

Macroinvertebrate seedbank composition in relation to antecedent duration of drying and multiple wet-dry cycles in a temporary stream

RACHEL STUBBINGTON*, JOHN GUNN†, SALLY LITTLE‡, THOMAS P. WORRALL§ AND PAUL J. WOOD¶

*School of Science and Technology, Nottingham Trent University, Nottingham, U.K.

†School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, U.K.

‡Department of Geography, Environment and Earth Sciences, University of Hull, Hull, U.K.

§APEM Limited, Cambridge, U.K.

¶Department of Geography, Centre for Hydrological and Ecosystem and Science, Loughborough University, Loughborough, Leicestershire, U.K.

SUMMARY

1. The aquatic invertebrate 'seedbank' comprises life stages that remain viable in the bed sediments of temporary freshwaters during dry phases. This seedbank promotes persistence of temporary-stream macroinvertebrates, but how its inhabitants respond to extended dry phases or repeated transitions between wet and dry phases remains unknown.
2. We rehydrated samples collected from the dry bed of a temperate-zone stream during a supra-seasonal drought, to examine the seedbank assemblage. Samples were first collected in autumn, from 12 sites along the ephemeral (4 sites), intermittent (2) and near-perennial (6) reaches, which had been dry for up to 8 months. Our first hypothesis was that assemblage composition would be related to the dry-phase duration preceding sampling, with longer dry phases reducing abundance and richness.
3. We revisited the same sites in three subsequent seasons, collecting and rehydrating sediments from all dry sites: five sites in early spring, three in late spring and four in late summer. Unpredictable flow resumption and redrying occurred between sampling dates. Our second hypothesis was that repeated wet-dry cycles would not degrade the assemblage because temporary-stream taxa would be adapted to fluctuating hydrological conditions.
4. Multiple individuals of only Chironomidae, Oligochaeta and *Pisidium* were present at sites that experienced the longest dry phases, providing some support for our first hypothesis. An additional 21 taxa were recorded across the remaining (shorter dry phase) sites in autumn, indicating that such sites act as refuges and potential recolonist sources following flow resumptions.
5. Although several insect orders first recorded in early spring were absent in later seasons, taxon-specific life cycles indicated that these absences were probably seasonal and not due to repeated wet-dry cycles.
6. We recorded 38 taxa in total, highlighting the seedbank as a dry-phase resistance mechanism for many temporary-stream macroinvertebrates. Our results also suggest that seedbank diversity may be threatened by increases in drought extent and duration.

Keywords: egg bank, intermittent river, resilience, streambed drying, supra-seasonal drought

Correspondence: Rachel Stubbington, School of Science and Technology, Nottingham Trent University, Clifton Campus, Nottingham NG11 8NS, U.K. E-mail: rachel.stubbington@ntu.ac.uk

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Introduction

The invertebrate 'seedbank' comprises the viable aquatic life stages that persist in bed sediments during dry phases in temporary freshwaters (Tronstad, Tronstad & Benke, 2005; Datry, Corti & Philippe, 2012; Stubbington & Datry, 2013). This seedbank is a source of recolonists following the return of surface water in temporary lentic waters, wetlands and floodplains, in particular for meiofauna (Boulton & Lloyd, 1992; Tronstad *et al.*, 2005). In addition, macroinvertebrates may persist in the 'dry' (i.e. lacking free water but retaining some moisture) bed sediments of temporary streams (Larned, Datry & Robinson, 2007; Stubbington *et al.*, 2009; Storey & Quinn, 2013). However, studies considering aquatic invertebrate community responses to drying have typically only sampled during wet phases before and/or after dry phases. Community resistance to drying (i.e. the capacity to persist within a dry reach; Bogan, Boersma & Lytle, 2015) may therefore have been underestimated in lotic ecosystems.

After surface water is lost, the persistence of seedbank inhabitants is promoted by interstitial humidity (e.g. Stanley *et al.*, 1994 cf. Datry *et al.*, 2012), and assemblage abundance and richness therefore decline as dry-phase duration increases, unless rainfall and/or upwelling groundwater sustain sediment moisture (Stubbington & Datry, 2013; Verdonschot *et al.*, 2015). Supra-seasonal droughts (*sensu* Lake, 2003) may increase the frequency, magnitude and duration of dry phases, potentially causing near-perennial reaches to dewater and extending the dry-phase duration in temporary reaches (Wood & Armitage, 2004; Bogan & Lytle, 2011). Supra-seasonal drought may therefore further reduce seedbank abundance and richness, as taxon-specific desiccation-tolerance thresholds are sequentially passed, with few aquatic macroinvertebrate taxa tolerating complete desiccation (Alpert, 2006; Bogan *et al.*, 2015).

Seedbank inhabitants emerge from resting states (aestivating eggs, juveniles or adults, *sensu* Strachan, Chester & Robson, 2015) in response to the return of free water and, potentially, other environmental cues such as temperature or photoperiod (Danks, 1978; Nordlie & Arthur, 1981). Emergence may be immediate, allowing organisms with rapid development to persist in systems with ephemeral flow (Jackson & Sweeney, 1995; Williams, 2006). However, brief flow events are a potential risk to seedbank integrity if developing organisms perish when sediments redry (Dieterich & Anderson, 1995; Strachan, Chester & Robson, 2016). Life-history strategies promoting persistence despite such hydrological unpredictability include delayed emergence following immersion

(Wickson, Chester & Robson, 2012) and asynchronous egg hatching (Zwick, 1996; Strachan *et al.*, 2016). Such 'bet-hedging' development strategies reflect taxon-specific adaptations to a historical flow regime (Lytle & Poff, 2004; Williams, 2006), be that seasonally intermittent, or ephemeral and unpredictable with repeated cycles of short flow events and subsequent dry phases (hereafter, 'wet-dry cycles'). With climate change predictions indicating that both heavy precipitation events and droughts may increase in some regions (Stocker *et al.*, 2013; Ledger & Milner, 2015), wet-dry cycles may become more frequent, but little is known about how lotic macroinvertebrate communities will respond to these transitions, particularly in temperate regions (Larned *et al.*, 2010; Dell, Alford & Pearson, 2014). Ledger *et al.* (2012) found that macroinvertebrate communities from a perennial chalk stream were less resilient to frequent than infrequent flow-cessation events in experimental mesocosms, but no study has examined the response of the seedbank to wet-dry cycles.

We rehydrated samples from the dry bed of the ephemeral, intermittent and near-perennial upper reaches of a temperate-zone stream. We first collected samples in autumn, during the latter stages of a prolonged and unusually widespread dry phase caused by a supra-seasonal drought (Marsh *et al.*, 2013). Our first hypothesis was that macroinvertebrate assemblage composition would be related to the duration of the dry phase preceding autumn sampling, with lower abundance and richness characterising sites with longer dry phases. We also compared the assemblage present to that recorded in previous studies, to identify potential supra-seasonal drought impacts. We resampled sites experiencing short dry phases the following spring and summer. Our second hypothesis was that repeated wet-dry cycles would not degrade the seedbank assemblage because taxa would be adapted to tolerate fluctuating hydrological conditions. Our third hypothesis was that macroinvertebrate taxa would persist in the seedbank as different life stages: eggs, juveniles and/or adults. To explore this hypothesis, we removed active individuals from samples pre-rehydration and at regular intervals during 56-day rehydration periods, then used our observations to infer the life stage of seedbank inhabitants, with delayed post-rehydration appearance of small individuals indicating persistence as eggs.

