Factors limiting the vertical movement and use of subsurface sediments by lotic macroinvertebrates in response to dewatering and surface drying

by

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Abstract

Climate change projections suggest increased frequency and duration of streambed drying in many regions across the globe. These predictions even apply to streams in temperate environments, which are typically characterised by perennial river flow. The subsurface sediments of streams are an important refuge for benthic invertebrates during streambed drying; this role may become more important given the predicted increased future extent and duration of stream drying. However the vertical movement of organisms into subsurface and hyporheic sediments, and factors affecting this movement, has received limited attention historically. The research in this thesis employed a series of laboratory experiments to examine the impact of sedimentological characteristics (particle size, porosity, sedimentation) on the vertical movement of macroinvertebrates. This research also used tightly controlled flume and field experiments to examine how the duration of drying events affected macroinvertebrate survival. Specifically, the laboratory studies used direct observation in transparent artificial mesocosms containing different sediment sizes and interstitial pore space volumes to examine the subsurface vertical movements of five lotic taxa displaying different biotic traits in response to water level reduction. The results indicated that lotic macroinvertebrates actively moved into subsurface sediments in response to water level reduction. The extent to which taxa moved vertically through sediments was: i) species-specific, and ii) restricted by sediment porosity. In addition, the research examined the effect of varying loads of fine sediment (particles < 2 mm) on the vertical movement of the freshwater shrimp, *Gammarus pulex,* through transparent mesocosms during water level reduction. The results demonstrate the limiting effect of fine sediment load and size on the ability of *G. pulex* to remain submerged as sand particles (0.5 - 1 mm) capable of bridging interstitial pathways into the subsurface impeded and prevented the movement of individuals into the subsurface. Finally, this thesis presents the results of flume and field mesocosm studies which examine the effect of surface water loss duration on the survivorship of *G. pulex* within the subsurface. These experiments demonstrate how increasing drying duration and variability in water quality can significantly reduce faunal survival within subsurface sediments. The results and synthesis illustrate the value of laboratory / mesocosm-based research and effective riverbed management to ensure instream ecology can access and utilise the vital hyporheic refuge in the face of increased drying due to climate change and anthropogenic management.

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Chapter 1 Literature Review & Introduction

1.1 Why are freshwater ecosystems important?

Approximately 70% of the Earth’s surface is covered by water (National Geographic Society, 2010). The majority of this water is saline, approximately 97.5%, leaving just 2.5% available as freshwater (Dudgeon et al., 2006; Fig. 1.1). Most of the freshwater is stored as ice in glaciers and snowfields leaving approximately 1% of the freshwater in an accessible form to species and only 0.006% of the Earth’s freshwater flowing in rivers (National Geography Society, 2010). Freshwater ecosystems cover just 0.8% of the world’s surface but support over 100,000 aquatic species (Dudgeon et al., 2006). Considering the relative scarcity of freshwater habitats and the aquatic biodiversity dependent on them, it is important to understand the structure and functioning of freshwater ecosystems and their role in sustaining global biodiversity.

Inland freshwater systems are important to human society, nation states and governments as they provide an economic, scientific, educational, aesthetic and cultural value (Dudgeon et al., 2006). However, the conservation and management of inland freshwater is critical as increasing anthropogenic demand for water is having detrimental effects on global freshwater biodiversity resulting in a marked increase in the rate of species loss (Dudgeon et al., 2006; Strayer & Dudgeon, 2010). The negative effect of anthropogenic activity on freshwater resources includes overexploitation, water pollution, flow modification, habitat destruction and non-native species invasion. These impacts have left freshwater systems vulnerable to severe degradation (Allan, 1995; Dudgeon et al., 2006; Strayer & Dudgeon, 2010; Vörösmarty et al., 2010). Furthermore, anthropogenic driven climate change is also affecting freshwater ecosystem structure and functioning via modifications to the hydrologic cycle and natural flooding and drying regimes, often causing an irreversible increase in the frequency and duration of extreme events (Grimm et al., 2013; Chadd et al., 2017).

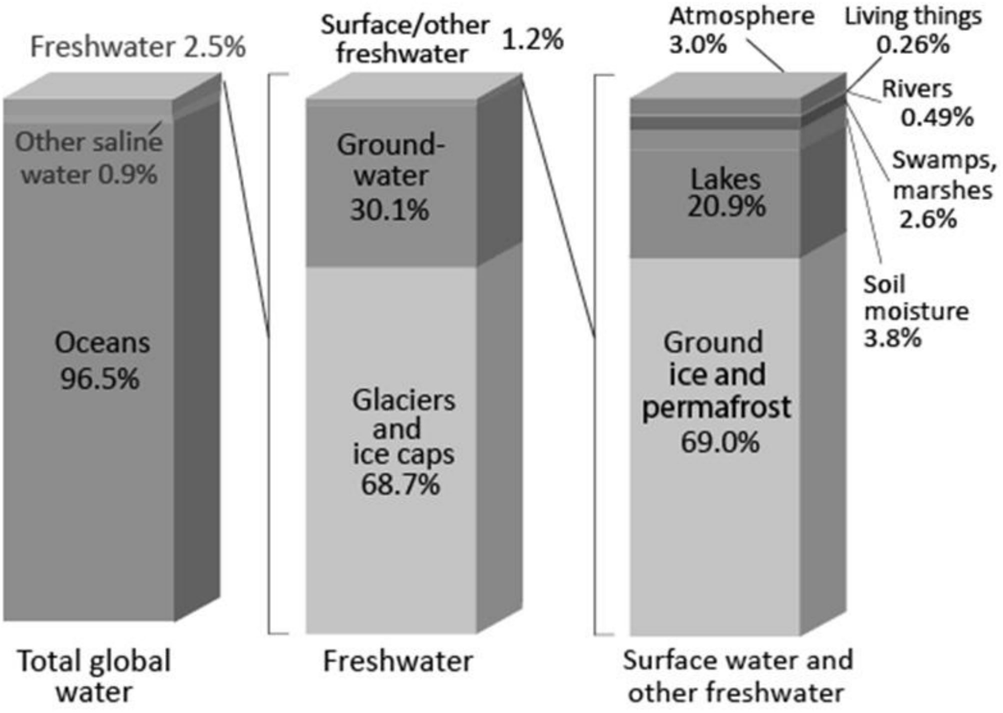


Fig. 1.1 Global distribution of water on Earth (sources: Shiklomanov, 1993; USGS, 2016). Percentages are rounded, so may not add to 100%.

1.2 Introduction to stream ecosystem connectivity

Stream systems are dynamic and can be conceptualised in a four-dimensional framework comprising a longitudinal, lateral, vertical and temporal dimension (Ward, 1989). The River Continuum Concept describes changes in both the physical characteristics, and ecosystem structure and functioning of streams from upstream to downstream reaches (longitudinal connectivity; Vannote et al., 1980). The River Continuum Concept predicts that headwaters are typically low order (small), cool and shaded by riparian vegetation with primarily allochthonous energy inputs from leaf litter (Vannote et al., 1980). The invertebrate community of stream headwaters, therefore, are expected to be composed of shredders and collectors which breakdown the coarse particulate organic matter of external vegetation into fine particulate organic matter (Vannote et al., 1980). As stream size increases in the mid-reaches of streams, shading and the importance of allochthonous inputs reduces and coincides with autochthonous primary production and a shift from shredder and collector dominated communities to collector and grazing dominated communities (Vannote et al., 1980). Finally, in the wide channels of the lower reaches, energy inputs are primarily received from upstream sources in the form of processed fine particulate organic matter which primarily supports invertebrate communities dominated by collectors (Vannote et al., 1980).

The lateral dimension of stream ecosystems encompasses the movements of organisms, nutrients and organic matter between the channel, riparian zone and floodplain (Ward, 1989). This connectivity is enhanced on the surface during flood events where the floodplain is inundated and directly connecting floodplain water bodies to the channel (Ward, 1989). The final physical dimension, the vertical dimension, of stream ecosystems encompasses ecological connectivity and processes between the surface water, hyporheic zone and groundwater (Ward, 1989). The fourth, temporal, dimension encompasses the changes in processes associated to the three physical dimensions over time (Ward, 1989). In addition to maintaining the vertical connectivity between the surface and groundwater of streams, the hyporheic zone in three-dimensions, depending on sediment characteristics, can maintain longitudinal and lateral connectivity through the subsurface hyporheic corridor (Stanford & Ward, 1993). The hyporheic zone and vertical connectivity is outlined in section 1.6. This thesis will focus on the vertical dimension of streams, examining the movement of organisms into, and the use of, subsurface sediments in response to drying over time (temporal dimension).

1.3 Introduction to streambed drying and temporary streams

Temporary streams are streams that cease to flow and often experience periods of streambed drying (Bogan et al., 2015; Leigh & Datry, 2017). More than half of the channels within river networks across the globe are temporary (Acuna et al., 2014; Datry et al., 2014a), with over 80% of streams being temporary in some regions (Sabater & Tockner, 2010; Bogan et al., 2015). The frequency and duration of drying events in temporary steams is expected to increase as a result of climate change and anthropogenic demand on freshwater systems (Jaeger et al., 2014; Ledger & Milner, 2015; Pyne & Poff, 2017). Consequently, historically perennial rivers of global significance including the Colorado River (USA) and the River Nile (Egypt) no longer flow continuously. The shift of perennial systems to intermittent systems in expected to increase (Datry et al., 2016a; Pyne & Poff, 2017). Temporary streams have, therefore, received increasing research interest, with the number of published research papers centred on this area increasing rapidly in recent times (Datry et al., 2016a; Leigh & Datry, 2017; Stubbington et al., 2017)

Seasonal disturbances, such as drying in temporary streams, are important processes which maintain the community structure of streams and rivers (Gasith & Resh, 1999; Datry et al., 2016b; Smith et al., 2017). Although the majority of temporary streams experience a cyclical and predicable dry-phase (Gasith & Resh, 1999), drying can occur over multiple seasons (supra-seasonally) leading to significant mortality of non-temporary water species (Lake, 2003; Williams, 2006). A range of studies from across the globe have demonstrated reduced ecological complexity (species richness and abundance) in streams which experience significant flow reductions or flow cessation (e.g., Aguiar et al., 2002; Smith & Wood, 2002; Meyer et al., 2003; Storey & Quinn, 2008; Datry, 2012), compared to perennially flowing streams (e.g., del Rosario & Resh, 2000; Rüegg & Robinson, 2004; Wood et al., 2005; Storey & Quinn, 2008). However, despite the often reduced macroinvertebrate abundance during increases in flow intermittency, a range of studies have reported species beta diversity being maintained (Boulton & Suter, 1986; Feminella, 1996; Storey & Quinn, 2008; Datry et al., 2014b). This is likely due to systems frequently affected by the loss of flow and surface drying promoting communities adapted to temporary stream conditions (Thompson & Townsend, 1999; Gibbins et al., 2001). The ways in which organisms are adapted to the conditions experienced in temporary streams are highlighted below in the section 1.4.

1.3.1 The effect of stream drying on stream connectivity and community

Temporary streams experience a loss of connectivity along three physical dimensions (lateral, longitudinal and vertical; Lake, 2000; Bogan et al., 2015). As discharge begins to decline, stream width constricts laterally within the channel, becoming isolated from the floodplain and riparian edges (Fig. 1.2; Lake, 2003). This disconnects the channel from riparian vegetation used as food, shelter or an emergence platform for aquatic macroinvertebrates, and may affect taxa dependent on lateral connectivity including Odonata and Lepidoptera (Boulton & Lake, 2008; Chadd et al., 2017). Following the loss of lateral linkages, streamflow typically disconnects longitudinally (Fig. 1.2), usually as a result of reduced discharge and streambed topography creating a series of connected and isolated pools (Lake, 2003; Verdonschot et al., 2015). This phase significantly affects taxa which require the current for respiration and food, and therefore, favours lentic macroinvertebrates causing taxa including Hydropsychidae and other filter-feeders to be eliminated (Boulton & Lake, 2008; Chadd et al., 2017). Finally remnant pools begin to dry; surface water constricts and recedes into the streambed, resulting in the loss of vertical connectivity between the surface water and groundwater (Fig. 1.2; Lake, 2003; Boulton, 2007). This loss in vertical connectivity typically causes the elimination of taxa sensitive to poor water quality and habitat decline (Boulton & Lake, 2008; Chadd et al., 2017).

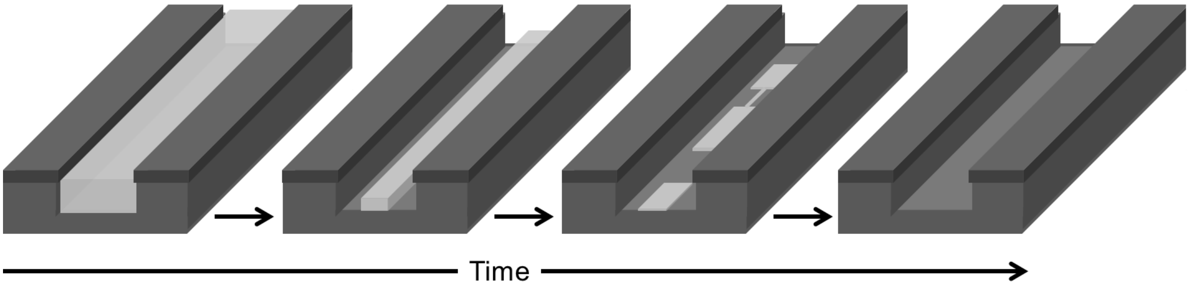


Fig. 1.2 Conceptual model showing how streams typically dry over time at the surface through the loss of the lateral, longitudinal and vertical dimensions.

During drying events, studies have demonstrated that rheophilic and desiccation-sensitive taxa are typically eliminated (e.g., Boulton & Lake, 2008; Bogan & Lytle, 2011; Graeber et al., 2013). Organisms tolerant of reduced flow velocities or zero flow conditions become constricted within remnant pools where biotic interactions (e.g., competition for space and predation) may gradually intensify (Bogan & Lytle, 2011; Stubbington et al., 2011). Furthermore, water quality may deteriorate in remnant pools as increased respiration and organic matter accumulation decreases dissolved oxygen and pH, and increased evaporation and the loss of stream flow increases conductivity and temperature (Lake, 2003; Boulton & Lake, 2008). Due to the deterioration in water quality, remnant pools become increasingly inhospitable and are often rendered inhabitable. Therefore, the latter stages of drying favour organisms which can tolerate poor water and habitat quality through life history adaptations (Strachan et al., 2015), are able to respire atmospheric oxygen (Chessman, 2015) or seek refuge in the saturated subsurface sediments (Stubbington, 2012).

1.4 Macroinvertebrate adaptations to drying in streams

Stream drying is widely perceived to have a negative effect on stream ecology; however, it can have a brief positive effect for predators in remnant pools as prey densities increase as a result of reduced wetted habitat forcing biota into close proximity (Lake, 2003; Boulton & Lake, 2008). Predation within remnant pools results in major changes to community structure (Stanley et al., 1997; Suren et al., 2003). During the decline and loss of surface water, particularly during the summer months, aquatic animals are often exposed to elevated temperatures and high levels of ultraviolet light (Williams et al., 2006; Alekeseev et al., 2007). High temperatures and increased sunlight have been reported to promote algal productivity in remnant pools which, in turn, provides an abundant food source for macroinvertebrates (Extence, 1981; Freeman et al., 1994; Suren et al., 2003). Increased feeding by macroinvertebrates may produce increased waste organic matter, which in turn may promote detritivore productivity (Extence, 1981). However, a reduction in water quality associated with the loss of hydrological connectivity may allow pollution tolerant taxa to persist (e.g. Coleopterans, Dipterans; Towns, 1983) and eliminate sensitive taxa (e.g. EPT; Extence, 1981), particularly in streams subject to supra-seasonal drying (Wood & Armitage, 2004).

1.4.1 Life history adaptations to drying in temporary streams

The timing and intensity of seasonal dry phases has shaped the development of temporary stream communities over time (Brock et al., 2003; Lytle & Poff, 2004; Lake, 2011). Temporary stream communities have, therefore, given rise to species which can avoid desiccation through life history, morphological, physiological and behavioural adaptation (Lytle & Poff, 2004), ultimately enhancing community resistance and resilience to drying.

The alteration / modification of an organism's life cycle to synchronise with regular features of the flow regime (e.g., summer drought) can result in life-history adaptations (Lytle & Poff, 2004). Such life-history adaptations include rapid development, continuous reproduction (Gray, 1981), prolonged hatching (Storey & Quinn, 2011), long emergence periods (Dieterich & Anderson, 1995), dormancy (diapause, aestivation and anhydrobiosis) (Gray, 1981; Watanabe, 2006; Wickson et al., 2012; Strachan et al., 2015) and extended flight periods (Sommerhäuser et al., 1995). Flow regime is therefore the primary driver of macroinvertebrate evolution (Lytle & Poff, 2004) and studies observing life-history adaptations to drying are described below.

Examining the life histories of invertebrates in a Sonoran Desert stream (Sycamore Creek, Arizona, USA) showed rapid development of 17 taxa and continuous reproduction to be dominant methods to avoid desiccation from drying events (Gray, 1981). For example, Ephemeroptera (Baetidae, Leptophlebiidae, Siphlonuridae and Tricorythidae), Hemipterans and Dipterans displayed rapid development rates from eggs to adults of 8-14 days, 21 days and 6-9 days, respectively. The life-cycle of blackflies (Simuliidae) in Switzerland have also been observed to display faster development rates of individuals within temporary streams compared to perennial streams (Rüegg & Robinson, 2004). Conversely, Trichoptera species displayed longer development rates, for example *Helicopsyche mexicana* (Helicopsychidae) averaged 50 days and *Polycentropus halidus* (Polycentropodidae) averaged 46 days. However, these Trichopterans reproduced continuously, potentially producing seven generations per year and increased the likelihood of breeding success when flow returned (Gray, 1981).

Prolonged hatching of eggs and emergence periods of mayflies (Siphlonuridae, Leptophlebiidae) and stoneflies (Nemouridae, Chloroperlidae, Perlodidae) in temporary streams of western Oregon, Canada, have been reported (Dieterich & Anderson, 1995). This resulted in asynchronous emergence, as adults, of taxa over a longer period, decreasing the risk of eradication from premature or prolonged droughts (Hynes, 1970). Similar findings were noted of Gripopterygidae stonefly and Hydrobiosidae caddisfly larvae (Storey & Quinn, 2011). However, prolonged hatching periods of *Soyedina interrupta* (Plecoptera: Nemouridae) eggs from Oak Creek (Oregon, USA) still resulted in synchronised adult emergence due to varying development rates, as smaller individuals at the beginning of flow periods displayed faster growth than larger individuals (Dietrich & Anderson, 1995).

Dormancy mechanisms, such as aestivation and diapause are used by organisms to supress macroinvertebrate metabolism (Danks, 1987; Williams, 1998; Wickson et al., 2012; Strachan et al., 2015). Dormancy tends to be geographically dominant in extreme regions (e.g. arid and polar) as macroinvertebrates use this mechanism to survive temperature extremes (Harper & Hynes, 1970; Williams, 1998). For example, Pugsley & Hynes (1986) found *Allocapnia pygmea* (Plecoptera: Capniidae) diapausing in benthic and hyporheic sediments of the Speed River, (Ontario, Canada). Studies have shown that rehydrating dry sediments can terminate dormancy and promote recolonization (e.g., Tronstad et al., 2005; Stubbington et al., 2016). Approximately 80% of aestivated caddisfly larvae *Lectrides varians* (Leptoceridae), following rewetting of individuals from dry and moist sediments, could survive at least 4 months with no surface water, even when the subsurface was completely dry in Deep Creek (Victoria, Australia; Wickson et al., 2012).

