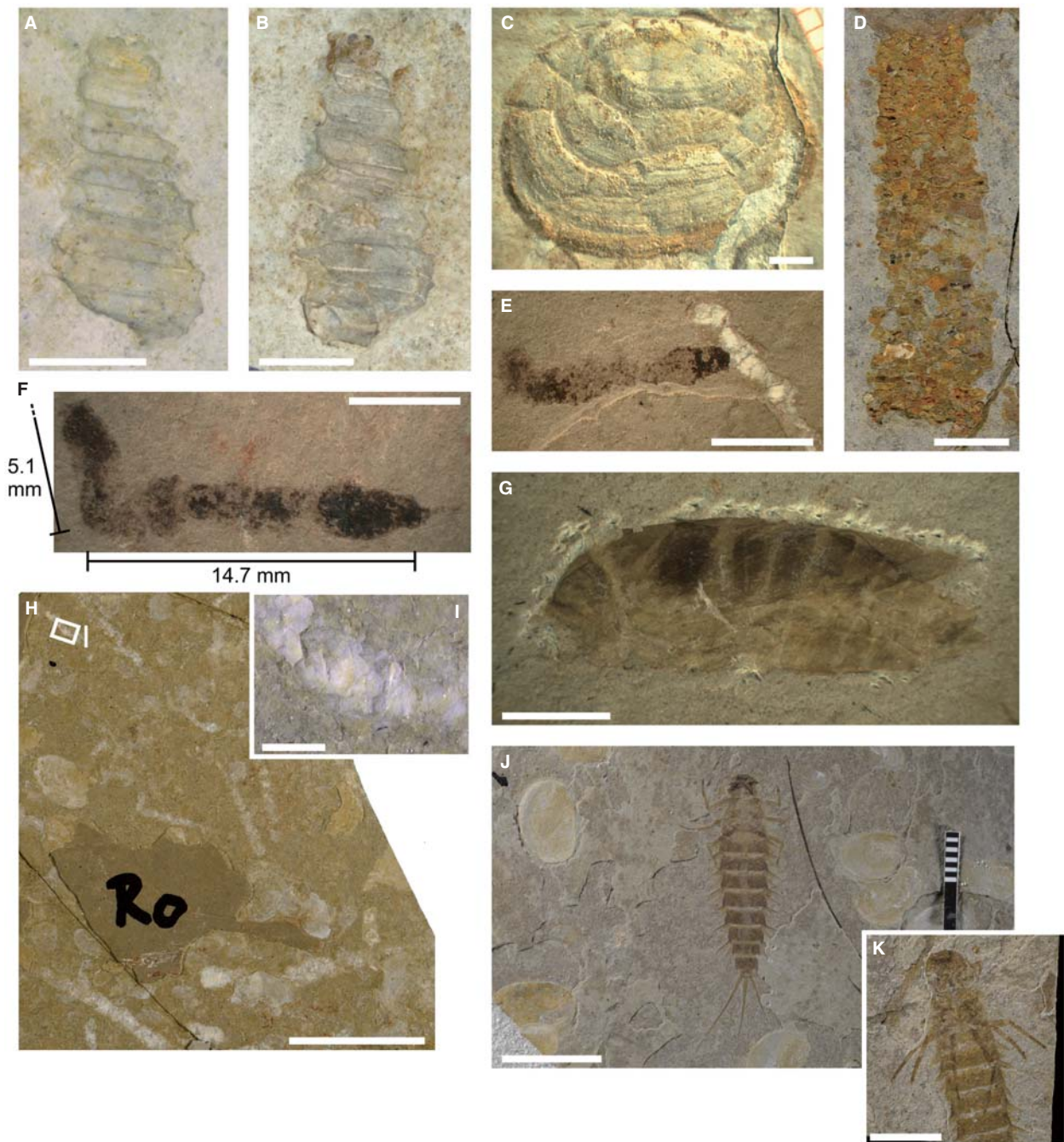
FIG. 5. Benthic fauna of excavation JSG. A–D, Bed 2 (SNSB-BSPG 2018 XIV_AP6, AP5, AP2, and AM3). E–K, Bed 3 (SNSB-BSPG 2018 XIV_O12, W3, X3, R10, J2, AA4). A–C, the three stratigraphically lowermost horizons AP-AN yield high-spired gastropods (*Probaicalia gerassimovi*) and, partially, bivalves (*Arguniella ventricosa*). D, caddisfly tubes occur in JSG AM and in horizons of the Transitional Fauna. E–G, oligochaetes (E, F) and G, *Karataviella* cf. *pontoforma* (G) are restricted to Bed 3 and dominate several horizons of the Transitional Fauna (cf. Fig. 3C). H–I, coprolites made up of fragmented clam-shrimp carapaces are common in numerous horizons of Bed 3 (Hethke *et al.* 2018*b*, S5). J–K, mayfly nymphs of the species *Ephemeropsis trisetalis* form the second-most abundant faunal component of this excavation; their relative abundance peaks in the Transitional Fauna; in contrast, a standardization to excavated area points to more evenly distributed mayfly larvae across associations; note that the preservation of Bed 3 mayfly larvae is fundamentally different to the iron sulphide replacement of Bed 2. Scale bars represent: 1 mm (A, B, I); 2 mm (C, G); 5 mm (D–F); 2 cm (H, J); 1 cm (K). Colour online.
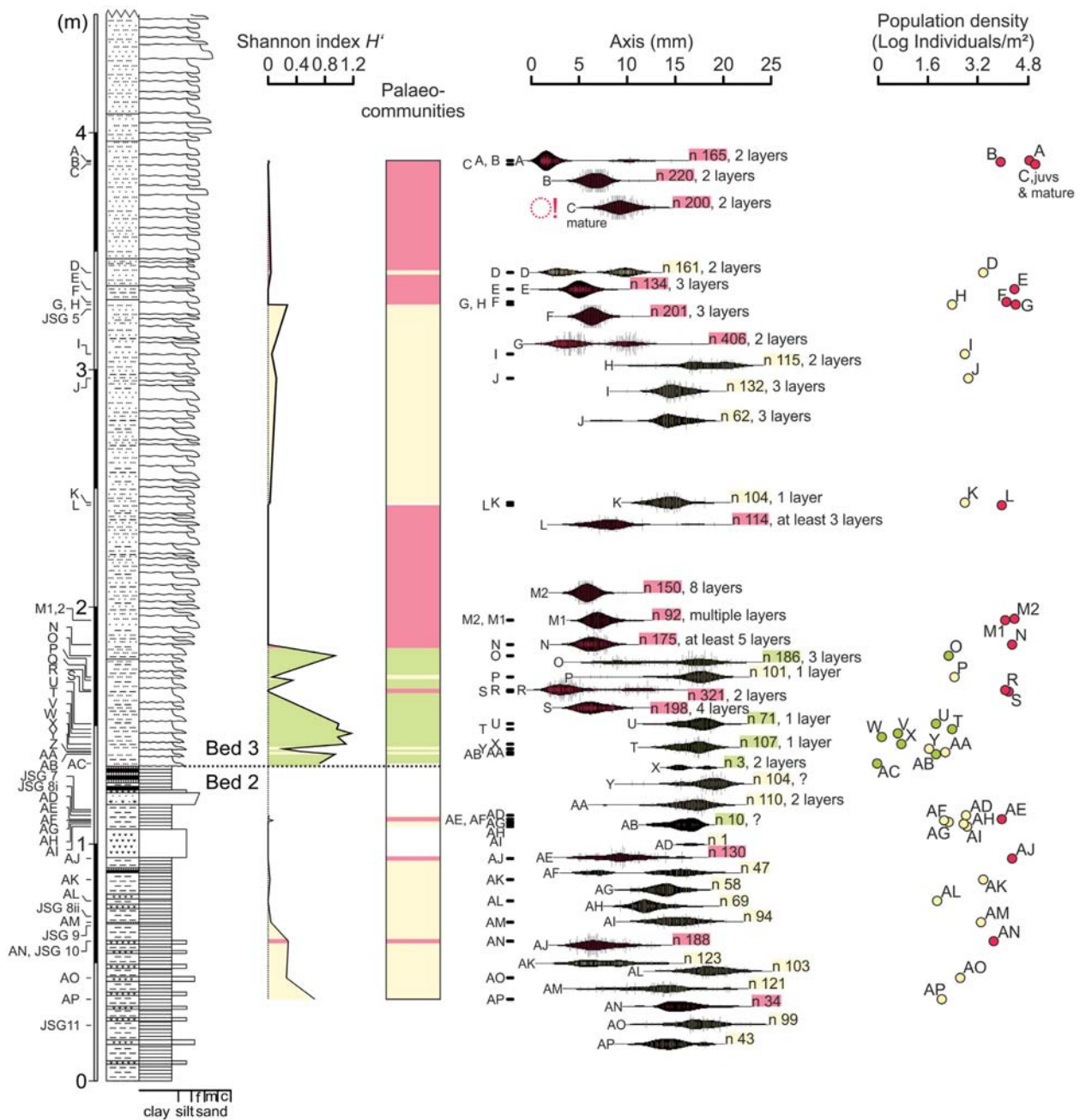
FIG. 6. Litholog of excavation JSG and associated environmental proxies. The four columns plotted against the litholog are diversity (Shannon index), palaeocommunities (Fig. 7), axis-measurements of *Eosestheria middendorfii*, and clam-shrimp density (individuals per m² on a log-scale). The diversity curve has not been smoothed, as horizons are well-constrained and a sudden drop in diversity is believed to be authentic. Palaeocommunities are colour-coded in red (low diversity, high density association), yellow (low to medium diversity, medium density association) and green (Transitional Fauna, comprising a medium to comparatively high diversity, low density association, and two assemblages). The population density of JSG C includes juveniles of ~1 mm, indicated by a red circle in the corresponding beanplot. The number of layers represented by a size distribution is indicated for each Bed 3 horizon (μm-scale laminae of Bed 2 did not allow exact counts). The bimodal size distribution of JSG D, for example, is an artefact of two closely adjacent layers that each yield individuals of a single size class.

A total of nine benthic taxa were recognized in the horizons of excavation JSG: the spinicaudatan *Eosestheria middendorfii*, nymphs of *Ephemeropsis trisetalis* and *Ephemeropsis* sp. B, the water boatman *Karataviella* cf. *pontoforma*, oligochaetes, caddisfly larvae, the malacostracan *Liaoningogriphus quadripartitus*, the gastropod

Probaicalia gerassimovi and the bivalve *Arguniella ventricosa* (Jones 1862; Eichwald 1864; Reis 1910; Martinson 1949; Lin 1976; Kolesnikov 1980; Chen 1999*a, b*; Pan & Zhu 1999; Shen *et al.* 1999; Jiang *et al.* 2007; Zhang *et al.* 2010; Pan *et al.* 2012*b*; Hethke *et al.* 2018*a*).

Specimens of the morphologically variable spinicaudatan *Eosestheria middendorfii* were identified based on ornamental features and shape according to Hethke *et al.* (2018*a*). Several exceptionally large clam shrimp of horizon JSG H (L = 20.6 mm) lack growth-band crowding, and ornamentation on the last four growth bands differs from the species description in exhibiting coarse reticulation. A possible explanation is that such ornamentation is commonly invisible due to crowding. We tentatively assign these large shrimp to *Eosestheria* cf. *middendorfii* pending further investigations into ornament variability using rearing experiments.