Methods

Study sites

Samples were collected from 12 sites along the upper reaches of a temperate-zone system in central England:

the River Lathkill and its tributary in Cales Dale (53.186 N, 1.740 W; Fig. 1), between autumn (November) 2011 and summer (August) 2012. The Lathkill rises from the centre of a limestone outcrop within the White Peak area of the Peak District National Park. The stream is groundwater-fed and hydrologically isolated, flowing through a steep-sided valley with no perennial upstream surface waters. The perennial source is *c.* 4 km downstream of the cave from which the stream emerges during high flows (i.e. the high-flow source, Fig. 1), and the Lathkill joins the perennial River Wye 6 km downstream of the study area. A tufa (calcium-carbonate encrusted) deposit underlies the channel *c.* 1 km downstream of the high-flow source, ending in a 2 m high cliff which creates a waterfall when the stream is flowing (Fig. 1). Land use upstream of Cales Dale is low-intensity grazing of unimproved grassland. The proportion of land covered by deciduous woodland increases downstream of site E, with trees lining both banks below the tufa cliff and also in Cales Dale (Fig. 1).

Study sites differed in their flow permanence and sediment characteristics (Table 1). Headwater sites (A–D,

Fig. 1) were ephemeral (Table 1), typically drying for a total of >6 months each year, but experiencing 2–4 flow resumptions per annum in response to high rainfall. The substrate of headwater sites comprised exposed limestone bedrock and finer organic-rich sediments, with scattered boulder- to gravel-sized clasts and patches of semi-aquatic and terrestrial vegetation. Further downstream (sites E–L, Fig. 1), the substrate was alluvium and flow was non-perennial. Some of these sites dry every summer ('intermittent', Table 1) and others dry only during supra-seasonal droughts ('near-perennial', Table 1). Near-perennial sites were considered perennial prior to this study (Stubbington, Wood & Reid, 2011). Further details of the study system are provided by Wood *et al.* (2005) and Stubbington *et al.* (2009).

Environmental conditions

A rainfall deficit with its origins in winter 2009–2010 was compounded by a further period of low precipitation in December 2010, resulting in a supra-seasonal drought extending over much of central England (Marsh

Fig. 1 The River Lathkill study reach and sampling sites: (A) the high-flow source (Lathkill Head Cave); (B) 50 m downstream of the source; (C) Holme Grove Risings; (D) Cales Dale; (E) Pudding Springs; (F) 400 m upstream tufa cliff; (G) directly upstream tufa cliff; (H) moss on tufa cliff; (I) directly downstream tufa cliff; (J) downstream Carter's Mill; (K) upstream Mandale Sough; (L) upstream perennial source.

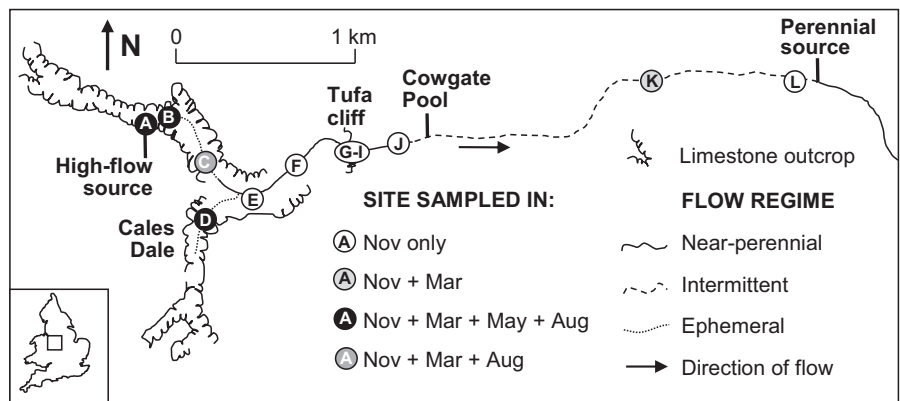


Table 1 Flow permanence regime, dry-phase duration prior to autumn (November 2011) sampling and sediment characteristics, for sites on the River Lathkill. Site locations are shown in Fig. 1. Assignment to groups 1–3 is described in the text.

| Site | Flow permanence | Dry phase (months) | Sediment characteristics | Group |
|------|-----------------|--------------------|---------------------------------------|-------|
| A | Ephemeral | 8 | Limestone clasts | 1 |
| B | Ephemeral | 8 | Limestone clasts | 1 |
| C | Ephemeral | 6.8 | Limestone clasts | 1 |
| D | Ephemeral | 7 | Limestone clasts, organic rich | 1 |
| E | Near-perennial | 3.3 | Mixed alluvium, abundant fines | 3 |
| F | Near-perennial | 5.5 | Mixed alluvium, limited fines | 2 |
| G | Near-perennial | 6 | Tufa + limited alluvium/fines | 2 |
| H | Near-perennial | 6 | Tufa cliff bryophytes | 2 |
| I | Near-perennial | 6 | Mixed alluvium, limited fines | 2 |
| J | Near-perennial | 2.4 | Mixed alluvium, abundant fines | 3 |
| K | Intermittent | 4.5 | Mixed alluvium, gravel/fines dominate | 3 |
| L | Intermittent | 4.8 | Mixed alluvium, abundant fines | 3 |

et al., 2013). In the Lathkill, the extent and duration of drying was greater than in any period since continuous measurement of flow began in 1998, affecting all reaches upstream of the perennial source (Fig. 1) and lasting for most of 2011 (Fig. 2). Winter flows from the high-flow source (Fig. 1) resumed in December 2011, 9 months after surface water had been lost from the ephemeral headwaters, but persisted for a shorter duration than in any previous monitored hydrometric year: the headwaters dried again in mid-March 2012, for *c.* 28 days (Fig. 2). Rainfall then exceeded the 1971–2001 long-term average (LTA) for the rest of the study (Met Office, 2014). In response to this precipitation, surface flow resumed throughout the near-perennial and intermittent reaches in April 2012 and continued for the remainder of the study. In the headwaters, flow remained ephemeral and surface water was lost from the main channel on two further occasions: for *c.* 39 days starting in mid-May and for *c.* 46 days starting in early August (Fig. 2). During the dry phase that began in mid-May, surface water was present at headwater site C, a groundwater-fed upwelling spring, with flow extending <10 m downstream.

Considering the period from April 2011 to August 2012 (i.e. preceding and during the study), regional air temperatures were 4.1 °C above the 1981–2010 LTA in April 2011, then were within 1.2 °C of the LTA between May and August 2011 (Met Office, 2014). The rest of the year was mild, with monthly mean temperatures 1.1–2.7 °C above the LTA from September to December 2011. In 2012, regional and catchment monthly mean temperatures were within 1.9 °C of the LTA from January until the end of the study (Met Office, 2014).

Field methods

Samples were first collected in autumn (11–12 November) 2011, from all 12 sites (Fig. 1). Further sampling was conducted in 2012, in early spring (24 March), late spring (19 May) and late summer (18 August, Fig. 2). On each of these further sampling dates, all dry sites were sampled: A–D and K in early spring; A, B and D in late spring and A–D in late summer (Fig. 1). On each sampling date, surface water had been lost within the previous 5–10 days (Fig. 2). In total, 24 samples were collected, each comprising *c.* 2.5 kg (2 L) sediment, except for the site H sample, which comprised the same volume of moss (Bryophyta) removed from the tufa cliff (Fig. 1). Prior to sediment excavation, larger surface clasts of the armour layer were removed. Fine sediments were then collected using a hand trowel, from a 0.25 m² area and to a depth of 5–10 cm, with all clasts >10 mm diameter discarded.