In addition to supressed metabolic rates, some temporary water taxa can switch their metabolism off via processes including anhydrobiosis where individuals dehydrate themselves during dry phases and rehydrate when flow returns (Strachan et al., 2015). Taxa capable of anhydrobiosis include Turbellaria, Nematodes, Rotifers, Tardigrades and Chironomid larvae, for example, *Polypedilum vanderplanki* (Diptera: Chironomidae) (Kikawada et al., 2005; Watanabe, 2006; Kriska, 2013; Walsh et al., 2014; Strachan et al., 2015). A range of Limnephilidae caddisfly can also exhibit extended flight periods (Crichton, 1971; Sommerhäuser et al., 1995) which often coincide with seasonal dry phases in temporary streams (Meyer & Meyer, 2000). This extended flight period of Limnephilidae can also include 6 to 8 weeks diapause in damp and cool karstic caves through the summer (Bouvet, 1978; Trim & Sommerhäuser, 1994). Rocky slopes, caves and riparian vegetation can therefore provide a refuge for Trichoptera taxa with prolonged flight periods (Meyer & Meyer, 2000; Greenwood et al., 2001). Life-history adaptations are therefore important for maintaining community diversity in temporary streams (Lytle & Poff, 2004).

1.4.2 Morphological and physiological adaptations to drying in temporary streams

Morphological adaptations to drying in streams primarily refers to desiccation-resistant life stages, for example, eggs of the mosquito *Aedes aegypti* (Diptera:

Culicidae; Juliano et al., 2002). Physiological adaptations to drying mainly refers to body functions which avoid the negative effects of drying, for example, the uptake of atmospheric oxygen in the absence of water by most aquatic beetles (Stanley et al., 1994; Fairchild et al., 2003) and the use of haemoglobin for oxygen uptake by bloodworms (Chironominae) (Hynes, 1970; Stanley et al., 1994). Stubbington et al. (2009) revealed *Agabus guttatus* (Coleoptera: Dytiscidae), *Helophorus brevipalpis* (Coleoptera: Helophoridae), *Diacyclops bicuspidatus* (Cyclopodia: Cyclopidae) and Chrionomidae from desiccation resistant life stages in temporary waters. The desiccation-resistant eggs of *Nemoura cambrica* (Plecoptera: Nemouridae) have also been reported within the dry riverbed sediments known as the 'macroinvertebrate seedbank' in the Peak District, UK (Stubbington et al., 2016). Morphological and physiological adaptations promote the persistence of aquatic macroinvertebrates during drying (Lytle & Poff, 2004).

1.4.3 Behavioural adaptations to drying in temporary streams

Behavioural adaptations to drying in temporary streams by macroinvertebrates primarily involves the use of refuges to avoid desiccation (e.g., Bogan & Lytle, 2007; Robson et al., 2011; Poznańska et al., 2015; Vadher et al., 2015). Habitats which promote biotic resistance (ability to withstand) and resilience (ability to recover) during biophysical disturbances are considered a refuge (Sedell et al., 1990). Stream features including standing pools (Stanley et al., 1994; Covich et al., 2003; Robson et al., 2011), woody debris (Sedell et al., 1990), perennial springs (Marshall et al., 2006; Sheldon & Thoms, 2006; Chester & Robson, 2011) and subsurface sediments (Stubbington et al., 2010; Vadher et al., 2015; Vander Vorste et al., 2016a) can provide temporary relief for macroinvertebrates from surface drying. The use of these refuges, for example in the case of moist subsurface sediments, can be either active (Vadher et al., 2015) or passive (Stubbington & Wood, 2013). This thesis will explore the behavioural response of stream macroinvertebrates to drying and the use of subsurface sediments as a refuge. Research into subsurface sediment refuge use in temporary streams is outlined in Section 1.6.

In addition to refuge use, organisms such as Dytiscid beetles, have been observed escaping drying streams entirely by taking flight following stream drying (Zimmerman, 1959; Smith, 1973; Kingsley, 1985). Lytle et al. (2008) also reported macroinvertebrates leaving drying streams when thousands of *Postelichus immsi* (Coleoptera: Dryopidae) were observed crawling upstream to avoid drought in a desert stream and high densities of the grey sand-dragon *Progomphus borealis* (Odonata: Gomphidae) (690 larvae per m^2) were found moving upstream through moist subsurface sediments. Upstream migration of macroinvertebrates in search of permanent water sources has also been recorded in Capniidae stonefly larvae, Chironomidae larvae (Delucchi, 1989) and *Parameletus chelifer* (Ephemeroptera: Siphlonuridae) larvae (Olsson & Soderström, 1978). Behavioural adaptations are therefore important mechanisms for community resistance to drying.

1.5 The role of disturbance

Ecological disturbances are defined as potentially damaging forces which are applied to habitat spaces occupied by a population, community or ecosystem (Lake, 2000). Disturbances are considered a primary determinant of community structure in streams (Lake, 2000). They often result in changes to the abiotic conditions important to aquatic organisms, such as, flow, sediment composition, temperature and oxygen concentration (Allan, 1995). Past research has not considered predictable / seasonal damaging forces as disturbances, for example seasonal drying and flooding associated with natural flow regimes, in order to allow for realistic comparisons between times, sites and rivers (Resh et al., 1988; Poff & Ward, 1989). The magnitude of ecological responses to a predictable or unpredictable disturbance event will vary due to evolutionary adaptation. Predictable disturbance events will still, however, always have ecological effects (Poff, 1992). Therefore, this thesis will also regard natural drying events in streams as a disturbance.

A disturbance of stream systems can be characterised as either a pulse, press or ramp disturbance (Fig. 1.3; Lake, 2000). Pulse disturbances are short-term and display a clear beginning and endpoint, for example, flooding (Fig. 1.3a; Lake, 2000; Collier & Quinn, 2003). Press disturbances often commence sharply and maintain disturbance strength, for example, following construction of a dam (Fig. 1.3b, Lake, 2000; Tullos et al., 2014). Ramp disturbances increase in strength steadily over time, sometimes without an endpoint, for example, drying in streams (Fig. 1.3c; Lake, 2000; Humphries & Baldwin, 2003).

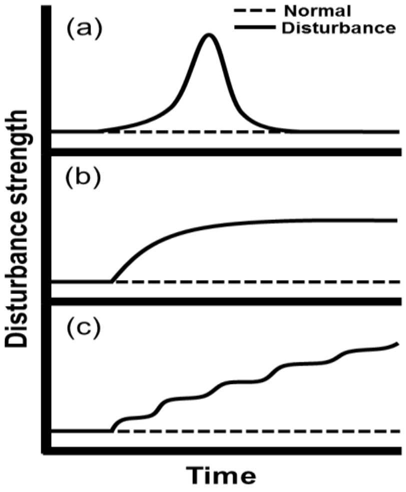


Fig. 1.3 Pulse (a), press (b) and ramp (c) disturbances (adapted from Lake, 2000). Solid lines show the disturbance strength in lotic systems, dashed lines show the normal system condition without a disturbance.

1.6 Introduction to the hyporheic zone and hyporheic refuge hypothesis

The hyporheic zone is the dynamic interface between the surface stream and groundwater where water, organic matter and biota vertically exchange (Orghidan, 1959; Boulton et al., 1998). Within the interstitial pore spaces of the hyporheic zone, water flows slowly and irregularly, often fed by upwelling and downwelling zones that result in biogeochemical heterogeneity (Boulton et al., 1998; Krause et al., 2013). Upwelling water typically displays lower dissolved oxygen, pH and temperatures but higher fine particulate organic matter and nutrient content compared to well-oxygenated, coarse particulate organic matter dominated downwelling water (Valett et al., 1990; Krause et al., 2013). The influence of upwelling and downwelling zones on biological productivity is controlled by the streambed sediment composition and porosity (Olsen & Townsend, 2003). This sedimentological and hydrological heterogeneity results in patches of high productivity, primarily where downwelling water often supports aerobic processes (Jones et al., 1995; Valett et al., 1990; Krause et al., 2013). Despite this biogeochemical patchiness within the subsurface, the hyporheic zone contains permanent residents, the ‘hyporheos’. These subsurface invertebrate taxa are typically less than 1 mm in body size (Ricci & Balsamo, 2000) and their biodiversity and distribution is often controlled by ground-water-surface-water exchanges (Stanley & Boulton, 1993; Malard et al., 2003) and sediment composition (Richards & Bacon, 1994; Olsen & Townsend, 2003).

The hyporheic zone can also be inhabited by temporary residents, usually by benthic macroinvertebrates during adverse surface conditions (high flow and drying events; Table 1.1; Table 1.2). Benthic macroinvertebrates were first reported to exist in the subsurface sediments following drying events in the 1960’s (Clifford, 1966). The hyporheic zone was then identified as a refuge for benthic macroinvertebrates by

Williams & Hynes (1974) as benthic taxa were abundant in the sediments following high flows and flooding of the Speed River, Ontario, Canada. Palmer et al. (1992) later formalised and tested this ‘hyporheic refuge hypothesis’ finding that the hyporheic zone did not effectively serve as a refuge for invertebrate taxa during flooding of Goose Creek, Virginia, USA. Tests of the hyporheic refuge hypothesis have since been equivocal, with many studies both supporting and rejecting the hypothesis (Table 1.1; Table 1.2). This equivocal evidence suggests the hyporheic zone is a patchy refuge primarily controlled by direction of hydrological exchange (Dole-Olivier et al., 1997), sediment characteristics (Richards & Bacon, 1994; Fowler & Death, 2001) and water quality (Lake, 2003).

Table 1.1 Research providing evidence for (a) and against (b) the hyporheic refuge hypothesis (HRH) during high flows and floods (adapted form Robertson & Wood 2010; Stubbington, 2012).

Location	Site	Substratum	Hydrological Conditions	Evidence of HR	Reference
(a) Evidence supporting HRH					
Ontario, Canada	Speed River	Mixed coarse substrate	Flood	Benthic taxa, inc. EPT were more abundant in the HZ following high flows.	Williams & Hynes (1974)
Texas, USA	Brazo River	Gravel and sand	Flood	*Cheumatopsyche* (Trichoptera) and *Neochoroterpes* (Ephemeroptera) occurred deeper in the HZ following a flood.	Poole & Stewart (1976)
Canada	Thompson River	Gravel, cobble and sand	High flow	Diptera larvae migrated into the HZ in response high flows.	Marchant (1988)
Lyon, France	Miribel Canal	Gravel (0.4-5cm) and pebble (5-20cm)	High flow, upwelling	EPT and chironomids present at 50cm subsurface following a flood.	Marmonier & Creuzé des Châtelliers (1991)
Lyon, France	Miribel Canal	Gravel (0.4-5cm) and pebble (5-20cm)	Spates (>300 m^3 s^1)	Ephemeroptera, Plecoptera, Ostracoda and *Gammarus pulex* (Amphipoda) abundance increased in the HZ (50cm into substratum).	Dole-Olivier & Marmonier (1992)
Virginia, USA	Goose Creek	Sand (1mm)	Flood	Rotifers vertical distribution increased into the substratum during a flood	Palmer et al. (1992)
Lyon, France	Rhône River	Gravel and cobble	High flow/flood, downwelling	Benthic taxa present in the HZ during low and medium magnitude flood events.	Dole-Olivier et al. (1997)
New Zealand	Laboratory study	Gravel and small cobble	Incrementally increased flow	Hydrobiidae, Leptophlebiidae and Leptoceridae moved into deeper, more stable sediments in response to high flows.	Holomuzki & Biggs (2000)
South Island, New Zealand	Waimakariri River	-	Historical disturbances	Unstable substrate resulted in increased benthic abundance in the HZ during disturbance.	Fowler & Death (2001)

(b) Limited or no evidence of HRH					
Virginia, USA	Goose Creek	Sand (1mm)	Flood	Subsurface taxa did not display significant vertical movement into the HZ during a flood.	Palmer et al. (1992)
Minnesota, USA	Experimental streams	Gravel (2-5cm) and sand	Experimental high flows (stepped)	Increase of mean number of taxa in the HZ was not significant during spates.	Imbert & Perry (1999)
France	Alpine stream	Gravel and cobble	High flow/flood (manipulated)	No significant change in benthic taxa inhabiting the HZ before and after spate onset.	Gayraud et al. (2000)
New South Wales, Australia	Never Never River	Gravel dominant	Flood and experimental increase in flow, downwelling	No significant increases of epigean water mites in the HZ.	Boulton et al. (2004)
Ontago, New Zealand	Kye Burn	Fine sand dominant	Flood	Benthic taxa abundances reduced in the HZ following a flood compared to pre-flood abundances.	Olsen & Townsend (2005)
Derbyshire, UK	River Lathkill	Sand to cobble	Spate (>5.6 $m^3 s^{-1}$)	Abundance of benthic invertebrates (*Polycelis felina* (Tricladida) and *Gammarus pulex* (Amphipoda)) declined in the HZ.	Stubbington et al. (2010)

Table 1.2 Research providing evidence for (a) and against (b) the hyporheic refuge hypothesis (HRH) during low flows and drying (adapted from Wood et al. 2010; Stubbington et al. 2012).

Location	Site	Substratum	Hydrological Conditions	Evidence of HR use	Reference
(a) Evidence supporting HRH					
Indiana, USA	Caldwell Hollow	Fine sediment	Streambed drying	Isopoda, amphipods and coleopterans survive in moist interstitial spaces.	Clifford (1966)
Ontario, Canada	Moser Creek and Kirkland Creek	Fine sediment (mean aggregate size = 3.5mm)	Streambed drying	Benthic taxa e.g. *Allocapnia vivipara* (Plecoptera) and *Heterocerus* species (Coleoptera) migrate into subsurface.	Williams & Hynes (1976)
New York, USA	Hurd Road Creek	Gravel and cobble dominant	Temporary stream (0.0054 - 0 m^3 s^{-1})	*Paraleptophlebia* (Ephemeroptera) and Chloroperlidae (Plecoptera) vertically migrated prior to drying in riffles.	Delucchi (1989)
Adelaide, Australia	Brachina Creek	Gravel and cobble	Streambed drying	High proportion of benthic taxa inc. Diptera and EPT found in the HZ	Cooling & Boulton (1993)
West Virginia, USA	Two Appalachian streams	Gravel (20-25mm)	Streambed drying	Benthic taxa, particularly two Plecoptera migrated into the HZ in response to dewatering. Many colonised the lower layer of migration baskets.	Griffith & Perry (1993)
New South Wales, Australia	Sassafras Creek (SC) and Coachwood Creek (CC)	Sand to gravel (2 - 10mm)	Low flows (median discharge: SC, 0.02 ML day^{-1}; CC, 0.07ML day^{-1})	Benthos abundance in dewatered riffles increased into the HZ, particularly filter-feeding taxa.	Hose et al. (2005)
North West Italy	Po River	Coarse substratum with sand	Streambed drying	Benthic taxa inc. Diptera, Plecoptera, Ephemeroptera, Chironomidae migrated into the HZ. *Agabus paludosus* (Coleoptera) found at depths of 90cm below surface.	Fenoglio et al. (2006)
Derbyshire, UK	River Lathkill	Sand to cobble	Low flows (<0.5 m^3 s^{-1})	*Polycelis felina* (Tricladida) and *Gammarus pulex* (Amphipoda) abundance increased into the HZ.	Stubbington et al. (2010)

Kent, UK	Little Stour River	Fine sediment and gravels	Low flows (0.165 - 0.053 m^3 s^{-1})	Significant *Gammarus pulex* (Amphipoda) proportions in the HZ during drying and increased water temperature.	Wood et al. (2010)
Derbyshire, UK and Lincolnshire, UK	River Lathkill (RL) and River East Glen (REG)	Cobble and pebble gravel with fine sediment	Low flows (RL) and streambed drying (REG)	*Gammarus pulex* (Amphipoda) and *Polycelis felina* (Tricladida) migrated into the hyporheic zone during dewatering.	Stubbington et al. (2011)
France	Eygues River	Gravel	Drying	Common benthic taxa found in the hyporheic zone during drying events.	Vander Vorste et al. (2016a)
France	Laboratory study	Gravel (10 - 14 mm)	Drying	*Gammarus pulex* moved into the subsurface sediments during water level reduction.	Vander Vorste et al. (2016b)
France	Drôme River	Cobbles, pebbles and coarse sand	Drying	Benthic abundance in the HZ increased seven-fold during drying.	Maazouzi et al. (2017)

(b) Limited or no evidence of HRH

New York, USA	Hurd Road Creek	Gravel and cobble dominant	Temporary stream (0.0054 - 0 m^3 s^{-1})	Insignificant migrations into the HZ of riffles by benthos during drying. Upstream migration dominated movement.	Delucchi (1989)
Sonoran Desert, USA	Sycamore Creek	Gravel (<30mm) dominant with sand	Streambed drying	Limited benthic taxa occurring in hyporheic community. HZ became dewatered.	Boulton & Stanley (1995)
California, USA	Cronin Creek	Gravel and cobble (median diameter 28mm), HZ = median 9mm gravel	Streambed drying	No increase of benthic taxa (e.g. Plecoptera and Trichoptera) into the HZ during dewatering/drying.	Del Rosario & Resh (2000)
North Island, New Zealand	Booths Creek, Kiriwhakapapa Stream and Reef Creek	Mixed with cobbles (64-256mm) and gravel (2-64mm) dominant	Experimentally reduced flows by 88-96%	Abundance of benthic taxa in colonisation chambers did not change significantly following 1 month flow reduction.	James et al. (2008)
South Island, New Zealand	Kaiapoi River	Gravel (30 - 40 mm)	Experimental flow reduction (~0.018 - 0.00125 m^3 s^{-1})	>85% benthos abundance retained in the top 7 cm of experimental baskets.	James & Suren (2009)
Poland	Laboratory study	Sand	Drying	Gammarids only migrated just under the surface with their backs still exposed.	Poznańska et al. (2013)

1.6.1 Effect of hydrology on the hyporheic refuge

Studies that have reported downwelling regions (i.e., the downward movement of surface water into the riverbed) promote the use of the hyporheic zone as a refuge by benthic macroinvertebrates, whereas upwelling water (i.e., upward flushing of water from the sediments into the surface water column) reduces refuge potential (e.g., Dole-Olivier et al., 1997; Stubbington et al., 2011; Fig. 1.4). Stubbington et al. (2011) conceptually described this, suggesting downwelling regions promote hyporheic refuge use as a result of high hyporheic oxygen availability, water chemistry parameters similar to the surface water, increased allochthonous organic matter input and enhanced migration from the physical downwelling force on macroinvertebrates (Fig. 1.4). Upwelling water from groundwater sources, however, typically contains lower dissolved oxygen concentrations, water chemistry parameters similar to that of groundwater and a lack of allochthonous nutrient inputs (Stubbington et al., 2011). Additionally, the physical resistance from upwelling forces on downward moving macroinvertebrates potentially impeding refuge use (Stubbington et al., 2011). In contrast to this, mesocosm experiments have demonstrated that upwelling flow supports movements by rheophilic benthic macroinvertebrates into the subsurface sediment (Mathers et al., 2014); however, this may be due to the unrealistic subsurface conditions of mesocosm experiments. The direction of hydrological exchange may, therefore, be a key influence of subsurface sediment use.

