Vertical movements through subsurface stream sediments by benthic macroinvertebrates during experimental drying are influenced by sediment characteristics and species traits.

Atish N. Vadher[1], Catherine Leigh[2], Jonathan Millett[1], Rachel Stubbington[3] and Paul J. Wood[1]

[1]Centre for Hydrological and Ecosystem Sciences, Department of Geography, Loughborough University, Loughborough, Leicestershire LE11 3TU, UK

[2]Australian Rivers Institute and the Griffith School of Environment, Griffith University, Nathan, QLD 4111, Australia

[3]School of Science and Technology, Nottingham Trent University, Clifton Campus, Nottingham NG11 8NS, UK

Author for correspondence:
Atish N. Vadher
Department of Geography
Loughborough University
Loughborough
Leicestershire
LE11 3TU
UK
Email: A.Vadher@Lboro.ac.uk

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Summary

1. Streambed drying is becoming more common due to climate change and increasing anthropogenic water resource pressures. Subsurface sediments are a potential refuge for benthic macroinvertebrates during drying events in temporary streams.
2. Sediment characteristics are important controls on the distribution of macroinvertebrates in subsurface habitats, but difficulties making observations impedes quantification of vertical movements. Species traits (e.g., subsurface habitat affinity) also influence vertical movements of macroinvertebrates into the subsurface sediments, but most species-specific responses remain uncharacterized.
3. Transparent artificial mesocosms were used to directly observe the vertical movements of individuals of three aquatic insect nymphs and two crustaceans. Mixtures of three types of transparent sediment of varying particle size were combined to produce six treatments with differing interstitial pore volumes and, hence, differing subsurface porosity. Macroinvertebrate vertical movements were measured during incremental reductions in water level from 5 cm above to 20 cm below the sediment surface. These species comprised a variety of trait categories including feeding group, species affinity to temporary streams and subsurface habitats. Active and passive vertical movements were determined by conducting experiments with both live individuals and their cadavers.
4. Sediment treatment influenced the vertical movements of individuals as reducing subsurface porosity decreased vertical movements for most species. Vertical movement into subsurface sediments in response to water level reduction was the result of active, not passive, movements for all species.
5. Species identity influenced the vertical movements made by individuals. *Nemoura cambrica* had the highest affinity for temporary streams and subsurface habitats and

its vertical movements were unaffected by sediment treatment, generally reaching depths between 20–25 cm. Most individuals of species with a weaker subsurface affinity (i.e. the benthic grazer *Heptagenia sulphurea* and the filter-feeder *Hydropsyche siltalai*) became stranded as water levels were reduced in all sediment treatments. Vertical movements of *Gammarus pulex* and *Asellus aquaticus* were restricted primarily by pore volume, these taxa becoming stranded most commonly in sediments with smaller interstitial volumes.

6. Our results highlight the need for the development and implementation of river management strategies that increase streambed porosity, allowing macroinvertebrates to access to the saturated subsurface habitat during stream drying.

Introduction

Temporary streams experience periods of no flow, often resulting in the loss of longitudinal hydrological connectivity and surface water (e.g. Boulton, 2003; Datry et al., 2014a; Bogan et al., 2015). Surface water loss can be detrimental and, in some instances, fatal to many aquatic species (Extence, 1981; Stanley et al., 1997; Wood et al., 2010). A wide range of species, however, display behavioural adaptations that facilitate their persistence in temporary streams (Lytle & Poff, 2004; Datry, 2012). Temporary streams are widespread and occur in every climatic zone from polar (e.g., McKnight et al., 1999) and temperate regions (Williams & Hynes, 1976; Stubbington et al., 2016) through to tropical and arid zones (Steward et al., 2012; Leigh 2013; Bogan et al., 2015). They, therefore, represent a widespread stream type supporting distinct species assemblages (Westwood et al., 2006; Bogan et al., 2013; Acuña et al., 2014).

Surface flow cessation and streambed drying are the primary drivers of temporary stream community structure (Bogan & Lytle, 2011; Datry et al., 2014a), taxonomic richness (Datry et al., 2014a; Stubbington et al., 2015; Leigh & Datry, 2016), population abundance (Smith & Wood, 2002; Rüegg & Robinson, 2004) and ecosystem functioning (Datry et al., 2011; Magoulick, 2014). Despite an increasing recognition of the wider value of ecosystem services provided by temporary streams and their biota (Acuña et al., 2014; Datry et al., 2014b), few studies have examined the response of individual lotic species to surface water loss, including their survival, and the ability of individuals to access and use subsurface habitats during drying (Imhof & Harrison, 1981; Vadher et al., 2015; Vander Vorste et al., 2016a). Addressing this knowledge gap is important because poor access to subsurface habitats (e.g., due to sedimentation/colmation) during streambed drying is likely to compromise the persistence of many aquatic macroinvertebrate species (Descloux et al., 2013; Jones et al., 2015; Vadher et al., 2015; Leigh et al., 2016).

After benthic sediments dry, subsurface water may persist within the hyporheic zone (Hose et al., 2005; Fenoglio et al., 2006). The potential for these subsurface sediments to function as a refuge has long been recognised (Williams & Hynes, 1974). The hyporheic zone is an important habitat and resource for aquatic fauna during streambed drying in many streams (Dole-Olivier, 2011; Vadher et al., 2015; Vander Vorste, 2016b). If individuals can access and persist in saturated subsurface sediments during periods of surface water loss, they may be able to return to the channel when flow returns (Stubbington, 2012; Vadher et al., 2015), thus facilitating the rapid recolonization and recovery of temporary stream communities (Vander Vorste et al., 2016b). However, not all streams have extensive hyporheic zones and it may be absent in streams where, for example, bedrock dominates the channel (Malard et al., 2002), or in other instances it may be inaccessible due to fine sediment deposition and clogging (Descloux et al., 2013; Vadher et al., 2015).