Laboratory methods

Each sample was distributed across multiple trays for an initial inspection of the fauna, potentially including terrestrial, semi-aquatic and aquatic invertebrates. All observed invertebrates were removed, to prevent subsequent loss of organisms through predation. The sample was then divided into two approximately equal halves, and one half was preserved for later examination and removal of all macroinvertebrates. Organisms removed during the initial and later examinations were combined and are hereafter termed ‘pre-rehydration’. The other half of the sample was placed into a 10-L container with a perforated lid, the holes being plugged with cotton wool to allow gas

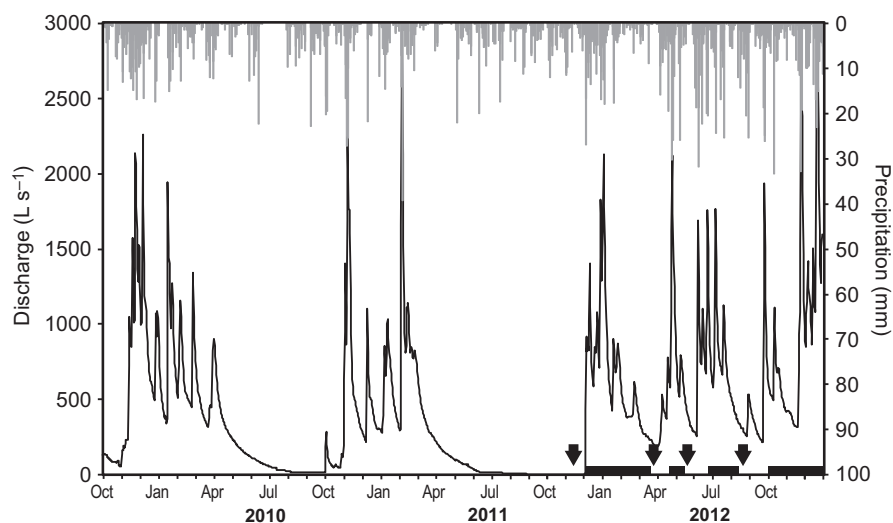


Fig. 2 Daily discharge (L s^{-1}) at Cowgate Pool within the intermittent section of the River Lathkill (53.189 N, 1.742 W; authors' data; Fig. 1) and daily precipitation (mm) at Wardlow Hay Cop, 8 km to the north (53.262 N, 1.734 W), October 2009 to December 2012. Arrows indicate sampling dates; horizontal black bars indicate wet phases during the study period.

exchange but prevent egg deposition and colonisation by external invertebrates, and to prevent escape of sample organisms. Samples were rehydrated with dechlorinated tap water and aerated continuously for 56 days, a sufficient duration to allow development of insects including chironomids, mayflies, stoneflies and caddisflies from eggs (Jackson & Sweeney, 1995; Huryn & Wallace, 2000). Samples were rehydrated outside away from direct sunlight in white plastic containers, and therefore experienced an ambient temperature regime with limited exposure to a natural photoperiod.

Samples were inspected 7, 14, 28 and 56 days after rehydration. Inspection involved manually disturbing the sediments to cause organisms to enter the water column, pouring the water into trays and removing (without replacement) all observed live invertebrates. Gastropods were left in samples until their activity had

been confirmed. A final inspection was conducted on day 56 to remove all remaining live invertebrates.

Animals were identified to the lowest taxonomic resolution practicable at 50 × magnification, or mounted on slides for identification at a higher magnification where required. Many taxa were identified to species level, but identification was to genus for *Pisidium* (Bivalvia: Sphaeriidae), *Limnebius* (Coleoptera: Hydraenidae) and *Laccobius* (Coleoptera: Hydrophilidae), and to family for larvae of Dytiscidae, Hydrophilidae and Scirtidae (Coleoptera) and all Diptera. Chironomidae (Diptera) were recorded at family level within analyses because most specimens were small, first- and second-instar larvae that could not be identified further; in addition, a representative selection of larger instars was identified to genus or species. Individual Oligochaeta were not identified further, although the families present were

Table 2 Macroinvertebrate taxa in samples extracted from the dry bed of the River Lathkill, indicating ecological status (aquatic, semi-aquatic /combination of both statuses) and the life stage of insect taxa. NA, not applicable.

| Ecological status | Group | Family | Genus/species | Insect life stage | | |
|-----------------------------|-------------------------------|---------------------------|-------------------------------|-------------------------------|---------------------------|---------------------------|
| Aquatic | Bivalvia | Sphaeriidae | <i>Pisidium</i> | NA | | |
| | | Coleoptera | Dytiscidae | <i>Agabus biguttatus</i> | Adult | |
| | <i>Agabus guttatus</i> | | | Adult | | |
| | <i>Hydroporus ferrugineus</i> | | | Adult | | |
| | Elmidae | | <i>Hydroporus pubescens</i> | Adult | | |
| | | | <i>Elmis aenea</i> | Larval | | |
| | | | | Adult | | |
| | | | <i>Limnius volckmari</i> | Adult | | |
| | | | <i>Oulimnius troglodytes</i> | Adult | | |
| | | | <i>Riolus subviolaceus</i> | Larval | | |
| | | | | Adult | | |
| | | | Helophoridae | <i>Helophorus brevipalpis</i> | Adult | |
| | | | | Hydraenidae | <i>Hydraena palustris</i> | Adult |
| | | | <i>Limnebius</i> | | Adult | |
| | | | <i>Ochthebius auriculatus</i> | | Adult | |
| | | | Hydrophilidae | | Larval | |
| | | | | <i>Laccobius</i> | Adult | |
| | | | | | Larval | |
| | | | Gastropoda | Scirtidae | | Larval |
| | Lymnaeidae | <i>Lymnaea truncatula</i> | | | NA | |
| Isopoda | | <i>Asellus aquaticus</i> | | | NA | |
| | | Plecoptera | | | <i>Nemoura cambrica</i> | Nymphal |
| | | | | | Trichoptera | <i>Micropterna sequax</i> |
| | <i>Stenophylax permistus</i> | Larval | | | | |
| Semi-aquatic or combination | Diptera | Ceratopogonidae | | Larval | | |
| | | Chironomidae* | | Larval | | |
| | | | | Pupal | | |
| | | | | Larval | | |
| | | | | Larval | | |
| | | Empididae | | Larval | | |
| | | | Psychodidae | | Larval | |
| | | | Stratiomyidae | | Larval | |
| | | Tipulidae | | Larval | | |
| | | | Oligochaeta† | | NA | |

*Including the semi-aquatic genera *Bryophaenocladus*, *Limnophyes* and *Metriocnemus* and the aquatic species *Brillia bifida* and *Rheocricotopus fuscipes*.

†Including the terrestrial family Lumbricidae and the aquatic taxa Naididae and tubificoid naids.

noted. Caution is required when interpreting results relating to taxa identified to a coarse resolution, in particular chironomids and oligochaetes, because patterns may reflect a single dominant species or multiple species with comparable or contrasting responses. Different life stages (i.e. larvae, pupae and adults) were counted as different taxa as they may respond differently to drying and rehydration (Williams, 1996).