Huang *et al.* (2007) suggested assigning the imago of the mayfly *Ephemeropsis trisetalis* to the genus *Epicharmeropsis*, which was erected in the same publication based on an imago. However, the original description of the species *Ephemeropsis trisetalis* is based on nymph specimens (Eichwald 1864), which is why we retain the genus *Ephemeropsis* pending further investigation of the type material. *Ephemeropsis* sp. B refers to a markedly larger and somewhat inflated mayfly larva. Individuals are restricted to horizons characterized by an overall abundance of mayfly larvae (AB, AA and Z), reaching body lengths of 5.8 cm (excluding cerci). Generally, the morphology of nymphs of different species might be very similar. It is possible that *Ephemeropsis* sp. B represents a different nymph stage of *Ephemeropsis trisetalis*. However, as the former is distinctly larger than larvae of all other horizons of this excavation (e.g. 4.1 cm in JSG J, Fig. 5J) we decided to treat the two morphotypes as separate species, corroborated by the presence of more than one imago in the Yixian Formation (Huang *et al.* 2007).

Water boatmen and oligochaetes are restricted to Bed 3 (Hethke *et al.* 2018*b*, S2) in our excavation and they are by far the most common in the Transitional Fauna. We identified one water-boatmen species (Fig. 5G), which we provisionally assign to *Karataviella* cf. *pontoforma*. The type material described by Lin (1976) is pictured in

TABLE 1. Test results for the orientation patterns of *Ephemeropsis* larvae

	n	Rayleigh's R	p(random)
JSG H	43	0.338	0.0068
JSG J	11	0.553	0.031
JSG AA	83	0.282	0.0012
JSG AB	49	0.143	0.370
JSG AP	14	0.143	0.758

ventral and dorsal view, while specimens of excavation JSG are preserved in lateral view. Yet, body sizes and shapes are comparable. Water boatmen are a recurrent group in Mesozoic lakes of eastern Asia, and they are especially abundant in the Middle to Upper Jurassic of north-eastern China (Zhang 2010; Yang *et al.* in press).

Although the preservation of the vermiform fossils (Figs 3A, C; 5E, F) does not allow any detailed taxonomic identification, body size, visible segmentation, and a thickened region at one end, interpreted as an annelid clitellum, allow a tentative assignment of the fossils to the Oligochaeta, which form ubiquitous elements of benthic communities in modern aquatic environments (e.g. Michiels & Traunspurger 2005). Figure 5F illustrates a specimen that is at least 19.8 mm long with a faintly visible outer body wall and a possible segmented anterior projection, reminiscent of a sucking organ of naidid oligochaetes. The latter is, however, rarely preserved in other individuals.

Molluscs, the gastropod *Probaicalia gerassimovi* (Reis, 1910) and the bivalve *Arguniella ventricosa* (mentioned in Kolesnikov 1980) are restricted to the clay–silt couplets of Bed 2. The gastropod species was originally erected as *gerassimowi* (Reis 1910) but spelled '*gerassimovi*' by Martinson (1949), which has been adopted in numerous subsequent publications. Our identification of the bivalve specimens follows Pan *et al.* (2012*b*), which is the common identification of this species; the original species diagnosis is difficult to trace. A definite identification was our intention but is beyond the scope of this paper.

The raw species abundance data are compiled in Hethke *et al.* (2018*b*, S2), which also lists species richness (S), rarefied richness and the Shannon index (H'), the latter of which is plotted against the litholog in Figure 6. The three diversity indices generally agree. Species richness, however, does not give a good representation of diversity with increasing clam-shrimp dominance, and there are 14 monospecific clam-shrimp horizons (disregarding coprolites or terrestrial insects within these horizons).

Common faunal components, or their traces, that have not been included in the palaeocommunity analysis are coprolites (Fig. 5E, H, I, Hethke *et al.* 2018*b*, S5) and fish fossils (lowermost horizon JSG 11; marked in Fig. 1). Coprolites are entirely made up of clam-shrimp fragments, indicating that spinicaudatans were the main food source of an unidentified predator (possibly fish). In addition to the remains of aquatic organisms, there are numerous excellently preserved terrestrial insect fossils in Bed 3, such as spittle bugs (JSG U) or snakeflies (JSG B, JSG Y; Fig. 4C).

Benthic palaeocommunities

Three associations and two assemblages have been identified using group-average sorting (Fig. 7A). Groupings are

driven by diversity, the ratio of *Ephemeropsis trisetalis* to *Eosestheria middendorfii*, and clam-shrimp population density (Fig. 6). Monospecific horizons of different population densities are well-separated by NMDS (Fig. 7B) but not in the CA plot (Fig. 8). The following groups can be distinguished:

Association 1. (n = 15, 'low diversity, high density association', red) Association 1 comprises mainly monospecific assemblages of small to medium-sized *Eosestheria middendorfii* with possible minor occurrences of mayfly larvae, water boatmen and gastropods. Diversity remains at, or near, $H' = 0$ and does not exceed $H' = 0.28$ (JSG AN; Fig. 6).

Association 2. (n = 18, 'low to medium diversity, medium density association', yellow) Association 2 is made up of 10 horizons of Bed 2 and 8 horizons of Bed 3. This association includes four monospecific horizons of *Eosestheria middendorfii* (AL, AH, AG, AD), all of which are marked by a distinctly lower clam-shrimp density (individuals per m²) than horizons of Association 1. The remaining 14 horizons are also dominated by *E. middendorfii*, but they are characterized by an increasing importance of the mayfly larva *Ephemeropsis trisetalis*. Except for one specimen count, Association 2 is devoid of water boatmen. It can be considered as an intermediate association that ranges between associations 1 and 3.

Association 3. (n = 8, 'medium to comparatively high diversity, low density association', green, part of the Transitional Fauna) Association 3 consists of horizons yielding varying proportions of large-sized *Eosestheria middendorfii*, the mayfly larva *Ephemeropsis trisetalis*, the water boatman *Karataviella* cf. *pontoforma* and oligochaetes. Oligochaetes are restricted to this association and form a dominant faunal component in several of its horizons.

Assemblage JSG Z. ('low diversity mayfly assemblage', green, part of the Transitional Fauna) Assemblage JSG Z is marked by an absence of clam shrimp and a dominance of mayfly larvae with minor occurrences of water boatmen and caddisfly larvae. *Ephemeropsis* sp. B is common in this assemblage.

Assemblage JSG Q. ('low diversity water-boatmen assemblage', green, part of the Transitional Fauna) Assemblage JSG Q is also marked by an absence of clam shrimp. This assemblage is dominated by the water boatman *Karataviella* cf. *pontoforma*. Mayfly larvae occur in smaller numbers.

Association 3 and assemblages JSG Z and JSG Q (Fig. 7A) occur immediately above the sedimentological transition of Bed 2 into Bed 3 (Fig. 6). Together, they are referred to as the 'Transitional Fauna', in which the two assemblages represent end members of Association 3. The presence of bivalves and gastropods does not have a large influence on the groupings. Instead, clam-shrimp population density forms an important determining factor, as illustrated by the density column of Figure 6, which clearly separates Association 1 (red) from Association 2 (yellow). Therefore, depending on their clam-shrimp population density, monospecific horizons may be allocated to either one of the two associations.

While all 14 monospecific clam-shrimp horizons plot in a single spot, horizons yielding the Transitional Fauna (green) are well delimited from all other samples by CA1 (Fig. 8). Horizons which are aligned along CA1 are characterized by a progressively higher relative abundance of mayfly larvae with respect to clam shrimp (mainly Association 2). Mollusc-yielding horizons are separated by CA2. They occur within the lowermost three horizons of the profile (Fig. 1). The third axis (Fig. 8B), which explains 14.3% of the variation in the data, is less informative, but it underlines the importance of less abundant taxa, such as caddisfly larvae or a second mayfly species.

Relay plot

Proposed response curves to the environmental gradient underlying CA1 are illustrated in the relay plot of Figure 9. Spinicaudatans form the only faunal component that occurs in monospecific assemblages, so they acted as initial colonists or they survived environmental conditions adverse to all other benthic taxa. In turn, mayfly larvae and water boatman dominate Assemblages JSG Z and JSG Q, respectively, to the exclusion of clam shrimp. Therefore, these two taxa were able to survive conditions that the clam shrimp *E. middendorfii* did not tolerate.

Figure 9 shows that the six unimodel response curves partly overlap. *Eosestheria middendorfii* displays the widest response curve, indicating the highest tolerance towards this variable. Conversely, that of oligochaetes is comparatively narrow and has nothing in common with that of the bivalve *Arguniella ventricosa*. Occurrences of *Ephemeropsis* sp. B, caddisfly tubes and *Liaoningogriphus* are too sporadic to position potential peaks of the respective response curves, which is why they are omitted from the relay plot. The extrapolation of the response curve for *E. middendorfii* is based on the fact that clam-shrimp abundances peak when they occur in nearly monospecific assemblages (Fig. 9A). This indicates that they tolerated a wider range than that picked up by the relay plot.
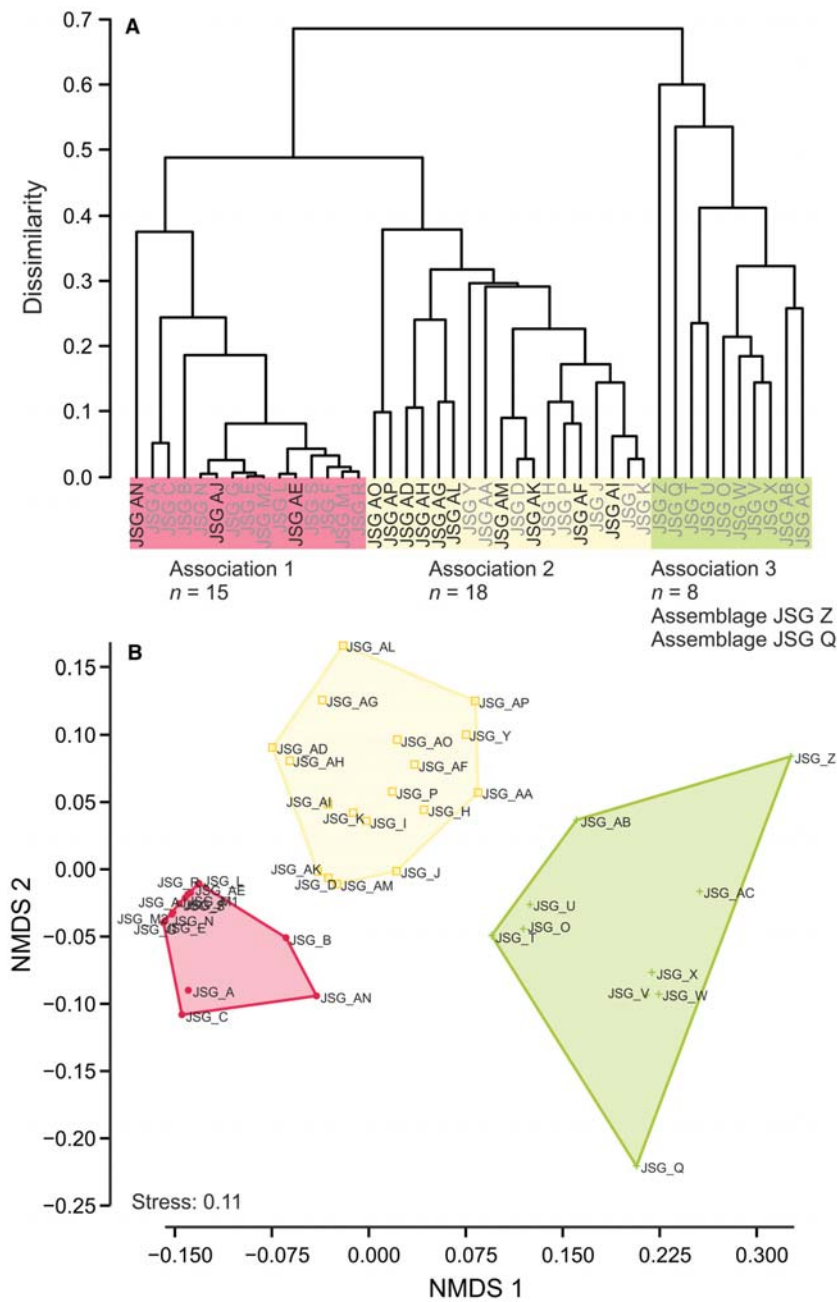
FIG. 7. A, hierarchical cluster analysis (group average sorting) identified three associations and two assemblages. B, non-metric multidimensional scaling (stress = 0.11) illustrates the separation of faunal associations; diversity is near monospecific at negative values of NMDS 1 and it increases towards positive values.