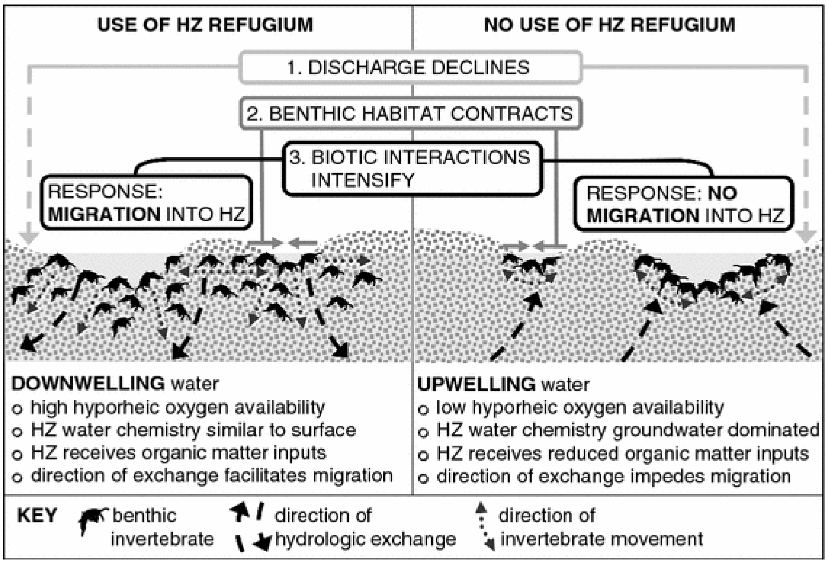


Fig. 1.4 Conceptual diagram of the spatial variability in the refuge potential of the hyporheic zone (HZ) during drying in relation to hydrologic exchange. Source: Stubbington et al. (2011).

1.6.2 Effect of sediment composition and fine sediment on the hyporheic refuge

Sediment composition and interstitial volume have been reported to directly impact the refuge potential of the hyporheic zone (Navel et al., 2010; Descloux et al., 2013; Vadher et al., 2015). Smaller sediments, in particular fine sediments (particles < 2 mm diameter), reduce sediment porosity and permeability by benthic macroinvertebrates (Bo et al., 2007; Descloux et al., 2013; Mathers et al., 2014; Vadher et al., 2015). This is likely due to the hyporheic zone presenting a series of maze-like pathways through the subsurface sediment in which macroinvertebrates are likely to be trapped if pathways become blocked and interstitial volumes are no longer favourable (Mathers et al. 2014; Vadher et al., 2015; Vander Vorste et al., 2016b). Consequently, larger particle sizes which produce a more open gravel framework for benthic macroinvertebrates to move through could potentially increase the use of the hyporheic zone as a refuge (Vadher et al., 2015; Vander Vorste et al., 2016b). Sediment porosity has also been reported to impact hyporheic processes by affecting the flow of water within the subsurface and consequently impacting aerobic and anaerobic processes due to the increased residence time of water (Nogaro et al., 2010). Sediment porosity may, therefore, be a key determinant of subsurface sediment use by benthic macroinvertebrates.

Fine sediments can occur both naturally in streams, usually as a result of within-river erosive processes and river bank erosion (Lawler et al., 1999; Smith et al., 2003), and as a result of anthropogenic activity, for example agricultural practices (Lamba *et al*., 2015), channel management (Dunbar *et al*., 2010) and urbanisation (Taylor & Owens, 2009). Excess fine sediment input as a result of anthropogenic activity can cause significant changes to the natural fine sediment dynamics in streams and result in ecological and habitat degradation (Owens *et al*., 2005). Vadher et al. (2015) demonstrated how fine sediment can bridge interstitial spaces in the sediment surface producing a physical barrier which prevents macroinvertebrate movements into the subsurface in response to drying. Furthermore, studies have reported fine sediment infiltration as a major influence on the movement of macroinvertebrates through the subsurface (Weigelhofer & Waringer, 2003; Mathers & Wood, 2016) and subsurface community structure (Bo *et al*., 2007; Larsen *et al*., 2011; Buendia *et al*., 2013; Descloux et al., 2013; Jones *et al*., 2015). Therefore, sediment composition and fine sediment affect the refuge potential of the hyporheic zone for benthic macroinvertebrates.

1.6.3 Effect of water quality on the hyporheic refuge

Oxygen availability has been identified as the main water quality parameter which controls hyporheic refuge potential for macroinvertebrates (Stubbington, 2012).

Dissolved oxygen levels within the subsurface sediments are typically lower compared to the surface stream and decrease with depth into subsurface sediments (Williams & Hynes, 1974; Marmonier et al., 2010), particularly in upwelling zones (Franken et al., 2001). Studies have found a positive correlation between dissolved oxygen concentration and macroinvertebrate use of the hyporheic zone (e.g., Williams & Hynes, 1974; Boulton et al., 1997; Franken et al., 2001).

During drying events, changes in water quality can increase macroinvertebrate use of the hyporheic zone. Temperature increases in the water column prior to drying has been identified as a potential trigger, alongside increased competition, for macroinvertebrates to use the hyporheic zone (Wood et al., 2010; Stubbington et al., 2011; Vander Vorste et al., 2017). The direct impacts of changes in water chemistry parameters and sediment characteristics on hyporheic refuge use by benthic macroinvertebrates has, however, been poorly studied. Therefore, laboratory-based research is needed to provide robust evidence of subsurface sediment refuge use by macroinvertebrates and factors limiting this use.

1.7 Research questions

Given the increasing global recognition of the importance of temporary streams (see section 1.3) there is a need to consider the abiotic factors (e.g., sediment characteristics and dry period duration) which influence the ability of aquatic organisms to persist in these challenging environments. This thesis specifically aims to examine: i) the effect of sedimentological characteristics on the use of subsurface sediments by specific benthic macroinvertebrate taxa during experimental and natural drying of riverine substrates and ii) the effect of the duration of drying events on the survival of a model benthic macroinvertebrate taxon (*Gammarus pulex* - Crustacea: Amphipoda) within the subsurface.

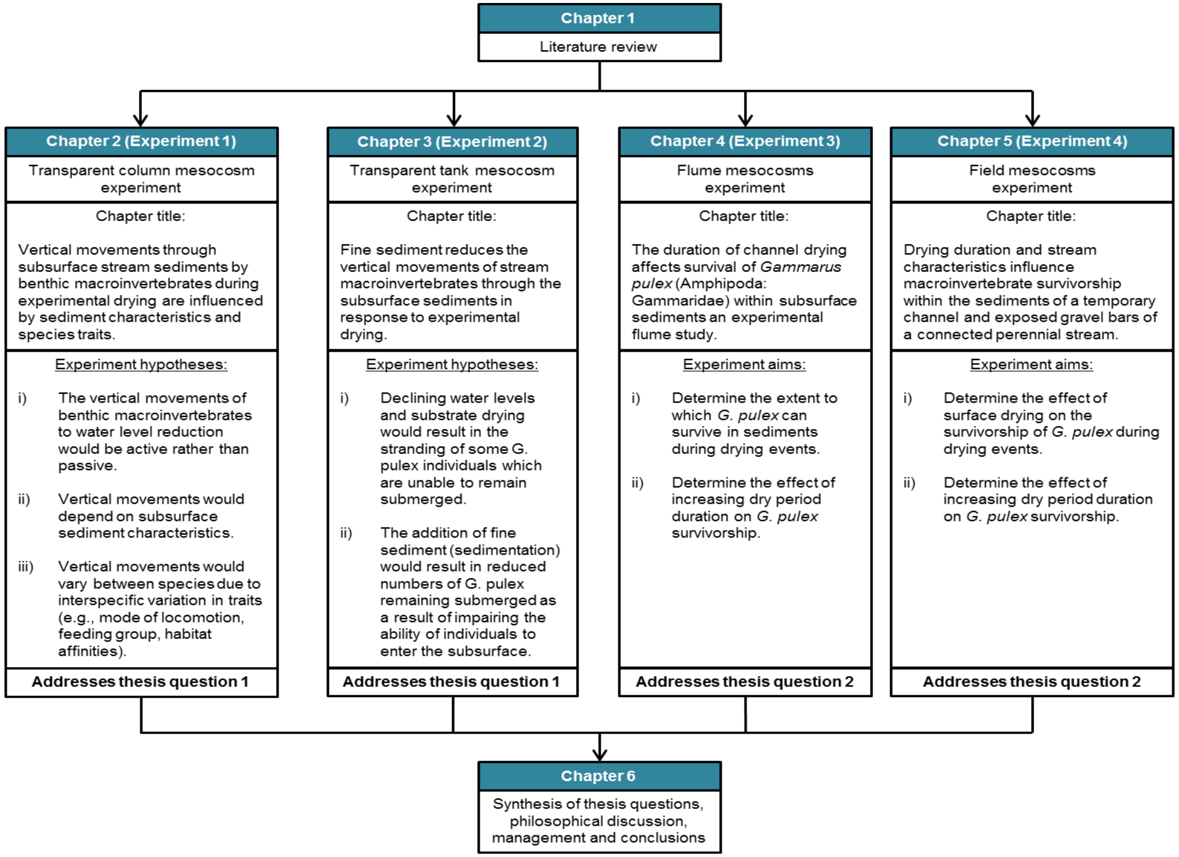
These aims were addressed in four mesocosm experiments (two laboratory, one flume and one field experiment) which examined: the vertical movement of individuals of different taxa to drying and sediment characteristics (Chapter 2); how multiple individuals of a model organism (*Gammarus pulex*) respond to the process of substrate drying, dewatering and sedimentation (Chapter 3); and how drying duration in a highly controlled flume (Chapter 4) and field conditions (Chapter 5) affects macroinvertebrate survivorship. This thesis addresses the following overarching research questions:

> Question 1: To what extent do sedimentological characteristics affect the use of subsurface sediments by benthic macroinvertebrate individuals in direct response to a reduction in water level?
>
> Question 2: How does the duration of drying events affect benthic macroinvertebrate survivorship within the subsurface sediments?

1.8 Structure of thesis

To address the aims and research questions outlined above, this thesis is comprised of six chapters (see Thesis map in Fig. 1.5). Chapter 1 provides an introduction to the field of research and specifically considers the key literature in the field of temporary streams, macroinvertebrate adaptation to drying, drying as a disturbance and the hyporheic refuge hypothesis. Chapter 2 – 5 presents the results of four mesocosm based experiments which directly address the thesis aims and research questions. The results of these experiments are presented in the form of four individual chapters. Each of these chapters is presented as submitted to relevant journals; the journal and current status of the manuscript is presented at the start of each chapter. To address the first research question, two ex-situ experimental studies were undertaken in a laboratory setting. The first quantified the vertical movements of specific benthic macroinvertebrate taxa in response to water-level reduction and changes in sediment characteristics using mesocosm columns containing transparent sediments (Chapter 2, experiment 1; Fig. 1.5). Five benthic macroinvertebrate species, with different life-history traits, were selected to examine their response to water level reduction. These species were: a stonefly nymph, *Nemoura cambrica* (Plecoptera: Nemouridae), which is a widespread inhabitant of seasonally dry headwater streams (Stubbington *et al*., 2009); two crustaceans, *Asellus aquaticus* (Isopoda: Asellidae) and *Gammarus pulex* (Amphipoda: Gammaridae), which have been widely recorded in benthic and hyporheic sediments in both perennial and temporary systems (Stubbington *et al*., 2015); a benthic caseless caddisfly larvae, *Hydropsyche siltalai* (Trichoptera: Hydropsychidae), and a benthic mayfly nymph, *Heptagenia sulphurea* (Ephemeroptera: Heptageniidae), typically associated with perennially flowing streams (Eyre *et al*., 2005; Wood *et al*., 2005) including the hyporheic zone (Datry, 2012). To examine how sedimentary characteristics influenced the ability of a model organism (*G. pulex*) to move vertically through subsurface sediments (research question 1), a second laboratory experiment was conducted to explore how the particle size of fine sediment (in varying proportions of fine and coarse sand) deposited onto the sediment surface influenced the ability of multiple individuals of *G. pulex* (10 per-experimental trail) to access and remain submerged in novel transparent sediment mesocosms during water level reduction (Chapter 3, experiment 2; Fig. 1.5). Experiments 1 and 2 demonstrated that benthic macroinvertebrates actively move into the subsurface sediments in response to drying, but species traits and sediment characteristics (porosity and fine sediment) influence their ability to move vertically.

To address the second research question, a flume and field experiment were conducted using subsurface mesocosms to examine the survivorship of *G. pulex* individuals within the sediments in response to the increasing duration of drying (1 day, 7 day, 14 day, 21 day) of artificial (Chapter 4, experiment 3) and natural (Chapter 5, experiment 4) drying events. The flume experiment (Chapter 4) examined the survival of *G. pulex* within 20 cm × 20 cm × 20 cm mesocosms containing a 10 cm depth of fluvial gravel in a recirculating laboratory flume following water level reduction and increasing dry period durations. The field experiment (Chapter 5) examined the survival of *G. pulex* within 25 cm deep subsurface mesocosms following increasing dry period durations of exposed gravel bars of a perennial stream and the drying of an adjacent temporary stream. Experiments 3 and 4 demonstrated the negative impact of dry period duration on the survival of *G. pulex* within the subsurface sediments. A final synthesis chapter (Chapter 6) provides a general discussion of the two thesis questions in the context of the four experiments, outlines the contribution of this thesis to theoretical knowledge, proposes management options for streams susceptible to drying, and suggests avenues for future research (Fig. 1.5).



Chapter 1

Literature review

Chapter 2 (Experiment 1)

Transparent column mesocosm experiment

Chapter title:

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

Experiment hypotheses:

i) The vertical movements of benthic macroinvertebrates to water level reduction would be active rather than passive.

ii) Vertical movements would depend on subsurface sediment characteristics.

iii) Vertical movements would vary between species due to interspecific variation in traits (e.g., mode of locomotion, feeding group, habitat affinities).

Addresses thesis question 1

Chapter 3 (Experiment 2)

Transparent tank mesocosm experiment

Chapter title:

Fine sediment reduces the vertical movements of stream macroinvertebrates through the subsurface sediments in response to experimental drying.

Experiment hypotheses:

i) Declining water levels and substrate drying would result in the stranding of some G. pulex individuals which are unable to remain submerged.

ii) The addition of fine sediment (sedimentation) would result in reduced numbers of G. pulex remaining submerged as a result of impairing the ability of individuals to enter the subsurface.

Addresses thesis question 1

Chapter 4 (Experiment 3)

Flume mesocosms experiment

Chapter title:

The duration of channel drying affects survival of *Gammarus pulex* (Amphipoda: Gammaridae) within subsurface sediments an experimental flume study.

Experiment aims:

i) Determine the extent to which *G. pulex* can survive in sediments during drying events.

ii) Determine the effect of increasing dry period duration on *G. pulex* survivorship.

Addresses thesis question 2

Chapter 5 (Experiment 4)

Field mesocosms experiment

Chapter title:

Drying duration and stream characteristics influence macroinvertebrate survivorship within the sediments of a temporary channel and exposed gravel bars of a connected perennial stream.

Experiment aims:

i) Determine the effect of surface drying on the survivorship of *G. pulex* during drying events.

ii) Determine the effect of increasing dry period duration on *G. pulex* survivorship.

Addresses thesis question 2

Chapter 6

Synthesis of thesis questions, philosophical discussion, management and conclusions

Fig. 1.5 Map of thesis structure*.* Chapter 1 provides a background and introduction to the literature around temporary streams. Chapter 2 – 5 are journal style papers presenting four experiments which address two overarching thesis questions. Chapter 6 presents a thesis synthesis discussing the research questions.

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Chapter 2 (Experiment 1)

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

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2.1 Abstract

Subsurface streambed sediments have been identified as a potential refuge for benthic macroinvertebrates during streambed drying in temporary streams. Sediment characteristics, including particle shape and size, and interstitial pore volume are important controls on the distribution of macroinvertebrates in subsurface habitats. The difficulties of direct sampling and observation within the subsurface zone, however, means that the movement of individuals within these sediments remains poorly understood. There is, therefore, a lack of understanding of the controls over individual movement within subsurface sediments. This study used novel transparent artificial mesocosms containing different sediment sizes resulting in varying interstitial pore volumes (six sediment treatments) to directly observe the subsurface movements of individuals of three aquatic insect nymphs—*Heptagenia sulphurea* (Ephemeroptera: Heptageniidae), *Hydropsyche siltalai* (Trichoptera: Hydropsychidae) and *Nemoura cambrica* (Plecoptera Nemouridae)—and two aquatic crustaceans—*Asellus aquaticus* (Isopoda: Asellidae) and *Gammarus pulex* (Amphipoda: Gammaridae)— in response to surface water loss and water-level reduction. The results demonstrated that faunal movements into subsurface sediments in response to surface water loss were the result of active, not passive, movements as water level declined. Both sediment treatment and species identity influenced vertical movements. Movements by individuals of *N. cambrica* were unaffected by sediment treatment. Most individuals of species with a weaker affinity for the subsurface (i.e. the benthic algal grazer *H. sulphurea* and the filter-feeder *H. siltalai*) became stranded in all sediment treatments. Vertical movements of *G. pulex* and *A. aquaticus* were restricted primarily by pore volume, stranding most commonly in sediments with smaller interstitial volumes. Streambed drying may become more common as a result of climate change and increasing pressure on water resources in some regions. The results highlight the need to quantify sediment characteristics and species-specific responses to surface water loss to understand the wider community and functional consequences of streambed drying.

Keywords: intermittent rivers; streambed drying; macroinvertebrates; sediment characteristics, mesocosm experiments.

2.2 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 *et al*., 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*., 2017), few studies have examined the response of individual species to the process of surface water loss including the ability of individuals to access and use subsurface habitats during drying (Poznańska *et al*., 2013; 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 persists at varying depths in the sediments of the hyporheic zone (Hose *et al*., 2005; Fenoglio *et al*., 2006). The potential for these subsurface sediments to function as a refuge that promotes community persistence during adverse conditions in steams has long been recognised (Williams and Hynes, 1974). The hyporheic zone is an important habitat and resource for aquatic fauna during streambed drying (Dole-Olivier, 2011; Vadher *et al*., 2015; Vander Vorste, 2016b). If individuals can access and persist in suitable subsurface habitats (e.g., fully saturated hyporheic interstices) throughout 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).

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*., 2010; 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 studies (Nogaro *et al*., 2006; Stumpp & Hose, 2013) have demonstrated that sediment characteristics including particle size, shape, heterogeneity and porosity can influence the distribution of benthic communities and populations. However, the direct response of individuals to drying and their ability to move into the hyporheic zone has rarely been studied in real time (exceptions being

Poznańska *et al*., 2013; Stumpp & Hose 2013; Vadher *et al*., 2015). This reflects the inherent difficulties of directly observing fauna within subsurface habitats.

This study aimed to examine the effects 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. This study 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. In this study it was hypothesised that the response of 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; iii) vary among species due to interspecific variation in traits (e.g., mode of locomotion, feeding group, habitat affinities); and iv) differ among individuals of the same species due to intraspecific trait variation.