Data analysis

Univariate analyses were conducted in IBM SPSS Statistics 22 (IBM Corporation, New York) using log + 1 transformed abundance and untransformed richness data. Multivariate analyses were conducted using square-root transformed abundance data in PRIMER 6 (PRIMER-E, Ivybridge).

Composition of the seedbank assemblage

Each taxon was classified as aquatic, semi-aquatic or terrestrial, being considered semi-aquatic where members of the taxon inhabit aquatic habitats, waterlogged riparian sediments and soils. Taxa with both aquatic and terrestrial representatives (e.g. Chironomidae and Oligochaeta) were included (Table 2) in further analyses, whereas terrestrial organisms were excluded. Our study focussed on the macroinvertebrate assemblage and therefore meiofauna (i.e. Cladocera, Copepoda, Nematoda and Ostracoda) were also excluded from analyses.

All records of each taxon found in a given sample (i.e. pre-rehydration and days 7–56) were combined in analyses of spatial variability and in analyses of temporal changes between seasons. To summarise seedbank composition, taxon richness (the number of taxa) and total abundance (the number of individuals) were calculated for each sample. The abundance of common taxa was also determined, 'common' being defined as comprising $\geq 1\%$ of the total assemblage and occurring in $\geq 50\%$ of all samples. To examine broad-scale spatiotemporal variability in assemblage composition, all 24 samples were ordinated using non-metric multidimensional scaling (NMDS) of a Bray–Curtis similarity matrix with 100 random restarts. Two-dimensional NMDS solutions with stress values < 0.2 were considered ecologically interpretable (Clarke, 1993).

Spatial variability in assemblage composition in autumn

To test our first hypothesis, we examined spatial variability in the composition of the assemblage emerging

from samples collected from the 12 sites in autumn. The 12 sites (A–L, Fig. 1) were assigned to one of three groups (1–3) based on the duration of the dry phase prior to sample collection, the flow permanence regime and sediment characteristics (Table 1; also see 'Study sites' descriptions); site assignment was also informed by previous research (e.g. Wood *et al.*, 2005). Analysis of similarities (ANOSIM) with pairwise comparisons (10 000 permutations) was used to compare assemblages at sites in groups 1–3 (Table 1). Similarity percentages (SIMPER) were used to identify the taxa responsible for differences between groups. A series of one-way ANOVAs with *post-hoc* Tukey tests was conducted to identify differences in richness, total abundance and the abundance of common taxa between groups 1–3; 'common' taxa were defined as above, but considering only autumn samples. Non-linear regression analyses were used to examine relationships between total abundance and taxon richness, and the dry-phase duration prior to autumn sampling.

Temporal changes in assemblage composition between seasons

To test our second hypothesis, ANOSIM with pairwise comparisons was used to examine differences in assemblage composition between seasons (autumn, early spring, late spring and late summer), using only data from the three sites sampled in all four seasons (A, B and D, Fig. 1). SIMPER was used to determine the taxa responsible for differences. One-way ANOVAs with *post-hoc* Tukey tests were conducted using data from these same three sites, with richness, total abundance and the abundance of each common taxon (defined as above but considering samples from the three sites only) as dependent variables, and with season as the factor.

Temporal changes in the observation of macroinvertebrates during the rehydration period

To test our third hypothesis, the days (from pre-rehydration, i.e. day 0 to day 56) on which live taxa were removed from samples were considered. For each common taxon, a one-way repeated-measures (RM) ANOVA test was conducted with days 0, 7, 14, 28 and 56 as within-subjects variables. Mauchly's tests were used to verify the assumption of sphericity and where this was violated, sphericity estimates (Greenhouse-Geisser epsilon statistics) were consulted.

Results

Composition of the seedbank assemblage

A total of 2009 individuals from 38 aquatic or semi-aquatic macroinvertebrate taxa were recorded from the 24 samples, either pre- or post-rehydration (Table 2). Richness ranged from 3 to 15 (mean \pm SE, 7.3 ± 0.7) taxa per sample and abundance ranged from 11 to 176 (84 ± 9.3) individuals per sample. Seven taxa each accounted for $\geq 1\%$ of the assemblage: Oligochaeta (38.1%), Chironomidae (36.4%), Stratiomyidae (Diptera; 5.5%), *Pisidium* (Bivalvia: Sphaeriidae; 5.5%), Tipulidae (Diptera; 1.7%), Hydrophilidae larvae (Coleoptera; 1.6%) and *Agabus guttatus* adults (Coleoptera: Dytiscidae; 1.0%). Of these, four taxa (Oligochaeta, Chironomidae, *Pisidium* and Hydrophilidae larvae) were common. The most diverse orders were Coleoptera, represented by adults and larvae of at least 14 species from six families, and Diptera, also represented by six families.

NMDS ordination indicated spatial differences in assemblage composition, with samples from the ephemeral headwaters (sites A–D, group 1) forming a dispersed cluster with low NMDS1 axis scores and limited overlap with other samples (Fig. 3). The only sample from site E (the last to dewater prior to the 2011 dry phase) was plotted on the periphery of the ordination with a high NMDS1 score (Fig. 3). Temporal variability between seasons was also apparent: early and late

spring samples plotted as a relatively tight, overlapping cluster with high NMDS2 axis scores, whereas autumn samples were spread along NMDS1 (Fig. 3). This variability partly reflects the additional sites sampled in autumn, but temporal variability along NMDS2 is also apparent when only the repeatedly sampled sites (A, B and D) are considered.

Spatial variability in assemblage composition in autumn

Assemblage composition of group 1 sites differed from that of group 2 (ANOSIM $R = 0.771$, $P = 0.029$) and group 3 sites ($R = 0.740$, $P = 0.029$), whereas no significant difference was found between group 2 and 3 assemblages ($R = -0.135$, $P = 0.743$). Group 1 assemblages were characterised by high *Pisidium* abundance (one-way ANOVA, $F = 6.208$, d.f. = 11, $P = 0.032$) and low Chironomidae abundance ($F = 5.211$, d.f. = 11, $P = 0.046$). Taxon richness was lowest at group 1 sites (mean \pm SE, 4.0 ± 1.0 taxa per sample) and higher and more variable at sites in groups 2 (9.0 ± 2.2 taxa) and 3 (8.8 ± 1.3 taxa); these differences were not statistically significant (one-way ANOVA, $F = 3.294$, d.f. = 11, $P = 0.084$). Richness peaked at 15 taxa at site L, directly upstream of the perennial source (Fig. 1). Total abundance was lowest at group 1 sites (mean \pm SE, 48 ± 24 individuals per sample), moderate at group 2 sites (80 ± 15 individuals) and highest at group 3 sites