A range of biological traits may enhance species resistance (ability to persist) and resilience (ability to recover) to stream drying, for example body size, locomotion and feeding habits (e.g., Bonada et al., 2007). Sedimentary characteristics that may also affect access to and movement through the hyporheic zone have been explored (e.g., Nogaro et al., 2006; Stubbington et al., 2011; Descloux et al., 2013; Mermillod-Blondin et al., 2015). Field (Duan et al., 2008; Gayraud & Philippe, 2003; Descloux et al., 2013) and laboratory (Nogaro et al., 2006; Navel et al., 2010; Vadher et al., 2015) studies have demonstrated that sediment characteristics including particle size, shape, heterogeneity and porosity can influence the distribution of benthic populations. However, the direct response of individuals to drying and their ability to move into subsurface sediments has rarely been studied in real time (exceptions being Stumpp & Hose 2013; Vadher et al., 2015). This reflects the inherent difficulties of directly observing fauna within subsurface habitats.

In this ex-situ study, we experimentally examined the effect of surface water loss and water level reduction within subsurface sediments of varying sediment characteristics (particle size, heterogeneity and interstitial volume) on the vertical movement of individuals of five benthic macroinvertebrate species. We used artificial sediments of known size and shape within laboratory mesocosms to directly control sediment characteristics. The use of different particle combinations allowed the volume of interstitial space to be quantified and controlled (Mathers et al., 2014). To facilitate direct observation of individuals and their behaviour within the subsurface sediments, transparent artificial sediments were used to allow the precise location of individuals to be observed throughout the experimental period. We hypothesised that the response of lotic benthic macroinvertebrates to water level reduction and their vertical movement through the subsurface would: i) be active rather than passive; ii) depend on subsurface sediment characteristics; and iii) vary between species due to interspecific variation in traits (e.g., mode of locomotion, feeding group, habitat affinities).

Materials and methods

Invertebrate collection and test species

Five species of benthic macroinvertebrate were chosen for examination of their response to surface water loss and water level reduction: a stonefly nymph, *Nemoura cambrica* (Plecoptera: Nemouridae); a caseless caddisfly larvae, *Hydropsyche siltalai* (Trichoptera: Hydropsychidae); a mayfly nymph, *Heptagenia sulphurea* (Ephemeroptera: Heptageniidae); and two crustaceans, *Asellus aquaticus* (Isopoda: Asellidae) and *Gammarus pulex* (Amphipoda: Gammaridae). These species consisted of one widespread inhabitant of seasonally dry headwater streams, *N. cambrica* (Stubbington et al., 2009); two species which have been widely recorded in benthic and hyporheic sediments in both perennial and temporary systems, *A. aquaticus* and *G. pulex* (Stubbington et al., 2015); and two benthic species typically associated with perennially flowing systems, *Hy. siltalai* and *H. sulphurea* (Eyre et al., 2005; Wood et al., 2005a; Datry 2012). These species were selected to represent a range of biological traits (Table 1).

All five species were collected from sites on Wood Brook (52°46’07.5”N 1°12’34.6”W) and Burleigh Brook (52°45’50.5”N 1°14’28.6”W) in Loughborough (Leicestershire, UK). *Gammarus pulex*, *Hy. siltalai*, *H. sulphurea* and *N. cambrica* were collected from shallow riffles and *A. aquaticus* was collected from a slow-flowing pool adjacent to Wood Brook. Individuals were collected using a gentle kick-sampling technique with a standard kick-net (900 μm mesh, 23 cm × 25.5 cm frame, 27.5 cm bag depth). Captured specimens were removed from the net individually and transferred to a 5-L container of stream water and transported to the laboratory for use in experiments on the same day.

Sediment-column mesocosms

Twelve sediment-column mesocosms were constructed from transparent acrylic pipes (35 cm × 4.6 cm internal diameter; Fig. 1a) to allow direct observation of individuals. Columns were sealed at the base by a rubber bung with a 5-mm glass tube (3 mm internal diameter) in the centre to allow drainage. A silicon tube was secured over the glass tube and a Hoffman clip allowed control of the water level to within 0.5 mm (Fig. 1a).

Columns were mounted onto retort stands (Fig. 1b) within an environmental cabinet (108 cm × 27 cm × 68 cm). The front wall of the cabinet was covered with a black cloth to maintain darkness and provide lighting conditions analogous to the subsurface streambed whilst allowing an observer to inspect the columns inside.

Sediment treatments

Mixtures of three transparent particle types were used to create six sediment treatments of varying pore-size volumes: small (10 – 15 mm) angular particles; large (20 – 25 mm) angular particles; and large (14 – 20 mm) smooth particles (Table 2). The difference in interstitial volume between sediment treatments allowed examination of the effect of sediment porosity on the vertical movement by species. The interstitial volume was calculated by measuring the amount of free water within the sediment-filled columns five times to provide a mean and standard error for each sediment treatment (Table 2). Sediment treatments containing multiple particle types were thoroughly mixed prior to use, randomly distributed throughout the columns and filled to a depth of 25 cm (Fig. 1b).

Water treatment and depth control

Tap water was pre-treated with AquaSafe® (Tetra®, Virginia) to neutralise any residual chemicals and cooled to 11°C over a 24-h period prior to the commencement of experiments. Dissolved oxygen was measured directly in the surface water at the start of experiments and oxygen saturation was maintained throughout each experiment using oxygen tablets, widely used in domestic aquaria.

