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EXPERIMENTAL INVESTIGATION OF INSECT DEPOSITION IN LENTIC ENVIRONMENTS AND IMPLICATIONS FOR FORMATION OF KONSERVAT-LAGERSTÄTTEN

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Abstract: Terrestrial insects are often remarkably well preserved in lacustrine Konservat-Lagerstätten. However, the assumption that carcasses should sink fast through the water column seems to contradict evidence that this scenario is unlikely due to excessive buoyancy and surface tension. The mechanisms that promote rapid and permanent emplacement onto the sediment surface (RPESS) of such terrestrial animal remains are not fully understood. Here we use taphonomic experiments to show that floating in water, growth of microbial biofilms and reception of rapid sediment load promote RPESS of terrestrial insect remains in lentic water bodies. Our results show that the optimum conditions for RPESS occur when terrestrial insects enter a lentic water body in articulation, experience brief decay in association with growth of microbes, then are buried rapidly by airborne volcanic ash. These results provide a model for preservation of articulated terrestrial insects and emphasize the

importance of microbial activity and volcanism for insect preservation in lacustrine Konservat-Lagerstätten.

Key words: experimental taphonomy, Konservat-Lagerstätten, decay, microbes, ashfall.

KONSERVAT-LAGERSTÄTTEN are characterized by a high quality of fossil preservation, notably the preservation of articulated skeletons, sometimes in association with soft tissues (Seilacher *et al.* 1985). These biotas are often likened to a series of rare snapshots of ancient life that provide much more complete data on the diversity of ancient communities than the remainder of the fossil record (Briggs 2001). Preservation of soft tissues has been a focus of recent research on such biotas; skeletal preservation (e.g. completeness, articulation) has received less attention. Early models considered the principal mechanisms leading to soft tissue preservation to include anoxia (stagnation), rapid burial (obrution), early diagenetic concretion growth, and microbial coverings, in addition to more localized decay-inhibitory media such as tar, permafrost, and amber (Seilacher *et al.* 1985). Over the past three decades, publication of a comprehensive body of experimental taphonomic studies has added nuance to these models and provided detailed information on how various processes operate. Experiments have shown that anoxia and obrution cannot inhibit degradation (Allison 1988) and thus cannot in isolation result in soft-tissue preservation. Instead, soft-tissue preservation is understood to reflect a complex interplay of factors, including decay, mineralization and/or maturation of organic material (Allison 1988; Briggs 1995; Briggs and McMahon 2016; Purnell *et al.* 2018 and references therein). Decay is a major factor in preservation via the generation of steep geochemical gradients necessary for replication of soft tissues in authigenic minerals (Sagemann *et al*. 1999) and for certain types of organic preservation (McNamara et al. 2016). The impact of sediment composition on soft tissue preservation has

also been investigated (Anderson *et al*. 2011; Wilson and Butterfield 2014; Murdock *et al.* 2016).

In contrast, controls on the preservation of articulated skeletons are less well understood. Taphonomic experiments have shown that freshly killed animals may remain articulated after long-distance transport, whereas they disarticulate readily if transported following a period of decay (Allison 1986; Bath Enright *et al.* 2017). Decay and subsequent disarticulation of animal remains on the water surface begins rapidly (commonly within 3–10 weeks; e.g. Duncan 1997; Peñalver 2002; Brand *et al.* 2003; Syme and Salisbury 2014), thus rapid and permanent emplacement onto the sediment surface (RPESS) of the animal remains is an important factor in the preservation of articulated skeletons under water.

Insects are of particular importance as they are the most diverse taxon in terrestrial ecosystems (Gaston 1991; Stork 2009) and are abundant in many lacustrine Konservat-Lagerstätten (Zherikin 2002). Decay sequences and taxonomic biases of insects have been studied (Martínez-Delclòs and Martinell 1993; Duncan 1997; Peñalver 2002; Duncan *et al*. 2003; Smith *et al*. 2006; Wang *et al*. 2013), but other aspects of their taphonomy are less well understood. Notably, depositional mechanisms – particularly those relating to sinking – have not been investigated systematically. Previous studies have suggested that in the absence of transport, turbulence and sediment influx, terrestrial insects rarely sink onto, and remain on, the sediment-water interface without first disarticulating at the water surface (Martínez-Delclòs and Martinell 1993; Duncan 1997; Duncan *et al.* 2003). Studies in natural lake systems also support the hypothesis that terrestrial insects rarely sink (Rolla *et al*. 2017). Transport increases rates of both sinking and disarticulation but does not impact the trend for sinking to occur after disarticulation of the abdominal or leg segments (Duncan et al. 2003; Smith et al. 2006).

Whether a floating animal carcass can sink to the sediment surface in standing water depends on the contrast between the downward force (a product of carcass mass *mg* plus any external downward force *F* exerted on it) and upward resistance force (a product of surface tension *ST* plus buoyancy *B*; Fig. 1): $F + mg$ *vs.* $B + ST$. The carcass will float until the downward force overcomes the resistance force. The force contrast varies depending on the biological characteristics of the floating carcass (e.g. size, shape, hydrophilicity and mass; Sansom 2014; Vella 2015), water conditions (e.g. pH, salinity, extent of microbial biofilms, temperature, and depth; Allison 1990; Allison *et al.* 1991; Raff *et al.* 2014), and the amount and nature of physical disturbances (e.g. wind, rainfall and sediment load; Martínez-Delclòs and Martinell 1993; Beattie 2007). After a carcass enters a lentic water body, several of these parameters (e.g. submerged volume, contact area, hydrophobicity and mass) change over time due to the effects of decay, absorption of water and development of biofilms (Martínez-Delclòs and Martinell 1993; Duncan 1997); this, in turn, could potentially reduce the force contrast, but could not overcome the resistance force alone. Extrinsic factors, such as waves, fungi and algae, have been suggested to promote sinking (Martínez-Delclòs and Martinell 1993).

Among these physical disturbances, rapid sediment load may significantly enhance the downward force and has been identified as a factor in the rapid burial of articulated carcasses (Smiley *et al*. 1975; Beattie 2007; Yang *et al*. 2019). Beattie (2007) investigated ash load on freshly killed insects, and most insects remained floating. In this study, we test whether: 1) development of microbial biofilms on floating carcasses can reduce the force contrast, and 2) rapid sediment load, mimicking volcanic ashfall, can promote RPESS of terrestrial insect remains. Our results are compared with lacustrine-hosted Lagerstätten that contain articulated preserved terrestrial insects. Other extrinsic factors, such as rainfall, wind, predation and attaching bivalve larvae, may contribute to external downward forces (Martínez-Delclòs and

Martinell 1993; Ilger 2011), but these are not tested here as they do not yield a sustained force, easily cause disarticulation or are not applicable widely.

MATERIALS AND METHODS

Experimental materials

Specimens. Our experiments used commercially available specimens of the cockroach *Periplaneta americana* (Blattodea: Blattidae; n = 580) and the butterfly *Danaus chrysippus* (Lepidoptera: Nymphalidae; n = 510). We selected these taxa because they differ strongly in mass, geometry and hydrophobicity (Table 1). Specimens were euthanized via asphyxiation as in Duncan *et al.* (2003). To ensure that specimens were dead, they were left within the airtight chambers for 24 h after their last movement. All specimens died in the wing-folded posture.