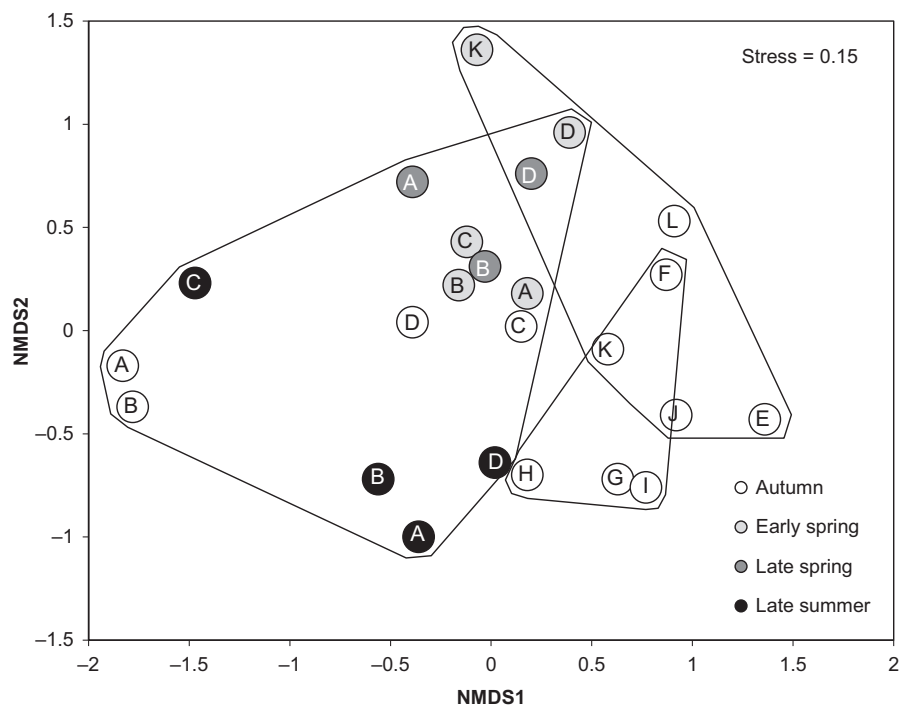


Fig. 3 Non-metric multidimensional scaling (NMDS) ordination of the macroinvertebrate assemblages in 24 samples collected from the River Lathkill in four seasons, day 0 to 56 data combined for each sample. Letters indicate sites A–L, as in Fig. 1. Polygons enclose sites in groups 1 (A–D), 2 (E, J–L) and 3 (F–I), as in Table 1.

(120 ± 22 individuals); these differences were not significant (one-way ANOVA, $F = 3.267$, d.f. = 11, $P = 0.086$). Abundance was highest at site E (176 individuals, Fig. 1), which had been dry for a relatively short duration (3.3 months, Table 1).

An exponential decline in richness occurred as the dry-phase duration increased ($R^2 = 0.485$, d.f. = 11, $P = 0.043$; Fig. 4a). An exponential model also best explained the reduction in abundance with increasing dry-phase duration ($R^2 = 0.492$, d.f. = 11, $P = 0.013$; Fig. 4b). The strength of this relationship increased ($R^2 = 0.676$) when a notable outlier was excluded: site J had only moderate abundance (76 individuals) despite a short preceding dry phase (Table 1).

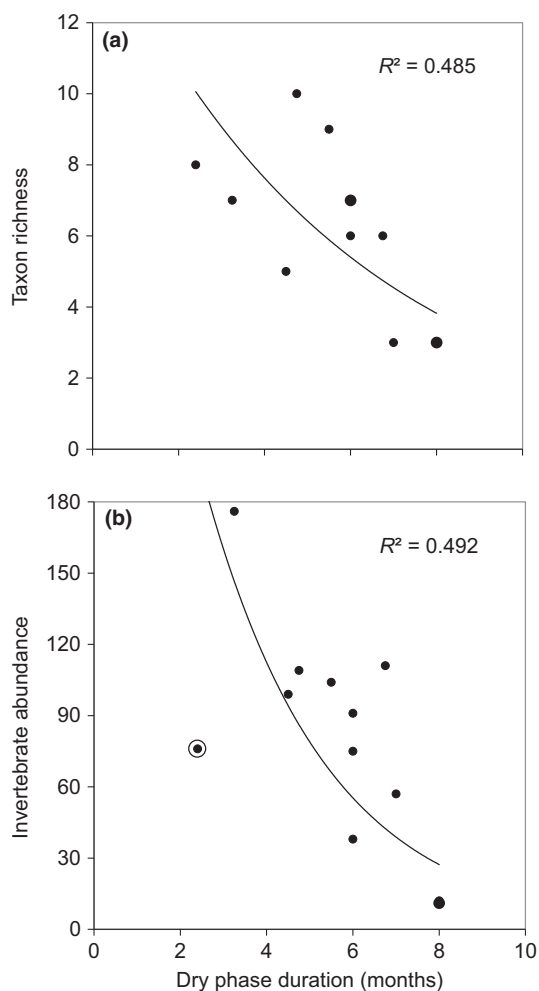


Fig. 4 Macroinvertebrate assemblage metrics for samples collected from the dry bed of the River Lathkill in autumn (November 2011) at 12 sites differing in the duration of the dry phase prior to sampling: (a) richness (taxa per c. 2 L sample) and (b) abundance (individuals per c. 2 L sample). The circled point on pane (b) indicates site J (as in Fig. 1), a notable outlier; larger symbols represent two points.

Temporal changes in assemblage composition between seasons

No significant differences were found in assemblage composition between seasons (ANOSIM $R \leq 1$, $P \geq 0.1$). Richness differed between seasons (one-way ANOVA, $F = 5.825$, d.f. = 11, $P = 0.021$), being lowest in autumn (mean \pm SE, 3.0 ± 0 taxa per sample), significantly higher in early spring (8.3 ± 1.9 taxa) and late spring (8.0 ± 1.0 taxa; Tukey test, $P \leq 0.04$) and then declining in late summer (5.0 ± 0 taxa). Taxa first recorded in early spring were all insects, including four Coleoptera genera, three Diptera families, *Nemoura cambrica* (Plecoptera: Nemouridae) and the limnephilids *Stenophylax permistus* and *Micropterna sequax* (Trichoptera: Limnephilidae, Table 3). With the exception of two Coleoptera and two Diptera families, loss of a comparable range of taxa was responsible for the reduction in richness in late summer.

Abundance differed between seasons (one-way ANOVA, $F = 8.588$, d.f. = 11, $P = 0.007$), and was lowest in autumn (mean \pm SE, 27 ± 15 individuals per sample), increased to a peak in late spring (140 ± 20 individuals, Tukey test, $P = 0.008$), then declined in late summer (40 ± 8.6 individuals). Taxa responsible for this pattern included Chironomidae, the abundance of which was low in autumn (mean \pm SE, 8.7 ± 6.7 individuals per sample), increased to a peak in late spring (88 ± 22 individuals) then declined in late summer (8.3 ± 0.7 individuals; one-way ANOVA, $F = 7.948$, d.f. = 11, $P = 0.009$). *Pisidium* also experienced temporal changes in abundance, increasing from autumn (mean \pm SE, 4.3 ± 0.9 individuals per sample) to late spring (7.3 ± 2.0 individuals) before disappearing in late summer (one-way ANOVA, $F = 9.951$, d.f. = 11, $P = 0.004$). Oligochaeta abundance was stable across seasons at 25–42 individuals per sample (one-way ANOVA, $P = 0.523$), whereas Hydrophilidae larvae were absent in autumn and occurred at low abundance (≤ 4 individuals per sample) in all subsequent seasons ($F = 0.987$, d.f. = 11, $P = 0.435$).