2.3 Materials and methods

2.3.1 Invertebrate collection and test species

Five species of benthic macroinvertebrates were chosen for examination of their response to surface water loss and water-level reduction: a stonefly nymph, *Nemoura cambrica* Stephens, 1836 (Plecoptera: Nemouridae); a caseless caddisfly larvae, *Hydropsyche siltalai* Döhler, 1963 (Trichoptera: Hydropsychidae); a mayfly nymph, *Heptagenia sulphurea* Müller, 1776 (Ephemeroptera: Heptageniidae); and two crustaceans, *Asellus aquaticus* (L.) (Isopoda: Asellidae) and *Gammarus pulex* (L.) (Amphipoda: Gammaridae). These species included 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, *H. siltalai* (Eyre *et al*., 2005) and *H. sulphurea* (Wood *et al*., 2005a) including the hyporheic zone (Datry 2012). These species were selected to represent a range of biological traits (Table 2.1).

Table 2.1 Biological traits of the five study taxa relevant to subsurface movement and habitat preferences (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

Individuals of the five species were collected from waterbodies draining 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); all sites were within 5 km of the laboratory at Loughborough University where the mesocosm experiments were conducted.

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 immediate use in experiments.

2.3.2 Sediment-column mesocosms

Twelve sediment-column mesocosms were constructed from transparent acrylic pipes (35-cm × 4.6-cm internal diameter; Fig. 2.1a) to allow direct observation of individuals. Columns were sealed at the base by a rubber bung with a 5-mm glass tube 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. 2.1a).

Columns were mounted onto retort stands (Fig. 2.1b) 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.

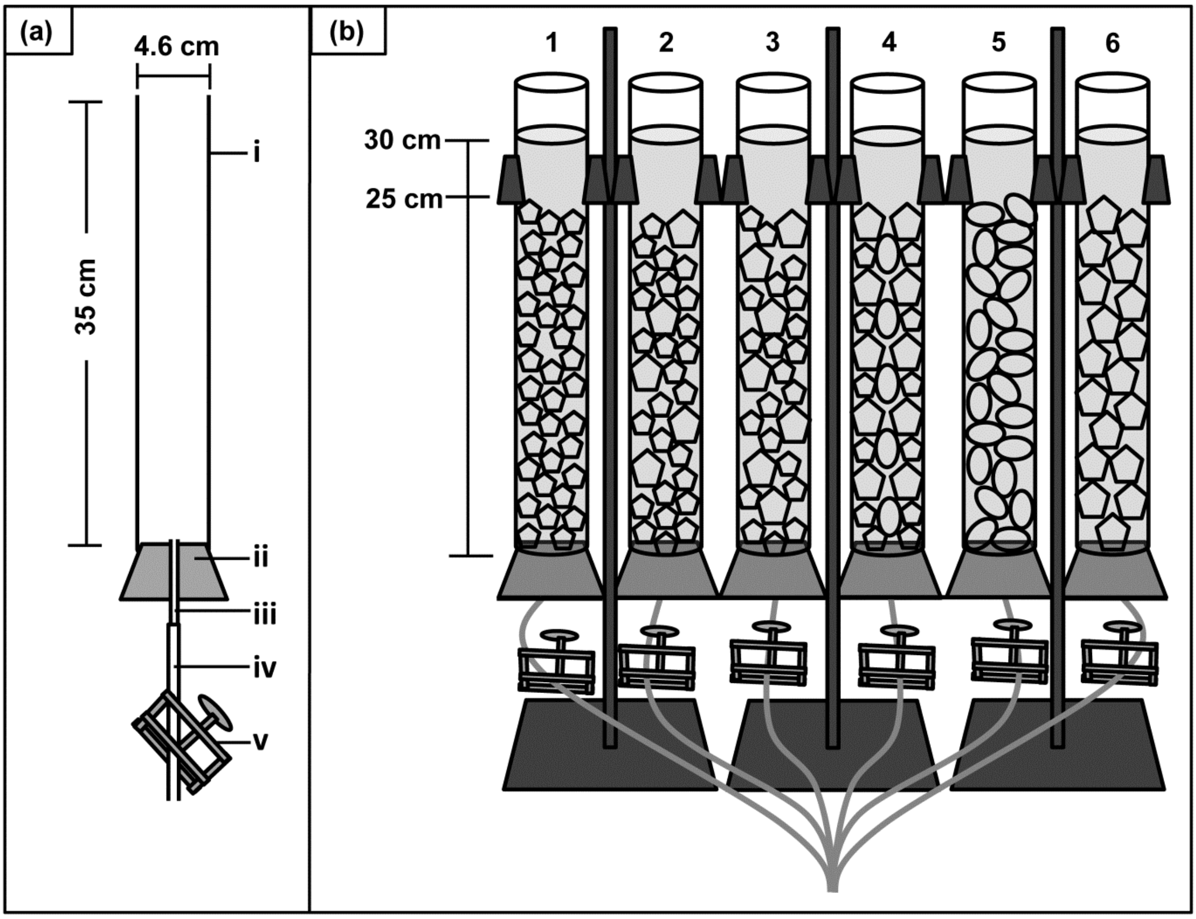

Fig. 2.1 Sediment column mesocosms. (a) Cross-section through a sediment column: i) acrylic pipe; ii) rubber bung; iii) 5 mm glass tube; iv) silicon tubing; v) Hoffman clip to control water drainage. (b) The six sediment treatments at the start of experiments (5 cm surface water). Sediment treatments are as described in Table 2.2. Not to scale.

2.3.3 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 2.2). 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 2.2). 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. 2.1b).

Table 2.2 Description of sediment treatment composition, pore-size volume and the mean depth from the sediment surface (± 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

2.3.4 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. Complete 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. 2.1b). 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 was retained in each column at the end of each trial as a refuge.

2.3.5 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 minutes were sufficient). Following acclimatisation, organisms 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 location of the organism was recorded and the experiment terminated. The sediment columns were deconstructed and their sediments removed and washed thoroughly to remove live test organisms, debris and biological waste. Organisms were then euthanized and preserved using 70% industrial methylated spirit (IMS).

To distinguish between active and passive movements associated with water-level reduction, the experiment was repeated using the cadavers, 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 individual tests per 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 *H. siltalai*, *N. cambrica* and *H. sulphurea*.

2.3.6 Data analysis

One-way ANOVA tests were used to compare the absolute depth moved by live individuals and by cadavers (i.e. to distinguish active versus passive movement; hypothesis i), where absolute depth moved was defined as the difference between the depth of an individual in its column at time = 0 and its depth in the column at the end of the experiment.

To examine hypotheses ii, iii and iv, macroinvertebrate responses to surface water loss and water-level reduction were defined in three ways: final depth reached, absolute depth moved, and likelihood of becoming stranded. A General Linear Model (GLM) was used to examine the effect of sediment treatment and species identity, and their interaction, on the final and absolute depth reached by live individuals (their depth at end of experiment; hypotheses ii and iii). To examine and account for the potential influence of individual body size on final depth reached (hypothesis iv), 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 or species were detected.

Binary Logistic Regression (BLR, using the logit link function) 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. *N. 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).

2.4 Results

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

Live individuals reached greater absolute depths (109 ± 4 mm SE) than cadavers which effectively remained close to the surface (2.13 ± 0.31 mm SE; one-way ANOVA, $F_{1, 1198} = 749.64$, $P < 0.01$). Movement by individuals into and through the subsurface sediments was active, not passive.

2.4.2 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 moved by individuals varied significantly between species (i.e. the interaction term was significant; GLM, $P < 0.001$). The final depth moved in each sediment treatment was dependent on species identity. The pattern of final depth moved for each species within each sediment treatment generally increased from sediment treatment 1-6 with increasing interstitial volume (Fig. 2.2), however, final depth moved in each sediment treatment decreased for each species from *N. cambrica*, *A. aquaticus*, *G. pulex*, *H. siltalai* to *H. sulphurea*, respectively (Fig. 2.3). Body size had no detectable effect on individuals' final depth moved for any species (GLM, $P = 0.179$).

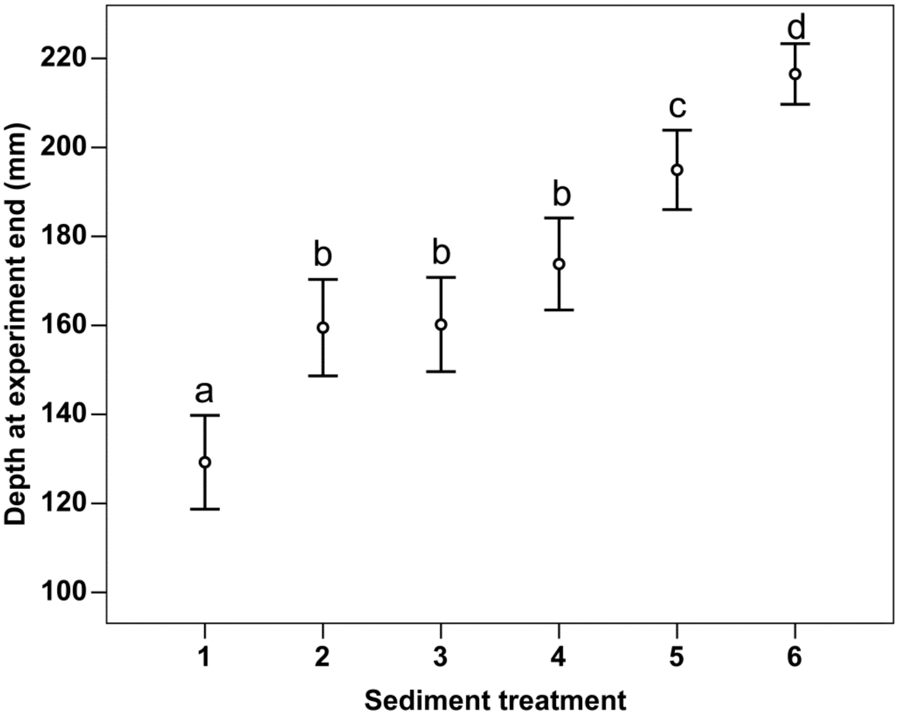

Fig. 2.2 Mean ±1 SE vertical depth moved by live individuals at the end of experiments in each sediment treatment. Sediment treatments are as described in Table 2.2; a-d indicate statistically different values (GLM, $P < 0.05$). Y-axis starts at 100 mm.

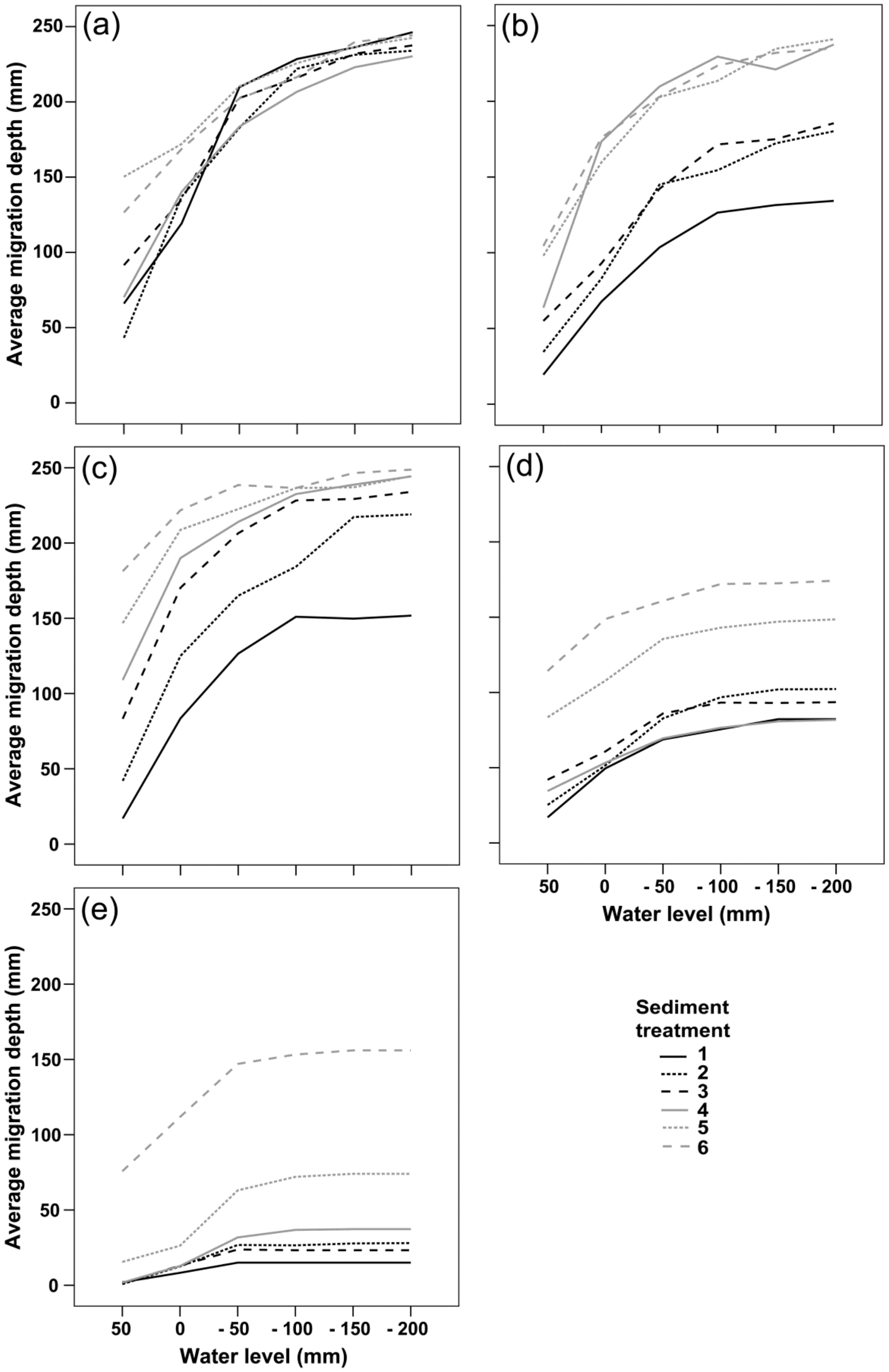

Fig. 2.3 Mean migration depth of live individuals in response to dewatering in each of six sediment treatments. (a) *Nemoura cambrica* (b) *Gammarus pulex* (c) *Asellus aquaticus* (d) *Hydropsyche siltalai* (e) *Heptagenia sulphurea*. Sediment treatments are as described in Table 2.2.

2.4.3 Effect of sediment treatment and species identity on the absolute depth moved by live individuals

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 migration depth of *N. cambrica* individuals was significantly greater than: *A. aquaticus* individuals in sediment treatments 1- 4 and 6; *H. 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 migration depth of *G. pulex* individuals was significantly greater than: *A. aquaticus* individuals in sediment treatments 4- 6; *H. 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, $P = 0.401$).

2.4.4 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 lastly treatment 6 (Fig. 2.4a). 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, $P > 0.05$).

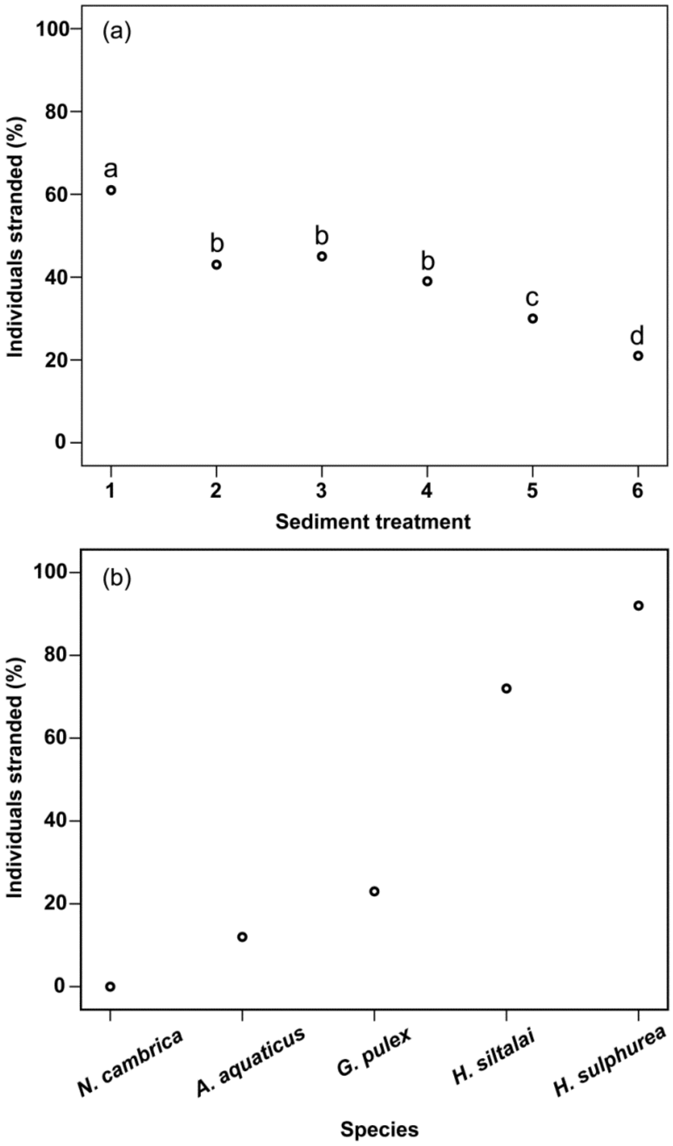

Fig. 2.4 Percentage of live individuals stranded in each sediment treatment (top) and by species (bottom). a-d indicate statistically different values (Binary Logistic Regression, $P < 0.05$). Sediment treatments are as described in Table 2.2.

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 *H. siltalai*, *G. pulex* and finally *A. aquaticus* (pairwise $P < 0.05$; Fig. 2.4b); no *N. cambrica* individuals became stranded in any treatment (Table 2.3 and Fig. 2.3a). The majority of *G. pulex* individuals stranded in sediment treatment 1

(70%) and < 50% were stranded in treatments 2 and 3 (Table 2.1 and Fig. 2.3b). Over half of *A. aquaticus* individuals stranded in sediment treatment 1 (Table 2.1 and Fig. 2.3c) and ≤ 10% became stranded in treatments 2 and 3. The majority of *H. siltalai* individuals became stranded during water-level reduction across all sediment treatments (except treatment 6 = 45%; Fig. 2.3d) and all *H. sulphurea* became stranded in sediment treatments 1 – 3 with ≤ 60% stranded in treatments 4 – 6 (Fig. 2.3e and Table 2.3).

Table 2.3 Percentage of individuals stranded above the water level during dewatering in each sediment treatment (see Table 2.2).

	% Stranded in sediment treatments*						
Taxa	1	2	3	4	5	6	All
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	61	43	45	39	30	21	

2.5 Discussion

2.5.1 Benthic macroinvertebrates actively move into the subsurface during dewatering

This study found support for the first hypothesis that faunal movement into the subsurface in response to dewatering was active. A range of studies have recorded benthic macroinvertebrates relatively deep (70-90 cm below the streambed surface) within the hyporheic sediments when surface sediments dry (e.g. Fenoglio *et al.*, 2006; Young *et al.*, 2011). 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 (organisms moving vertically to remain submerged) or passive (being drawn down with the receding water-line). The comparison of 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.

2.5.2 Effect of sediment treatment on the vertical movements of benthic macroinvertebrates

This study found support for the second hypothesis and found subsurface sediment characteristics influenced the ability of individuals to move vertically in response to water-level reduction. Smaller particle sizes with lower interstitial volume (sediment porosity) reduced vertical movements of individuals of all species except *N. cambrica*. Sediment characteristics have been reported to 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 implicated sediment characteristics such as interstitial pore volume as the primary factors influencing 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 severely limited 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). The observations of this study provide direct evidence to support studies which have inferred that certain 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).