Experimental media. Two different media were used in our experiments to evaluate the effect of microbial activity: natural eutrophic lake water and commercially available distilled water (Table 2). The former contains aquatic microbes in contrast to the latter, which contains no detectable microbes (Table 2). The lake water was collected from Lizhao Lake at the Xianlin campus of Nanjing University one or two days prior to the experiments and filtered through a 150 μm mesh to remove macroscopic debris. Filtered lake water was stored at 20–25°C until the experiments commenced; no other treatment was applied.

Sediment. Tuffs and tuffaceous sediments are a common component of the sedimentary successions hosting many lacustrine Konservat-Lagerstätten (e.g. Yixian Formation, Haifanggou and Tiaojishan Formations and Florissant Formation; Hethke *et al.* 2012; Yang *et al.* 2019; McLeroy and Anderson 1966) and volcanic ashfall has been proposed as a death

mechanism for some biotas (Sinichenkova and Zherikhin 1996; Beattie 2007; Jiang *et al.* 2011; Beattie and Avery 2012; Yang *et al.* 2019; Wang *et al.* 2019). Commercially available volcanic tephra (grain size 10–150 μm, mean 40 μm (Hess pumice, Idaho, USA)) was used in this study.

Experimental setup

Our experiments used plastic basins 258 mm in diameter, each containing ca. 4500 ml water. Basins with lake water were covered by 1 mm mesh to avoid potential physical disturbance, while those with distilled water were washed by hot water (> 80°C) prior to experiments and covered by saran wrap to minimize contamination by environmental microbes. All basins were placed in the basement of the School of Earth Sciences and Engineering, Nanjing University and were not moved during the experiment. Water temperature was maintained at 20–25°C using ambient air-conditioning units. In each experiment, ten carcasses of either cockroaches or butterflies were placed in each basin. The resulting carcass density was 145 insects/m^2, which is in the range of observations during mass mortality events (e.g. 10–200 cicadas/m^2, Nowlin *et al.* 2007; 200–500 ants/m^2, Carlton and Goldman 1984). During emplacement in the basins, carcasses were positioned carefully to prevent mutual contact. During the experiment, 78% of carcasses (calculated based on photographs of total 540 specimens) were spaced > 10 mm apart, which is ca. three times the capillary length for an air-water interface (2.7 mm; Cooray 2014). As lateral capillary forces between floating objects decay approximately exponentially over the capillary length (Velev *et al.* 1993), interactions among most of the carcasses were therefore negligible. The other 22% of carcasses (n = 540) were spaced < 10 mm apart. The antennae and body margins of 13% of carcasses (n = 540) locally came into contact, but no overlap of body parts was observed.

Four independent experiments were designed (Fig. 2A). All experiments were run with cockroaches and butterflies separately.

Experiment 1 was designed to make continuous taphonomic observations (of indices including decay state, articulation, sinkage and presence of microbial films, etc.) on both taxa placed in different media without treatment. Observations were recorded daily for one month, and then after an additional two weeks.

Experiment 2 was designed to test the effects of media type (predictor variable 1 (nominal) with two levels: lake and distilled water) and flotation duration (predictor variable 2 (ratio) with 8 and 7 levels for cockroaches and butterflies respectively; T_2 in Table 3) on mass (response variable 1 (ratio)) and resistance force (response variable 2 (ratio)) of the insects. The experimental design incorporated a longer flotation duration for butterflies: pilot experiments revealed that their wet mass continued to increase for a longer time than cockroaches.

Experiment 3 was designed to test the effects of media type (predictor variable 1 (nominal) with two levels of lake and distilled water) and flotation duration before introduction of 200 g sediment (predictor variable 2 (ratio) with 12–16 levels; T_3 in Table 3) on the flotation state (sink / not sink (response variable (nominal))) of the insects. Intervals of flotation duration were more closely spaced in the early part of the experiment in order to better define the shortest duration required for sinking.

Experiment 4 was designed to test the effects of mass of sediment introduced (predictor variable 1 (ratio) with 8 and 7 levels for cockroaches and butterflies respectively; Q in Table 3) on the flotation response (sink / not sink (response variable (nominal))) of the insects that had already floated for 14 days in lake water. Among the fifteen basins, more basins were treated with only 10–70 g of sediment in order to identify the minimum thickness

required for sinking. The flotation duration of 14 days was selected because most carcasses sank by that time in Experiment 3.

Measurements, sediment loading, observations, and data processing

In Experiment 2, measurements of resistance force (buoyancy and surface tension) were based on the physical principles used by Hughes (2005) and Wang *et al.* (2015) (Fig. 2B). Individual carcasses (including any attached microbial films) were transferred from the basins to a paper cup of water on a scale for weighing using sterile tweezers. To reduce confounding influences on surface tension, carcasses were placed horizontally onto the water surface in the cup to replicate as closely as possible the orientation of the insect in the basin relative to the air-water interface. All specimens remained floating after transfer. Each carcass was then pushed downward at a speed of 0.05 mm/s by a slider that was automatically manipulated by a wire bracket until fully submerged. The scale readings and the slider block positions were recorded once per second by G&G electronic balance sampling software. Carcasses that disarticulated or failed to submerge during the measurements were excluded from subsequent analyses.

After subtracting the resistance force exerted on the wire bracket, a resistance force-submerged depth curve was obtained for each carcass (Fig. 1B shows a schematic curve; the full dataset is available in Tian *et al*. 2019, S6–S9). The maximum resistance force was always reached when the insect broke through the water surface. The mean values and 95% confidence intervals of (1) wet weight, (2) buoyancy, (3) the force contrast between wet weight and buoyancy and (4) the contrast between wet weight and the maximum resistance force were plotted versus flotation duration using OriginPro (OriginLab, Northampton, MA). Linear regression using the Ordinary Least Squares algorithm was performed in PAST (Hammer *et al*. 2001) to test the effects of flotation duration on the force contrast between wet

weight and buoyancy, and the contrast between wet weight and the maximum resistance force, respectively.

In Experiment 3 and 4, sediment was introduced through a 150 μm sieve, which was held by hand at a distance of ca. 0.3 m above the water surface (Fig. 2C). This distance is sufficient to accelerate all clasts in our experimental sediment to speeds of over 88% of the maximum falling speed for that grain size in natural ashfall (Tian *et al*. 2019, S2). Sediment was introduced in pulses of 10 g, with each pulse lasting 26 ± 7 s (n = 25) and an interpulse interval of 33 ± 7 s (n = 25). The mean mass flux in each treatment (6.7 ± 0.7 kg/m^2/h, n = 5, calculated based on the area of water surface 6.88 × 10^{-2} m^2, and 30% loss of sediment from the vessel) has a similar magnitude to that of a natural volcanic ash fallout (1–2 kg/m^2/h; Scheidegger *et al.* 1982). Introduction of 200 g sediment took 18 ± 2 min (n = 5) in total and resulted in the formation of a lamina ca. 4 mm thick.