Temporal changes in the observation of macroinvertebrates during the rehydration period

Several patterns of temporal change were recorded for taxa removed from samples between the start and end of the rehydration experiment (i.e. from pre-rehydration to day 56, Table 4). Many live taxa were found only or predominantly pre-rehydration and not

Table 3 Seasonal change in the macroinvertebrate taxa present in sediments extracted from the dry bed of the River Lathkill. Data from three ephemeral headwater sites (A, B and D, Fig. 1) sampled in four seasons are included. Letters in parentheses indicate life stage: (a) = adult; (l) = larval; (p) = pupal. Additional taxonomic detail is provided in Table 2.

| Group | Taxon | Autumn | Early spring | Late spring | Late summer |
|-------------|-----------------------------------|--------|--------------|-------------|-------------|
| Bivalvia | <i>Pisidium</i> | ✓ | ✓ | ✓ | |
| Coleoptera | <i>Agabus guttatus</i> (a) | | ✓ | ✓ | ✓ |
| | <i>Hydroporus ferrugineus</i> (a) | | ✓ | | |
| | <i>Helophorus brevipalpis</i> (a) | | ✓ | | |
| | <i>Hydraena palustris</i> (a) | | | | ✓ |
| | Hydrophilidae (l) | | ✓ | ✓ | ✓ |
| | <i>Laccobius</i> (a) | | ✓ | ✓ | |
| | Scirtidae (l) | | | | ✓ |
| Crustacea | <i>Asellus aquaticus</i> | | | ✓ | |
| Diptera | Chironomidae (l, p) | ✓ | ✓ | ✓ | ✓ |
| | Empididae (l) | | ✓ | | ✓ |
| | Psychodidae (l) | | ✓ | | ✓ |
| | Tipulidae (l) | | ✓ | ✓ | ✓ |
| Oligochaeta | | ✓ | ✓ | ✓ | ✓ |
| Plecoptera | <i>Nemoura cambrica</i> | | ✓ | ✓ | ✓ |
| Trichoptera | <i>Micropterna sequax</i> (l) | | ✓ | ✓ | |
| | <i>Stenophylax permistus</i> (l) | | ✓ | ✓ | |

after day 14, including most Coleoptera, for example Hydrophilidae larvae (RM ANOVA, $F = 12.779$, d.f. = 2, $P < 0.001$), large Dytiscidae adults and Elmidae; *Asellus aquaticus* (Isopoda: Asellidae); larvae of several Diptera families; and late instars of the caddisfly genera *Stenophylax* and *Micropterna*. Declining numbers of *Pisidium* (RM ANOVA, $F = 23.890$, d.f. = 1.565, $P < 0.001$; Table 4), Tipulidae larvae and small *N. cambrica* nymphs were also removed from rehydrated sediments over the course of the experiment.

Different patterns of temporal change were observed for Chironomidae, Oligochaeta and Dytiscidae larvae. Of 732 chironomids, 121 were removed pre-rehydration, abundance then dipped on day 7, before increasing on each subsequent inspection (RM ANOVA, $F = 3.428$, d.f. = 2.048, $P = 0.04$; Table 4). Large chironomid larvae were observed pre-rehydration, including the semi-aquatic genera *Bryophaenocladus*, *Limnophyes* and *Metriocnemus* (Orthoclaadiinae). Chironomids recorded on day 7 were typically small, with individuals then increasing in size until day 56, when larvae ranged in size and were dominated by the aquatic species *Brillia bifida* and *Rheocricotopus fuscipes* (Orthoclaadiinae). Oligochaeta abundance peaked pre-rehydration, then declined until day 14 before increasing on each subsequent inspection (RM ANOVA, $F = 3.454$, d.f. = 2.295, $P = 0.033$; Table 4). Qualitative observations indicated a shift in the taxonomic composition of Oligochaeta, from larger, terrestrial Lumbrici-

dae individuals pre-rehydration to smaller, aquatic Naididae and tubificoid naids on subsequent days. Dytiscidae larvae were recorded only after rehydration, with 1–2 small individuals removed on every inspection from day 7 to day 56.

Discussion

Seedbank assemblage composition in relation to dry-phase duration and supra-seasonal drought

In total, 38 macroinvertebrate taxa from 19 families were recorded in the seedbank of the low-order, temperate-zone River Lathkill, including at least 24 taxa recorded near the end of an unusually long dry phase resulting from a supra-seasonal drought (Marsh *et al.*, 2013). Despite this richness, we found some support for our first hypothesis (that assemblage composition at sites with longer dry phases would be characterised by lower abundance and richness), with sediments from ephemeral headwater sites being inhabited by multiple individuals of only three taxa (*Pisidium*, Chironomidae and Oligochaeta). Chironomidae and Oligochaeta were mainly identified with coarse resolution but included multiple species, and caution is therefore necessary when interpreting patterns for these taxa: richness could have varied considerably between sites, although our qualitative observations do not support this suggestion. Regardless of chironomid and oligochaete richness, the assemblage was less taxonomically diverse compared to

Table 4 Number of individuals of live macroinvertebrate taxa collected from samples pre-rehydration (day 0) and on days 7–56 following experimental rehydration. Genus names are provided in Table 2; letters in parentheses indicate life stage: (a) = adult; (l) = larval; (p) = pupal. Total = the total number of individuals recorded; numbers in parentheses indicate the number of samples from which organisms were removed on each day; samples = the total number of samples (out of 24) in which the taxon occurred.

| Group | Taxon | Day | | | | | | Samples |
|---------------------|--------------------------------|---------------------|----------|----------|----------|----------|----------|---------|
| | | Total | 0 | 7 | 14 | 28 | 56 | |
| Bivalvia | <i>Pisidium</i> | 111 | 72 (14) | 29 (14) | 8 (4) | 1 (1) | 1 (1) | 14 |
| Coleoptera | Dytiscidae (l) | 6 | | 2 (1) | 1 (1) | 2 (1) | 1 (1) | 2 |
| | <i>A. biguttatus</i> (a) | 7 | 7 (2) | | | | | 2 |
| | <i>A. guttatus</i> (a) | 21 | 19 (8) | 2 (2) | | | | 8 |
| | <i>H. ferrugineus</i> (a) | 7 | 7 (5) | | | | | 5 |
| | <i>H. pubescens</i> (a) | 1 | 1 (1) | | | | | 1 |
| | <i>E. aenea</i> (a) | 8 | 6 (2) | 2 (2) | | | | 2 |
| | <i>E. aenea</i> (l) | 6 | 5 (2) | 1 (1) | | | | 2 |
| | <i>L. volckmari</i> (a) | 2 | 2 (1) | | | | | 1 |
| | <i>O. troglodytes</i> (a) | 2 | 1 (1) | 1 (1) | | | | 1 |
| | <i>R. subviolaceus</i> (a) | 10 | 8 (2) | | | 2 (1) | | 2 |
| | <i>R. subviolaceus</i> (l) | 2 | 1 (1) | 1 (1) | | | | 1 |
| | <i>H. brevipalpis</i> (a) | 6 | 4 (4) | 1 (1) | | 1 (1) | | 4 |
| | <i>H. palustris</i> (a) | 29 | 23 (8) | 6 (1) | | | | 9 |
| | <i>Limnebius</i> (a) | 3 | 3 (1) | | | | | 1 |
| | <i>O. auriculatus</i> (a) | 1 | | | | 1 (1) | | 1 |
| | Hydrophilidae (l) | 32 | 23 (15) | 7 (7) | 2 (2) | | | 17 |
| | <i>Laccobius</i> (a) | 5 | 2 (2) | | | 3 (2) | | 2 |
| | Scirtidae (l) | 10 | 6 (3) | 2 (2) | 2 (1) | | | 3 |
| | Diptera | Ceratopogonidae (l) | 6 | 5 (4) | | 1 (1) | | |
| Chironomidae (l, p) | | 732 | 121 (19) | 106 (19) | 131 (21) | 155 (20) | 219 (21) | 24 |
| Empididae (l) | | 5 | 5 (4) | | | | | 4 |
| Psychodidae (l) | | 12 | 7 (4) | 4 (1) | 1 (1) | | | 5 |
| Stratiomyidae (l) | | 111 | 92 (4) | 15 (2) | 4 (1) | | | 4 |
| Gastropoda | Tipulidae (l) | 35 | 20 (11) | 8 (7) | 4 (3) | 2 (2) | 1 (1) | 11 |
| | <i>L. truncatula</i> | 8 | 1 (1) | 1 (1) | 1 (1) | 3 (2) | 2 (1) | 2 |
| Isopoda | <i>A. aquaticus</i> | 5 | 5 (2) | | | | | 2 |
| Oligochaeta | Oligochaeta | 765 | 236 (22) | 112 (23) | 97 (22) | 141 (19) | 179 (20) | 24 |
| Plecoptera | <i>N. cambrica</i> | 12 | 5 (3) | 5 (3) | 1 (1) | 1 (1) | | 3 |
| Trichoptera | <i>Micropterna/Stenophylax</i> | 43 | 32 (8) | 11 (5) | | | | 8 |