2.5.3 Species-specific vertical movements of individuals through sediments

This study found support for the third hypothesis as the results presented in this study demonstrated that vertical movement varied between species, reflecting differences in traits and habitat affinities. Although the response of the species examined to sediment porosity was similar, the overall vertical distance moved by the five species was different as the temporary-water specialist *N. cambrica,* known to inhabit subsurface sediments (Stubbington *et al*., 2009), was able to move deeper in response to water-level reduction. *N. cambrica* has a small body size compared to the other species used in the present experiment 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. However, the present study did not detect an effect of body size on the vertical migration by other species, which suggests that trait combinations could influence movements.

Most *G. pulex* and *A. aquaticus* individuals moved vertically in all sediment treatments except the smallest particle size treatment (1). This observation advances the experimental findings of Vander Vorste *et al*. (2016a), who observed that *G.*

pulex use 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, *H. 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 2.1) may mean that *H. siltalai* and *H. sulphurea* lack behavioural adaptations to move strongly into the subsurface sediments in response to drying.

The net-spinning caseless caddisfly larvae *H. siltalai* and the free-living mayfly nymph *H. sulphurea*, became stranded in most sediment treatments during water-level reduction. *H. siltalai* larvae are benthic filter-feeders and *H. sulphurea* larvae are benthic grazers (Table 2.1). 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 *H. siltalai* individuals moved to the column base in the coarsest sediments with the largest interstitial pore space (treatment 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 migration ability of some species typically associated with benthic habitats.

2.5.4 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. This study highlights the need to understand species-specific responses in relation to differences in sediment characteristics among streams. The results of this study 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. This is essential if the future management of ‘drying refuges’, such as the hyporheic zone, is to increase community resistance and resilience to stream drying. However, the ability of organisms 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 process of streambed drying.

2.6 Acknowledgements

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2. 8 Supporting information

Table S1. Fisher’s LSD post-hoc pairwise comparison of absolute depth moved by live individuals between each species for each sediment treatment. Significant depths (P ≤ 0.05) are emboldened. ‘↑’ indicates the taxon listed is significantly higher than the taxa being compared. Sediment treatments are as described in Table 2.

Sediment Treatment 1	*N. cambrica*	*G. pulex*	*A. aquaticus*	*H. 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
H. siltalai					0.238
H. sulphurea					

Sediment Treatment 2	*N. cambrica*	*G. pulex*	*A. aquaticus*	*H. 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
H. siltalai					0.267
H. sulphurea					

Sediment Treatment 3	*N. cambrica*	*G. pulex*	*A. aquaticus*	*H. 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
H. siltalai					0.388
H. sulphurea					

Sediment Treatment 4	*N. cambrica*	*G. pulex*	*A. aquaticus*	*H. 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
H. siltalai					0.581
H. sulphurea					

Sediment Treatment 5	*N. cambrica*	*G. pulex*	*A. aquaticus*	*H. 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
H. siltalai					0.612
H. sulphurea					

Sediment Treatment 6	*N. cambrica*	*G. pulex*	*A. aquaticus*	*H. 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
H. siltalai					0.910
H. sulphurea					

Chapter 3 (Experiment 2)

Drying induced vertical movement of *Gammarus pulex* (Amphipoda: Gammaridae) through the subsurface is impeded by fine sediment characteristics

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Author contributions

Atish N. Vadher:	Experiment design, experiment set up and data collection, data analysis, author of complete first draft of chapter and revision of drafts following supervisor feedback.
Jonathan Millett:	Advised on experiment design, data analysis and provided feedback on chapter drafts. PhD supervisor.
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3.1 Abstract

Benthic macroinvertebrates inhabit the streambed sediments of temporary streams during drying events. Fine sediment (< 2000 µm in diameter) deposition and clogging of interstitial pathways reduces the connectivity between benthic and subsurface habitats, potentially inhibiting macroinvertebrate vertical movement. However, direct observations within subsurface sediments are inherently difficult and confirmation of macroinvertebrate vertical movement, and the effect of fine sediment, is limited. We used laboratory mesocosms containing transparent gravel sized particles (10 – 15 mm) to facilitate the direct observation of vertical movements by *Gammarus pulex* (L.) (Amphipoda: Gammaridae) in response to water level reduction and sedimentation. Six sediment treatments comprising two fine sediment fractions (small sand; 125 µm – 500 µm and coarse sand; 500 µm – 1000 µm) were deposited onto the surface of the substrate and the movements of individuals tracked during water drawdown. We found that *G. pulex* moved into the subsurface gravel sediments in response to drying, but their ability to remain submerged during water level reduction was impeded by fine sediment deposition. In particular deposition of the coarser sand fraction clogged the sediment surface, limiting vertical movements. Our results highlight the potential effect of sedimentation on *G. pulex* resistance to drying events in streams.

Keywords: Sedimentation; hyporheic zone; streambed drying; intermittent rivers; mesocosm; stranding; drying; invertebrate

3.2 Introduction

Streambed drying as a result of climate variability and anthropogenic pressures on water resources is an increasing global phenomenon (Acuña *et al*., 2014; Leigh *et al*., 2016), even in historically perennial systems (Datry *et al*., 2014; Pyne & Poff, 2017). As streams dry, flow becomes restricted within the channel, often forming a series of disconnected pools prior to complete drying and desiccation of the channel bed (Boulton *et al*., 2003). Habitat conditions typically become increasingly unfavourable for most aquatic organisms during drying events, often resulting in the complete elimination of lotic taxa, causing major changes to macroinvertebrate community structure and composition (Bunn & Arthrington, 2002; Bogan *et al*., 2015; Verdonschot *et al*., 2015; Leigh *et al*., 2016).

Many macroinvertebrate populations persist during dry events by employing a range of survival strategies including behavioural adaptations, such as vertical movement into the saturated sediments (Stubbington, 2012; Vander Vorste *et al*., 2016a; Vadher *et al*. Accepted), or physiological adaptations to desiccation (Strachan *et al*., 2015; Stubbington *et al*., 2016) and declining water quality (van Vilet & Zwolsman, 2008). Lotic macroinvertebrate taxa have been recorded and observed in the saturated subsurface sediments of drying streams, indicating that they may serve as a habitat where fauna may persist (Hose *et al*., 2015; Fenoglio *et al*., 2006), and from which populations may recolonize waterbodies following the resumption of flow (Vander Vorste *et al*, 2016a). However, confirmation of the vertical movement and the tracking on individuals has been hampered by the inherent difficulties associated with making direct observations within subsurface sediments (Vadher *et al*., 2017).

Sedimentation and the resulting loss of vertical connectivity between surface and subsurface sediments is widely considered a major cause of instream degradation globally, and may impede subsurface ecological functioning (Navel *et al*., 2010; Descloux *et al*., 2013). Fine sediments (typically referred to as particles < 2 mm in size) can infiltrate into subsurface sediments limiting the vertical movement of instream fauna (Weigelhofer & Waringer, 2003; Mathers & Wood, 2016) through the reduction of porosity and surface-groundwater hydrological exchange (Hartwig & Borchardt, 2014). Considering the widely acknowledged increasing input of fine sediment into rivers as a result of agricultural practices (Lamba et al., 2015), channel management (Dunbar et al., 2010) and urbanisation (Taylor & Owens, 2009; Naden *et al*., 2016) alongside the increased frequency of stream drying events (Pyne & Poff, 2017), there is a need to examine the combined effects of sedimentation and drying on faunal populations. Consequently, a growing number of field experiments have demonstrated the deleterious effects of increasing fine sediment content within the subsurface on faunal community structure and function within lotic systems (Richards & Bacon 1994; Bo *et al*., 2007; Larsen *et al*., 2011; Buendia *et al*., 2013; Jones *et al*., 2015). The direct effects of surface (Navel *et al*., 2010; Vadher *et al*., 2015) and subsurface (Mathers *et al*., 2014) clogging / colmation on the vertical movement of macroinvertebrates has, however, only been characterised and quantified more recently, using *ex-situ* experiments. These have demonstrated that sedimentation has a limiting and deleterious effect on macroinvertebrate vertical movements within subsurface sediments (Navel *et al*., 2010; Mathers *et al*., 2014; Vadher *et al*., 2015).

Previous field (e.g., Descloux *et al*., 2013) and laboratory investigations (e.g., Mathers *et al.* 2014) have largely inferred faunal responses to sedimentation by measuring the effect on their final position. This reflects the inherent difficulty of making direct, real-time, observations within subsurface sediments. Recent studies using individual organisms in mesocosms filled with transparent sediments, however, have facilitated direct observations of invertebrate movements and stranding within subsurface sediments in response to a reduction in water level and drying (e.g., Stumpp & Hose, 2013; Vadher *et al*., 2017). This greatly improves the ability to quantify and qualify movement behaviours in a more precise way.

Gammarus pulex (L.) (Amphipoda: Gammaridae) is a common benthic amphipod throughout north-western Europe (Crane, 1994; MacNeil *et al*., 1997). Where abundant, *G. pulex* is ecologically important in processing organic matter (Navel *et al*., 2010), and as a predator and prey for fish and other invertebrate species (Kelly *et al*., 2002; Kelly *et al*., 2006; MacNeil *et al*., 1997). *Gammarus pulex* have been reported to migrate into subsurface sediments in response to biotic competition (McGrath *et al*., 2007), elevated temperatures (Wood *et al*., 2010) and water level reduction (Vander Vorste *et al*., 2016b; Vadher *et al*., 2017). They are, therefore, a useful model organism. This study experimentally examined the effect of fine sediment deposition on the vertical movement of *G. pulex* within transparent subsurface sediments during water level reduction. The aim was to determine the extent to which coarse and fine sand deposition affects the vertical movement of *G. pulex* through subsurface sediments in response to water level reduction. We hypothesised that: i) declining water levels and substrate drying would result in the stranding of *G. pulex* individuals which were unable to remain submerged; and ii) the addition of fine sediment (sedimentation) would reduce the number of *G. pulex* remaining submerged as a result of impairment in the ability of individuals to enter the subsurface.

3.3 Materials and methods

3.3.1 Sediment tank mesocosms

Experiments were conducted using two transparent sediment tanks constructed using 1 cm thick clear acrylic panels (50 cm length × 35 cm height × 5 cm width) to create an internal volume of 5250 cm^3 (Fig. 3.1). To allow drainage, a 7 mm hole was made centrally into the base of the tank and a 5 mm silicone tube inserted. Drainage of water was controlled using a Hoffman clip, providing control of water depth to 1 mm precision (Fig. 3.1). To aid observation and water drawdown, five horizontal lines were marked onto the tank every 5 cm from the base (highest horizontal line at 25 cm from the base). The tanks were filled with a transparent gravel sediment substrate to a depth of 25 cm (Fig. 3.1) and were held vertically using wooden mounts 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.

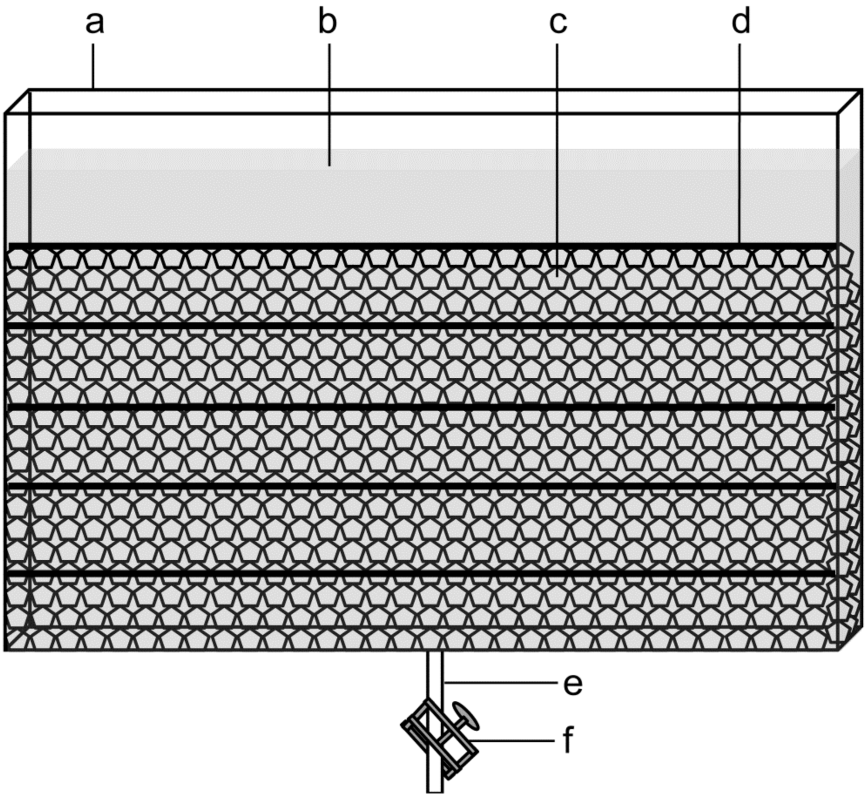

Fig. 3.1 Sediment tank mesocosm. a) acrylic tank (50 cm × 35 cm × 5 cm); b) water level at experiment start (5 cm above the sediment surface); c) 25 cm of transparent sediment (10 – 15 mm); d) line marked onto the tank at 5 cm intervals; e) 5 mm silicone tube; f) Hoffman clip.

3.3.2 Sediment treatments

Angular transparent gravel particles (10 –15 mm diameter) were used as the substrate onto which fine sediment treatments were applied. Two sand size fractions were used in the experiments, small (125 µm – 500 µm) and coarse (500 µm – 1000 µm), to create fine sediment treatments. These size fractions were chosen based on preliminary experiments which indicated that the smaller size fraction infiltrated into the substrate, under gravity, to the bottom of the tanks whilst the coarser sand particles bridged the spaces between the transparent gravel substrate particles, resulting in clogging of the substrate surface (Fig. 3.2). The interstitial volume within the top 5 cm of the substrate in each tank was determined by recording the volume of water drained from between the transparent particles (mean ± SE: tank 1 = 337 ± 1 ml; tank 2 = 339 ± 0.5 ml) in the top 5 cm. These interstitial volumes determined the amount of fine sediment required to fill all interstitial spaces within the 5 cm of the substrate. The two fine sediment sizes were thoroughly mixed in varying proportions of the total interstitial volume (100%, 87.5%, 75%, 50%, 25% and 12.5%) to create six fine sediment treatments (Table 3.1).

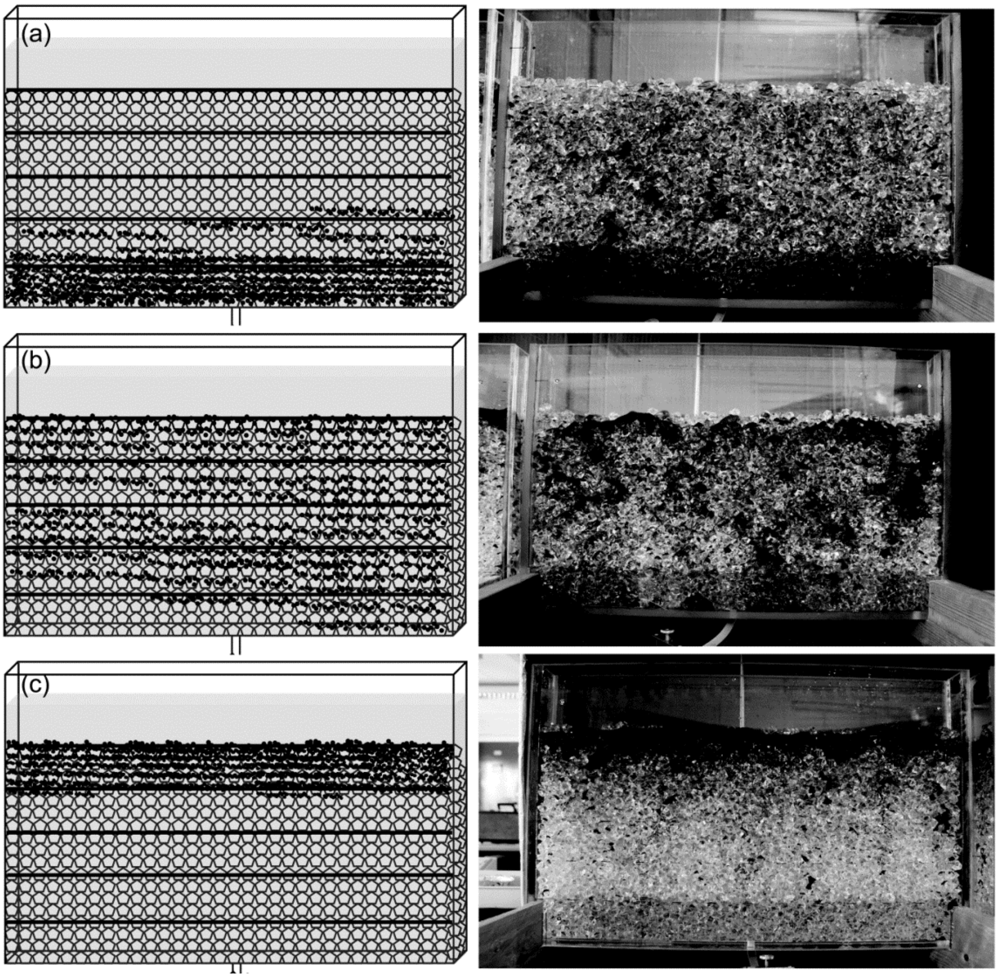

Fig. 3.2 Fine sediment infiltration through tank mesocosms using mixtures containing small (125 µm – 500 µm) and coarse (500 µm – 1000 µm) fine sediment particles. (a) 100% small fine sediment deposition; (b) 50% small and 50% coarse sediment mixture deposition; (c) 100% coarse fine sediment deposition.

Table 3.1 The proportion and volume of fine sediments used in each sediment treatment.

		Volume of small fines (500–1000 µm) (ml)		Volume of coarse fines (125–500 µm) (ml)	
Treatment	Fine sediment proportions	Tank 1	Tank 2	Tank 1	Tank 2
1	Control	-	-	-	-
2	100% small	337	339	-	-
3	75% small, 25% coarse	253	254	84	85
4	50% small, 50% coarse	169	169	169	169
5	25% small, 75% coarse	84	85	253	254
6	12.5% small, 87.5% coarse	42	42	295	297
7	100% coarse	-	-	337	339

3.3.3 Water treatment

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 the experiments. Complete oxygen saturation was maintained throughout each experiment using oxygen tablets, widely used in domestic aquaria. Immediately prior to the start of each experimental run, water was added to the tanks to 5 cm above the substrate surface (Fig. 3.1).

3.3.4 Test organisms

Gammarus pulex (>5 mm in length) were collected using a standard kick net (900 µm mesh, 230 mm × 255 mm frame, 275 mm bag depth) from a riffle on Black Brook (52°45’46.7”N 1°19’19.1”W) west of the town of Loughborough (Leicestershire, UK). Individuals were carefully removed from the net using tweezers and transported to the laboratory in 5-l containers of stream water for immediate use in experiments.