In Experiments 3 and 4, carcasses that sank onto the sediment surface were monitored, and those that remained there three days after the treatment were considered as having experienced RPESS. The effects of flotation duration and microbial activity on RPESS of individual specimens were tested by performing binary logistic regression analyses and likelihood ratio tests (the null hypothesis is that the probability of RPESS is independent of one predictor variable) in R using the functions glm {stats} (R Core Team 2018) and lrtest {lmtest} (Zeileis and Hothorn 2002). The proportion of specimens experiencing RPESS in each basin was plotted versus (1) duration before sediment loading (Experiments 3; with logistic regression lines for different water types) and (2) sediment thickness calculated from the mass of introduced sediment (Experiment 4) using OriginPro.

Representative sedimentary laminae containing carcasses from Experiments 3 and 4 were dried and embedded in epoxy resin; polished and thin sections were prepared from the resin blocks. For comparison, a typical laminated tuffaceous mudstone with fossil insects

from the Jurassic Haifanggou Formation was also sectioned. The sections were examined with a Nikon SMZ25 stereomicroscope and a Nikon ECLIPSE LV100N POL polarizing microscope.

Scanning electron microscopy

To test for the presence of microbial films on butterfly carcasses in lake water, small fragments (ca. 5 mm × 5 mm) of the hind wing between veins M_3 and Cu_1 were dissected from representative butterfly specimens using sterile tools after performing the measurements in Experiment 2. Samples were mounted on carbon tape, dried and sputter-coated with Au and examined with a Zeiss Supra 55 scanning electron microscope (SEM) at 15 kV and a working distance of 8.8–9.1 mm.

Lacustrine-hosted Lagerstätten

Terrestrial insect fossil records from deposits in lentic environments ("lacustrine", "crater lake" and "pond") were downloaded from the Paleobiology Database (PaleoDB: http://paleobiodb.org) on 19th May 2019 (Tian *et al*. 2019, S10). Among these deposits, 27 Lagerstätten hosted in laminated sediments and with well-studied sedimentary backgrounds and fossil records were selected for investigation. Data collected include location, age, lithology, and the thickness, organic carbon content and microbial component of the fossil-bearing laminae (Tian *et al*. 2019, S4). For "large-winged" insects (adult Lepidoptera, Ephemeroptera, Odonata, Neuroptera and Tricoptera) such as butterflies, RPESS is rare in normal situations (Martínez-Delclòs and Martinell 1993; Duncan 1997). The presence or absence of articulated "large-winged" insects in each Lagerstätte was also noted. Specimens assigned to "body" in "specimen_part" in the PaleoDB records were counted as articulated. The proportion of "body" specimens was calculated for each Lagerstätte as a proxy for the

proportion of articulated specimens in the fossil assemblage. As the PaleoDB includes only published specimens, the stated articulated proportion may be artificially inflated, but all Lagerstätten should have similar biases (Karr and Clapham 2015). The diversities of the articulated insect fossils were calculated for each Lagerstätte. The Lagerstätten were grouped into “microbe-bearing laminae” or “not determined” based on the presence / absence of microbial fossils.

The data were analysed as follows. Welch’s t test was performed to compare the proportion and diversity of articulated specimens in the two groups of Lagerstätten (“microbe-bearing laminae” and “not determined”). Fisher's exact test was performed to assess the association between the preservation of articulated “large-winged” insects (“present” or “not determined”) and the presence of microbial fossils (“microbe-bearing laminae” or “not determined”). The statistical analyses were performed in PAST (Hammer *et al*. 2001).

RESULTS

Experiment 1: observations

In lake water, the decay sequences for both cockroaches and butterflies are similar to sequences reported previously (Fig. 3; Martínez-Delclòs and Martinell 1993; Duncan 1997; Duncan *et al.* 2003):

Decay stage 1 (0–5 days): For both taxa, the legs and antennae became flaccid and the abdomen, swollen; microbial films were apparent on the carcass. Microbial films also appeared on the water surface in the basin with cockroaches. Most of the butterfly wings lost their iridescence.

Decay stage 2 (5–21 days): For both taxa, the antennae began to disarticulate. Microbial films covered the surface of the water in the basin with cockroaches and appeared on butterfly wings (Fig. 3C, 3F, 3H).

Decay stage 3 (> 21 days): Cerci of cockroaches, and heads, abdomens and distal segments of legs of butterflies started to disarticulate.

In distilled water, for both taxa, the decay sequence was identical to that in lake water but the rate of decay was slower. Microbial films were relatively rare on cuticles and were not observed on the water surface (Fig. 3B, 3E). Decay stage 1 lasted ca. 10 days. In both types of water, no RPESS of articulated carcasses was observed during the experiment.

Experiment 2: measurements

During decay, the wet weight and resistance force of specimens of both taxa showed similar trends in both types of water. Wet weight and buoyancy increased progressively for 9–18 days, then stabilized or decreased slightly (Fig. 4A–D). Buoyancy exceeded wet weight for all specimens except one cockroach (in distilled water, 12 days), but the contrast between buoyancy and wet weight has a negative relationship with flotation duration in water (Fig. 4E–F). The maximum resistance force exceeded the wet weight for each specimen and the contrast between the two forces does not correlate with duration except for the cockroaches in distilled water (Fig. 4G–H).

Experiment 3: flotation duration and sediment load

Sediment influx did not lead to RPESS of the freshly killed carcasses. During sediment influx, some carcasses were submerged, but refloated in less than 20 seconds when the overlying sediments slipped away from the wing surface (Fig. 5A, 5D). The initial submergence is consistent with the force contrast calculations, which show that the calculated maximum value of the external force generated by the sediment load exceeds the mean value of the force contrast for the carcasses floating on the water surface, thus submerging the carcasses (Fig. 4E–H).

In lake water, some butterfly carcasses sank after one day of flotation and subsequent sediment loading, and for cockroaches after three days (Fig. 6A–B). The proportion of carcasses that sank following sediment influx increased progressively with increased flotation duration; after 14 days of flotation, almost all carcasses of both taxa sank after sediment loading (Fig. 6A–B, 7). In distilled water, butterfly carcasses sank with sediment load after three days of flotation, and cockroaches after seven days (Fig. 6A–B). The proportion of carcasses of both taxa that sank increased progressively with increased flotation duration but more slowly than in lake water (Fig. 6A–B). The likelihood ratio tests showed that flotation duration and media type both had significant effects on the probabilities of RPESS of both cockroaches (flotation duration, $\chi^2 = 160.5$; media type, $\chi^2 = 91.4$; both p (independent) < 0.001) and butterflies (flotation duration, $\chi^2 = 58.3$; media type, $\chi^2 = 70.5$; both p (independent) < 0.001). The resulting sedimentary laminae were ca. 4 mm thick (Fig. 7).

Experiment 4: minimum sediment load

For the carcasses that had floated in lake water for 14 days, carcasses of cockroaches and butterflies sank after only 10 g and 100 g of sediment influx, respectively; the resulting sedimentary laminae were ca. 0.2 mm and 2 mm thick, respectively (Fig. 6C–D).