Carapace size and clam-shrimp population density

General observations. Columns 3 and 4 next to the litho-log in Figure 6 illustrate size and population densities of *Eosestheria middendorfii*. As indicated for each size distribution, single horizons are often made up of several sub-millimetric fossiliferous layers. Sampling distributions that are tightly clustered around the mean reflect a smaller amount of variability due to simultaneous hatching (a cohort), and distributions reflecting multiple fossil layers indicate the presence of various cohorts.

An inspection of horizons comprising a distinct bimodal size distribution and two layers (JSG A, C, D and G) shows that larger-sized clam shrimp are restricted to the lower layer and smaller clam shrimp to the upper layer. This indicates that the larger-sized generation was embedded slightly earlier than the smaller-sized generation.

Individuals of Association 2 and the Transitional Fauna are generally large. The largest individual of excavation JSG is from horizon JSG O (L = 22.94 mm; Association 3) followed by two individuals of Association 2 (JSG Y, L = 20.96 mm; JSG H, L = 20.62 mm). By far the
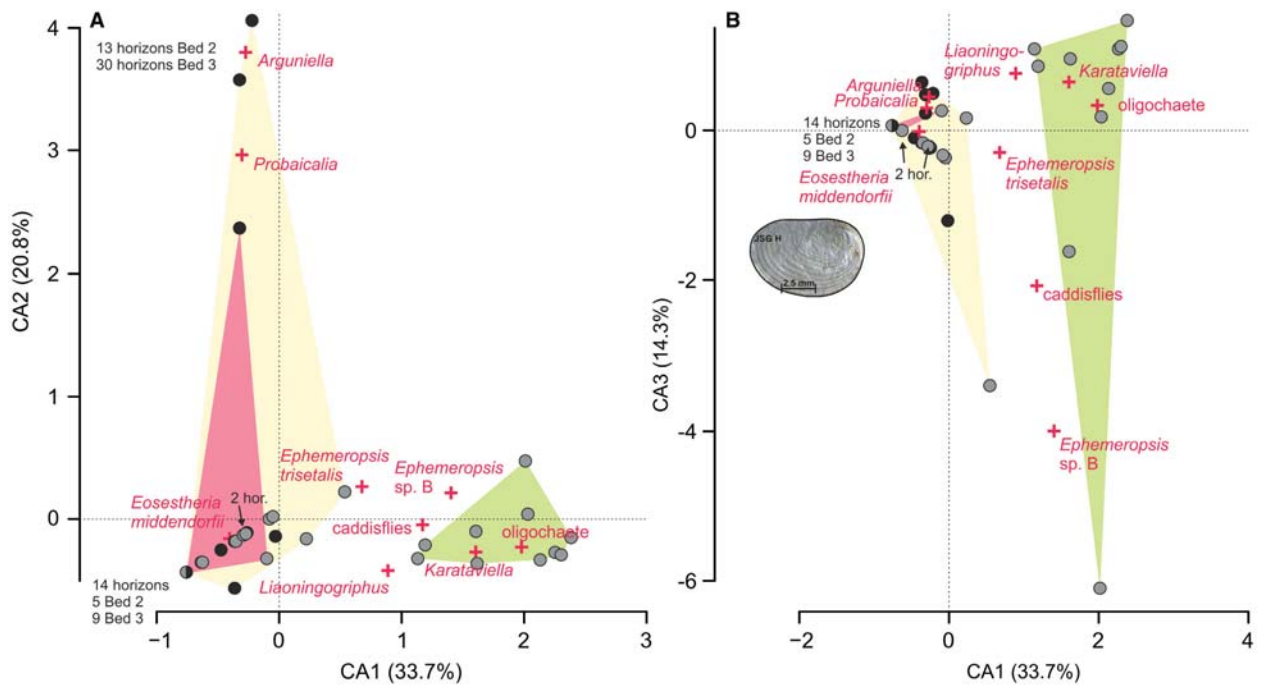
FIG. 8. Biplots of horizons and taxa based on correspondence analysis. Black dots indicate horizons of Bed 2, while grey dots mark those of Bed 3. Associations 1, 2 and the Transitional Fauna are colour-coded in red, yellow and green, respectively. A, CA1 vs CA2; horizons aligned along CA1 are driven by an increasing relative abundance of *Ephemeropsis* larvae, while CA2 is governed by the presence of molluscs. B, CA1 vs CA3; less common taxa, such as caddisflies or *Ephemeropsis* sp. B, span CA3.

highest clam-shrimp densities correlate with the overall smallest individuals of Association 1, exemplified by JSG B and F (Fig. 4B, E).

JSG D does not seem to follow these generalizations, as individuals seem small, yet they belong to Association 2. However, we chose to restrict the community data of JSG D to the lower layer that exhibits larger clam shrimp and several mayfly larvae. The only exception to the above generalizations that seems puzzling is JSG AN (Association 1; Fig. 2C), which exhibits large clam-shrimp sizes comparable to those of Association 2 but with high population densities. However, JSG AN was separated from all other horizons of Association 1 by CA2 (Fig. 8A) and grouped with JSG AO and JSG AP due to the occurrence of the gastropod *Probaicalia*.

Distributions of mean carapace size and population density in monospecific and higher-diversity associations. Monospecific (Y) and higher-diversity associations (N) yield significantly different distributions of population density and mean carapace size of *Eosestheria middendorfii*, respectively (Fig. 10; Kruskal–Wallis test results for equal medians $p < 0.001$, respectively; Hethke *et al.* 2018*b*, S6). Specifically, the population density of *Eosestheria middendorfii* is significantly higher in monospecific layers than in higher-diversity assemblages (non-parametric Wilcoxon test, one-

way chi square approximation with 1 degree of freedom = 12.7655, $p = 0.0004$). The test yielded a median population density of 129.5 individuals per 100 cm^2 in monospecific assemblages and 2.5 individuals per 100 cm^2 in assemblages with a diversity >0.

Furthermore, there is a significant difference between carapace sizes of higher-diversity assemblages and monospecific assemblages, yielding median sizes of 15.2 and 6.8 mm, respectively (Wilcoxon/Kruskal–Wallis Test, one-way chi square = 11.2, $p = 0.0008$). Hence, the response to increasing diversity in clam-shrimp yielding associations was: (1) a decrease in clam-shrimp population density; and (2) an increase of mean carapace size.

Outliers in Figure 10 can be explained by exceptionally low-diversity assemblages such as horizon JSG A, which yields a population density of 694 individuals per 100 cm^2 and a mean axis of 3.1 mm. It fell into the non-monospecific category due to the presence of just one mayfly larva.

Stepwise regression analysis. The stepwise approach settled on a model with two terms in addition to the intercept term (Fig. 11; Hethke *et al.* 2018*b*, S7). According to the model, variables that impact carapace size are diversity (Shannon) and the population density of *Eosestheria*
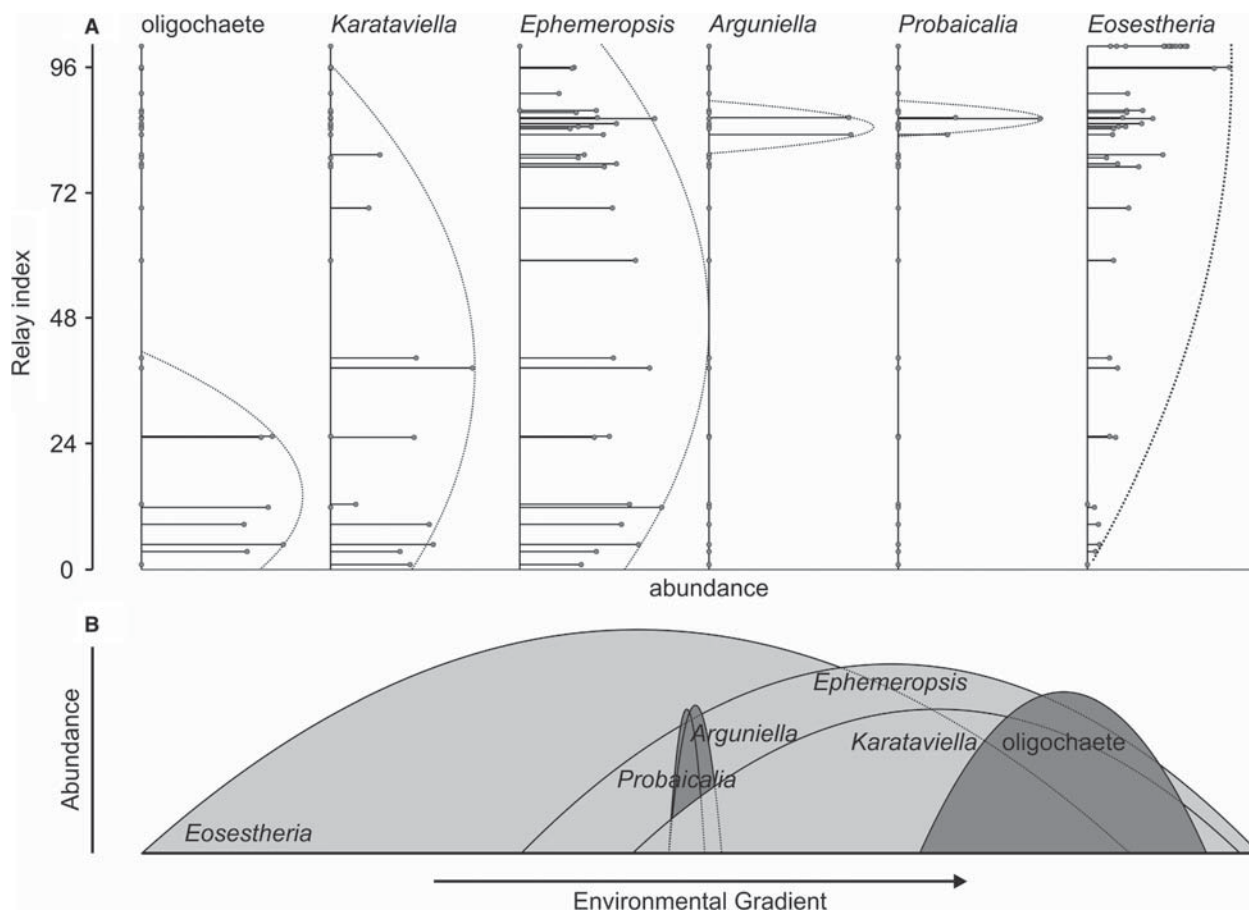
FIG. 9. A, relay plot based on the correspondence analysis of Figure 8; the six principal species are considered; vertical axes of the relay plot give CA1 row scores and the horizontal axes reflect original abundances in a given column, standardized to an excavation area of 10 m^2 and subsequently double-square-root transformed; this transformation strongly decreases the amplitudes of the ideally unimodal distributions; taxa are ordered according to their position on the environmental gradient; peaks are not entirely unimodal, but they give good representations of the respective response curves (dotted lines). B, the resultant schematic response curves (amplitudes arbitrary) are overlapping; clam shrimp, mayfly larvae and water boatmen were tolerant towards the environmental variable underlying CA1 (generalists), while oligochaetes, bivalves and gastropods can be regarded as specialists.

middendorfii, in line with the results presented in Figure 10. The latter relationship was fitted with linear and natural log regression in Figure 12 (Hethke *et al.* 2018*b*, S8; ANOVA p < 0.0001 for both the linear and natural log fit).