Water was added to each column to 5 cm above the sediment surface (Fig. 1b). Water level was then reduced in 12.5 mm increments every 15 min over a 5 h experimental period until a 5 cm depth of water (i.e., 20 cm below the sediment surface) was retained in each column at the end of each trial as a refuge. This rapid rate of drying is analogous to that experienced on topographic high points (riffles and marginal gravel bars) in streams with permeable sediments where upstream anthropogenic structures (e.g., weirs and spillways) control the volume of discharge and flow can be effectively cut off when a low flow threshold is crossed.

Experimental procedure

One individual from the same species was introduced into each water-filled column. Individuals were left to acclimatise within the columns and environmental cabinet prior to the start of an experiment until they ceased active exploration and burrowing behaviour (preliminary experiments indicated approximately 20 min were sufficient). Following acclimatisation, macroinvertebrates were observed within the cabinet using an LED light to minimise disturbance to each column during the experiment. Initial vertical movement from the sediment surface (depth = 0) into the subsurface was recorded (1 mm accuracy) at this time (time 0 = 5 cm surface water). Individuals within the water column or on the sediment surface were recorded as having a vertical movement of 0 mm. Prior to each water level reduction, the vertical position of the individual in each column (mm below depth 0) was recorded.

Once the water level had been reduced to 20 cm below the sediment surface for 15 min, the final depth reached by the macroinvertebrate beneath the substrate surface was recorded and the experiment terminated. The difference between the depth of individuals at the start of the experiment (time = 0) and the final depth reached beneath the substrate surface at the end of the experiment (time = 5 h) determined their absolute depth moved (i.e., total vertical movement during experiment). The sediment columns were deconstructed and their sediments removed and washed thoroughly to remove live test macroinvertebrates, debris and biological waste. Macroinvertebrates were then euthanized and preserved using 70% industrial methylated spirit (IMS).

To distinguish between active and passive movements associated with water level reduction, experiments were repeated using the cadavers of the same individuals as used in live experiments, which were rinsed thoroughly in tap water prior to reintroduction into reconstructed sediment treatments. If a cadaver became stranded above the water-line during the water level reduction, the depth was recorded, the experiment terminated, and the individual retrieved from the column and preserved in IMS. Each experiment (live and cadaver) was replicated 20 times for each of the six sediment treatments, providing 240 experimental trials using 120 individuals of each species.

Head widths of individuals were measured and calibrated using Motic® Images Plus 2.0 software (Motic®, Hong Kong), as a surrogate for body size (Smock, 1980). Head width measurements were standardised within species, being taken from the base of the antenna to the posterior margin of the head carapace for *G. pulex* and *A. aquaticus*, and as the widest cross section of the head capsule for *Hy. siltalai*, *N. cambrica* and *H. sulphurea*.

Data analysis

We tested our first hypothesis, that movement into the subsurface would be active rather than passive, using paired sample t-tests to compare the absolute depth moved (difference between depth of an individual at time = 0 and at the end of the experiment) by live and cadavers of the same individuals for each species and sediment treatment.

We tested our second and third hypotheses, that vertical movements through the subsurface would be influenced by sediment characteristics and would vary between species, using a General Linear Model (GLM) and a Binary Logistic Regression (BLR, using the logit link function). We defined macroinvertebrate responses to surface water loss and water level reduction in three ways: final depth reached, absolute depth moved, and likelihood of becoming stranded. A GLM was used to examine the effect of sediment treatment, species identity, and their interaction, on the final depth reached and absolute depth moved by live individuals. To examine and account for the potential influence of individual body size on final depth reached, head width was included in the model as a covariate, nested within species. The model was fitted using Maximum Likelihood Estimates (MLE). Fisher’s LSD *post-hoc* tests were used where significant effects of sediment treatment (hypothesis ii) or species (hypothesis iii) were detected.

BLR analysis was used to determine the effect of sediment treatment and species identity on the likelihood of live individuals becoming stranded above the water-line. Individuals were classified into two groups: stranded (coded as ‘0’) or below the water-line (not stranded; coded as ‘1’). In all cases, the BLR model had a good predictive capacity (correct classification rate 84.4%) and fit (Nagelkerke $R^2 = 0.672$). The BLR model was run multiple times to create a pairwise BLR model. *Nemoura cambrica* was excluded from the BLR analysis because no individual of this species became stranded during the live experiments, resulting in no variation in the response for the BLR to model. All analyses were performed using IBM SPSS Statistics (version 23, IBM Corporation, New York).

Results

Active vs passive movement of benthic macroinvertebrates into the subsurface sediments in response to water level reduction

Live individuals of each species reached greater absolute depths than their cadavers, which remained close to the sediment surface (Table 3). Live individuals within each sediment treatment reached greater absolute depths compared to their cadavers (Table 3).

Effect of sediment treatment, species identity and body size on the final depth reached by live individuals

The effect of sediment treatment on the final depth reached beneath the substrate surface by individuals varied significantly between species (i.e. the interaction term was significant; GLM, $P < 0.001$). The final depth reached in each sediment treatment was dependent on species identity. The pattern of final depth reached for each species within each sediment treatment generally increased from sediment treatment 1-6 with increasing interstitial volume (Table 2), however, final depth reached in each sediment treatment decreased for each species from *N. cambrica*, *A. aquaticus*, *G. pulex*, *Hy. siltalai* to *H. sulphurea*, respectively (Fig. 2). Within each species, body size had no effect on final depth reached (GLM, $P = 0.179$).

Effect of sediment treatment and species identity on the absolute depth moved

The effect of sediment treatment on the absolute depth moved by individuals varied between species (i.e. the interaction term was significant; GLM, $P < 0.01$). Specifically, the absolute depth moved by *N. cambrica* individuals were significantly greater than: *A. aquaticus* individuals in sediment treatments 1-4 and 6; *Hy. siltalai* individuals in sediment treatments 1, 2 and 4; and *H. sulphurea* individuals in sediment treatments 1-4 (see Table S1 in Supporting Information). The absolute depth moved by *G. pulex* individuals were significantly greater than: *A. aquaticus* individuals in sediment treatments 4-6; *Hy. siltalai* individuals in sediment treatments 3 and 4; and *H. sulphurea* individuals in sediment treatments 1-4 (see Table S1 in Supporting Information). Body size had no effect on the absolute depth moved (GLM, $P = 0.401$).