Analyses of lacustrine Lagerstätten

Analyses of the PaleoDB terrestrial insect fossil records from the “lacustrine”, “crater lake” and “pond” environments showed that 48% of all specimens (total n = 20947) are from the 27 selected Lagerstätten. Among these Lagerstätten, seven are associated with tuffaceous deposits, and eight formed in pre-existing calderas or maars where most fossiliferous laminae are not tuffaceous (Tian *et al.* 2019, S4). Statistical analyses show that Lagerstätten with microbe-bearing laminae have a higher proportion of articulated preserved terrestrial insects

compared with those from the ‘not determined’ group (Welch's t = 2.252, p (same mean) = 0.037). Further, the presence of microbe-bearing laminae is positively associated with the that of articulated “large-winged” insects (Fisher’s exact test, p (no association) = 0.004). Lagerstätten with microbe-bearing laminae do not have a significantly higher diversity of articulated preserved specimens compared with the others (Welch's t = 1.701, p (same mean) = 0.112).

DISCUSSION

Our experiments are subject to several potential confounding variables: temperature (20–25°C) and humidity during the experiments; abundance of microbes in the lake water prior to the experiments; and sedimentation rate in each pulse of sediment loading (15 ± 5 kg/m^2/h, n = 25). However, these variables do not undermine our results because (1) temperature does not significantly affect decay sequence between 15–25°C (Sansom *et al*. 2010) and there was a statistically robust sample size in each experiment; (2) experimental results of different taxa and water types had similar patterns; (3) mean flux during each treatment was similar among basins (6.7 ± 0.7 kg/m^2/h, n = 5) and calculations suggest that the momentary impact provides little downward force on floating insects (Tian *et al.* 2019, S3).

Our results show that floating in water can reduce the force contrast of fully submerged carcasses but – critically – in isolation cannot lead to RPESS. Rapid sediment loading can significantly increase the downward force, submerging carcasses, but does not usually result in RPESS of freshly killed carcasses, as observed previously (Beattie 2007). Rapid sediment loading can, however, trigger widespread RPESS where carcasses have floated in water for as little as 1–3 days. Most carcasses experience RPESS following flotation in natural lake water for two weeks, followed by sediment loading. Floating in water thus plays an important role in RPESS by reducing the force contrast exerted on the

submerged carcasses as measured in this experiment. This suggests that most well-preserved terrestrial insects in lacustrine sediments, though articulated, are likely to have experienced a phase of floating (and decaying) at the water surface.

Growth of microbial films on the carcasses promoted RPESS; this likely reflects enhanced retention of sediment via adhesion to the biofilm surface (Gerbersdorf and Wieprecht 2015) and, for the cockroaches, increased surface area for sediment adhesion on the extensive regions of biofilm surrounding carcasses (Fig. 5C). It is plausible that surficial biofilms (i.e. biofilms at the air-water interface) trapped additional sediment when the carcass was submerged, resulting in larger downward forces if the biofilms were better developed. These aspects of the biofilms may contribute to the positive association between the probability of specimens experiencing RPESS and flotation duration, and to the higher probability of specimens experiencing RPESS in lake water than in distilled water (where fewer microbial films were observed on carcasses; Figs. 3C, 3H, 6A–B). The highest probabilities of RPESS are associated with flotation in natural eutrophic lake water for over two weeks and with sediment load > 0.2 mm thick for cockroaches and > 2 mm for butterflies prior to substantial disarticulation.

The probability of RPESS was also influenced by certain biological characteristics of the insects, particularly their geometry and hydrophobicity. The higher wing surface-body mass index (wing surface/(body mass)$^{0.67}$; Wagner *et al.* 1996; Table 1) and enhanced hydrophobicity of the butterflies relative to the cockroaches (Table 1) results in a greater contrast between the downward and resistance forces applied to the carcasses (Fig. 4E–H), thus requiring a higher mass of sediment to overcome the force contrast than for the cockroaches (Fig. 6C–D). Further, the high surface area:volume ratio (relating to the large flat wings) of the butterflies relative to the cockroaches (Table 1; Fig. 3) mean that the overlying

sediment is less likely to slip away, so the sediment load can submerge butterflies after shorter flotation durations than the cockroaches in both types of water (Fig. 6A–B).

Collectively, these results demonstrate that RPESS of insect remains that are floating in static water bodies depends on their biological characteristics, flotation duration in water, intensity of microbial activity, and availability of sufficient sediment load. RPESS contributes to the preservation of articulated terrestrial insects in lacustrine Konservat-Lagerstätten. For the carcasses of terrestrial insects that remain articulated and floating on a eutrophic or hypereutrophic water surface for a sufficient duration (e.g. two weeks in our experiments), a rapidly deposited sediment load on the insect carcasses as little as 0.2 mm thick may enable RPESS. This process may have contributed to the preservation of articulated terrestrial insects in lacustrine Konservat-Lagerstätten with surficial biofilms and sediment load deposits over 0.06 mm thick, considering the density of compacted deposits as ca. 1.7 g/cm^3 (Duncan and Vucetich 1970). This is consistent with our statistical results that show that the presence of microbe-bearing laminae is associated with articulated preservation of terrestrial insects and the presence of articulated “large-winged” terrestrial insects in lacustrine Lagerstätten. The presence of microbe-bearing laminae suggests that microbial films existed around floating carcasses, which promote RPESS of terrestrial insects, especially the “large-winged” ones, as shown in our experiments. Microbial films not only make sediments adhere to the carcass to increase the external force for sinking, but they also delay decay and bind carcasses to reduce disarticulation before burial (Iniesto *et al.* 2016; Martínez-Delclòs and Martinell 1993).

All factors being equal, the likelihood of RPESS depends on the availability of sediment load. Carcasses decaying at the air-water interface in lakes that frequently receive rapid airborne sediment loads of silt and fine sand are more likely to experience RPESS than carcasses in lakes that rarely receive rapid airborne sediment load; frequent RPESS is likely to result in more abundant and diverse insect faunas. This may contribute to the abundance of

articulated terrestrial insects preserved in some lacustrine Konservat-Lagerstätten, as over half of the listed lacustrine Lagerstätten were formed in a volcanic background and about 25% contain tuff or tuffaceous deposits. Volcanic eruptions may contribute to the abundance of articulated fossils in three main ways. First, strong eruptions can cause mass mortalities (Elizalde 2014) that increase the number of articulated carcasses available for burial, and second, volcanic eruptions can trigger eutrophication of adjacent lake waters (Baross *et al.* 1982) which may promote biofilm development and sinking of insects in these lakes. Third, volcanic ashfall or remobilization of fresh pyroclastic sediments by wind (Wilson *et al.* 2011) can dump airborne sediment loads on the carcasses.