3.3.5 Experimental procedure

Sediment treatments were poured slowly onto the surface of the transparent gravel substrate through the 5 cm of surface water and left for 30 minutes to allow any natural settlement and vertical movement, due to gravity, of fine sediment through the substrate to occur. Ten *G. pulex* individuals were then introduced into each tank and left to acclimatise for 20 minutes prior to the start of experiments. During preliminary experiments, a 20 minute period was sufficient for exploratory and burrowing activity to subside. To minimise disturbance to organisms during the experiment, observation of the vertical position of *G. pulex* were made within the dark environmental cabinet using a low level LED light prior to each water level reduction. Water level was reduced in 12.5 mm increments every 15 min until a depth of 20 cm below the substrate surface was reached (a total duration of 300 minutes until drawdown was complete). A 5 cm depth of water was retained in each mesocosm at the end of each experiment as a refuge for organisms. Observations began at ‘time = 0’ (depth = 5 cm) and were made by counting the number of individuals in each 5 cm horizontal section. When water had been drawn down to 20 cm below the substrate surface, the number of *G. pulex* below the waterline (within the 5 cm refuge) was recorded and experiments terminated. Following the termination of experiments, the contents of each tank was carefully excavated and thoroughly washed to separate the transparent sediment, *G. pulex* individuals, coarse and small fine sediment particles. Each experimental trial was replicated seven times for the control (no fine sediment) and for each of the six sediment treatments (Table 3.1), providing 49 individual trials, each observed 21 times following water level reduction (total observations = 1,029).

3.3.6 Data analysis

We tested our first hypothesis, that declining water levels and substrate drying would result in some *G. pulex* individuals being unable to remain submerged, and our second hypothesis, that the addition of fine sediment (sedimentation) would result in reduced numbers of *G. pulex* remaining submerged, using full-factorial 2-way Repeated Measures ANOVA (RMANOVA) analyses. The percentage of *G. pulex* that remained submerged throughout the experiments were defined as the dependent variable, water depth was defined as the repeated measure (within-subject factor) and sediment treatment was defined as the between subject factor. Mauchly's tests were used to verify the RM ANOVA assumption of sphericity and the results of Greenhouse-Geisser tests used when this assumption was violated. We also tested the second hypothesis using a General Linear Model (GLM) to determine differences in the percentage of *G. pulex* that remained submerged at the end of experiments as the dependent factor with the sediment treatment defined as a fixed factor. *Post hoc* Fisher's Least Significant Difference (LSD) tests were used for both the RM ANOVA and GLM models to examine the effect of sediment treatment on the percentage of *G. pulex* that were submerged. All analyses were conducted in IBM SPSS Statistics (version 23, IBM Corporation, New York).

3.4 Results

3.4.1 Effect of water drawdown and fine sediment on vertical movement and stranding of G. pulex

The proportion of submerged *G. pulex* declined as water depth reduced in all sediment treatments, but decreased to a greater extent in treatments comprising higher proportions of coarse sand; there was a significant interaction between water depth and sediment treatment (RM ANOVA, Greenhouse-Geisser, $F_{20.992,\ 146.943}$ =

10.431, P = < 0.001; Fig. 3.3). The effect of water level reduction on the ability of *G. pulex* to move vertically and remain submerged was similar for treatments 1-3, for which a consistent reduction in the ability of *G. pulex* to remain submerged under the reducing water levels was observed during experiments. For treatments 5-6 the percentage of individuals submerged declined quickly for the first 5 cm of drawdown before a more gradual decrease of individuals remaining submerged was observed during experiments. Treatment 4 was intermediate between these two other groups. For treatment 7 (100% coarse sand addition) nearly all *G. pulex* became stranded following relatively minor reduction (10 cm) in water level (Fisher's LSD, P < 0.001; Fig. 3.3).

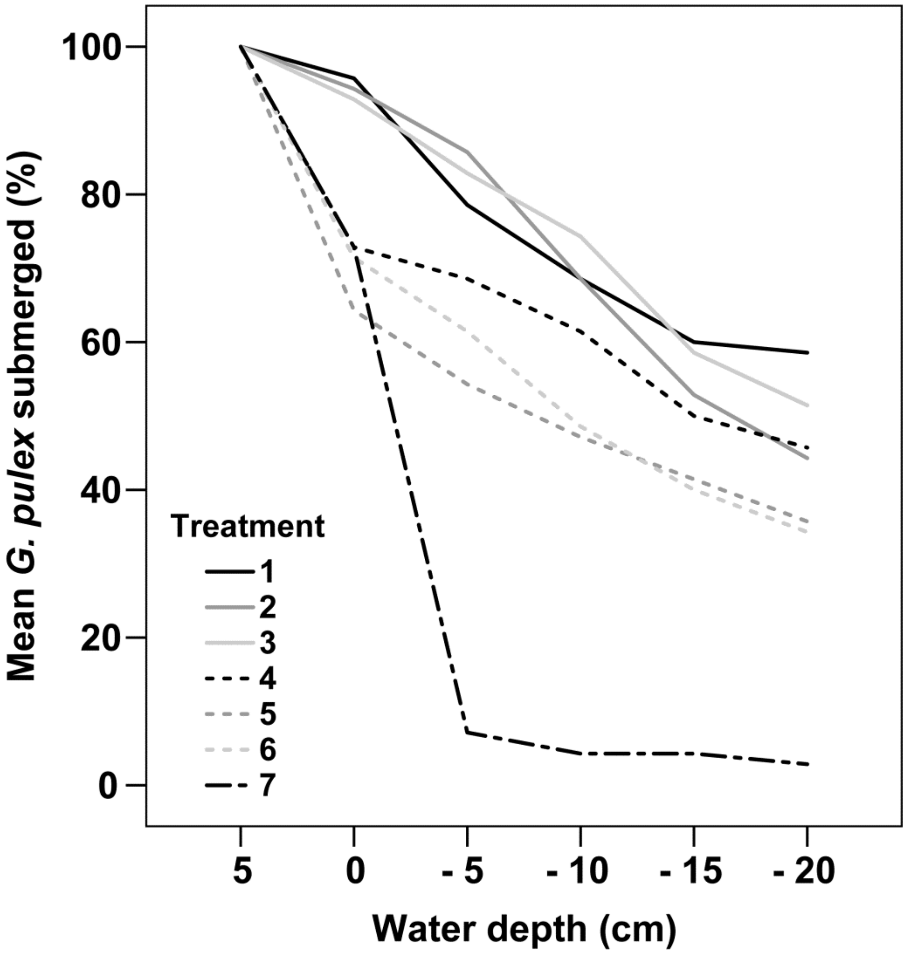

Fig. 3.3 Sediment treatment and water depth effect on the percentage of submerged *Gammarus pulex* during experiments. Sediment treatment 1–7 are defined in Table 3.1. *G. pulex* survivorship during water depth reduction was similar in treatments 1–3 and in 5–6 (Fisher's LSD, $P > 0.05$).

3.4.2 Effect of sediment treatment on the percentage of submerged G. pulex *at experiment end*

Sediment treatments comprising greater proportions of coarser sand particles reduced the percentage of *G. pulex* submerged at the end of experiments (GLM, $F_{6, 42} = 17.061$, $P < 0.001$; Fig. 4), although less *G. pulex* remained submerged at the end of experiments in treatment 2 compared to treatment 3 (Fig. 3.4). A markedly reduced proportion of *G. pulex* (2.9% ± 4.4% SE) remained submerged at the end of experiments for sediment treatment 7 (100% coarse sand addition) compared to all other treatments 1–6 (Table 3.2; Fig. 3.4).

Table 3.2 Fisher's Least Significant Difference *post hoc* pairwise comparison of *Gammarus pulex* survivorship at the end of experiments in treatments 1–7 (see Table 3.1). Significantly different *G. pulex* survivorships (P ≤ 0.05) are emboldened.

	***Post hoc* Fisher's (LSD) tests**						
Treatment	1	2	3	4	5	6	7
1		**0.026**	0.254	**0.043**	**0.001**	**<0.001**	**<0.001**
2			0.254	0.818	0.172	0.113	**<0.001**
3				0.360	**0.015**	**0.008**	**<0.001**
4					0.113	0.071	**<0.001**
5						0.818	**<0.001**
6							**<0.001**
7							

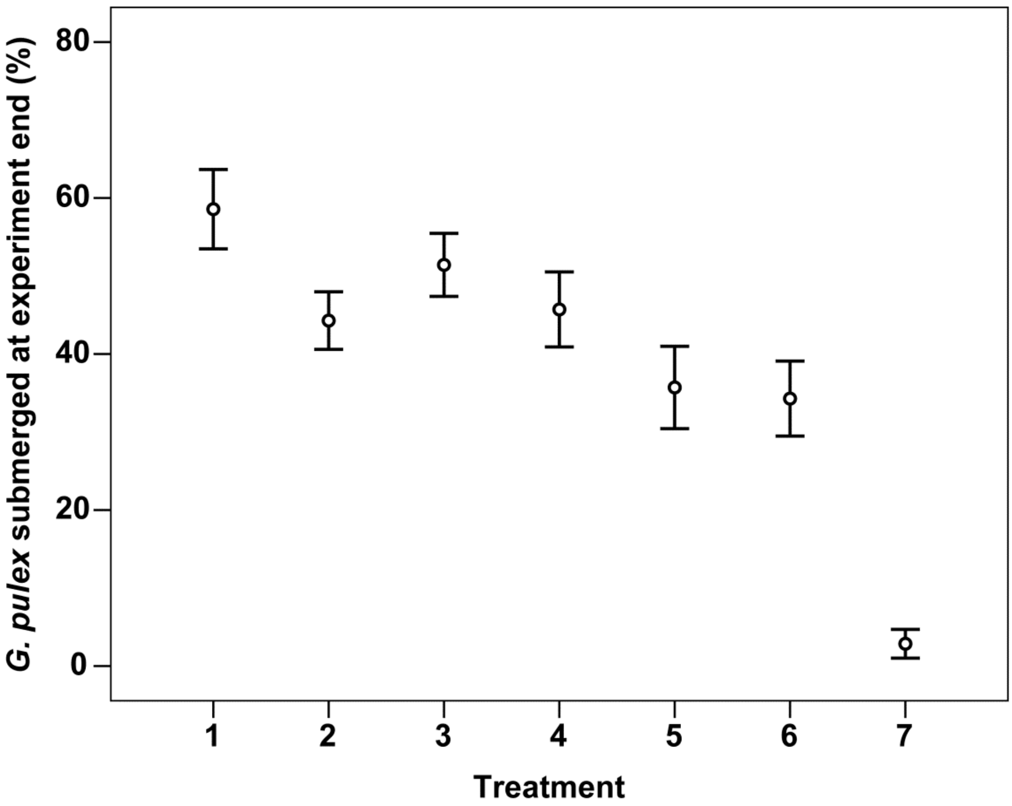

Fig. 3.4 Mean percentage of *Gammarus pulex* submerged (±1 SE) in sediment treatments at the end of experiments. Treatments 1–7 are defined in Table 3.1 with statistically different values highlighted in Table 3.2.

3.5 Discussion

Some *G. pulex* individuals were unable to remain submerged during dewatering, supporting our first hypothesis. These results also support the observations of Stumpp & Hose (2013) and Vadher et al. (2017) that reducing water level in artificial mesocosm experiments resulted in the stranding of individual invertebrates within subsurface sediments. Therefore, water level reduction as an environmental stressor may negatively affect faunal populations within the subsurface sediments. In this laboratory study, water quality parameters were kept relatively stable compared to the changes in water quality that occurs during the natural drying of streams (Boulton

& Lake, 2008). When combined with deteriorating water quality in natural streams, the effects of water level reduction on mortality may be significantly greater in temporary streams (Lake, 2003; Chadd et al., 2017) than those recorded in this study. A number of studies have inferred that subsurface clogging by fine sediment reduces the potential for vertical movement within subsurface riverbed sediments by invertebrates (e.g., Weigelhofer & Waringer, 2003; Descloux et al., 2013), but none have previously observed these effects. The use of transparent sediments within mesocosms has the potential to enhance understanding of faunal responses to drying in temporary streams, for example, Vadher *et al*. (2017) demonstrated a gradient of vertical movements through sediments due to different sediment characteristics. Without the use of transparent sediments only binary, presence / absence confirmation would have been possible. As a result, we were able to directly observe how decreased porosity, as a result of sedimentation, reduced the ability of *G. pulex* to migrate vertically and remain submerged. *Gammarus pulex* individuals actively moved through subsurface sediments in response to drying (Vadher et al., 2017). Therefore, this study shows how the extent of vertical movements made by *G. pulex* in response to surface water loss and drawdown into the subsurface was impeded by sedimentation.

We found support for our second hypothesis, that the addition of fine sediment (sedimentation) would result in reduced numbers of *G. pulex* remaining submerged because the ability of individuals to enter the subsurface was impaired. This reflects the high clogging potential of 500–1000 µm particles and their ability to bridge the interstitial spaces between particles, blocking pathways within the subsurface and reducing sediment porosity / permeability (Boulton et al. 1998; Bo et al. 2007; Vadher et al. 2015). The deposition of fine sediment particles (< 2000 µm) within riverbeds has been previously reported to reduce the vertical movement of macroinvertebrates into subsurface sediments (Richards & Bacon, 1994; Mathers et al. 2017) and our data demonstrates that these responses are due to the impairment and limitation of the ability of the individuals to move through the substrate. Our results showed a marked stepped / threshold effect of fine sediment on the vertical movement of *G. pulex* between treatment 6 (12.5% small and 87.5% coarse fine sediment) and treatment 7 (100% coarse fine sediment) which indicates that particle size and the heterogeneity of deposited sediments strongly influenced the ability of individual invertebrate to access the subsurface interstitial habitat.

The extent to which fine sediment and sediment composition affects individual species has been reported to be species-specific (Descloux et al., 2013; Vadher *et al*., 2017). For example, Descloux et al. (2013) reported a linear decline in macroinvertebrate abundance of species with increasing fine sediment within streambeds with the exception of the Ephemeropterans *Caenis* spp. and Heptageniidae; which displayed an exponential reduction and were completely absent when fine sediment content exceeded 30 and 50% respectively. However, while the physical effects of fine sediments on benthic habitat and organisms have been widely recognised (Richards & Bacon, 1994; Descloux et al., 2013), it is also important to acknowledge the effects that fine sediment deposition has on interstitial flow and the transport of nutrients and dissolved oxygen through the subsurface habitats (Olsen & Townsend, 2003). However, some taxa actively utilise fine sediments as a habitat (e.g., tubificid worms and Chironomidae) and in some instances construct galleries creating hyporheic flow paths and increasing connectivity (Nogaro et al., 2006; Nogaro et al., 2008). Therefore, further species-specific experiments are needed to quantify the effect of sedimentation on macroinvertebrate fauna.

This study has demonstrated that sedimentation affects *G. pulex* movement and stranding within subsurface habitats. Therefore, the vertical movement responses to sedimentation reported here will likely impact community resistance and resilience to drying as sedimentation reduces the subsurface refuge potential. We therefore highlight the need for effective refuge management through the enhancement of streambed porosity. Such management strategies should include measures to reduce fine sediment inputs into streams using sediment detention ponds / wetlands and planting riparian vegetation to stabilise river banks (Verstraeten & Poesen, 2000; Hughes, 2016). Where high river flows are insufficient in flushing fine sediment from streambeds, management techniques such as gravel jetting (Bašić et al., 2017), replenishing depleted coarser grained sediments (Merz & Ochikubo Chan, 2005; McManamay et al., 2010) and the use of in-stream structures to enhance hydraulic efficiency to transport fine sediments (Palm et al., 2007; Michel et al., 2014) should be considered.

In conclusion, our study highlights the importance of streambed permeability and fine sediment to allow for the vertical movements of macroinvertebrates during drying events. We particularly highlight the need to quantify the effect of deposited fine sediment composition on faunal community structure within temporary streams. With the frequency of drying events in streams increasing as a result of climate change (Ledger & Milner, 2015; Pyne & Poff, 2017) and anthropogenic pressure on water resources (Datry, 2014) alongside fine sediment increasing in streams (Lamba et al., 2015; Naden *et al*., 2016), mesocosm experiments may prove particularly useful in quantify the effects of multiple stressors on ecosystem structure and functioning.

Future research should therefore consider approaches which combine field and laboratory / mesocosm-based observations to facilitate greater understanding of streambed drying processes.

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Chapter 4 (Experiment 3)

The duration of channel drying affects survival of *Gammarus pulex* (Amphipoda: Gammaridae) within subsurface sediments: an experimental flume study.

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Atish N. Vadher:	Experiment design, experiment set up and data collection, data analysis, author of complete first draft of chapter and revision of drafts following supervisor feedback.
Jonathan Millett:	Advised on experiment design, data analysis and provided feedback on chapter drafts. PhD supervisor.
Paul J. Wood:	Advised on experiment design, data analysis and provided feedback on chapter drafts. PhD supervisor.
Rachel Stubbington:	Advised on original experimental design (Vadher et al., 2015). Commented on final chapter drafts used for journal submission.

4.1 Abstract

A range of field studies have demonstrated that benthic fauna use hyporheic sediments during drying events in temporary rivers, but the factors influencing the survival of fauna in subsurface sediments remain poorly quantified. Laboratory mesocosm experiments were conducted to determine how the length of drying events (1, 7, 14 and 21 d) influenced the survivorship of *Gammarus pulex* (L.) (Amphipoda: Gammaridae). Water level was reduced to 5 cm below the substrate surface during drying experiments and held at 5 cm above the sediment surface during control experiments. The results demonstrate that *G. pulex* survivorship was reduced with increasing length of the experiment period, particularly in the drying treatment compared to the control treatment. This study shows that *G. pulex* can use the subsurface habitat to persist during surface drying. Interacting climatic drivers and water resource pressures are increasing the spatial and temporal occurrence of streambed drying. The results from this study highlight the value of mesocosm-based studies for elucidating the abiotic controls of macroinvertebrate survival during drying events.

Keywords: Intermittent rivers; ephemeral streams; streambed drying; mesocosm experiment; drought; survivorship.

4.2 Introduction

Temporary streams experience periods of reduced hydrological connectivity as surface water levels decline, often resulting in sections of dry streambeds (Boulton, 2003; Larned et al., 2010). The ecological effects of surface water level reduction and drying on aquatic organisms potentially increases over time as abiotic conditions become increasingly unfavourable (Dewson et al., 2007; Lake, 2003; Lake, 2011). Flow cessation and surface drying events typically result in a reduction in the abundance of rheophilic taxa (Chessman, 2009; Verdonschot et al., 2015), an increased dominance of generalist taxa able to persist in isolated pools (Robson et al., 2011) and the presence of a limited number of desiccation-tolerant taxa (Datry, 2012; Stubbington et al., 2009). An increased duration of flow intermittence and channel drying can reduce benthic and hyporheic faunal density and richness as taxa reach their physiological limits (Datry et al., 2007; Arscott et al., 2010; Lancaster & Ledger, 2015), although recovery following the resumption of flow may occur quickly depending on refuge location (Chester & Robson, 2011). Studies investigating temporary streams have indicated that the duration of drying represents a primary control on the structure and diversity of communities (e.g., Feminella, 1996; Datry et al., 2014a; Storey, 2016). Adaptations of temporary stream fauna that enhance resistance and resilience to surface drying include behavioural responses, for example movement into saturated subsurface sediments (Vander Vorste, 2016a), life history adaptations, for example aestivation and diapause (Strachan et al., 2015) and physiological adaptations including desiccation-tolerance of juvenile or adult life stages (Stubbington et al., 2016).