Aquarium experiments using extant species. We identified a strong density effect on carapace size in two extant species, *Eulimnadia texana* Packard, 1871 and *Eocyzicus argillaquus* Timms & Richter, 2009 (Fig. 13). Both, the linear fit and the transformed fit to natural log are highly significant for both species (all ANOVAs highlighted in Hethke *et al.* (2018*b*, S9); p < 0.0001, except for *Eocyzicus* linear fit, which is p = 0.0004). As for the fossil species *Eosestheria middendorfii*, natural log regression provided the best fit for both extant species. Hence, spinicaudatan size decreases with density, but levels off after a certain density.

DISCUSSION

Sedimentological analysis and lake depth

The sediments of the Jianshangou Member are generally interpreted as representing comparatively deep and perennial conditions (Hethke *et al.* 2013*a*; Wang *et al.* 2016). This interpretation contrasts older works that interpret the lake as shallow based on the presence of spinicaudatans (<2 m; Wang 1999; Fürsich *et al.* 2007).

Generally, the very fine laminae of Bed 2 (clay–silt couplets; Hethke *et al.* 2013*a*) represent holomictic, eutrophic conditions interrupted by episodic meromictic interludes (Pan *et al.* 2012*a*; Hethke *et al.* 2013*b*) and show little evidence of higher-energy conditions. The sediments were deposited well below the storm wavebase, which is a function of lake fetch to depth and has been inferred to have occupied depths from <10 m to 5 m in the large Devonian
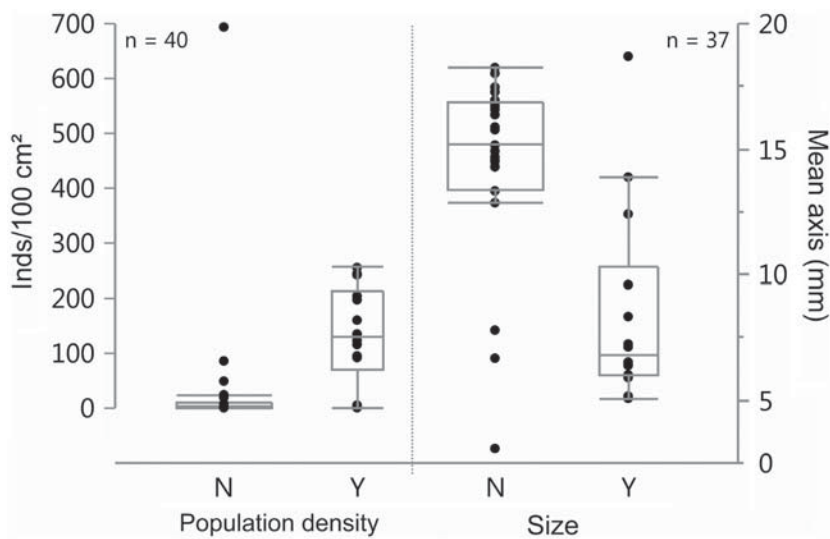
FIG. 10. Relationship between the categorical variable 'monospecific' (Y = monospecific/N = Shannon > 0) and (left) population density (individuals/100 cm^2) and (right) mean carapace size of *Eoestheria middendorfii*, respectively. See Hethke *et al.* (2018*b*, S6) for JMP output.

lakes of Shetland (Allen 1981). Although allochthonous clam shrimp have been identified in Bed 2 (Hethke *et al.* 2013*a*, fig. 8) their bulk is interpreted as parautochthonous based on biostratinomic data (see below). With the possible exception of segment 9 (Hethke *et al.* 2018*b*, S1) there are no clear signs of emergence (desiccation cracks or salt pseudomorphs) within Bed 2 of excavation JSG. Fibrous gypsum layers that mark the transition between the beds (Fig. 1) are interpreted as secondary products of sulphide oxidization.

The depositional units of Bed 3 are characterized by occasional cross-bedding (Hethke *et al.* 2018*b*, S4) and general pervasive meiofaunal bioturbation, an indicator of oxygenated conditions within the sediments and, correspondingly, within the bottom waters of a fully holomictic lake that was governed by hyperpycnal influx (lake turbidites). Spinicaudatans are mostly associated with the top layers of single depositional units that are less disturbed and formed by biofilms, implying tranquil conditions during reduced influx within a season. Hence, Bed 3 sediments correspond to an alternation between higher-energy conditions, triggered by increased rainfall during the wet season, which resulted in the deposition of normal-graded units that were quickly colonized by a meiofauna, and a dry season marked by the formation of fine-grained mat-like structures associated with benthic aquatics (Hethke *et al.* 2013*a*). The general coarsening and thickening-upwards trend within Bed 3 of the investigated section (Fig. 1) indicates an overall shallowing during Phase 3.

The rapid sedimentological transition between beds 2 and 3 of excavation JSG (Fig. 1) was widespread in the Sihetun area (Jiang *et al.* 2012; Wang *et al.* 2016) but interpretations remain ambiguous. While they agree on the existence of a holomictic, perennial lake, it has been

variously proposed that the onset of Phase 3 was marked by either shallowing or deepening:

1. A shallowing is supported by biotic evidence of the Transitional Fauna (green, Fig. 6) that is marked by a high abundance of water boatmen, whose respiration requires shallower waters (Oscarson 1987). This interpretation is in accord with that of Wang *et al.* (2016), who interpreted the Hengdaozi Bed, which includes the facies of Bed 3, as shallower than the Anjiagou Bed (Bed 2). According to this model, holomictic conditions fully established due to shallowing and Bed 3 would represent a more proximal setting.

2. The interpretation of an initially deeper lake during Phase 3 is based on an increased sediment influx due to more humid conditions following a change in climate from dry (Phase 2) to humid (Phase 3; Jiang *et al.* 2012; Hethke *et al.* 2013*a*). A similar transition has been identified in the deposits of Lake Khubsugul (Mongolia; Fedotov *et al.* 2003) in which the Pleistocene (cold and arid) clay–carbonate-dominated sedimentation was abruptly succeeded by a Holocene silt-dominated sedimentation. Shallowing during the arid intervals of the Pleistocene was accompanied by a reduction in surface area and an estimated reduced lake volume of 30–40 times the present-day level. At the onset of the Holocene, lake waters rapidly became diluted, putting an end to the prior Pleistocene carbonate build-up and marking the onset of fluvial sedimentation (Fedotov *et al.* 2003). Accordingly, the sedimentological shift between beds 2 and 3 of Lake Sihetun would imply lower water levels, a smaller surface area and lake volume, as well as higher salinities during Phase 2, corroborated by repeated intervals of carbonate precipitation (Hethke *et al.* 2013*a*, fig. 9). The onset of Phase 3 would be marked by deepening
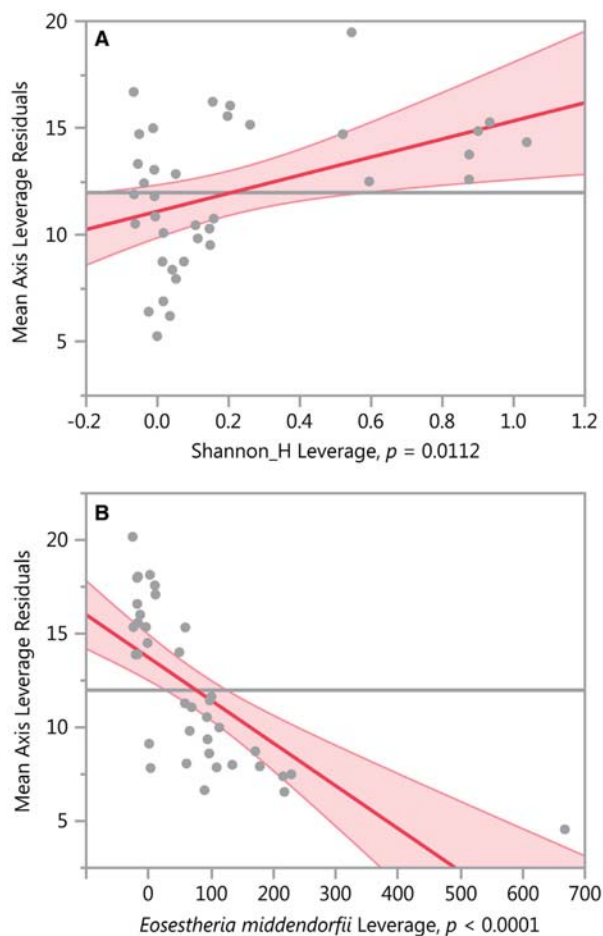
FIG. 11. Leverage plots for the two terms contributing to the regression model based on stepwise regression (Hethke *et al.* 2018*b*, S7). A, diversity; B, population density of *Eosestheria middendorfii* per 100 cm^2. Colour online.

FIG. 12. Bivariate fit of mean carapace size by population density for *Eosestheria middendorfii* (n = 37). Both linear and natural log regression were highly significant at $p < 0.0001$ (Hethke *et al.* 2018*b*, S8), hence carapace size seems dependent on density. Juvenile mass mortalities (JSG A; arrowed), differences in cohort age between different horizons, and time averaging influence the pattern, which why we use time-constrained rearing experiments to corroborate this palaeontological observation (Fig. 13).

and an increased fluvial influx, followed by (and this is true for both scenarios) shallowing.

Biostratinomic observations. In addition to small-scale cross-bedding (Hethke *et al.* 2018*b*, S4), we analysed orientation patterns of *Ephemeropsis* larvae to infer currents or wave action (Fig. 3D; Table 1). According to Rayleigh's test, no preferred orientation has been identified for JSG AP (Bed 2). The pattern resembles that of *Ephemeropsis trisetalis* and *Liaoningogriphus quadripartitus* from Bed 2 of excavation ZJG, for which Fürsich *et al.* (2007) reported invariably random orientations. However, three of nine test statistics listed in their study actually indicate directional patterns. Therefore, the lake floor remained generally calm during Phase 2 but was occasionally interrupted by light currents. In addition, we identified preferred orientations in three of four Bed 3 horizons (JSG H, J, and AA; general orientation of NE–SW). The presence of current cross bedding in Bed 3 indicates a
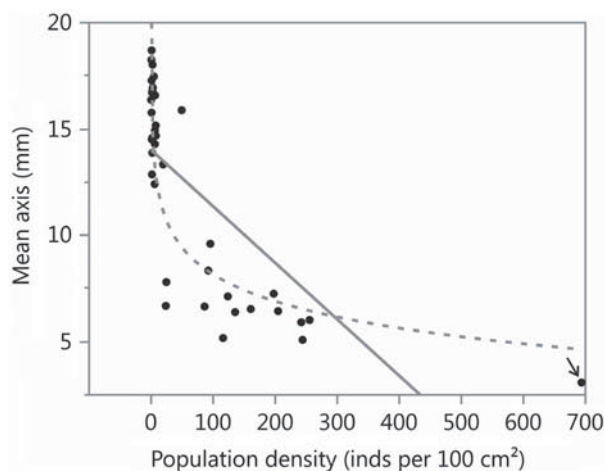
directional transport of larvae in contrast to wave action. Hence, the lake remained below storm wavebase during both phases (at outcrop position). Although there is evidence for transport in Bed 3, we conclude that it must have been minor to account for the excellent preservation of insect fossils, such as the snakefly *Alloraphidia* (Fig. 4C). Thin-section analysis revealed allochthonous clam shrimp in distal turbidites of both beds. However, this microfacies is comparatively rare in Bed 2, and most Bed 3 clam shrimp were embedded in the background sediments of normal graded sub-millimetric layers, rendering most of the benthic fauna parautochthonous.