Apart from tuffaceous lacustrine Lagerstätten, some diatomites, oil shales and laminated limestones also produced diverse and highly-articulated terrestrial insect fossil assemblages, which include articulated "large-winged" insects (Tian *et al.* 2019, S4). It is possible that microbial films on the water surface adhere other terrestrial detritus or suspended grains around floating carcasses as sediment load to promote RPESS (Gall 2001). The mechanism was supported by experiments on floating plastic pieces (Chen *et al.* 2019) but the efficiency on insects requires further study.

As different taxa have various conditions for RPESS, preservation biases will exist in a fossil assemblage. If the flotation duration is between 1–3 days in lake water, only butterflies can experience RPESS while cockroaches cannot (Fig. 6A–B); if the sediment load is less than 1 mm thick, only articulated cockroaches will be preserved but butterflies will not (Fig. 6C–D). With a longer floating period (but before disarticulation) or thicker sediment load, more diverse insects will be preserved in articulation in a fossil assemblage. Thus sedimentary conditions may skew our reconstructions of the community diversity.

CONCLUSIONS

Here, we address the conundrum of the excellent preservation of insects in Lagerstätten despite evidence that their carcasses generally do not sink in an undamaged condition (potentially because of resistance forces of the water). Our experiments show that the force contrast between the downward and resistance forces of fully submerged carcasses reduces with prolonged floating in lentic water body. We focus on the finding that rapid sediment loads can significantly increase the downward forces exerted on carcasses and pushes them fully beneath the water. In contrast to freshly killed carcasses that were buoyed up again, rapid sediment loads can lead to RPESS of carcasses that had floated for 1–3 days in natural eutrophic lake water and 3–7 days in distilled water. The proportion of RPESS triggered by rapid sediment load gradually increased with prolonged floating over 1–2 weeks, after which rapid sediment load dumping commonly led to RPESS of almost all carcasses. The proportion of RPESS increased with prolonged floating in both lake and distilled water but remained lower in the latter than in the former with the same flotation duration. For the carcasses that had floated in natural eutrophic lake water for 14 days, RPESS occurred when the experimental vessels received a rapid sediment load of as little as the amount that could form deposits about 0.2 mm thick for cockroaches and 2 mm for butterflies. Biological characteristics of the animal, flotation duration, intensity of microbial activity, and availability of sufficient and rapid sediment load are important factors that determine whether RPESS will happen or not to carcasses floating in static water bodies. These experimental results are supported by analysis of the spatiotemporal occurrences and relative abundances of articulated terrestrial insects in lacustrine Konservat-Lagerstätten.

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DATA ARCHIVING STATEMENT

Data for this study are available in the Dryad Digital Repository: https://datadryad.org/review?doi=doi:10.5061/dryad.6c2fk58

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FIGURES

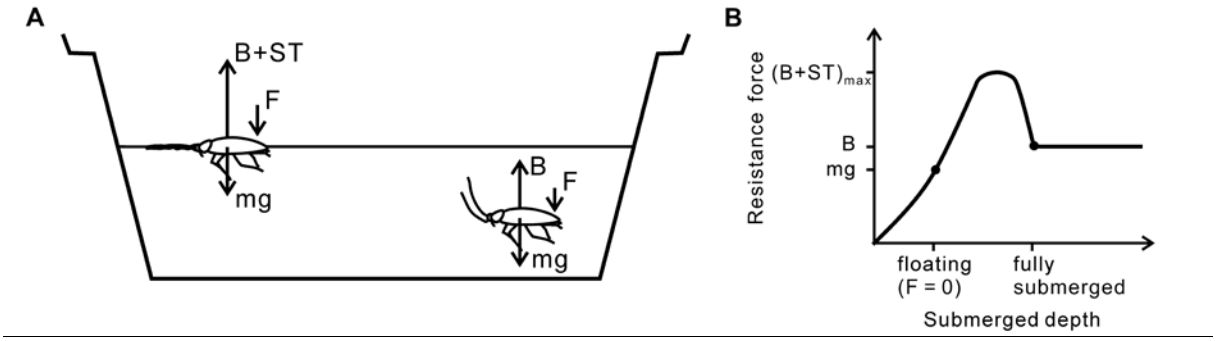


FIG. 1. Schematic showing the forces exerted on floating and submerged carcasses (A) and change in resistance force exerted by water on a carcass versus submerged depth (B). B: buoyancy; ST: surface tension; F: external force; mg: weight.

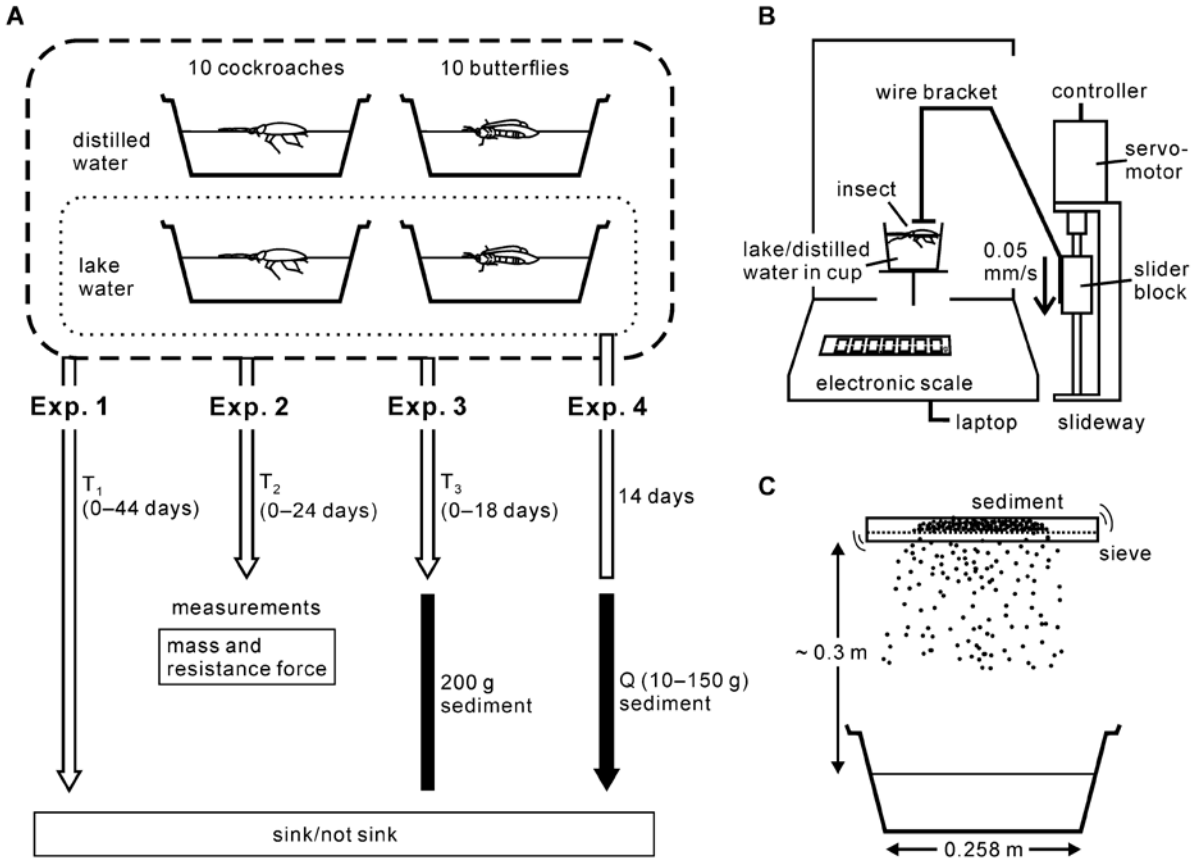


FIG. 2. Schematic diagrams of the experimental setup (A) and apparatuses for measurements (B) and sediment loading (C). Exp., Experiment. White and black arrows or bars represent floating in water and sediment loading, respectively. Predictor variables are beside the arrows:

T_1 is flotation duration prior to each observation; T_2 and T_3 are flotation durations prior to treatment for each basin in Experiments 2 and 3; Q is mass of sediment introduced to each basin in Experiment 4. Values of T_2, T_3 and Q for each basin are listed in Table 3. Response variables are in rectangles.