Subsurface riverbed sediments have been identified as a potential habitat and refuge for benthic macroinvertebrates during adverse environmental conditions (Williams &

Hynes, 1974; Stubbington, 2012). Some studies have demonstrated that benthic macroinvertebrate lotic fauna move into saturated subsurface sediments in response to severe low flows and surface water loss (e.g. Fenoglio et al., 2006; Wood et al., 2010; Vander Vorste et al., 2016a) and others have reported limited or no evidence of subsurface refuge use (e.g. del Rosario & Resh, 2000; Young et al., 2011). These equivocal results illustrate that abiotic conditions in subsurface sediments are heterogeneous and that their use by benthic fauna depends on factors including substrate characteristics (Descloux et al., 2013) and water quality (Stubbington, 2012; Lawrence et al., 2013). This variability makes generalisation difficult, as predicting lotic community responses to drying is currently constrained by a lack of species-specific information. This lack of understanding is becoming increasingly problematic given the need to understand the effects of predicted future increases in the frequency and duration of stream drying associated with climate change and anthropogenic pressures (Ledger & Milner, 2015; Pyne & Poff, 2017). In an attempt to control for the heterogeneity of temporary streams experienced in the field, *ex-situ* laboratory flume and mesocosm investigations have been undertaken to examine the effects of surface drying on aquatic invertebrate fauna (e.g., Ledger et al., 2011; Poznańska et al., 2013; Vadher et al., 2015; Vander Vorste et al., 2016b).

Gammarus pulex (L.) (Amphipoda: Gammaridae) is an ecologically important amphipod which functions as a processor of coarse particulate organic matter (Navel et al., 2010), a predator of other invertebrates, and as prey of both invertebrates and fish across northwest Europe (MacNeil et al., 1997). It has been used in laboratory mesocosm investigations (e.g., McGrath et al., 2007; Mathers et al., 2014; Stubbington et al., 2017) and occurs widely in benthic and subsurface habitats of perennial and temporary streams (Stubbington et al., 2009; Wood et al., 2010;

Verdonschot et al., 2015). *G. pulex* has been observed to actively migrate into subsurface sediments in response to surface drying in both field (e.g., Vander Vorste et al., 2016a) and laboratory (e.g., Vadher et al., 2015; Vander Vorste et al., 2016b) investigations.

This study examined the effect of increased duration of surface water loss and sediment surface drying duration on the survivorship of *G. pulex* within mesocosms in a recirculating flume. The mesocosms allowed flow velocity, water level, water quality (conductivity, dissolved oxygen, pH and temperature) and sediment characteristics to be controlled and held constant. The aim was to determine the extent to which *G. pulex* can survive in sediments during drying events and the effect of dry period duration on survivorship.

4.3 Materials and methods

4.3.1 Development of the flume mesocosm

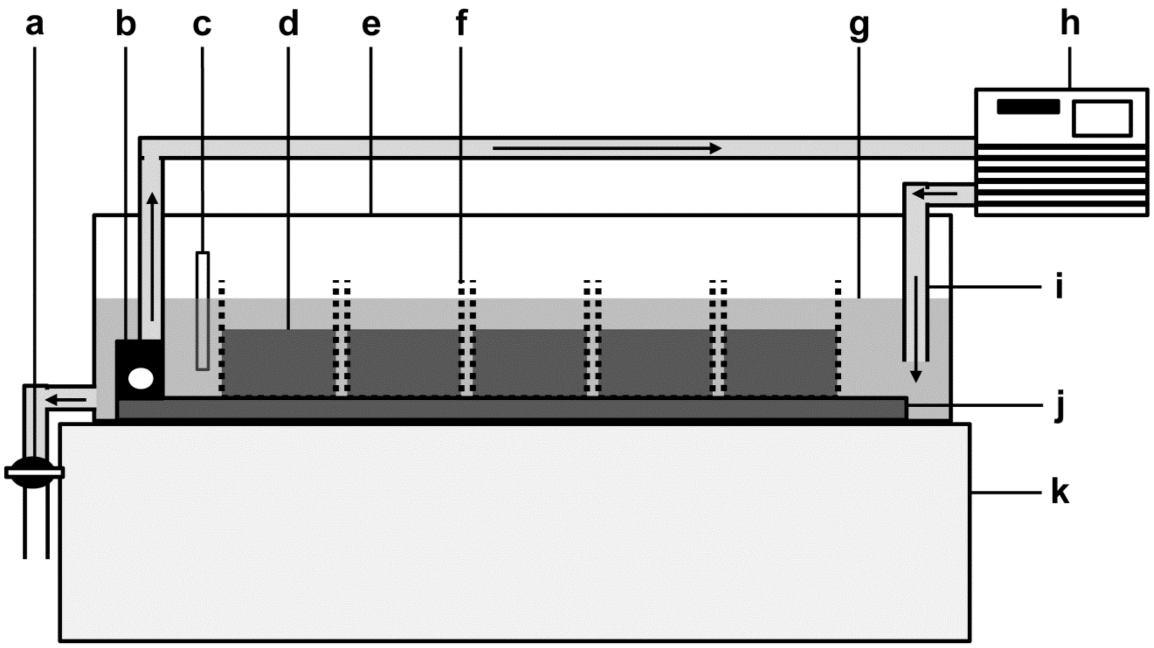

Fig. 4.1 Schematic cross-section through the experimental flume. a) ball valve to control water level; b) water pump; c) ruler; d) coarse gravel (10 – 20 mm) matrix; e) experimental tank; f) mesocosm (20 cm × 20 cm × 20 cm); g) water level (5 cm above coarse gravel matrix); h) water cooler; i) pipe; j) gravel base; k) platform. Arrows represent direction of flow. Not to scale. (Adapted from Vadher et al., 2015).

The experiment was conducted using an experimental flume (219 cm long × 56 cm wide × 56 cm deep; adapted from Vadher et al., 2015). The flume was constructed using a 300 l capacity tank linked to a water cooler and pump to create a closed recirculating system held at a temperature of 12 ± 0.5°C (Fig. 4.1). Five 20 cm × 20 cm × 20 cm mesocosms made using 3 mm-aperture steel mesh were lined with 150 µm-mesh nylon netting and filled to a depth of 10 cm with pre-washed coarse fluvial gravel (10 – 20 mm diameter). The mesocosms were placed adjacent to each other on a fine gravel base (2 – 4 mm diameter; Fig. 4.1). Water level was controlled using a ball valve and monitored (0.25 mm accuracy) using a ruler fixed to the inside wall of the flume (Fig. 4.1).

4.3.2 Experimental procedure

Twenty-four hours prior to an experiment, each mesocosm and its sediment were washed and the experimental tank filled with dechlorinated tap water to 5 cm above the substrate surface (Fig. 4.1) before being circulated until a temperature of 12°C was recorded. *G. pulex* were collected using a standard kick net (900 µm mesh, 230 mm × 255 mm frame, 275 mm bag depth) from a riffle on Black Brook (52°45’46.7”N 1°19’19.1”W) in Loughborough (Leicestershire, UK). Specimens used in experimental trials were carefully removed from the net using tweezers and those > 5 mm in length placed into a 5-l container of stream water for immediate transport to the laboratory. Mixed pre-conditioned native leaf litter from the stream was thoroughly washed in the stream channel to remove attached stream fauna and returned to the laboratory to serve as a food source during experiments. Between four and six leaves (depending on size) were shredded and distributed through the gravel subsurface of each mesocosm. Ten *G. pulex* were then transferred into each of the five mesocosms (population density in each mesocosm = 50 *G. pulex* m^{-2}) and left to acclimatise for approximately 30 minutes prior to water level reduction. Previous experiments have demonstrated this acclimation period is sufficient to allow individuals to complete initial exploratory movements and seek refuge between sediment clasts (Vadher et al., 2015).

To examine the effect of drying duration (1, 7, 14 and 21 days) on *G. pulex* survivorship, three replicate experiments were conducted for each duration in both drying (water level reduction) and control conditions (water level maintained at 5 cm above the coarse gravel surface), providing a total of 24 experiments (5 mesocosms per experiment; n = 120 mesocosms). A water pump recirculated water through the tank at a constant rate of 41.7 l min^{-1} for the duration of all experiments (drying and control conditions). After the initial faunal acclimation period, water level was reduced over a 2 h period from 5 cm above the gravel surface to 5 cm below the surface (dewatering rate of 5 h cm^{-1}) by opening the ball valve and reducing water level by 1.25 cm every 15 minutes to trigger vertical movement into the sediment by *G. pulex*. On completion of the water level reduction, the flume was maintained at the same water level (5 cm saturated gravel), temperature and flow rate for the duration of each experiment.

4.3.3 Assessing G. pulex survivorship

Upon termination of each experiment, mesocosms were individually removed and the number of surviving *G. pulex* determined. The contents of the mesocosms were carefully excavated into a steel tray (50 cm diameter) and rinsed so that all *G. pulex* could be removed and transferred into a beaker of dechlorinated tap water (Fig. 4.2). The survivorship of *G. pulex* was determined by the number of live (moving) *G. pulex* present. Whole, non-mobile individuals were considered to have died during the experiment and absent individuals or body parts were assumed to reflect predation (McGrath et al., 2007) or decomposition following stranding above the waterline. Body parts (e.g., legs, antennae) were not recorded unless the head was observed.

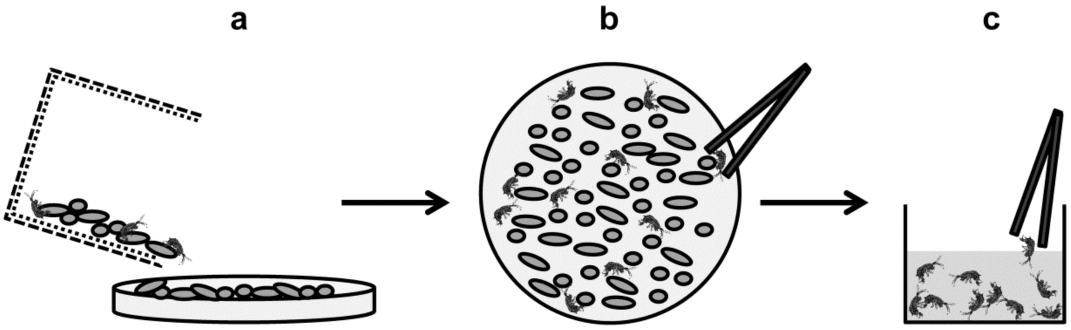

Fig. 4.2 Mesocosm excavation procedure to determine *Gammarus pulex* survivorship. a) Mesocosm contents excavated onto a steel tray; b) *G. pulex* individuals removed using tweezers; c) *G. pulex* transferred to a beaker of water to determine survival. Arrows indicate order of procedure.

4.3.4 Abiotic parameters

Prior to the start of each experiment, pH, dissolved oxygen (mg l^{-1}), temperature (ºC) and conductivity (µS cm^{-1}) were measured in the free water around each mesocosm using laboratory meters (Hanna Instruments, Bedfordshire, UK). At the end of each experiment, abiotic parameters were re-measured to determine the stability of abiotic conditions throughout the experiment.

4.3.5 Statistical analysis

To determine if each mesocosm could be regarded as independent, a preliminary Repeated Measures (RM ANOVA) analysis was used to examine the effect of both experiment condition (control / drying) and duration (1, 7, 14, 21 days) on the percentage survival (survivorship) of *G. pulex*. Mesocosm (1-5) was defined as a within-subject effect and experiment condition, duration, and their interaction, were both defined as between-subject effects. Mauchly’s tests were used to verify the RM ANOVA assumption of sphericity and the results of Greenhouse-Geisser tests consulted when this assumption was violated. Mesocosm (RM ANOVA, Greenhouse-Geisser, $F_{2.6,\ 41.53} = 2$, $P = 0.136$) and its interaction with condition ($F_{2.6,\ 41.53} = 2.348$, $P = 0.095$), duration ($F_{7.8,\ 41.53} = 2.348$, $P = 0.065$) and their interaction ($F_{7.8,\ 41.53} = 2.348$, $P = 0.086$) did not affect *G. pulex* survivorship and could be considered independent. As a result, General Linear Models (GLM) were used to examine the effect of experiment condition, duration and the change in abiotic parameters (end – start) on *G. pulex* survivorship. Experiment condition and duration were defined as fixed factors with survivorship defined as the dependent variable. The change in pH, dissolved oxygen, water temperature and conductivity were defined as covariates. The effect of experiment condition and duration on these abiotic covariates was examined using GLM models by defining the mean abiotic parameters as dependent variables and experiment condition and duration as fixed factors. All analyses were conducted in IBM SPSS Statistics (version 23, IBM Corporation, New York).

4.4 Results

Overall, abiotic parameters during the experiments were similar (Table 4.1). Water pH and dissolved oxygen did not varying significantly between control and drying experiments (Table 4.2). Water temperature was marginally higher and conductivity was lower in drying experiments (Table 4.1; Table 4.2). Water temperature, conductivity and pH varied among experiment durations (Table 4.2).

Table 4.1. Mean values (± SE) of pH, dissolved oxygen, water temperature and conductivity after experiments in the control and drying condition.

	Duration (days)			
Mean parameter	1	7	14	21
	Control condition			
pH	8.4 ± 0.0	8.4 ± 0.0	8.2 ± 0.0	8.2 ± 0.0
Dissolved oxygen (mg l^{-1})	7.4 ± 0.0	7.3 ± 0.0	7.2 ± 0.0	7.3 ± 0.0
Water temperature (ºC)	12.1 ± 0.0	12.1 ± 0.0	12.4 ± 0.0	12.3 ± 0.0
Conductivity (µS cm^{-1})	513 ± 1.2	509 ± 2.0	572 ± 6.7	572 ± 2.3
	Drying condition			
pH	8.3 ± 0.0	8.3 ± 0.0	8.1 ± 0.0	8.3 ± 0.0
Dissolved oxygen (mg l^{-1})	7.1 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	6.9 ± 0.1
Water temperature (ºC)	12.2 ± 0.0	13.1 ± 0.1	13.0 ± 0.1	12.7 ± 0.1
Conductivity (µS cm^{-1})	512 ± 3.5	520 ± 6.4	485 ± 4.5	507 ± 1.9

Table 4.2 General linear model (GLM) analysis of variation in abiotic parameter between control and drying experiments (experiment condition) and duration (1, 7, 14 and 21 days). Significant terms are emboldened.

	Experiment condition			Duration		
Abiotic parameter	*df*	*F*	*P*	*df*	*F*	*P*
pH	1	2.714	0.102	3	6.896	**<0.001**
Dissolved oxygen	1	1.517	0.221	3	1.580	0.198
Temperature	1	44.458	**<0.001**	3	9.894	**<0.001**
Conductivity	1	38.873	**<0.001**	3	10.598	**<0.001**

Gammarus pulex survivorship decreased with increasing duration for both experiment conditions, but was reduced to a greater extent during the drying experiments (significant interaction term; GLM, $F_{3,\,112}$ = 5.562, P = 0.001; Fig. 4.3).

Gammarus pulex survivorship was similar after 1 and 7 days for control conditions (Fishers LSD, $P = 1$). Mean survivorship (± 2.08% SE) following control experiments was 100% after both 1 and 7 days and was reduced to 93% and 85% after 14 and 21 days. Mean survivorship following drying experiments was 100% after 1 day, but reduced to 89%, 79% and 70% after 7, 14 and 21 days respectively. When individual abiotic parameters were considered, change in pH (GLM, $F_{1,\,108} = 0.701$, $P = 0.404$), dissolved oxygen ($F_{1,\,108} = 0.114$, $P = 0.736$), water temperature ($F_{1,\,108} = 0.167$, $P = 0.684$) or conductivity ($F_{1,\,108} = 1.929$, $P = 0.168$) did not significantly influence *G. pulex* survivorship.

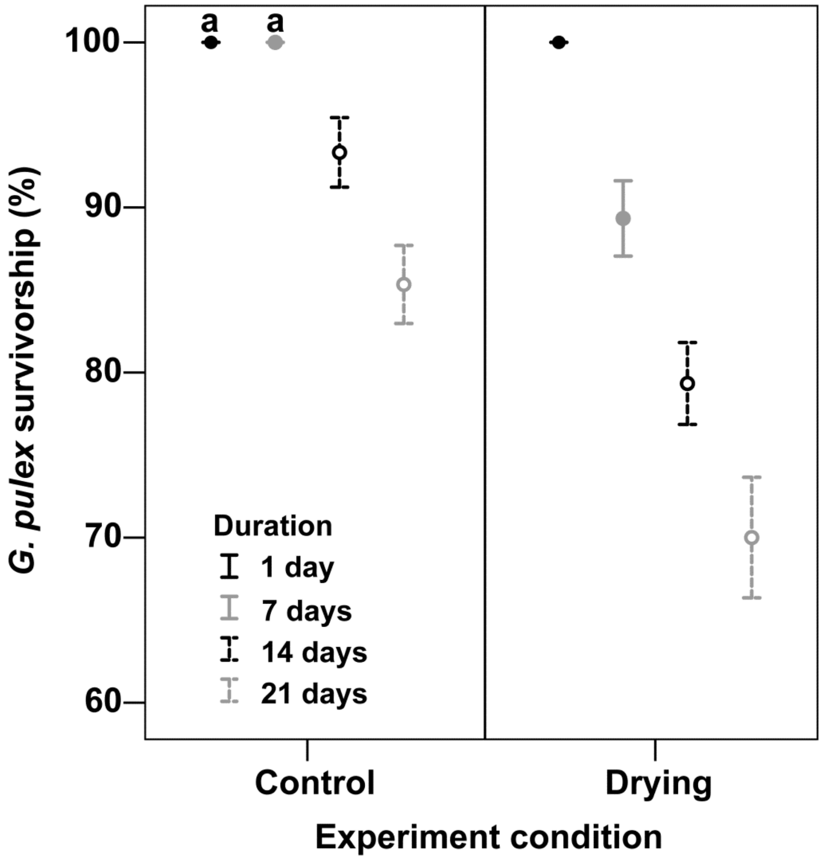

Fig. 4.3 *Gammarus pulex* survivorship (± 1 SE) after each experiment duration. The left panel shows the control condition, and the right panel shows the drying condition. Letter ‘a’ indicates values which are not statistically different in the control experiment (Fisher’s LSD, $P > 0.05$). *Y* axis starts at 60%.

4.5 Discussion

Survivorship was lower for drying experiments compared to experiments in which surface water persisted. The findings of this study support those of previous laboratory studies which have reported a reduction in macroinvertebrate survival in response to drying (Poznańska et al., 2013; Vander Vorste et al., 2016b). The limitation of these studies has often been the short experimental durations, from hours (Vadher et al., 2015) to approximately a week (e.g., Poznanska et al., 2013; Vander Vorste et al., 2016b), leaving the effect of longer drying durations on macroinvertebrate survival uncharacterized. Vadher et al. (2015) observed limited vertical movements and stranding above the water line in 2-hour dewatering experiments as fine sediment reduced sediment porosity in mesocosms of identical design to those used in this experiment. By extending the experimental duration, the current study extends our understanding of faunal response to surface water loss. The longer duration may potentially have allowed individuals to re-submerge into the moist subsurface sediments following any initial stranding and move vertically into the saturated subsurface over a longer time period. *G. pulex* survivorship remained relatively high following 7 days of surface water loss compared to Vander Vorste et al. (2016b), who reported survival of up to 71% after 7 days in mesocosms experiencing water level reduction to 30 cm below the substrate surface. Survivorship in the experiments presented in this study may have been higher due to the relatively short vertical distance required by *G. pulex* to remain submerged.