Response curves predict community composition

A previous study recognized ten taxa in eight fossil communities within Bed 2 of excavations ZJG and LXBE (Pan *et al.* 2012*a*), including fish, fish coprolites and plant fragments. In the present study, we restrict the palaeocommunity analysis to (nekto)benthic taxa that are exclusively representative of the lake bottom waters. Nevertheless, excavation JSG yielded abundant coprolites made up of clam-shrimp carapaces (especially Bed 3; Fig. 5H, I; Hethke *et al.* 2018*b*, S5) which indicate an important predator–prey relationship and that predators were at least part-time members of the demersal community.

Fürsich *et al.* (2007) identified three low diversity arthropod associations in excavation ZJG, characterized
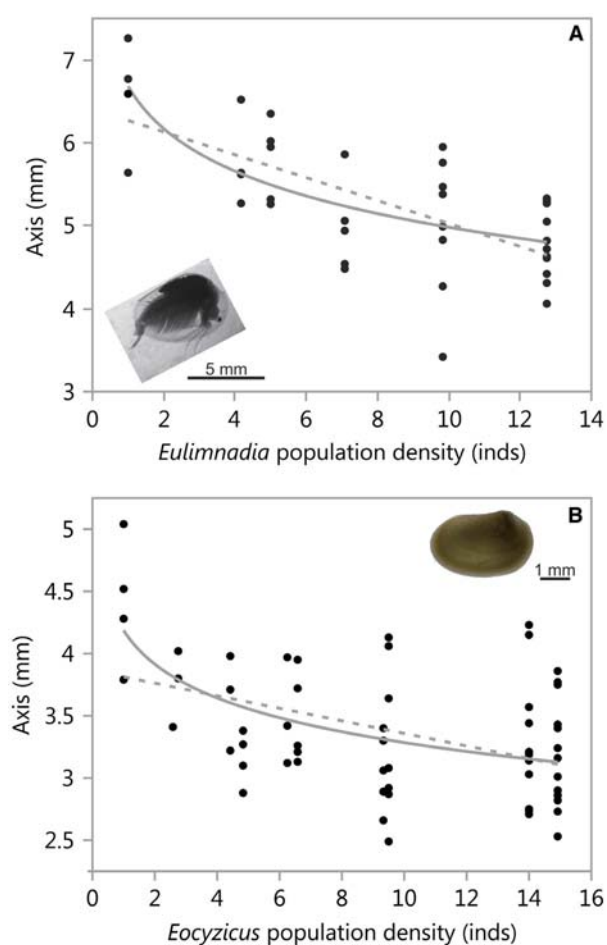
FIG. 13. Bivariate fit of mean carapace size by population density. A, *Eulimnadia texana* (n = 37) measured 14 days after hydration. B, *Eocyzicus argillaquus* (n = 59) measured 16 days after hydration. Both linear and natural log regression are highly significant, but the latter provides the best fit at p < 0.0001 (Hethke *et al.* 2018*b*, S9). *Abbreviation*: inds, individuals.

by *Ephemeropsis trisetalis*, *Liaoningogriphus quadripartitus* and *Eosestheria middendorfii*, respectively. These three associations are partly represented by the three associations and two assemblages of this study (Fig. 7). Assemblage Z, for example, equals the *E. trisetalis*-Association of Fürsich *et al.* (2007). The fact that the former belongs to Bed 3 and the latter to Bed 2 implies that *E. trisetalis* was not restricted to a particular lake phase. Neither was the clam shrimp *E. middendorfii*. Both species were generalists with wide response curves towards environmental gradients (Fig. 9). This explains why associations 1 and 2 are distributed throughout beds 2 and 3 (column 2 next to the litholog in Fig. 6). Association 1 is, however, especially abundant in the upper part of Bed 3.

Excavation JSG reveals long-term temporal patterns in the community composition of Lake Sihetun (Fig. 6). Association 3 and assemblages Z and Q (Transitional

Fauna) are restricted to the basal part of Bed 3. The drastic change in community composition from the clam-shrimp dominated associations of Bed 2 to the Transitional Fauna reflects environmental changes connected to a change in water depth (unresolved whether shallowing or deepening). Apart from *E. trisetalis* and exclusively adult individuals of *E. middendorfii*, the main taxa adapted to this environmental change were the water boatman *Karataviella* cf. *pontoforma* (Fig. 5G) and oligochaetes (Fig. 5E, F). Sporadic occurrences of water boatmen in clam-shrimp dominated associations render their response curve wider than that of the oligochaetes (Fig. 9). A gradual shallowing followed the onset of Phase 3, expressed by fluctuating community compositions and a return to the lower-diversity associations 1 and 2, which dominated the benthic fauna during the later Phase 3.

The response curves of Figure 9 should predict the community composition along the environmental gradient underlying CA1, but there are limitations. In horizon JSG AC (Hethke *et al.* 2018*b*, S3) oligochaetes occur with mayfly larvae in about equal numbers and only one clamshrimp specimen has been counted, placing this horizon at the far right side of the environmental gradient depicted. However, even though the response-curve overlap suggests that water boatmen should be present as well, they did not co-exist with the fauna governing this horizon. This can be explained by seasonality or some other factor that led to the local absence of water boatmen. Therefore, the proposed overlap of response curves (Fig. 9B) provides potential scenarios.

The identified prominent shallowing during Phase 3 renders temperature fluctuations a determining factor for community composition (see Life-history Patterns of *Eosestheria middendorfii*, below). Substrate preferences, in contrast, were less important, as fossil communities of beds 2 and 3 are similar, with the exception of the Transitional Fauna. The significant shift between phases 2 and 3 can be ascribed to a shift from an oxygen-controlled lake floor environment (Phase 2; Hethke *et al.* 2013*b*) to a temperature-controlled environment (Phase 3).

In addition to the response of species to environmental variables, interspecific competition and predation may be considered to explain the separation of species. The malacostracan *Liaoningogriphus quadripartitus* assumed a nektobenthic life style, similar to *Eosestheria middendorfii*, and there is an obvious decline in the abundance of clam shrimp in *Liaoningogriphus*-rich horizons and vice versa (excavation ZJG, Phase 2; Fürsich *et al.* 2007; Pan *et al.* 2012*a*). In turn, the mayfly *Ephemeropsis trisetalis* co-occurred with both species in larger numbers. Therefore, it is possible that *Eosestheria middendorfii* excluded *L. quadripartitus* competitively, accounting for the (near) absence of the latter in excavation JSG. Niche differentiation was probably responsible

for the coexistence of *E. middendorfii* and *E. trisetalis* (Figs 8, 9).

Population ecology of benthic invertebrates

Aquatic insects and molluscs. In addition to spinicaudatans, mayfly larvae played a key role in the communities of Lake Sihetun and in modern freshwater communities alike. Extant taxa are accepted as bioindicators for water quality, and mayfly diversity directly correlates with habitat variety (Bauernfeind & Moog 2000); undisturbed river sections may yield more than 30 species. Therefore, mayfly species occur in various environments. While one species is dependent on deep pools with macrophytic vegetation, other species are found on dead wood and submerged roots; yet others colonize steep clay banks (Bauernfeind & Moog 2000). This shows that a comparison with modern species does not assist the palaeoecological interpretation of *Ephemeropsis trisetalis*. The presence of *E. trisetalis*-dominated associations in Bed 2 (ZJG; Fürsich *et al.* 2007) and Bed 3 (Assemblage Z; Fig. 7A; Hethke *et al.* 2018*b*, S2) shows that this species was a generalist and tolerant towards environmental variables related to water depth (Fig. 9).

The fossil oligochaete of Lake Sihetun (Fig. 5E, F) resembles the modern naidid oligochaete *Stylaria lacustris*, which is 3.5–12 mm long (Kaliszewicz 2003) and slightly smaller than the Early Cretaceous oligochaete. Most Naididae feed on detritus and epiphytic algae (Kaliszewicz 2003) and are common in shallow to medium depths (5.5–18.5 m in Lake Michigan, Hiltunen 1967). The modern species *S. lacustris* dominates oligochaete assemblages associated with *Phragmites* (reed) stems in the littoral zones of northern German lakes (Löhlein 1996). Individuals do not seek refuge in sediments or self-constructed tubes (Kaliszewicz 2003). Meiofaunal bioturbation within Bed 3 indicates the presence of an infauna. However, the Early Cretaceous oligochaetes are about 1.2–2 mm thick and therefore not responsible for these trace fossils. Instead, they probably assumed a similar epibenthic lifestyle to their modern analogue *S. lacustris*.

Adult water boatmen, such as *Karataviella* cf. *pontoforma* (Figs 4D, 5G), are good flyers, and colonize temporary waters by immigration rather than by desiccation-resistant cysts (Batzer & Wissinger 1996). Modern water boatmen generally occur in shallower waters (e.g. 8.5 m water depth; Oscarson 1987) although habitat preferences (proximal vs distal) are species-specific. The reproductive cycle of modern water boatmen is annual and their eggs are typically oviposited on submerged plants, sticks or rocks. Nymphs develop through five growth stages with incomplete metamorphosis. Water boatmen swim near the bottom of ponds or lakes and mostly feed on algae and aquatic plants. They lack gills, and frequently carry an air bubble on their body surface or under their wings to draw oxygen from while under water. Also, they are particularly abundant in seasonally flooded and semi-permanent marshes (along with midges, beetles and mosquitoes; Batzer & Wissinger 1996).

Less common taxa of excavation JSG are gastropods (Fig. 5A, B), bivalves (Fig. 5C), caddisfly larvae (Fig. 5D) and the malacostracan *Liaoningogriphus quadripartitus*. In contrast to excavations ZJG and LXBE (Pan *et al.* 2012*a*), gastropods are more abundant than bivalves in excavation JSG. CA2 separates horizons yielding molluscs from others (Fig. 8A) and the response of both mollusc species to environmental variables seems to be narrow (Fig. 9). However, both response curves are based on small sample sizes and should be considered preliminary.

In contrast to localities ZJG and LXBE, *Liaoningogriphus quadripartitus* plays only a minor role in excavation JSG. Also, modern caddisfly larvae are widely used in water quality assessments (Bonada *et al.* 2004). However, the high variability in the ecological profiles of modern taxa (very intolerant to fairly tolerant; Bonada *et al.* 2004) and their rarity in excavation JSG render the fossil caddisfly larvae of Lake Sihetun less significant for the environmental characterization of the lake.

Life-history patterns of Eosestheria middendorfii. Specific environments are known to induce different combinations of life-history traits in organisms (Stearns 1992; Roff 1992, 2002) and the life history of *Eosestheria middendorfii* has been summarized as opportunistic (Fürsich *et al.* 2007). Relevant life-history traits identified in the extant clam shrimp *Eulimnadia texana* are growth rate, egg production, moult frequency, age at maturity, and lifespan (Weeks *et al.* 1997). *Eulimnadia texana* is characterized by rapid growth, quick maturation (5–6 days), an emphasis on reproduction and then early senescence (Weeks *et al.* 1997). These crustaceans have a significant drop in the rate of growth with the onset of egg production, underscoring a substantial trade-off between growth and egg production. This combination of life history traits is indicative of an early colonist life history. *Eulimnadia texana* is adapted to life in short-lived, smaller water bodies, which pose strong natural selection for rapid growth, leading to early reproduction before the pool dries and an overall short lifespan of little more than 20 days with an average moulting periodicity was about 22 h (~1.1 moults per day; Weeks *et al.* 1997). In general, growth continues in surviving individuals, even if it slows with the onset of egg production.

The Early Cretaceous *Eosestheria middendorfii* lived in an environment that induced considerable variation in

individual size (Fig. 6). The leverage plots in Figure 11 suggest that the rate of growth in *E. middendorfii* was mainly a function of population density and species diversity (Shannon; Hethke *et al.* 2018b, S7): fast growth was triggered by lower clam-shrimp population densities and higher diversities. In turn, smaller individuals tend to occur when populations are monospecific and con-specifics are numerous.