sites with shorter dry phases, with at least eight Coleoptera species and four Diptera families absent from ephemeral sites (excluding site C, as discussed below), but present at intermittent and near-perennial sites in autumn. As in previous studies, exponential declines in abundance and richness occurred as the dry period duration increased; such reductions have previously been attributed to declining sediment moisture, which is considered a primary driver of spatial variability in seedbank assemblages (Larned *et al.*, 2007; Datry *et al.*, 2012; Stubbington & Datry, 2013).

Comparison of Group 1 assemblages with those collected in the same ephemeral headwater reach by Stubbington *et al.* (2009) and reported in Stubbington & Datry (2013) provides evidence of supra-seasonal drought impacts on the seedbank. Both previous studies collected 38–42 kg of dry sediment from across seven sites, whereas we collected 37.5 kg from four of these

sites, with the remaining three sites being within the same reach and having comparable environmental characteristics; we therefore consider the sampling effort to be comparable between studies. Stubbington *et al.* (2009) recorded 24 *Gammarus pulex* (Amphipoda: Gammaridae) individuals across four sites, however, all were observed after a dry period of <7 days. The absence of this taxon from this study indicates that the supra-seasonal drought eliminated *G. pulex* from the ephemeral headwater seedbank, probably within a few days, although our own unpublished data show that the taxon was present at low abundance in wet-phase samples from early spring, indicating rapid recolonisation from local refuges (see below). Stubbington & Datry (2013) also reported 26 *Phagocata vitta* (Tricladida: Planariidae) from across six headwater sites. In contrast to *G. pulex*, *P. vitta* was absent from both this study and from our unpublished post-drought seedbank and wet-phase samples, suggest-

ing that the drought eliminated this taxon from the headwaters. In addition, both previous studies recorded adult *Anacaena globulus* (Hydrophilidae), larval Ceratopogonidae and Dixidae (Diptera) and *Polycelis* (Planariidae) at low abundance; further sampling would be required to determine whether drought eliminated these less-abundant taxa from the headwaters.

After flow resumes, temporary-stream community recovery is facilitated by refuges, in particular upstream perennial waters (Boulton, 1989; Chester & Robson, 2011; Bogan, Boersma & Lytle, 2013), which were absent in the Lathkill. Other refuges include downstream perennial reaches, but triclads and other taxa apparently eliminated by drying may not have had time during this study to recolonise the headwaters by upstream migration, in particular due to the barrier presented by the tufa cliff. Community recovery may also have been facilitated by recolonists from groundwater refuges, such as the fissures and caves within the karst limestone bedrock (e.g. *P. vitta* and *G. pulex*: Wood, Gunn & Rundle, 2008; Stubbington *et al.*, 2009), and by insects with adults capable of terrestrial flight (Diptera: Tronstad, Tronstad & Benke, 2007) or overland dispersal (*A. globulus*; Sanderson *et al.*, 2005).

At near-perennial sites, the extent and duration of the dry phase preceding autumn sampling were greater than in any period since measurement of flow began in 1998. Despite the rarity of this drying event, taxa recorded in autumn included many typical temporary-stream inhabitants with adaptations promoting desiccation tolerance and persistence in humid interstices. For example, Coleoptera including active *Agabus* adults were observed pre-rehydration in sediments that had dried 4.8 months previously; these dytiscid adults typically obtain oxygen from air and have a relatively impermeable cuticle that reduces water loss (Holdgate, 1956). Sphaeriids were common and are also typical temporary-water inhabitants (Williams, 2006), their desiccation tolerance being facilitated by valve closure to minimise water loss and by entrance into a dormant state in which gas exchange occurs through minute pores (McKee & Mackie, 1980; Williams, 2006).

Assemblage composition at intermittent and near-perennial group 2 and 3 sites was comparable and distinct from ephemeral group 1 sites. Sites in both groups 2 and 3 had likely passed the critical environmental thresholds of surface and hyporheic water loss (Boulton, 2003; Bogan *et al.*, 2015), but retained enough moisture in inhabitable interstices to support comparable assemblages. In contrast, the longer dry-phase duration and finer sediments in the ephemeral headwaters (group 1

sites) limited seedbank inhabitation to a less taxonomically diverse range of invertebrates capable of tolerating extended drying in restricted interstices. A lack of shading potentially hastened drying at headwater sites, as found by Storey & Quinn (2013) and Verdonschot *et al.* (2015), although upwelling groundwater may have maintained moisture levels locally (i.e. at site C). The occurrence of single individuals of four taxa (one Diptera and three Coleoptera families) at site C but at no other group 1 site provides limited evidence of the influence of sediment moisture content on assemblage composition. However, controlled experiments would be required to disentangle the effects of environmental variables that may co-vary and concurrently influence biotic responses in field investigations, such as dry-phase duration and sediment characteristics in this study (Stewart *et al.*, 2013).

Temporal changes between seasons: effects of wet-dry cycles

Richness increased five-fold between autumn and early spring, with 11 Coleoptera, Diptera, Plecoptera and Trichoptera being first recorded in the latter season. These insect taxa may have been present in autumn as undetected dormant eggs (as discussed below for *Stenophylax* and *N. cambrica*), or may have been absent in autumn, recolonising from local refuges during the winter wet phase. For example, adult *A. guttatus* and *Helophorus brevipalpis* (Coleoptera: Helophoridae) beetles were present in downstream (groups 2 and 3) seedbank assemblages in autumn and may have recolonised the headwaters by overland flight (Jackson, 1952). *Hydroporus ferrugineus* (Coleoptera: Dytiscidae) adults also occurred at downstream sites, although this flightless beetle inhabits groundwater-dominated habitats and may have recolonised the headwaters by downstream migration from the limestone conduit system that supplies the Lathkill Head Cave (Jackson, 1958; Wood, Gunn & Perkins, 2002). Our second hypothesis predicted that these recolonist taxa would have adaptations promoting their survival of the wet-dry cycles they subsequently experienced in the headwaters, but three taxa disappeared by late spring and a further six were absent in late summer. However, rather than indicating impacts of repeated wet-dry cycles, we suspect these 'losses' were seasonal transitions between life stages that reflected insect life cycles, as detailed below for two example taxa, *Stenophylax* and *N. cambrica*. Therefore, although we find no evidence to reject our second hypothesis, further field and experimental work is needed to confirm

the relative contributions of desiccation tolerance and recolonisation to taxon-specific survival of repeated wet-dry cycles.