Effect of sediment treatment and species identity on the stranding of live individuals

Sediment treatment affected the likelihood of individuals being stranded (BLR, $P < 0.001$). Individuals were most likely to become stranded in sediment treatment 1 followed by 2-4 (not significantly different), then 5, and finally treatment 6 (Fig. 3a). More specifically, the likelihood of individuals becoming stranded differed between sediment treatments 1, 5 and 6 (pairwise BLR, $P < 0.05$). In addition, the likelihood of stranding in sediment treatments 1, 5 and 6 differed (pairwise BLR, $P < 0.05$) from the likelihood of stranding in treatments 2, 3 or 4 (for which the likelihood of stranding was comparable; pairwise BLR, $P > 0.05$).

Species identity also had a significant effect on the likelihood of individuals becoming stranded (BLR, $P < 0.001$). Individuals of *H. sulphurea* were most likely to become stranded followed by *Hy. siltalai*, *G. pulex* and finally *A. aquaticus* (pairwise $P < 0.05$; Fig. 3b); no *N. cambrica* individuals became stranded in any treatment (Table 4 and Fig. 2a). The majority of *G. pulex* individuals stranded in sediment treatment 1 (70%) and < 50% were stranded in treatments 2 and 3 (Table 4 and Fig. 2b). Over half of *A. aquaticus* individuals were stranded in sediment treatment 1 (Table 4 and Fig. 2c) and ≤ 10% became stranded in treatments 2 and 3. The majority of *Hy. siltalai* individuals became stranded during water level reduction across all sediment treatments (except treatment 6 = 45%; Fig. 2d) and all *H. sulphurea* became stranded in sediment treatments 1-3 with ≤ 60% stranded in treatments 4-6 (Table 4 and Fig. 2e).

Discussion

Benthic macroinvertebrates actively move into the subsurface during dewatering

We found support for our first hypothesis, that faunal movement into the subsurface in response to dewatering would be active. Studies have recorded benthic macroinvertebrates relatively deep within the hyporheic zone when surface sediments dry (e.g., Fenoglio et al., 2006; Young et al., 2011). *Agabus paludosus* (Coleoptera: Dytiscidae), for example, has been recorded at 70 – 90 cm below the streambed surface in response to drying (Fenoglio et al., 2006). It has been suggested that individuals recorded deep within riverbed sediments have moved there (actively or passively), via interstitial pore spaces. However, in the absence of direct observation and being able to track individuals, it has not previously been possible to determine if these movements were active (macroinvertebrates moving vertically to remain submerged) or passive (being drawn down with the receding water-line). Comparison of our direct observations of live individuals and cadavers in response to water level reduction in transparent sediment-column mesocosms provide the first definitive evidence that vertical movements are active, not passive.

Effect of sediment treatment on the vertical movements of benthic macroinvertebrates

We found support for our second hypothesis, that subsurface sediment characteristics would influence the ability of individuals to move vertically in response to water level reduction. Sediments with lower interstitial volume (sediment porosity) due to smaller particle sizes reduced the vertical movements of individuals of all species except *N. cambrica*. This supports previous studies which found that sediment characteristics influence the use of subsurface sediments by a range of benthic macroinvertebrate species (Stubbington et al., 2011; Descloux et al., 2013; Vadher et al., 2015).

Previous studies have indicated that sediment characteristics such as interstitial pore volume influence the ability of macroinvertebrate taxa to move into subsurface sediments and that movement would reflect species traits (Boulton et al., 1998; Gayraud & Philippe, 2003; Vadher et al., 2015; Mathers & Wood, 2016). Small particles with reduced sediment porosity (i.e. fine sediment deposited on the sediment surface or those subject to sedimentation within the sediment matrix) potentially limit the function of the subsurface as a refuge for macroinvertebrates following surface water loss (Navel et al., 2010; Descloux et al., 2013; Vadher et al., 2015) and studies have reported the absence or reduced use of subsurface sediments due to the limited interstitial pore spaces available (Boulton 1989; Richards & Bacon, 1994; Smock et al., 1994; James et al., 2008). Our observations provide direct evidence to support studies which have inferred that sediment characteristics limit the movement of benthic macroinvertebrates into the subsurface during adverse conditions (e.g. Stanley et al., 1994; Smock et al., 1994; Olsen & Townsend, 2005; Stubbington et al., 2011).

Species-specific vertical movements of individuals through sediments

Our results demonstrate that vertical movement varies between species, reflecting differences in traits and habitat affinities. This supports our third hypothesis, that the vertical movement of species through the subsurface would be influenced by their traits. The response to sediment porosity of the species examined was similar; however, the absolute vertical distance moved by the five species differed. The family-level trait designation of *Nemoura* suggests that species in this genus have no affinity with the subsurface (Table 1; Tachet et al., 2010), but our results suggest that this species *is* able to move into the sediments in response to water level reduction. In our study *N. cambrica* was able to freely move into the subsurface, in response to water level reduction and has also been recorded in temporary streams (Stubbington et al., 2009). *Nemoura cambrica* has a small body size compared to the other species used in our experiments and can burrow and excavate itself from fine sediment deposits (Wood et al., 2005b). A reduced size of mature nymphs can promote invertebrate resistance in subsurface habitats (Gayraud & Philippe, 2001; Navel et al., 2010; Vander Vorste et al., 2016b) and may explain why *N. cambrica* did not become stranded above the water-line and moved freely through interstitial pore spaces in all experimental treatments. Although we did not detect an effect of body size on the vertical movement within individual species, the body size differences between species probably influences vertical movements.