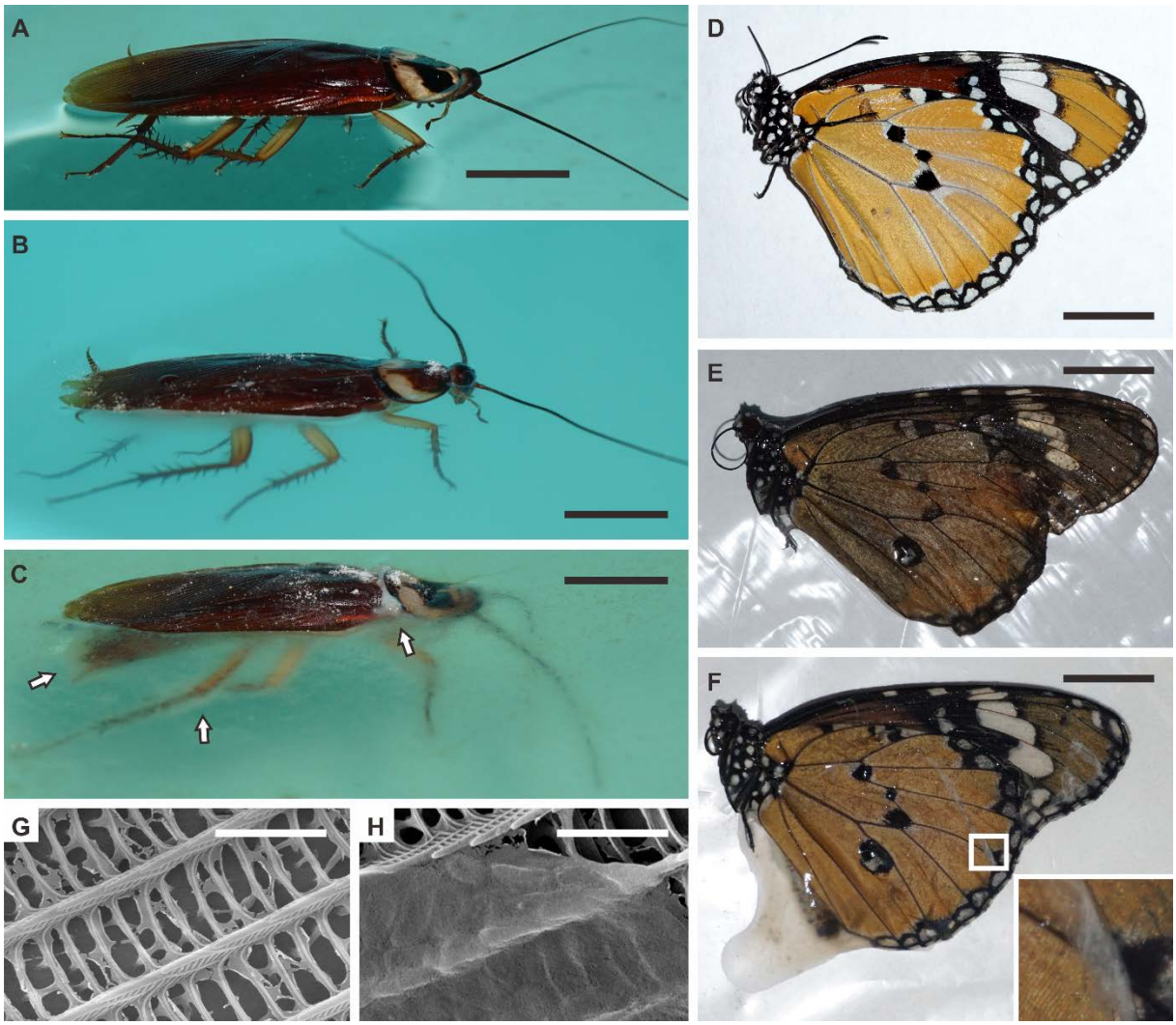


FIG. 3. Morphology and flotation of decaying carcasses. A–C: Selected cockroaches in Experiment 1, photographed in the experimental basins. Freshly killed (A), 14 days in distilled water (B), 14 days in natural lake water (C; the same specimen as in (A)). Note microbial film at the water surface and enveloping the carcass in (C; marked by arrows). D–F: Submerged surfaces of selected butterflies in Experiment 2, photographed following removal from the experimental basin. Freshly killed (D), 12 days in distilled water (E), 12 days in

natural lake water (F). Note microbial films, associated with the abdomen, and film-like structure on the wings (boxed area enlarged in inset) in (F). G–H: Scanning electron micrographs showing wing scales on freshly killed (G) and decayed (for 12 days in natural lake water; H; Experiment 2) butterfly carcasses. Note the microbial film in (H). Scale bars: 10 mm for A–F and 2 μm for G and H.