Hence, we propose a strong population density effect on carapace size (Fig. 12) that produced fossil horizons with small numbers of large-sized shrimp (low population density; Fig. 3) or large numbers of small-sized shrimp (high population density; Fig. 4B, E, G). An investigation of this trend, involving the two extant species *Eulimnadia texana* and *Eocyzicus argillaquus* (Fig. 13), corroborates this trend. High densities lead to slow growth, and vice versa, while time to maturity varied only slightly with density. Thus, spinicaudatans can mature at a small size in high population densities. Individual size rapidly decreases with increasing population density but levels off at a certain density, which seems to be accompanied by increased mortality (SCW, MH, pers. obs.)

In addition to the population-density effect, we noticed that the carapace size of *E. middendorfii* is significantly smaller in monospecific than in higher-diversity associations, and that population densities in monospecific horizons are significantly higher than in higher-diversity horizons (Fig. 10). We conclude that *E. middendorfii* reacted with smaller carapace sizes to extreme values of a gradient (e.g. oxygen, temperature) under which other species could no longer exist (Fig. 9). A strong temperature effect on clam-shrimp size has been identified in extant spinicaudatans (Huang & Chou 2017) but the proposed oxygen effect has not been tested yet. Environmental conditions might also have influenced hatching propensity of *E. middendorfii* (thus indirectly population size).

Size distributions of Eosestheria middendorfii *indicate both senescence and mass-mortality events in Lake Sihetun*

Possible proxies for the recognition of clam-shrimp mass mortality events (in contrast to death after reproductive senescence) are narrow size distributions and a lack of growth-band crowding at the carapace margin, which can be observed in some of the largest clam shrimp of our excavation (e.g. JSG H, P and V).

A comparatively wide range of clam-shrimp sizes in a death assemblage of a cohort indicates favourable environmental conditions, as growth continues in surviving individuals during population decline. Hence, death after reproductive senescence should be indicated by a higher standard deviation in size distributions (normal age distribution), exemplified by JSG K and T (1.53 and

1.55 mm, respectively; Fig. 6). In turn, a low standard deviation in a size distribution should be an estimate for an abrupt death of a cohort of juveniles or young adults, indicating environmentally-induced mass mortality; exemplified by the small spread of the juvenile fraction in the bimodal size distribution of JSG A.

Hence, the axis measurements in Figure 6 show that most of the layers studied do not represent mass mortality events, as previously suspected (Fürsich *et al.* 2007), but death from senescence. However, there are horizons that record both: the bimodal size distribution of JSG A (Fig. 6), with larger individuals occurring in the stratigraphically lower layer and smaller individuals in the upper layer, indicates a seasonal trigger for mass mortalities during Phase 3. The first cohort of the year lived to sexual maturity and died before the second hatched. Even though temperatures (as the main trigger for hatching; Mattox 1950; Brendonck 1996) were suitable for the hatching of both cohorts, environmental conditions worsened for the second within days after hatching, when the individuals had just completed their naupliar phase. The size of the final naupliar stage of extant species is a little less than 1 mm (Olesen & Grygier 2004) which is comparable to the smallest clam shrimp of JSG A (length ~1 mm). This second population therefore suffered from a seasonal mass mortality event. Such events seem to have been comparatively common in communities of Association 1.

Modern clam-shrimp population ecology in comparison with Eosestheria middendorfii

Much of the extrapolation of modern clam-shrimp habitats to fossil ones seems ill-founded (Webb 1979; Orr & Briggs 1999) as the ecology of many living species is still insufficiently studied. The general interpretation of sediments yielding spinicaudatans is that of a 'temporary pond filled with freshwater' (e.g. Todd 1991; Wang 1999). In the present study, we challenge the general palaeoenvironmental interpretation by comparing the population ecology of *E. middendorfii* with ecological parameters of modern spinicaudatans. Generally, *E. middendorfii* exhibits a high ecophenotypic variability, indicating a high tolerance towards environmental variables.

The main ecological factors influencing the development of clam shrimp are temperature, population density, salinity, turbidity and the relative permanency of sites (Mattox 1950; Horne 1967; Timms & Richter 2002; Stoch *et al.* 2016; Huang & Chou 2017).

Life style. Clam shrimp adopt a general life style as occasional swimmers, but habits vary among groups. Limnadiids seem to be active swimmers, while modern cyzicids are

generally benthic, non-selective deposit feeders that burrow into the mud to filter out ingestible material (Frank 1988; Vannier *et al.* 2003; Smith & Gola 2001), and it is often required to disturb the sediment to recognize their presence (e.g. *Ozestheria*). In other cases, cyzicids can be associated with the water surface (*Eocyzicus*; Schwentner *et al.* 2015). Little is known about spinicaudatan feeding strategies, but they appear to be omnivores, feeding on detritus, algae and zooplankton (Pennak 1978; Martínez-Pantoja *et al.* 2002).

Following the close affinities between eosestherioids and cyzicids (Hethke *et al.* 2017), it is likely that *Eosestheria middendorfii* was nektobenthic and closely associated with the lake floor.

Depth. According to Wang (1999), clam shrimp of the Yixian Formation lived in quiet, shallow waters near the lake coast, in depths of about 2–50 cm to a maximum 2 m. This interpretation is clearly based on an analogy with modern clam shrimp. Most pools of *Limnadia stanleyana* were less than 20 cm deep (Bishop 1967a), and in Australia, pool depth for *Paralimnadia badia* ranged from 17.7 to 68.3 cm (Calabrese *et al.* 2016). However, excavation JSG yielded coprolites in multiple horizons of Bed 3 (Fig. 5E, H, I; especially abundant in JSG R) as well as fish body fossils in Bed 2 (JSG 11; Fig. 1), implying somewhat deeper conditions to accommodate fish populations (Pan *et al.* 2012a) and connectivity to a drainage system yielding fish. In addition, the proposed episodic meromixis during Phase 2 (Hethke *et al.* 2013b; Wang *et al.* 2016) required a certain water depth below storm wavebase.

Light intensity. Eggs are more likely to hatch in zones of light penetration in the mud (Bishop 1967b; Belk 1972; Brendonck 1996). In addition to hatching, growth is also accelerated by permanent illumination (Goretzki 2003; SCW, pers. obs.). In contrast, Horne (1971) noticed that photoperiod was not the primary environmental stimulus for hatching, as eggs were found to hatch during winter if temperatures permit it.

Oxygen concentration. Clam shrimp are exceptionally tolerant to low oxygen conditions (Horne 1971; Eriksen & Brown 1980), yet oxygen concentration can be a limiting factor in extant branchiopod associations (Moore & Burn 1968). Controlled experiments of Bishop (1967b) showed that eggs of *Limnadia stanleyana* hatch more readily at higher oxygen concentrations that are close to the equilibrium with air. Oxygen depletion reduces the amount of nauplii hatching. Therefore, the deeper eggs are buried in the mud the more likely it is that they will not hatch, as oxygen concentrations commonly decrease (Bishop 1967b). Rainwater saturated with air may stimulate hatching. In contrast, *Caenestheriella*

setosa withstands oxygen concentrations as low as 0.1 ppm (Horne 1971; tap water: 4–7 ppm dissolved oxygen). Therefore, there are clam-shrimp taxa that are exceptionally tolerant to hypoxia. Experiments show that low oxygen concentrations stimulate active swimming, whereas at higher concentrations *C. setosa* lies motionless on the substrate. Concentrations below 0.1 ppm lead to the death of the clam shrimp within 30 min (Horne 1971). Furthermore, Horne (1971) noticed that at oxygen levels as low as 0.6 ppm no other branchiopods (tadpole shrimp and fairy shrimp) were co-occurring with clam shrimp.

Moore & Burn (1968) surveyed hypoxic ponds (<1 ppm; 1 m maximum depth) in Louisiana. One instance of high water temperatures led to the depletion of subsurface oxygen for more than a week, but the pond fauna was not entirely eliminated. Instead, the behaviour of some animals changed during this anoxic interval. Anostracans (fairy shrimp) were concentrated in the upper cm of the surface waters, while comparatively weak swimmers such as the clam shrimp *Eulimnadia inflecta* were eliminated (Moore & Burn 1968).

Eosestheria middendorfii was similarly adapted to very low lethal oxygen thresholds, as hypoxia frequently occurred in Lake Sihetun during Phase 2 (Hethke *et al.* 2013b), which was marked by low-diversity associations (Fig. 6; Fürsich *et al.* 2007). Some of the clam-shrimp-free layers in Bed 2 might indicate inhibited hatching during recurring anoxic conditions. The situation was more relaxed during the oxygenated Phase 3, when factors other than oxygen governed community composition.

pH. According to Goretzki (2003), nauplii only hatch in neutral to weakly alkaline conditions, but there is no report of which or how many species were tested. Tasch (1969) mentioned that the preferred pH of clam shrimp ranges between 7 and 9. Spinicaudatans of Lake Cuitzeo prefer more alkaline waters (pH 7.9–9.6), while they are absent from other parts of the lake (Martínez-Pantoja *et al.* 2002).

These observations correspond to the alkaline interpretation for the bottom waters of Lake Sihetun (Hethke *et al.* 2013a, b). Also, alkalinity has been proposed as one of the determinant habitat factors separating two Late Carboniferous species of the eastern Illinois Basin (Petzold & Lane 1988).

Precipitation, nutrients and population density. Our rearing experiments support a strong density effect on carapace size for both *Eulimnadia texana* and *Eocyzicus argillaquus* (Fig. 13), which is corroborated by field data: heavy rainfall resulting in a high food supply and

larger, more permanent pools, greatly reduces population density and intraspecific competition. Individuals of *Cyzicus gynecia* (formerly *Caenestheriella gynecia*) hatching during such wetter times were distinctly larger with higher numbers of growth lines (10.6 mm as opposed to 7.3 mm in dryer years; Mattox 1950).

The onset of Phase 3 produced some of the largest clam shrimp of this study, which lived in exceptionally low-density communities (Transitional Fauna; Figs 6–8). This would fit the interpretation of an increased food supply and lake volume due to increased precipitation (scenario 2, Sedimentological Analysis and Lake Depth, above). However, as carapaces are similarly large in excavation ZJG (Phase 2; Fürsich *et al.* 2007), this interpretation does not capture all of the size variation in *Eosestheria middendorfii*.

Salinity. Most species of the Paroo catchment in Australia dwell in fresh to subsaline waters (<250–5000 μS/cm; Timms & Richter 2002). Hence, clam shrimp are generally indicators for freshwater conditions. There are, however, exceptions such as the halophilic species *Eocyzicus parooensis*, which occurs in hyposaline lakes (up to 15 g/l; Timms & Richter 2002). Also, experiments on *Cyzicus californicus* demonstrate that some species are able to survive hyposaline conditions of up to 7 ppt for at least a couple of days (Eriksen & Brown 1980).

Exceptions such as the morphologically variable species *E. parooensis* (in number of growth lines and varying lengths and heights; Richter & Timms 2005) show that it is not possible to predict what levels of salinity *Eosestheria middendorfii* was able to tolerate. Carbonate precipitates might indicate increased salinities during Phase 2. Yet, carbonate layers are comparatively rare in Bed 2, indicating predominantly freshwater conditions for Lake Sihetun.