Most *G. pulex* and *A. aquaticus* individuals moved vertically in all sediment treatments except the smallest particle size treatment. This observation advances the experimental findings of Vander Vorste et al. (2016a), who observed that *G. pulex* used the subsurface as a refuge in response to water level reduction, and Vadher et al. (2015), who found that *G. pulex* were unable to use the subsurface when sediment porosity was reduced. In marked contrast, *Hy. siltalai* and *H. sulphurea* displayed limited ability to move vertically into the subsurface in response to water level reduction. Both these taxa are primarily associated with benthic habitats in perennial streams, and a low affinity to intermittence (Eyre et al., 2005; Wood et al., 2005a, Table 1) may mean that *Hy. siltalai* and *H. sulphurea* lack behavioural adaptations to move strongly into the subsurface sediments in response to drying.

The net-spinning caseless caddisfly larvae *Hy. siltalai* and the free-living mayfly nymph *H. sulphurea*, became stranded in most sediment treatments during water level reduction. *Hydropsyche siltalai* larvae are benthic filter-feeders and *H. sulphurea* larvae are benthic grazers (Table 1). These feeding traits mean that these taxa typically occupy microhabitats close to, or on, the sediment surface where algal growth is most abundant and filter-feeding is most efficient. As a result, they may not typically move vertically into the subsurface as trophic resources would be reduced. However, even for these species, around half of *Hy. siltalai* individuals moved to the column base in the coarsest sediments with the largest interstitial pore space (treatments 5 and 6) and 60% of *H. sulphurea* remained submerged in the largest sediments (treatment 6) suggesting that in rivers with coarse sediments and open gravel frameworks, stranding may be reduced and vertical movement possible to enhance the use of the subsurface refuge during streambed drying. These results provide evidence to support studies indicating reduced invertebrate species diversity within streams which have experienced surface water loss and drying (Extence, 1981; Feminella, 1996; Datry, 2012; Bogan et al., 2013) and clearly highlights the reduced vertical movement of some species typically associated with benthic habitats.

Conclusions and future directions

Hydrological extremes within streams may become increasingly common as climate change (Ledger & Milner, 2015; Pyne & Poff, 2017) and water resource pressures interact to increase the duration of dry phases in some regions (Datry et al., 2014b). This study highlights the variation in species responses to simulated water level reduction in sediments with different characteristics. We highlight the need to understand species-specific responses in relation to differences in sediment characteristics among streams. Although the subsurface sediments of the hyporheic zone can be an extensive refuge in gravel-bed rivers (Vander Vorste et al., 2016c), they are naturally heterogeneous and can also be a patchy refuge (Dole-Olivier et al., 1997). In some places, the hyporheic zone can be limited in spatial extent due to reduced interstitial habitat availability as a result of small particle sizes limiting access to the subsurface for many taxa (Gayraud & Philippe, 2003). Furthermore, anthropogenic activity (e.g., mining and flow regulation) may result in enhanced fine sediment deposition, further reducing the ability of subsurface sediments to function as a refuge (Descloux et al., 2013; Vadher et al., 2015).

Our results also highlight the need for effective refuge management and maintenance of sediment porosity in streams as active movements made by macroinvertebrates into the subsurface sediments could potentially enhance recovery from drying events and may maintain species abundance and diversity. Such management strategies should include measures of reduce fine sediment inputs to river channels via the use of sediment detention ponds/wetlands and more effective planting of riparian vegetation (buffer strips) to reduce sediment transport and help stabilize river banks, especially in agricultural areas (Verstraeten & Poesen, 2000; Hughes, 2016). In some instances, where fine sediment inputs are high and river flows are insufficient to flush fines from the interstices of the riverbed, gravel cleaning may be required to reconnect benthic and hyporheic habitats, improve subsurface water quality and ultimately increase sediment porosity and hydrological connectivity (Meyer et al., 2008). Developing effective management strategies is essential if the future of ‘drying refuges’, such as the hyporheic zone, is to increase community resistance and resilience to stream drying. However, the ability of macroinvertebrates to migrate back to the surface and recolonize benthic habitats as water levels rise remains uncharacterized. Future research should, where possible, combine field and laboratory mesocosm-based approaches to validate observations and facilitate a greater understanding of community and individual responses to the processes of streambed drying and flow resumption.

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Figure legends

Fig. 1. Sediment column mesocosms. (a) Cross-section through a sediment column: i) acrylic pipe; ii) rubber bung; iii) 5 mm glass tube; iv) silicon tubing; v) Hoffman clip to control water drainage. (b) The six sediment treatments at the start of experiments (5 cm surface water). Sediment treatments are as described in Table 2. Not to scale.

Fig. 2. Mean vertical movement of live individuals in response to water level reduction in each of six sediment treatments. (a) *Nemoura cambrica*; (b) *Gammarus pulex*; (c) *Asellus aquaticus*; (d) *Hydropsyche siltalai*; (e) *Heptagenia sulphurea*. Sediment treatments are as described in Table 2.

Fig. 3. Percentage of live individuals stranded (a) in each sediment treatment and (b) by species. a-d indicate statistically different values (Binary Logistic Regression, $P < 0.05$). Sediment treatments are as described in Table 2.