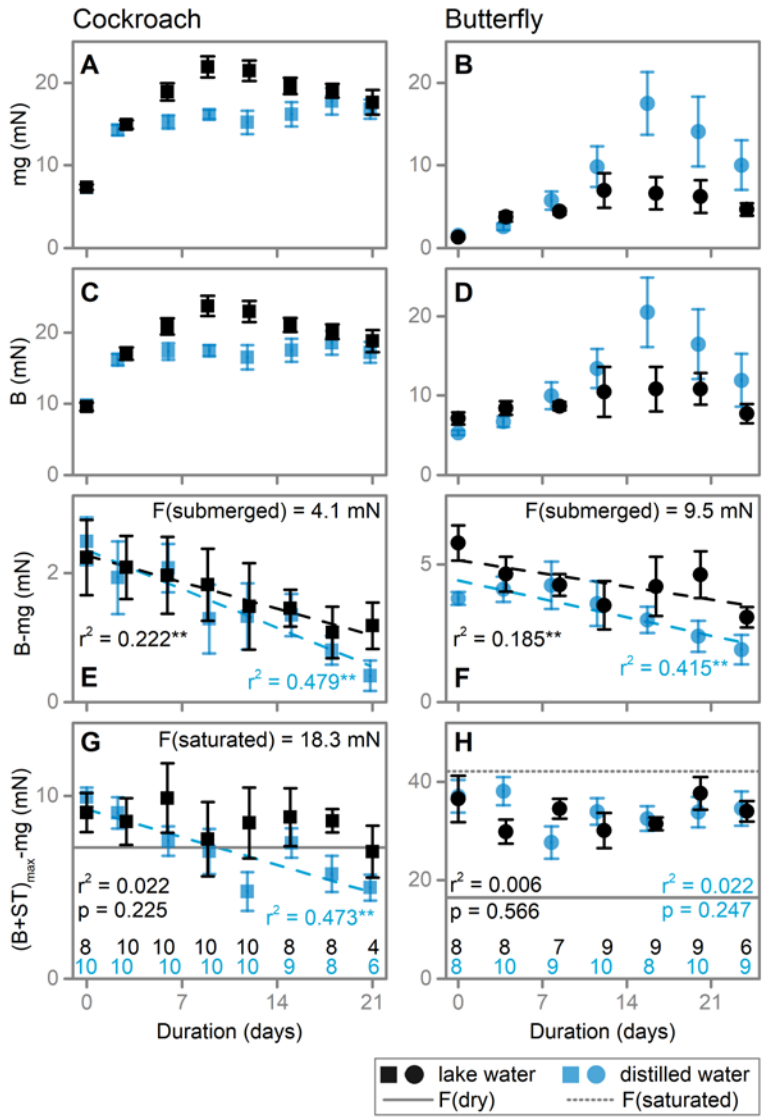


FIG. 4. Changes in wet weight (A, B), buoyancy (C, D), force contrast exerted by water on submerged carcasses (E, F) and maximum force contrast (G, H) during decay. B: buoyancy; mg: wet weight; ST: surface tension. The number of specimens represented by each whisker

is annotated in G or H for corresponding water type and duration. Points are mean values and error bars are 95% confidence intervals. Dashed lines are linear regression models for significantly correlated variables; **: p (uncorrelated) < 0.001. F(dry), F(saturated) and F(submerged): the calculated maximum external downward force exerted by 200 g rapid sediment load on a carcass when the sediment is under different conditions (calculations in Tian *et al*. 2019, S3).

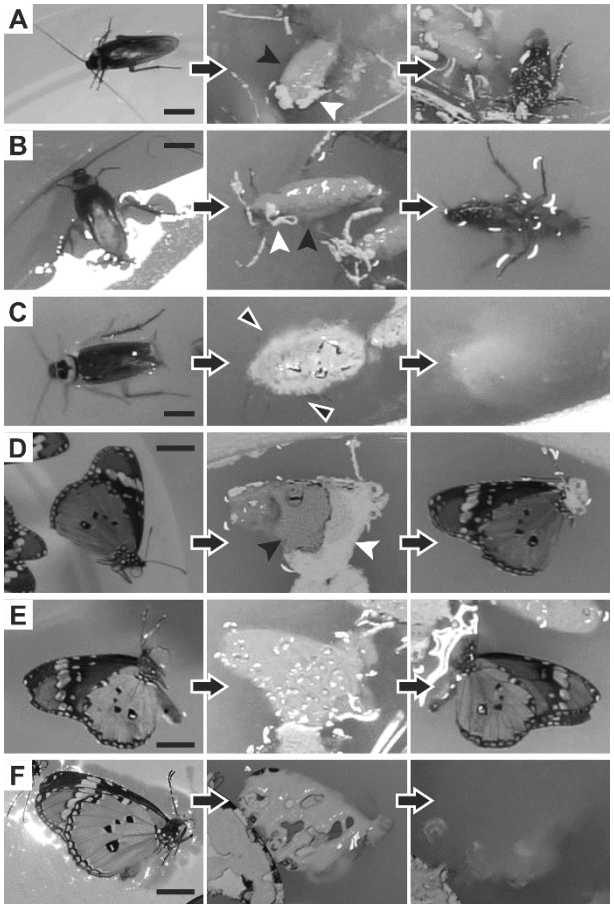


FIG. 5. Photographs showing the impact of sediment load on carcasses in Experiment 3. A–C, Cockroaches. Freshly killed (A), 13 days in distilled water (B), 13 days in lake water (C). D–F, Butterflies. Freshly killed (D), 10 days in distilled water (E), 10 days in lake water (F). Images in each row show the condition of the carcass before sediment influx (left), 2–10 seconds before submergence (middle), and 0–20 seconds after submergence (right). The carcasses in (A, B, D, E) refloated after submergence, while the carcasses in (C) and (F) sank

to the bottom. Saturated sediment (black arrow heads) shows darker colour compared with adjacent dry sediment (white arrow heads). More sediment is attached to the carcasses in (C) presumably due to the presence of microbial films around the cockroach (marked by triangles) in the natural lake water. Scale bars, 10 mm.

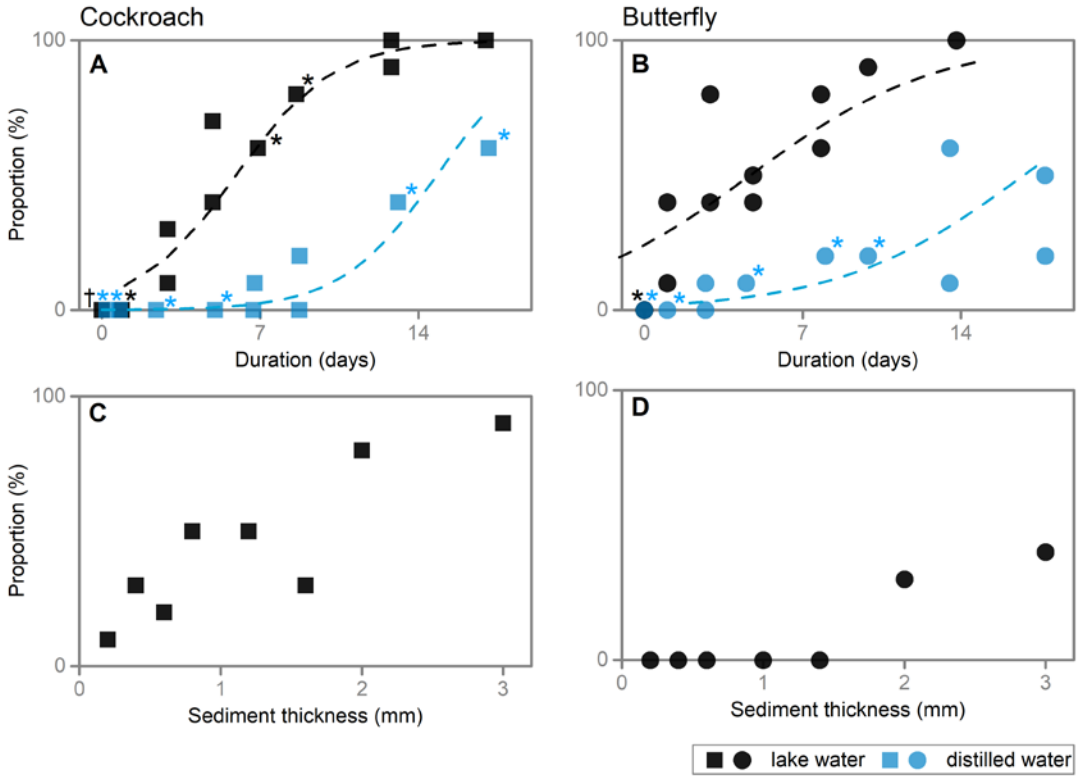


FIG. 6. Proportion of carcasses experiencing RPESS following sediment influx (Experiment 3; A–B) and sediment quantities introduced to carcasses that had floated in lake water for 14 days (Experiment 4; C–D). A, C: cockroach; B, D: butterfly. Dashed lines in (A) and (B) are the binary logistic regression models. The sediment thickness in (C) and (D) is based on the mass of sediment introduced (200 g of sediment forms a lamina ca. 4 mm thick in the basin). Each datapoint represents one basin (datapoints with an asterisk or a dagger represent two or three overlapping points, respectively).