Stenophylax larvae were first recorded in the headwaters in early spring. Larval development of this caddisfly starts when desiccation-resistant eggs break dormancy after winter flows resume (Ruiz-García & Ferreras-Romero, 2007) and, in the Lathkill, development probably began after autumn sampling, with any eggs present in rehydrated autumn samples potentially not developing if species-specific hatching cues such as flowing water were absent (Stubbington & Datry, 2013). Late-instar larvae were then observed in late spring, with desiccation-resistant individuals probably persisting in humid interstices during short dry phases (Bouvet, 1977; Ruiz-García & Ferreras-Romero, 2007). The *S. permistus* flight period extends from spring to autumn in England (Crichton, 1971) and in summer adults aestivate in terrestrial habitats including caves (Bournaud, 1971); the caves present at the high-flow source and in Cales Dale potentially provided suitable adult habitat in the Lathkill catchment.

Nemoura cambrica stonefly nymphs may also have completed their life cycle despite repeated wet-dry cycles. *Nemoura* adults are active in spring (April–June) and oviposit on water, with eggs of some species breaking dormancy months later in response to short photoperiod and low temperature (Lehmkuhl, 1971; Hynes, 1976). In the Lathkill, the absence of nymphs in autumn (November) and the presence of a small number of early-instar nymphs in spring (March and May) suggest oviposition during a short wet phase in spring 2011, with desiccation-resistant eggs remaining dormant until after autumn sampling. Any eggs present in autumn samples may not have developed following experimental rehydration if hatching cues were lacking. Subsequent, possibly asynchronous egg hatching (Dieterich & Anderson, 1995) may have resulted in the nymphs observed in spring samples. Small *Nemoura* nymphs may tolerate desiccation (Lehmkuhl, 1971; Jacobi & Cary, 1996) and the viability of nymphs observed pre-rehydration in spring samples confirms persistence of *N. cambrica* in humid interstices. The absence of nymphs in late summer is typical (Mackereth, 1957), the species being present instead as dormant eggs. Our observations in the Lathkill are based on relatively few individuals in one system, and the generality of these patterns therefore requires confirmation.

Our study suggests that aquatic insect occurrence in the seedbank is taxon-specific, reflecting timing and duration of adult flight periods as well as dormancy and

desiccation tolerance of eggs and juveniles. Where flight periods span several months and extend into autumn (e.g. many British limnephilid caddisflies; Crichton, 1971), repeated colonisation by successive cohorts of egg-laying females may be possible even when repeated wet-dry cycles occur. Such repeated colonisation may be unnecessary if juveniles tolerate short-term drying, as we observed for *S. permistus* and *M. sequax*. However, aerial adults decline as a recolonist source if dry phases persist into autumn as adult activity subsides, unless dormant eggs deposited on dry sediments remain viable. Insects with short flight periods (e.g. New Zealand Conoesucidae and Oeconesidae caddisflies: Winterbourn & Crowe, 2001) or single oviposition events (e.g. the nemourid stonefly *Nemurella pictetii*: Elliott, 1988) may not survive repeated wet-dry cycles, if all eggs develop in response to ephemeral surface water and desiccation-sensitive juveniles subsequently perish (Stewart & Anderson, 2010). Timing may therefore be as critical as frequency in determining dry-phase effects, although asynchronous egg hatching can allow populations to repeatedly reestablish following multiple inundations (Strachan *et al.*, 2016).

Pisidium bivalves were common at the three repeatedly sampled sites between autumn and late spring, but were absent in late summer. However, sphaeriids tolerate desiccation (McKee & Mackie, 1980) and were not eliminated from the headwaters by repeated wet-dry cycles: in late summer, some were recorded at site C, the groundwater-fed spring that had been wet in late spring. Sphaeriid persistence may have been enhanced by relatively high interstitial humidity in such localised refuges. Reproductive activity in summer (Holopainen & Hanski, 1986) may also have caused seasonal changes in *Pisidium* abundance, with undetected embryos rather than mature individuals present in late summer, as reported for other temperate-zone sphaeriids (Mouthon, 2004).

Temporal changes in the observation of macroinvertebrates during the rehydration period

Most taxa recorded in this study were observed pre-rehydration, indicating persistence in humid interstices as active or dormant juveniles or adults. These taxa included caddisfly larvae, stonefly nymphs, Elmidae larvae and adults, Hydrophilidae larvae and Dytiscidae adults, adaptations of which are considered above. In contrast, taxa for which multiple individuals were observed exclusively post-rehydration included small Dytiscidae larvae. Adults observed in this and previous studies (Stubbington *et al.*, 2009) suggest that dytiscid

larvae probably included *Agabus*, which may have developed from desiccation-resistant dormant eggs following rehydration (Nilsson & Holmen, 1995); this suggestion is based on few individuals and requires confirmation by further taxon-specific sediment rehydration experiments.

Chironomid abundance was lower on day 7 than pre-rehydration, then increased on every inspection between days 14 and 56. This family included representatives with contrasting ecological preferences, for example the semi-aquatic Orthoclaadiinae genera *Bryophaenocladus*, *Limnophyes* and *Metriocnemus*, and the aquatic orthoclads *B. bifida* and *R. fuscipes* (Armitage, Pinder & Cranston, 2012). Although our results require cautious interpretation due to the predominance of early instars that could not be identified further (Lancaster & Ledger, 2015), temporal changes can probably be explained by concurrent, contrasting taxon-specific responses to rehydration: semi-aquatic larvae present pre-rehydration were removed, while dormant eggs of aquatic species developed following rehydration, resulting in numerous small chironomids from day 7. This inferred persistence as eggs provides support for our third hypothesis, that seedbank assemblages would comprise multiple life stages. Although our interpretations are again tentative, due to the coarse taxonomic resolution preventing confirmation of species-specific responses, a combination of terrestrial and aquatic responses probably also explain changes in oligochaete abundance, with the loss of terrestrial Lumbricidae preceding the appearance of aquatic Naididae and tubificoid naidids, most likely from dormant eggs (Tronstad *et al.*, 2005).

Maintaining a diverse seedbank in a time of global change

Our results contribute to recent recognition of the seedbank as a mechanism by which many lotic aquatic macroinvertebrates persist in streams during dry phases, thereby contributing to community resistance and resilience to drying. Our study also provides some support for the previous finding that the seedbank becomes less diverse as the dry-phase duration increases (Stubbington & Datry, 2013). In addition, our results indicate that severe supra-seasonal droughts may cause local elimination of desiccation-sensitive taxa.

The arrival of multiple insect taxa in spring suggested that aerial adults are an important recolonist source in the Lathkill, as in other temporary streams (Robson, Chester & Austin, 2011; Bogan *et al.*, 2015). Their contribution to community resilience is particularly pronounced in catchments, such as the Lathkill, that are hydrologically iso-

lated and/or lack perennial upstream waters (Chester *et al.*, 2015). Management strategies and restoration projects seeking to promote invertebrate persistence in headwater streams should therefore look beyond instream conditions and seek to sustain and enhance terrestrial habitats of adult insects (Leigh *et al.*, 2016a). With extreme events including droughts and heat waves becoming more common (Stocker *et al.*, 2013; Ledger & Milner, 2015), radical interventions, such as riparian reforestation to increase instream shading and promote sediment moisture retention, may be needed to mitigate climate change impacts on temporary-stream communities and promote invertebrate persistence (Leigh *et al.*, 2016b).

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