Temperature. There are important temperature effects that influence hatching, the size of nauplii, and the post-larval development (Mattox & Velardo 1950; Belk & Belk 1975; Eriksen & Brown 1980; Brendonck 1996; Huang & Chou 2017). Hatching takes place after a species-specific temperature has been reached, for example 13°C for wet and 17°C for dried eggs of *Cyzicus gynecia*. The optimum temperature for egg development of *C. gynecia* lies between 24 and 37°C and nauplius size decreases when hatched above this optimum. Post-embryonic development of *Limnadia stanleyana* is strongly dependent on temperature, taking 440 h at 15°C, but only 160 h at 25°C (Bishop 1967*a*). Belk & Belk (1975) reared *Caenestheriella setosa* and report an optimum of 25°C for number of eggs hatching. Accelerated growth at the same optimum in comparison to lower temperatures has also been identified in *Eulimnadia braueriana*

(Huang & Chou 2017). In addition, the growth of *C. gynecia* was found to be distinctly slower in autumn populations (lower temperatures) than in their summer equivalents, resulting in more closely-spaced growth lines. Emberton (1980) suggested a channelling of energy into egg production as the reason for reduced growth in autumn populations. The appearance of *Caenestheriella setosa* in ponds is driven by temperature as the main factor (Horne 1971), as populations hatched during particularly warm spells in January (in Texas). Normally this species does not hatch during December to February, even if the respective pools are repeatedly filled.

Water depth and temperature are linked and the inferred shallower waters during the deposition of the upper part of excavation JSG certainly experienced enhanced temperature fluctuations. Considering the influence of temperature on the post-embryonic development of modern clam shrimp, temperature fluctuations must have had a strong effect on the size of *E. middendorfii*. We noticed that the second hatch of a season often suffered from mass-mortality events (e.g. JSG A). High temperatures during summer stagnation and the formation of oxygen-restricted habitats could have been responsible for the elimination of competing taxa and, occasionally, repeated seasonal mass mortality events.

Turbidity. Turbidity is one of the factors that keep species apart. Some spinicaudatans prefer clear, well-oxygenated waters, others do well in highly turbid waters, such as *Caenestheria lutraria* (Timms & Richter 2002). As benthic, non-selective deposit feeders, modern cyzicids burrow into the mud and filter out ingestible material (Vannier *et al.* 2003). The dislodging of detritus creates enough turbidity to identify ponds carrying clam shrimp and those without (Frank 1988). Experimental studies show that turbidity increases with clam-shrimp population density (Luzier & Summerfelt 1997).

Co-occurrences. The frequency of co-occurrence of large branchiopods has been debated over the years. In his seminal book, Pennak (1978) suggested that branchiopods were most commonly found in single-species pools. Since then, there have been many studies noting multi-species assemblages of large branchiopods (Maeda-Martínez *et al.* 1997; Broeck *et al.* 2015; Mabidi *et al.* 2016; Stoch *et al.* 2016; Alfonso 2017; Machado *et al.* 2017). However, co-occurrence of large branchiopods appears less common than the presence of monospecific assemblages, especially within (sub)orders. Also, congeneric co-occurrences of freshwater large branchiopods are rare or even absent in some regions (Machado *et al.* 2017; 505 surveyed sites in Portugal). In Mexico, out of 183 localities with branchiopods of all types (fairy shrimp, clam shrimp and tadpole shrimp), 122 were monospecific (67%) (Maeda-

Martínez 1991). Hamer & Martens (1998) also found that most of their samples from South Africa contained a single branchiopod species.

Within the Spinicaudata, monospecific assemblages are common, as suggested by several hundred surveyed ponds in Kansas and Oklahoma (Tasch 1969). There are, however, numerous reported cases of co-occurrence, but coexisting species are commonly from different families and are almost always from differing genera. Pools in Aruba contained one spinicaudatan species, *Leptestheria venezuelica* (Belk *et al.* 2002). In Mexico, Martínez-Pantoja *et al.* (2002) reported *Leptestheria compleximanus* and *Eocyzicus digueti* in the ephemeral part of Lake Cuitzeo, the second largest lake of the country. In a larger survey from Mexico and Arizona, Maeda-Martínez *et al.* (1997) noted comparatively frequent spinicaudatan co-occurrences of: (1) *Caenestheriella setosa* and *Leptestheria compleximanus*; (2) *C. setosa* and *Eulimnadia texana*; (3) *Eocyzicus digueti* and *L. compleximanus*; and (4) *L. compleximanus* and *E. texana*; but in no case was more than one species from the same genus found to co-occur. In Morocco, clam-shrimp species co-occurred in only 4 out of 43 spinicaudatan-yielding sites (9%; *Cyzicus bucheti* and *Maghrebestheria maroccana*) (Broeck *et al.* 2015). In Apulia, Italy, the two identified spinicaudatans, *Cyzicus tetracerus* and *Leptestheria mayeti* were found to co-occur with other large branchiopods, but not with each other (Alfonso 2017). In Austria, Eder *et al.* (1997) reported co-occurrence of *Imnadia yeyetta* and *Limnadia lenticularis*. In former Yugoslavia, Petrov & Cvetković (1997) reported *Leptestheria saetosa* and *Imnadia banatica* co-occurring. In an extensive reported case of spinicaudatan co-occurrence, Mabidi *et al.* (2016) surveyed 22 water bodies in South Africa and found *Cyzicus australis* occurring with *Eulimnadia* sp., a three clam-shrimp species assemblage of *C. australis*, *Eocyzicus obliquus* and *Leptestheria rubidgei*, and a single instance of two species of one genus co-occurring: *Leptestheria rubidgei* and *L. striatoconcha*. These two species were also found to co-occur in Zimbabwe (Nhiwatiwa *et al.* 2014). Thus, in all of these surveys across three continents and the Caribbean, only a single instance of two species within the same genus has been reported.

In contrast, spinicaudatan multispecies assemblages and the co-occurrence of congeners of *Eulimnadia*, *Ozesthteria* and *Limnadopsis* seem exceptionally common in Australia (Timms & Richter 2002; Weeks *et al.* 2006; Schwentner *et al.* 2015). Syntopic co-occurrences of two or more clam-shrimp genera (spinicaudatans and *Lynceus*) were found in 21% of the investigated pools from the Paroo catchment (Australia; Timms & Richter 2002).

Co-occurrences of two species are comparatively common in creek pools, as they are connected to other localities, and they are least common in hyposaline lakes (Timms & Richter 2002). Species are probably kept separate through salinity, high turbidity, clear short-lived water, different feeding strategies, and season (Eder *et al.* 1997; Petrov & Cvetković 1997; Timms & Richter 2002; Schwentner *et al.* 2015). For example, both taxa *Caenestheria* spp. and *Caenestheriella* spp. occur in turbid sites with salinities of <5000 μS/cm that tend to fill for 4–8 weeks a year. Both can be found at any time during the year, although *Caenestheria* prefers the warm season and *Caenestheriella* the cooler one. Therefore, one species persists until the late stages of filling, whereas the other occupies the early stages of filling (Timms & Richter 2002).

The identification of fossil co-occurrences is difficult, as taphocoenoses are commonly preserved. Alleged co-occurrences of fossil spinicaudatans with a diverse marine fauna (e.g. Kummerow 1939) might merely be the result of allochthonous depositions (Vannier *et al.* 2003). Two Late Carboniferous species of the Illinois Basin are mostly mutually exclusive and one alleged co-occurrence in fact represents a transported fauna (Petzold & Lane 1988). Co-occurrences of two different clam-shrimp species have been proposed for a late Norian to early Rhaetian bedding plane (Barth *et al.* 2014). In the case of Lake Sihetun, a taxonomic revision demonstrated that five of the ten species reported from the Yixian Formation of the Sihetun area belong to a single species, *Eosestheria middendorfii* (Hethke *et al.* 2018a). Excavation JSG yielded no evidence for co-occurrences of this variable species with any other clam-shrimp species.

Predators. The abundance of coprolites entirely made up of spinicaudatan carapace fragments (Fig. 5E, H, I; Hethke *et al.* 2018b, S5) shows that *Eosestheria middendorfii* was prey to higher trophic levels, probably fish, which can be abundant in the Yixian Formation (Fig. 2D; Pan *et al.* 2015). Our excavation yielded two fish fossils in layers underlying horizon JSG AP. Modern spinicaudatans can be (episodic) food sources for fish (e.g. *Caenestheriella belfragei* in fish stomachs; Donald 1989) and observations of extant branchiopod occurrences indicate that an often proposed absence of predators is not essential for branchiopod success (Retallack & Clifford 1980). Water bodies inhabited by modern spinicaudatans are generally too alkaline or temporary to support fish (Pennak 1978). Yet, co-occurrences of fish and spinicaudatans are possible when temporal and perennial habitats connect, as has been reported from Lake Cuitzeo (Mexico), an astatic lake in an endorheic basin, in which spinicaudatans are confined to the ephemeral part (Martínez-Pantoja *et al.* 2002). In any case, the coprolites show that the food web of Lake Sihetun frequently became more complex than in most modern spinicaudatan-yielding habitats.

Eggs, hatching and early growth. Generally, reproductive adaptations of species restricted to small bodies of standing water are sexual systems such as unisexual reproduction as well as dormant and rapidly hatching eggs (Barnes & Harrison 1992). Branchiopod hatching is triggered by temperature, light and the absorbing of water until the breakage of the outer shell (Mattox 1950; Brendonck 1996). Only a limited proportion of eggs is commonly subjected to a favourable micro-environment when the pool fills and many eggs remain unhatched in the mud, forming a reserve (Bishop 1967*a*; Simovich & Hathaway 1997; Brendonck & De Meester 2003). Hatching is prevented by a lack of oxygen or light (Bishop 1967*b*) and it may occur after several years (to decades) of drought (Dumont & Negrea 2002).

However, even though hatching is commonly associated with the filling of a pool, eggs may hatch as readily without having been dried beforehand (Bishop 1967*b*; Belk 1972). Therefore, temperature, oxygen and light are considerably more important stimuli for hatching. Moreover, Mattox & Velardo (1950) pointed out that drying might even retard the development and hatching of eggs. Therefore, a resting period marked by desiccation or freezing does not seem to be necessary for egg development in all clam-shrimp species (Brendonck 1996).

At the time of hatching, nauplii of *Cyzicus gynecia* are 0.37 mm long, growing rapidly to 0.48 mm within three hours. Four days after hatching, their body length averages 1.1 mm, enclosed in a carapace of 1.5 mm in length (Mattox 1950), which is comparable with the seventh naupliar stage of *Caenestheriella gifuensis* (686–854 μm; Olesen & Grygier 2004). The development of adults takes several days and is shorter for limnadiids (Bishop 1968) and a bit longer for cyzicids (Frank 1988). Temperature strongly influences the post-embryonic development. *Cyzicus mexicanus* hatches from May to August (late spring to summer; Mattox 1939).

The onset of warm conditions in Lake Sihetun probably triggered the hatching of nauplii of *Eosestheria middendorfii* in adequately oxygenated waters during late spring. There is no need to postulate the desiccation of larger areas of Lake Sihetun on a seasonal basis, as dehydration is not required for egg development (Mattox & Velardo 1950; Bishop 1967*b*). Furthermore, sedimentary features that may corroborate repeated desiccation have not been observed in excavation JSG. Therefore, the hatching of nauplii was probably triggered by temperature (Hethke *et al.* 2013*b*) rather than by flooding after desiccation. The egg production of females (Hethke *et al.* 2017) was probably high, considering the production of 1–2 egg clutches per day in extant species (Weeks *et al.* 1997). *Eosestheria middendorfii* was a species capable of hatching at least twice a year, with the first population of a season growing to maturity, while the second hatch,

although triggered by a similarly favourable micro-environment, often suffered from the rapid formation of unfavourable conditions and seasonal mass-mortality events (e.g. JSG A, C, D and G; size measurements in Fig. 6). The main hatching stimulus for Bed 3 populations must have been temperature, as light intensity was probably the same or higher when the second wave was triggered.