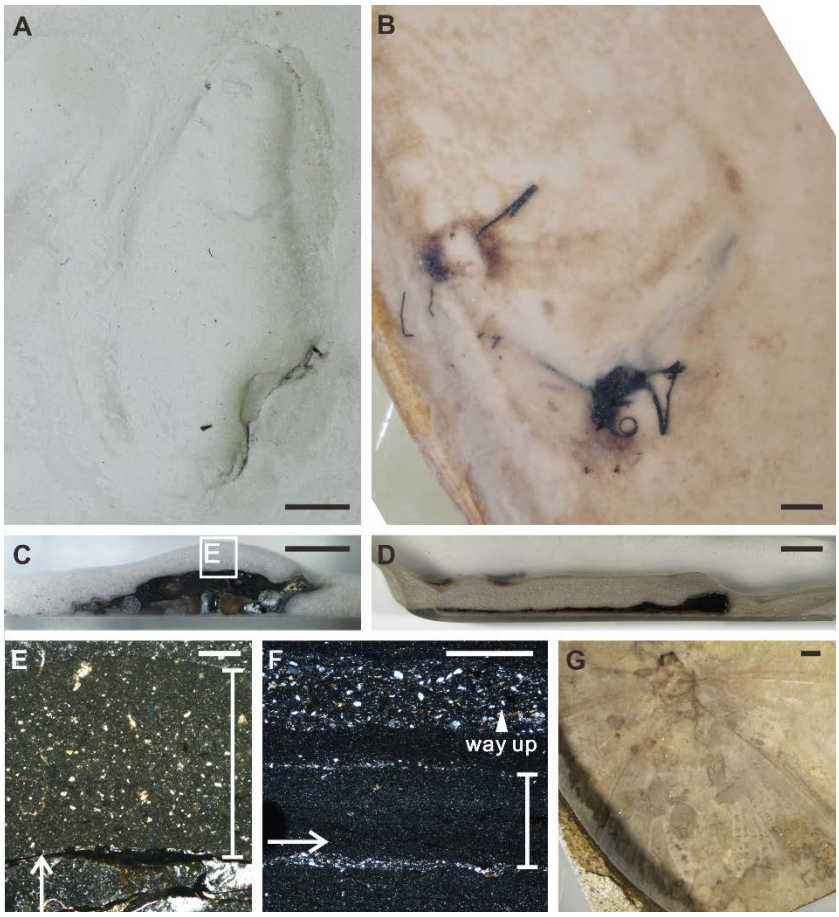


FIG. 7. Comparison of sediment hosting experimental and fossil insects. A–B, Cockroach (A) and butterfly (B) in plan views; carcasses had floated in lake water for 17 days (Experiment 3, 200 g sediment) and 14 days (Experiment 4, 150 g sediment), respectively; the butterfly antennae and the legs disarticulated during resin block preparation. C–D, Vertical sections of the carcasses in (A) and (B). E, Cross-polarized photomicrograph of the region indicated in (C), with the overlying lamina and cockroach carcass indicated by a white whisker and an arrow, respectively. F–G, Cross-section and plan views of an articulated neuropteran fossil (NJU-DHG-NH48) from the Jurassic Haifanggou Formation. F, Cross-polarized photomicrograph, the fossil is indicated by a white arrow. Scale bars represent: 5 mm (A-D, G); 0.5 mm (E, F).

TABLES

TABLE 1. Characteristics of experimental specimens.

Taxa	Mass (g)	Dorsal surface area (mm^2)	Static contact angle (°)
Cockroach	0.76 ± 0.04, n = 180	358 ± 34, n = 7	92 ± 1, n = 2
Butterfly	0.15 ± 0.02, n = 150	827 ± 144, n = 7	130 ± 6, n = 4

Cockroaches were all adult male. Butterflies were all adult of both sexes. Errors are standard deviations. Measuring methods are available in Tian *et al.* 2019 S1.

TABLE 2. Properties of the water used in the experiments.

Water properties	Natural lake water	Distilled water
Density* (kg/m^3)	998	996
Total dissolved solids (mg/L)	76	9
pH	7.52	5.91
Ammoniacal nitrogen (mg/L)	0.2	Not detected
Nitrate nitrogen (mg/L)	1.1	0.006
Orthophosphate (mg/L)	0.056	Not detected
Chemical oxygen demand (mg/L)	73.5	8.2
Microorganisms	Diatoms (*Navicula cryptocephala*, *Melosira*, *Pinnularia*, *Synedra*, *Rhopalodia gibba*), cryptomonads (*Cryptomonas rostrate* and *C. erosa*) and green algae (*Marimo*)†	Colonies number 0 cfu/mL; coliform <3 MPN/100mL; *Salmonella*, *Shigella, Staphylococcus aureus,* moulds and yeasts not detected‡

* Measured with a glass hydrometer (Shanghai Loikaw, China).

† From an unpublished report (GAO, P., JI, J., JIANG, K. and ZENG, L. 2015. Study Report of Water Environment of Tianlai River. School of the Environment, Nanjing University).

‡ From an unpublished test report (2008; No. W08061004188) by Shanghai Institute of Quality Inspection and Technical Research.

TABLE 3. Flotation duration prior to treatment for each basin in Experiment 2 (T_2) and Experiment 3 (T_3) and mass of sediment introduced to each basin in Experiment 4 (Q).

T_2 (days)				T_3 (days)				Q (g)	
lake water		distilled water		lake water		distilled water		lake water	
cc	bt	cc	bt	cc	bt	cc	bt	cc	bt
0	0	0	0	0	0	0	0	10	10
3	4	3	4	0	0	0	0	20	20

6	8	6	8	0	1	1	1	30	30
9	12	9	12	1	1	1	1	40	50
12	16	12	16	1	3	3	3	60	70
15	20	15	20	3	3	3	3	80	100
18	24	18	24	3	5	5	5	100	150
21		21		5	5	5	5	150	
				5	8	7	8		
				7	8	7	8		
				7	10	9	10		
				9	14	9	10		
				9		13	14		
				13		13	14		
				13		17	18		
				17		17	18		

Abbreviations "cc" and "bt" represent "cockroach" and "butterfly", respectively.

1