Tables

Table 1. Family-level biological traits of the five study taxa relevant to subsurface movement and ecological traits (adapted from Tachet et al., 2010)

	Biological Traits						
Genus	Max. body size (mm)	Locomotion	Habitat type	Flow velocity	Feeding group	Temporary stream affinity	Subsurface affinity
Nemoura	5 – 10	Crawler	Lotic	Medium – fast	Shredder	Moderate	None
Asellus	10 – 20	Crawler	Lentic / Lotic	None – slow	Shredder	None	Low
Gammarus	20 – 40	Swimmer / Crawler	Lotic	Slow - medium	Shredder	Low	Low
Hydropsyche	20 – 40	Crawler	Lotic	Medium	Filter-feeder	None	None
Heptagenia	10 – 20	Crawler	Lotic	Medium – fast	Scraper	None	None

Table 2. Description of sediment treatment composition, pore-size volume and the mean depth (± SE) which macroinvertebrates reached at the end of experiments

Sediment treatment	Particle size composition	Pore-size volume (ml)	Mean species depth at experiment end (mm)
1	100% small angular particles	145.6 ± 1.29	129.3 ± 10.5
2	60% small angular and 40% large angular particles	150 ± 0.63	159.5 ± 10.9
3	50% small angular and 50% large angular particles	151.6 ± 1.21	160.2 ± 10.5
4	33% small angular, 33% large angular and 33% large smooth particles	155.2 ± 1.46	173.8 ± 10.3
5	100% large smooth particles	158.6 ± 1.08	195 ± 8.9
6	100% large angular particles	186.4 ± 1.57	216.5 ± 6.8

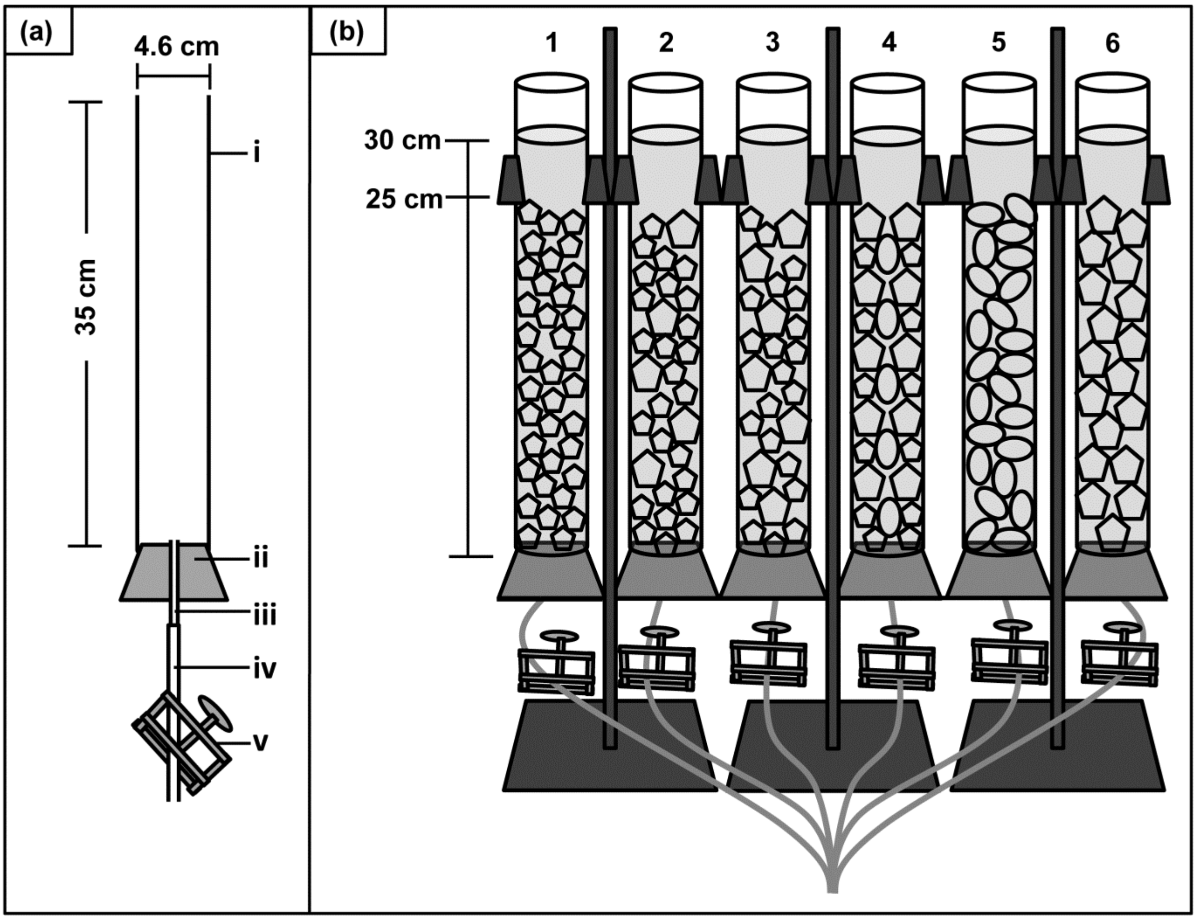
Table 3. Paired sample t-test analysis between the absolute depth moved by live individuals and cadavers for each species and within each sediment treatment (see Table 2).