Temporary vs perennial water bodies

Spinicaudatan population ecology has been proposed as static from the Devonian to the Recent (Gueriau *et al.* 2016). However, this exclusive 'shallow and temporary' interpretation has been challenged by various authors (Orr & Briggs 1999; Vannier *et al.* 2003; Olsen 2016). There are numerous examples of temporary settings yielding clam shrimp recognized by the presence of desiccation cracks, footprint horizons, algal laminae and stromatolites in the associated sediments (e.g. Olempska 2004; Lucas & Milner 2006). Other palaeoenvironmental interpretations, however, are entirely based on an analogy with the realized ecological niche of extant spinicaudatans. Spinicaudatans of the Westphalian Coal Measures, for example, are associated with diverse aquatic habitats, which have been proposed to have been temporary by alluding to extant forms (Todd 1991).

Vannier *et al.* (2003) paralleled the Late Carboniferous Montceau biota with that of temporary aquatic environments, marked by a low diversity, high density crustacean fauna, a narrow size range of spinicaudatan assemblages suggesting cohorts, the presence of resting eggs, the dominance of crustaceans as a whole, and a high insect diversity in contrast to a relatively low abundance. However, the presence of fish and syncarid crustaceans at Montceau, which are commonly absent from modern shallow and isolated temporary pools, led Vannier *et al.* (2003) to reconsider water depth. In addition, they also recognized that sedimentological features of temporary environments were not associated with the clam-shrimp bearing horizons of Montceau. The same arguments can be put forward for Lake Sihetun (Fig. 1). Vannier *et al.* (2003) reconciled this discrepancy between the observed 'perennial' features and the common 'temporary' interpretation of clam shrimp by proposing flooding and the transportation of faunal remains to the depositional area. Thus, the Late Carboniferous Montceau Lagerstätte has been characterized as a lake ecosystem with both permanent and temporary ecological niches, reminiscent of extant spinicaudatans of Lake Cuitzeo (Martínez-Pantoja *et al.* 2002).

The presence of various sub-environments has been proposed for Lake Sihetun (e.g. with regard to oxygen availability, Hethke *et al.* 2013*b*; Wang *et al.* 2016). However,

the commonly used argument that clam-shrimp eggs require a dry phase cannot be upheld, and we propose temperature, light and oxygen availability as the most important hatching stimuli for clam-shrimp populations in Lake Sihetun (Mattox & Velardo 1950; Bishop 1967*b*; Horne 1971; Brendonck 1996). This interpretation is not entirely in contrast to realized niches of extant branchiopods; a close phylogenetic relative of the spinicaudatans, *Cyclestheria hislopi*, dwells in permanent waters (Schwentner *et al.* 2013). Therefore, instead of interpreting clam shrimp as transported to fit the common ecological niche of extant taxa, it is herein proposed that *Eosestheria middendorfii* was adapted to life in permanent waters.

CONCLUSIONS

The population ecology of the Early Cretaceous *Eosestheria middendorfii* is, in parts, comparable to that of modern relatives, yet sedimentological evidence suggests that this species assumed an ecological niche not realized by most extant species. The key difference between modern species and this Early Cretaceous form is an adaption to life in the permanent waters of the volcanic Lake Sihetun, characterized by a comparatively complex food web including higher trophic levels that fed on clam shrimp.

Abiotic environment during lake phases 2 and 3

Phase 2 was markedly deeper than inferred in previous studies. Indicators for higher-energy conditions in the lake during Phase 3 are small-scale cross-bedding and orientation patterns of *Ephemeropsis* larvae, which indicate a preferred direction of NE–SW. A gradual coarsening upwards trend within Bed 3 implies overall shallowing during Phase 3. Phase 2 marked a time of dysoxic to temporary anoxic bottom waters, while Phase 3 was governed by alternations of wet and dry intervals with a well-oxygenated lake floor during the wet season that was quickly colonized by a meiofauna. The dry season was represented by background sedimentation and the formation of biofilms. Spinicaudatans mostly died during this tranquil phase. No evidence of repeated emergence has been identified in sediments of either phase.

Community development

We find that standardization to excavated area and subsequent double-square-root transformation best facilitates the recognition and interpretation of palaeocommunity patterns in successions governed by mass occurrences of one species.

The long-term community development across the transition between phases 2 and 3 yielded three associations and two assemblages, which were mostly arthropod-dominated, of high numerical abundance, and of low diversity. The environmental changes in Lake Sihetun taking place between phases 2 and 3 are reflected by a sharp modification in the palaeocommunity composition from the clam-shrimp dominated associations 1 and 2 to the insect- and oligochaete-dominated Transitional Fauna, the latter being marked by comparatively high diversities but low population densities.

Community composition

Response curves to environmental variables are overlapping but of various widths, explaining the absence of taxa from certain fossil horizons. The clam shrimp *Eosestheria middendorfii* exhibits the widest response curve, expressed by a pronounced ecophenotypic variability. Larvae of *Ephemeropsis trisetalis* and the water boatman *Karataviella* cf. *pontoforma* can also be considered to be generalists. In contrast, oligochaetes, bivalves and gastropods were less tolerant towards environmental variables, manifested in narrower response curves.

Life history of Eosestheria middendorfii

Hatching of *Eosestheria middendorfii* was triggered several times per year, but the chances of survival were significantly smaller for the second generation of a season, whose individuals often barely survived the naupliar stage. The species did not depend on the drying of eggs prior to hatching and subsequent flooding. Instead, temperature, light and oxygen are proposed as the main hatching triggers. The population dynamics of *E. middendorfii* were dominated by a sudden recruitment and a subsequent progressive population decline, which was repeatedly interrupted by population crashes driven by oxygen depletion during Phase 2 and possibly high temperatures in shallower waters during the later stage of Phase 3. However, most of the fossil layers do not represent mass-mortality events, but instead death after reproductive senescence.

The observed decrease in spinicaudatan size with increasing population density in *E. middendorfii* seems to reflect a pattern true for several members of the group. Rearing experiments indicate a significant population density effect on carapace size, with natural log regression providing the best fit for all three: the fossil and the two modern species studied.

DATA ARCHIVING STATEMENT

Data for this study are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.9387k41

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REFERENCES

ALFONSO, G. 2017. Diversity and distribution of large branchiopods (Branchiopoda: Anostraca, Notostraca, Spinicaudata) in Apulian ponds (SE Italy). *The European Zoological Journal*, **84**, 172–185.

ALLEN, P. A. 1981. Devonian lake margin environments and processes, SE Shetland, Scotland. *Journal of the Geological Society, London*, **138**, 1–14.

AMIOT, R., WANG, X., ZHOU, Z., WANG, X., BUFFETAUT, E., LÉCUYER, C., DING, Z., FLUTEAU, F., HIBINO, T., KUSUHASHI, N., MO, J., SUTEETHORN, V., WANG, Y., XU, X. and ZHANG, F. 2011. Oxygen isotopes of East Asian dinosaurs reveal exceptionally cold Early Cretaceous climates. *Proceedings of the National Academy of Sciences*, **108**, 5179–5183.

BARNES, R. D. and HARRISON, F. W. 1992. Introduction. *In* HARRISON, F. W. and HUMES, A. G. (eds). *Microscopic anatomy of invertebrates. Vol. 9, Crustacea*. Wiley-Liss.

BARTH, G., FRANZ, M., HEUNISCH, C., KUSTATSCHER, E., THIES, D., VESPERMANN, J. and WOLFGRAMM, M. 2014. Late Triassic (Norian-Rhaetian) brackish to freshwater habitats at a fluvial-dominated delta plain (Seinstedt, Lower Saxony, Germany). *Palaeobiodiversity & Palaeoenvironments*, **94**, 495–528.

BATZER, D. P. and WISSINGER, S. A. 1996. Ecology of insect communities in nontidal wetlands. *Annual Review of Entomology*, **41**, 75–100.

BAUERNFEIND, E. and MOOG, O. 2000. Mayflies (Insecta: Ephemeroptera) and the assessment of ecological integrity: a methodological approach. *Hydrobiologia*, **422/423**, 71–83.

BELK, D. 1972. The biology and ecology of *Eulimnadia antlei* Mackin (Conchostraca). *The Southwestern Naturalist*, **16**, 297–305.

—— and BELK, M. S. 1975. Hatching temperatures and new distributional records for *Caenestheriella setosa* (Crustacea, Conchostraca). *The Southwestern Naturalist*, **20**, 409–411.

—— —— and READING K. A. L. 2002. Survey of large branchiopods on Aruba and observations on taxonomic characters in *Leptestheria* (Spinicaudata). *Hydrobiologia*, **486**, 115–118.

BISHOP, J. A. 1967a. Seasonal occurrence of a branchiopod crustacean, *Limnadia stanleyana* King (Conchostraca) in eastern Australia. *Journal of Animal Ecology*, **36**, 77–95.

—— 1967b. Some adaptations of *Limnadia stanleyana* King (Crustacea: Branchiopoda: Conchostraca) to a temporary freshwater environment. *Journal of Animal Ecology*, **36**, 599–609.

—— 1968. Aspects of the post-larval life history of *Limnadia stanleyana* King (Crustacea: Conchostraca). *Australian Journal of Zoology*, **16**, 885–895.

BONADA, N., ZAMORA-MUÑOZ, C., RIERADEVALL, M. and PRAT, N. 2004. Ecological profiles of caddisfly larvae in Mediterranean streams: implications for bioassessment methods. *Environmental Pollution*, **132**, 509–521.

BRAAK, C. J. F. TER 1985. Correspondence analysis of incidence and abundance data: properties in terms of a unimodal response model. *Biometrics*, **41**, 859–873.

BRENDONCK, L. 1996. Diapause, quiescence, hatching requirements: what we can learn from large freshwater branchiopods (Crustacea: Branchiopoda: Anostraca, Notostraca, Conchostraca). *Hydrobiologia*, **320**, 85–97.

—— and DE MEESTER, L. 2003. Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment. *Hydrobiologia*, **491**, 65–84.

BROECK, M., VAN DEN WATERKEYN, A., RHAZI, L. and BRENDONCK, L. 2015. Distribution, coexistence, and decline of Moroccan large branchiopods. *Journal of Crustacean Biology*, **35**, 355–365.

CALABRESE, A., McCULLOUGH, C., KNOTT, B. and WEEKS, S. C. 2016. Environmental characteristics of ephemeral rock pools explain local abundances of the clam shrimp, *Paralimnadia badia* (Branchiopoda: Spinicaudata: Limnadiidae). *Journal of the Royal Society of Western Australia*, **99**, 9–15.

CHANG, S., ZHANG, H., RENNE, P. R. and FANG, Y. 2009. High-precision ^{40}Ar/^{39}Ar age for the Jehol Biota. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **280**, 94–104.

CHEN, J. 1999a. A study of nonmarine bivalve assemblage succession from the Jehol Group (U. Jurassic and L. Cretaceous). *Palaeoworld*, **11**, 92–113. [in Chinese]

CHEN, P. 1999b. Fossil conchostracans from the Yixian Formation of western Liaoning, China. *Palaeoworld*, **11**, 114–130. [in Chinese]

CLARKE, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, **18**, 117–143.

COLWELL, R. K., MAO, C. X. and CHANG, J. 2004. Interpolating, extrapolating, and comparing incidence-based species accumulation curves. *Ecology*, **85**, 2717–2727.

DAVIS, J. C. 1986. *Statistics and data analysis in geology*. John Wiley & Sons.

DONALD, D. B. 1989. First Canadian record for the clam shrimp *Caenestheriella belfragei* (Crustacea: Conchostraca). *The Canadian Field-Naturalist*, **103**, 593–594.

DUMONT, H. J. and NEGREA, S. V. 2002. *Introduction to the class Branchiopoda*. Backhuys, Leiden.

EDER, E., HÖDL, W. and GOTTWALD, R. 1997. Distribution and phenology of large branchiopods in Austria. *Hydrobiologia*, **359**, 13–22.