	Live	Cadaver			
	Mean absolute depth moved (mm)	Mean absolute depth moved (mm)	d.f.	t	*P*
Species					
Nemoura cambrica	158.7 (± 8.2)	1 (± 0.3)	119	19.139	<0.001
Asellus aquaticus	150 (± 8.5)	4 (±1.3)	119	17.038	<0.001
Gammarus pulex	131.7 (± 8.7)	1.8 (± 0.5)	119	14.719	<0.001
Hydropsyche siltalai	62.8 (± 6.3)	2.3 (± 0.5)	119	9.623	<0.001
Heptagenia sulphurea	39.4 (± 5)	1.6 (± 0.4)	119	7.606	<0.001
Sediment treatment					
1	105 (± 9.5)	0.2 (± 0.1)	119	10.999	<0.001
2	130.4 (± 9.9)	1 (± 0.4)	119	12.954	<0.001
3	106.6 (± 9.6)	0.3 (± 0.1)	119	11.094	<0.001
4	118 (± 9.9)	0.6 (± 0.2)	119	11.895	<0.001
5	96.2 (± 9.3)	4 (± 0.7)	119	9.959	<0.001
6	96.1 (± 8.6)	6.8 (± 1.6)	119	10.374	<0.001

Table 4. Percentage of individuals stranded above the water level during dewatering in each sediment treatment (see Table 2)

	% Stranded in sediment treatments						
Species	1	2	3	4	5	6	All treatments
Nemoura cambrica	0	0	0	0	0	0	0
Asellus aquaticus	55	10	5	0	0	0	12
Gammarus pulex	70	30	40	0	0	0	23
Hydropsyche siltalai	80	75	80	95	55	45	72
Heptagenia sulphurea	100	100	100	95	95	60	92
All species	61	43	45	39	30	21	

Figure 1



687 Figure 2

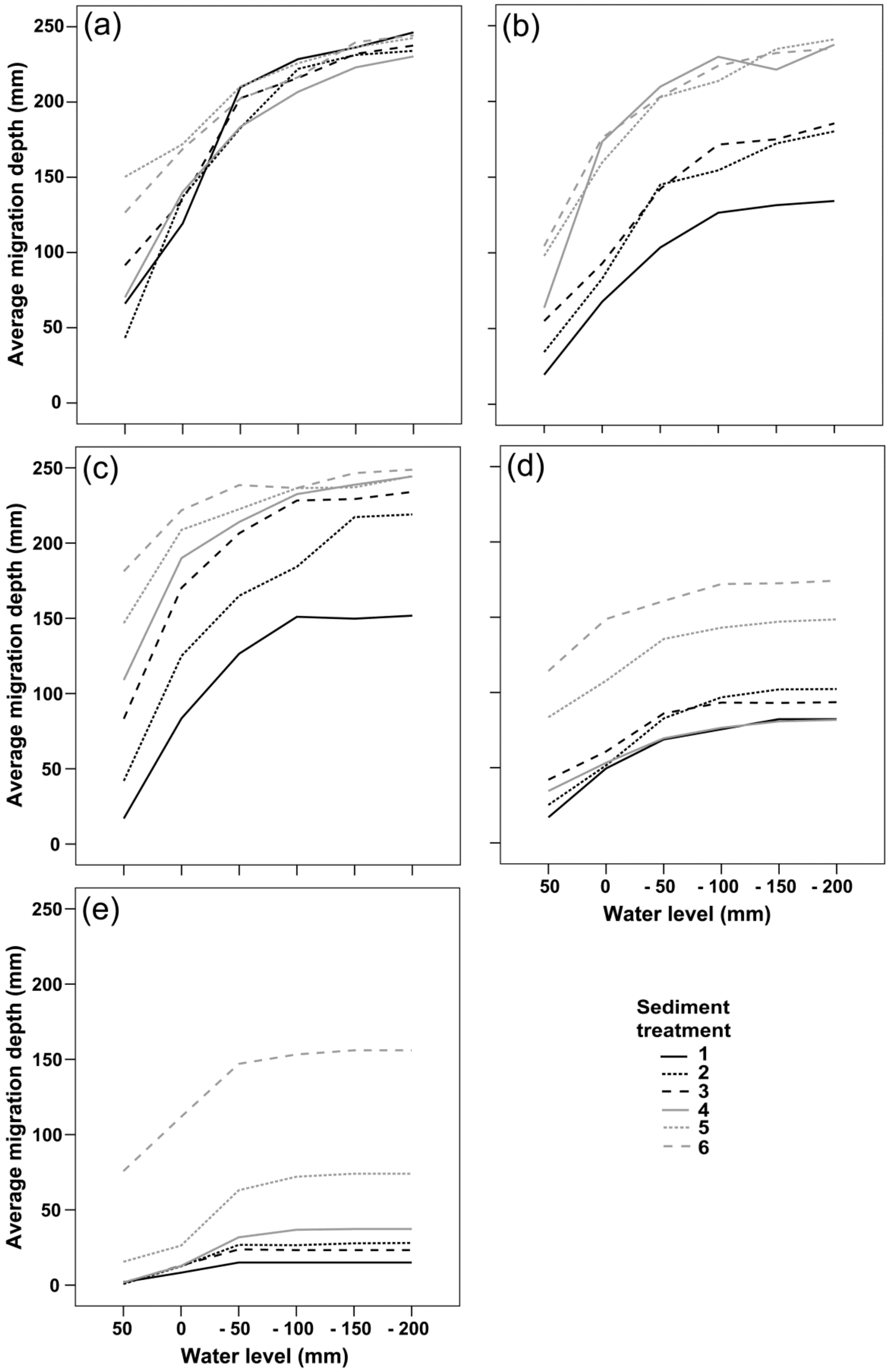


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Figure 3

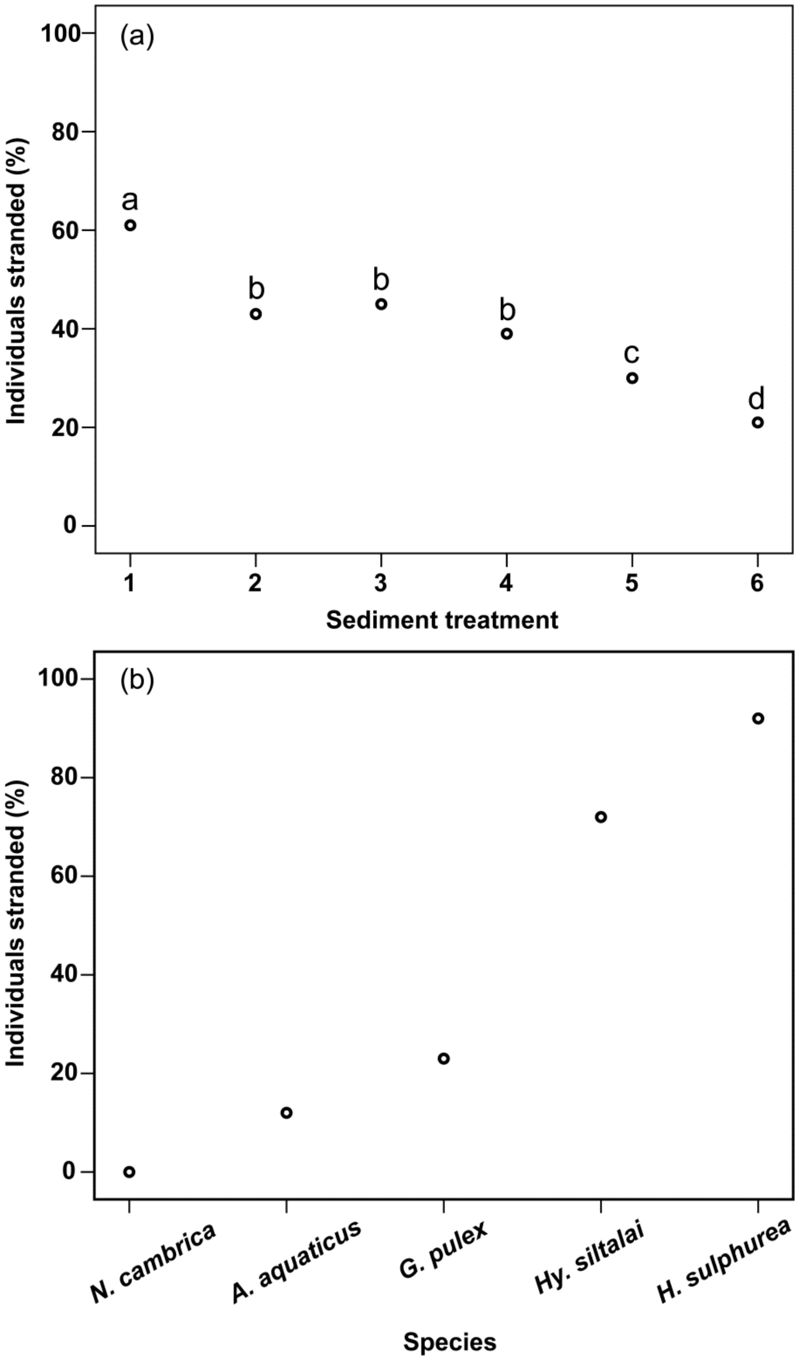
(a)
Individuals stranded (%)
100
80
60
40
20
0
a
b
b
b
c
d
1
2
3
4
5
6
Sediment treatment
(b)
Individuals stranded (%)
100
80
60
40
20
0
N. cambrica
A. aquaticus
G. pulex
Hy. siltalai
H. sulphurea
Species

Table S1. Fisher's LSD post-hoc pairwise comparison of absolute depth moved by live individuals between each species (*Nemoura cambrica*, *Gammarus pulex*, *Asellus aquaticus*, *Hydropsyche siltalai* and *Heptagenia sulphurea*) for each sediment treatment. Significant depths ($P \leq 0.05$) are emboldened. '↑' indicates the taxon listed is significantly higher than the taxa being compared. Sediment treatment 1) 100% small angular 2) 60% small angular 40% large angular 3) 50% small angular 50% large angular 4) 33% small angular, 33% large angular and 33% large rounded 5) 100% large rounded 6) 100% large angular.

Sediment Treatment 1	*G. pulex*	*A. aquaticus*	*Hy. siltalai*	*H. sulphurea*
N. cambrica	0.148	↑ **0.016**	↑ **0.050**	↑ **0.009**
G. pulex		0.073	0.261	↑ **0.034**
A. aquaticus			0.256	0.835
Hy. siltalai				0.238

Sediment Treatment 2	*G. pulex*	*A. aquaticus*	*Hy. siltalai*	*H. sulphurea*
N. cambrica	0.197	↑ **0.028**	↑ **0.042**	↑ **0.007**
G. pulex		0.093	0.135	0.016
A. aquaticus			0.430	0.948
Hy. siltalai				0.267

Sediment Treatment 3	*G. pulex*	*A. aquaticus*	*Hy. siltalai*	*H. sulphurea*
N. cambrica	0.446	↑ **0.043**	↑ **0.070**	↑ **0.025**
G. pulex		0.060	↑ **0.050**	↑ **0.019**
A. aquaticus			0.409	0.873
Hy. siltalai				0.388

Sediment Treatment 4	*G. pulex*	*A. aquaticus*	*Hy. siltalai*	*H. sulphurea*
N. cambrica	0.637	↑ **0.019**	↑ **0.032**	↑ **0.021**
G. pulex		↑ **0.011**	↑ **0.003**	↑ **0.006**
A. aquaticus			0.370	0.670
Hy. siltalai				0.581

Sediment Treatment 5	*G. pulex*	*A. aquaticus*	*Hy. siltalai*	*H. sulphurea*
N. cambrica	0.876	0.051	0.369	0.244
G. pulex		↑ **0.010**	0.066	0.064
A. aquaticus			0.121	0.298
Hy. siltalai				0.612

Sediment Treatment 6	*G. pulex*	*A. aquaticus*	*Hy. siltalai*	*H. sulphurea*
N. cambrica	0.800	↑ **0.013**	0.203	0.236
G. pulex		↑ **0.004**	0.069	0.164
A. aquaticus			0.061	0.112
Hy. siltalai				0.910