The medium to coarse gravel particle sizes used in this study were unlikely to have impeded the vertical movement of *G. pulex* into the subsurface (see chapter 2) and the excess leaf litter provided a plentiful food source. Reduced survivorship in drying conditions may therefore reflect intraspecific predation (McGrath et al., 2007;

Stubbington et al., 2011). The results demonstrate survivorship was lower in drying experiments, suggesting that increased density-dependent biotic interactions may have occurred as the wetted habitat constricted (Stubbington et al., 2011), a suggestion supported by the significant interaction recorded between experiment condition (control / drying) and duration.

The experiments provide clear evidence that increasing drying duration reduces survivorship. This finding supports observations made as part of a mesocosm-based field experiment investigating the effect of identical drying durations (7, 14 and 21 days) on the survivorship of *G. pulex* (see chapter 5). Under field conditions, *G. pulex* survivorship was approximately 65%, 60% and 51% following 7, 14 and 21 day dry periods, respectively (see Chapter 5). In the current investigation, the effect of drying duration on survivorship was reduced compared to this field study (lowest mean survivorship recorded was 70% after 21 days of drying in the current experiment), almost certainly reflecting the relative stability of other abiotic variables within the laboratory flume compared to field conditions. This study provides supporting evidence that the hyporheic zone functions as a refuge for benthic invertebrates if environmental parameters (water quality, food sources) remain favourable (Hose et al., 2005; Fenoglio et al., 2006; Vander Vorste et al., 2016a). However, the relationship between survivorship and drying duration seems to be linear (Fig. 4.3), indicating that subsurface sediments may be limited in their long-term refuge potential.

In conclusion, the results of this study support field investigations highlighting the effect of streambed drying on instream faunal densities (e.g., Clarke et al., 2010; Datry et al., 2014a; Bogan et al., 2015; Verdonschot et al., 2015). Specifically, the results of this study show that surface drying reduces survivorship within subsurface sediments, and that this reduction increases over time. However, field studies often highlight the effect of other, usually interacting, abiotic parameters associated with channel drying (e.g., low dissolved oxygen, high conductivity and elevated water temperatures; Bond et al., 2008; Verdonschot et al., 2015). Therefore, this laboratory-based mesocosm study demonstrates that *ex-situ* experiments can facilitate understanding of the influence of abiotic factors by specifically manipulating them or holding them constant (Vadher et al., 2015; Vander Vorste et al., 2016b; Vander Vorste et al., 2017).

The predicted effects of climate change and increasing demand on water resources are likely to increase the frequency and duration of stream drying events (Ledger & Milner, 2015; Pyne & Poff, 2017). This study shows the potentially negative effect of drying and increasing drying duration on *G. pulex* survivorship, which could have consequences that reverberate throughout the food webs if processing of coarse organic matter is reduced (Ledger et al., 2011). Given the linear relationship between *G. pulex* survivorship and drying duration, longer experiments are needed to further understand the effects of drying duration on macroinvertebrate survivorship. This study provides further evidence of the need for effective refuge management in streams and the maintenance of streambed sediments to enhance vertical connectivity (Boulton et al., 2010). Quantifying the effects of drying and refuge use is therefore important, and future research should consider combined field and mesocosm-based approaches to facilitate a better understanding.

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Chapter 5 (Experiment 4)

Drying duration and stream characteristics influence macroinvertebrate survivorship within the sediments of a temporary channel and exposed gravel bars of a connected perennial stream.

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Author contributions

Atish N. Vadher:	Experiment design, experiment set up and data collection, data analysis, author of complete first draft of chapter and revision of drafts following supervisor feedback.
Jonathan Millett:	Advised on experiment design, data analysis and provided feedback on chapter drafts. PhD supervisor.
Paul J. Wood:	Advised on experiment design, assisted in the field, data analysis and provided feedback on chapter drafts. PhD supervisor.
Rachel Stubbington:	Commented on final chapter draft for journal submission.

5.1 Abstract

Intermittent rivers, which experience periods of flow cessation and streambed drying, occur globally. Given that the frequency and duration of stream drying events is likely to increase as a result of anthropogenic pressures and global climate change, riverbed sediments may become increasingly important as refuge habitat for benthic macroinvertebrates. This study aimed to examine the effect of surface water loss and increasing drying duration on the survivorship of *Gammarus pulex* (L.) (Amphipoda: Gammaridae) within exposed gravel bars of a perennial stream and a temporarily flowing side channel. *G. pulex* survivorship declined more over time during drying conditions compared to control conditions (flowing water present). More individuals survived in the temporary channel compared to the exposed gravel bars. These results illustrate that subsurface sediments may facilitate *G. pulex* persistence during drying events and highlight the potential need for effective refuge management and conservation for instream fauna during drying events.

Keywords: Intermittent river; streambed drying; dry period; hyporheic refuge; faunal survival; *Gammarus pulex*

5.2 Introduction

Temporary streams experience periods of surface flow cessation and typically the drying of some or all of the river bed (Bogan et al., 2015; Datry et al., 2016). These streams comprise a large proportion of the total channel length across the globe, occurring throughout climatic zones from the poles to the equator (Acuña et al., 2014; Leigh et al., 2016). In some instances, historically perennial streams now experience intermittent flow and channel drying due to anthropogenic activities such as water abstraction, which captures some or all of the surface flow (Mackay et al., 2014; Arroita et al., 2017). The frequency and duration of 'no-flow' events including streambed drying may increase in some global regions based on future climate change predictions (Bonada et al., 2007a; Verdonschot et al., 2010; Ledger & Milner, 2015; Pyne & Poff, 2017), therefore, a greater understanding of the response of lotic ecosystems to drying duration would help guide future management options.

Drying events are typically conceptualised as 'ramp' disturbances which intensify over time as environmental conditions become increasingly unfavourable for the majority of organisms (Lake, 2011). However, the biotic response to drying events and recovery may be characterized by 'stepped' changes in faunal diversity and abundance as critical thresholds of habitat connectivity are transcended (Boulton, 2003; Bogan et al., 2015). Drying events result in major changes to instream communities (Leigh et al., 2016), most notably the loss of rheophilic (Graeber et al., 2013) and desiccation-sensitive taxa (Bogan & Lytle, 2011; Storey, 2016). Drying events may, therefore, be the primary driver of community structure and functioning in temporary streams (Poff et al., 1997; Bunn & Arthington, 2002; Leigh & Datry, 2017). As a result, there is a need to quantify the effects of stream drying on population structure and functioning in lotic ecosystems (Dewson et al., 2007).

The ability of fauna to persist during drying events may be achieved through behavioural adaptations, for example burrowing into the saturated sediments of the hyporheic zone (Stubbington, 2012; Vander Vorste et al., 2016a), or physiological adaptation such as desiccation-tolerant juvenile or adult life stages (Strachan et al., 2015; Stubbington et al., 2016) and tolerance of declining water quality as discharge declines (van Vilet & Zwolsman, 2008; Whitworth et al., 2012). Such changes generally include increased conductivity as a result of the concentration of solutes due to evaporation (e.g. Caruso, 2002) and decreased dissolved oxygen (e.g. Boulton & Lake, 1992; Sprague, 2005). Adaptation to drying enhances community and population resistance (ability to persist during an event) and resilience (ability to recover after flows resume; Lake, 2000; Bogan et al., 2014).

Subsurface sediments have been demonstrated to function as a refuge for invertebrate fauna during drying events by both field studies (Hose et al., 2005; Fenoglio et al., 2006; Vander Vorste et al., 2016a) and laboratory investigations (Vadher et al., 2015; Vander Vorste et al., 2016b). Following the resumption of surface flow, these sediments may be the primary source of stream recolonists, if individuals persist and migrate back to the surface sediments (Vander Vorste et al., 2016a). A range of studies have examined the effect of dry periods and flow permanence (perennial, intermittent, and ephemeral streams) on community structure, often demonstrating that different dry phase durations control community composition (e.g. Feminella, 1996; Bonada, 2007b; Arscott et al., 2010; Datry, 2012; Storey, 2016). Increasing dry phase duration may also decrease macroinvertebrate abundance (Storey, 2016).

Gammarus pulex (L.) (Amphipoda: Gammaridae) is a predominantly benthic organism widespread across much of north-western Europe (Crane, 1994; MacNeil et al., 1997). Where abundant, *G. pulex* is an ecologically important crustacean due to its role in processing coarse particulate organic matter (Navel et al., 2010), as a predator of other invertebrates (Kelly et al., 2006), and as a prey for predatory invertebrates, fish and birds (MacNeil et al., 1997; Kelly et al., 2002). *G. pulex* occurs in the benthic and hyporheic sediments of perennial and temporary streams (Stubbington et al., 2009; Wood et al., 2010), migrating into the subsurface sediments in response to increased predation pressure (McGrath et al., 2007) and channel drying (Vander Vorste et al., 2016a).

In this study, we examined the effect of increasing duration of surface water loss (drying) on the survivorship of *G. pulex* using mesocosms within the bed of a temporary channel and exposed gravel bars of a directly adjacent, perennial channel. The aim of this study was to quantify the effect of surface drying and the duration of drying events on *G. pulex* survivorship.

5.3 Materials and methods

5.3.1 Study site

Black Brook is a small regulated stream located west of Loughborough (Leicestershire, UK). The study sites were located 950 m downstream of Black Brook reservoir (52°45’53.1”N 1°19’16.8”W) where the channel divides into two parallel branches, each approximately 3 m wide (Fig. 5.1). The primary channel sustains perennial flow and the secondary channel is subject to temporary flow, experiencing complete streambed drying during base flow conditions. In the perennial channel, marginal gravel bars were exposed as discharge declined. This allowed the investigation of the effect of increasing duration of drying on faunal survival within both exposed gravel bars of the perennial channel and within the temporary channel

(Fig. 5.1). Both channels were shaded by deciduous trees and drained pastoral agricultural land.

Preliminary surveys were conducted to quantify the sediment composition and organic matter content of both channels. The substrate of both channels was sampled five times in representative areas using a McNeil sampler (McNeil & Ahnell, 1964), indicating that the subsurface sediments were primarily composed of cobble-gravel sized clasts: 90.2% in the perennial channel and 79.7% in the temporary channel. The proportion of fine sediment (< 2 mm) comprised 9.8% in the perennial channel and 20.3% in the temporary channel. Particulate organic matter content was 14.4% in the perennial channel and 17.8% in the temporary channel.

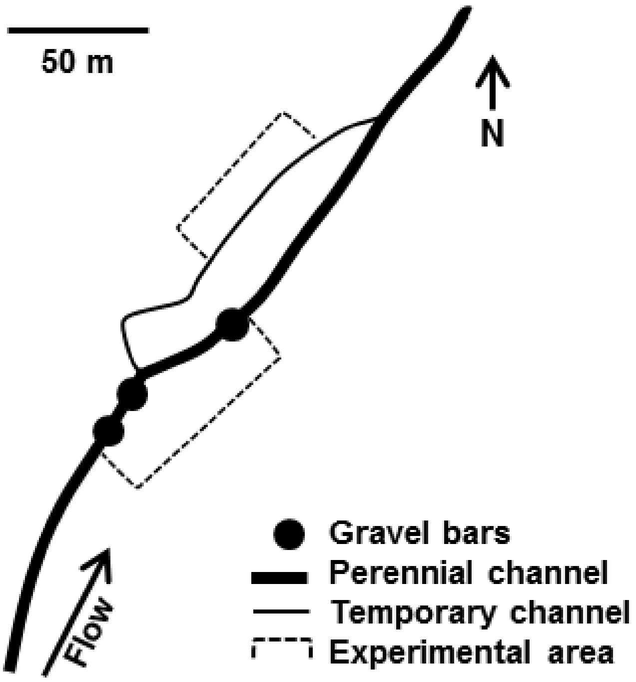

Fig. 5.1 Diagram of the Black Brook study sites. The experimental area in the perennial channel (containing three gravel bars) and the temporary channel are shown.

5.3.2 Subsurface mesocosm column description and installation

Open-ended PVC pipe sections (6.8 cm internal diameter × 25 cm length) were used as subsurface columns to house mesocosms (adapted from Mathers & Wood, 2016). Columns were open-ended to allow movement of downwelling and upwelling water, and perforated with 16 0.6-cm diameter holes to allow subsurface water to flow through the columns horizontally (Fig.5. 2). The columns were inserted to a depth of 25 cm into the streambed by driving a steel pipe (6 cm diameter) vertically into the sediment and threading a column over the pipe and into the subsurface (Fig. 5.2). The steel pipe was then extracted, leaving a subsurface void within the columns (Fig. 5.2).

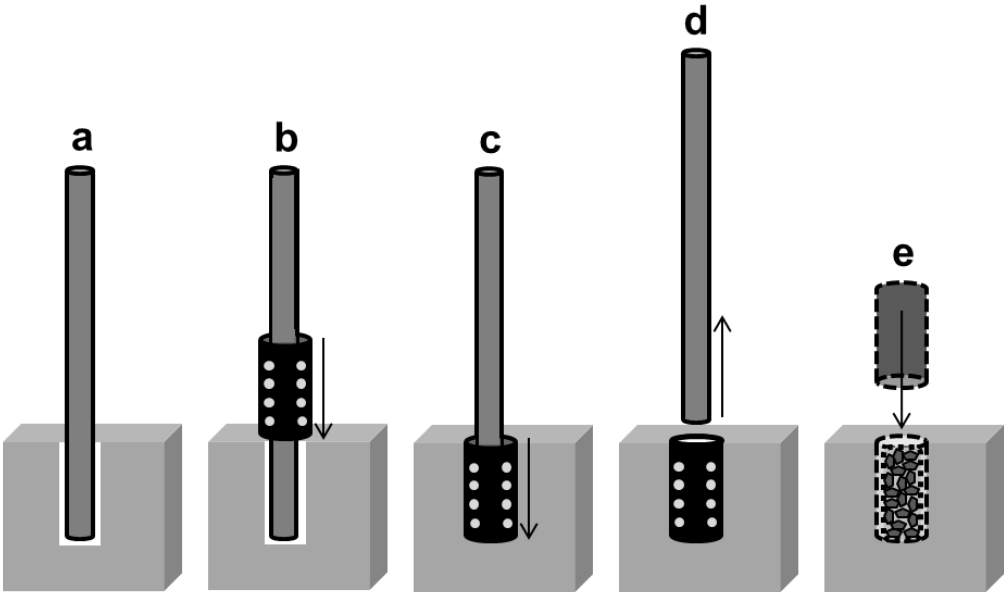

Fig. 5.2 Schematic diagram of subsurface column installation. A) Steel pipe (6cm diameter) driven 25 cm into the streambed; b) subsurface column thread over the pipe; c) column driven into the streambed around the pipe; d) pipe removed leaving the column in place; e) blank sediment bag inserted into subsurface column. Not to scale.

Twelve columns were inserted at the margin of each of three submerged gravel bars (*n* = 36) in the perennial channel (Fig. 5.1) with ≥ 50 cm between each to avoid any influence of adjacent columns during installation and the experiments. A total of 36 subsurface columns were also inserted into the temporary channel, ≥ 50 cm apart (Fig. 5.1). To allow the sediment matrix around the columns to settle and to avoid the subsurface voids created by the columns filling with fine sediment, 'blank' sediment bags (0.5 cm aperture mesh) containing medium-sized gravel were inserted into the columns until the experiment commenced, when they were replaced by experimental mesocosms.

The experimental mesocosms were constructed from 60 cm^2 sections of 250 µm aperture mesh. Each mesocosm was filled with medium-sized gravel particles (10 – 20 mm size range) and mixed pre-conditioned native leaf litter from the channel upstream. *G. pulex* were collected from a riffle > 200 m upstream of the study sites using a standard kick net (1 mm mesh, 230 mm × 255 mm frame, 275 mm bag depth) and 10 individuals (> 5 mm in size) were placed into each mesocosm. Each mesocosm was securely sealed with a cable tie to contain the contents throughout the experiment.

To examine the effect of drying duration, mesocosms were left *in situ* for 7, 14 or 21 days during both flowing (control) conditions and during drying of the marginal gravel bars and temporary channel. Given that the temporary channel and perennial stream gravel bars did not experience surface water loss at exactly the same time, control and drying experiments were conducted when the conditions were appropriate in each channel. Twelve replicate mesocosms × 3 durations × 2 conditions × 2 channels yielded a total of 144 mesocosms.

At the end of the experimental period (7, 14 or 21 days), mesocosms were extracted from subsurface columns and submerged into a container of stream water for immediate transport to the laboratory for determination of survivorship. To examine variability in abiotic parameters, dissolved oxygen (using a dissolved oxygen meter, Hannah Instruments HI-9142), pH and temperature (using a handheld pH/temperature tester, Hannah Instruments pHep®4 HI-98127), conductivity (using a handheld conductivity sensor, Hannah Instruments HI-98311), and water level from the surface of the sediment were measured. These were measured *in situ* in the free water within subsurface columns before and after mesocosms were deployed.

5.3.3 Laboratory assessment of G. pulex *survivorship*

The contents of individual mesocosms were carefully placed into a large white tray containing stream water, inspected, and survivorship determined by counting the number of live (active) *G. pulex* present. Inactive whole and parts of *G. pulex* individuals were recorded as dead, and absent *G. pulex* were assumed to have been cannibalised (McGrath et al., 2007) or decomposed as a result of stranding above the waterline. Individual body parts were not counted unless the head was observed.

5.3.4 Statistical analysis

A General Linear Model (GLM) was used to examine the effect of experiment condition (flowing surface water / surface drying), experiment duration (7, 14 and 21 days) and site (temporary channel / marginal gravel bars) on *G. pulex* survivorship using a full-factorial 3-way combination of these factors, with each as a fixed effect. *Post hoc* Fisher's Least Significant Difference tests were used to examine the effect of duration on *G. pulex* survivorship. A second GLM was used to determine the influence of these factors (condition, duration and site main effects) plus the change (start vs end) in abiotic parameters (pH, dissolved oxygen, water temperature, conductivity and water level) defined as covariates on *G. pulex* survivorship. A third multivariate GLM was used to compare the mean abiotic parameters (defined as dependent variables) between the two sites (defined as a fixed factor). A final multivariate GLM was used to compare the mean abiotic parameters (dependent variables) between each duration and condition (fixed factors) within each site. All analyses were conducted in IBM SPSS Statistics (version 23, IBM Corporation, New York).

5.4 Results

5.4.1 Abiotic parameter variability

Water level was lower during the drying experiments compared to the control conditions in both the temporary channel and marginal gravel bars (Table 5.1). Water level (GLM, $F_{1, 142} = 4.213$, $P = 0.042$), pH ($F_{1, 142} = 166.584$, $P < 0.001$), dissolved oxygen ($F_{1, 142} = 14.558$, $P < 0.001$) and water temperature ($F_{1, 142} = 121.712$, $P < 0.001$) were lower, and mean conductivity ($F_{1, 142} = 603.017$, $P < 0.001$) was higher, in the temporary channel compared to the perennial channel (Table 5.1). Within the temporary channel, pH (GLM, $F_{1, 68} = 13.274$, $P = 0.001$) and dissolved oxygen ($F_{1, 68} = 85.609$, $P < 0.001$) were higher during drying conditions, and the mean pH ($F_{2, 68} = 12.690$, $P < 0.001$), dissolved oxygen ($F_{2, 68} = 4.582$, $P = 0.014$), temperature ($F_{2, 68} = 16.398$, $P < 0.001$) and conductivity ($F_{2, 68} = 6.515$, $P = 0.003$) displayed varied responses to each duration (Table 5.1). Within the gravel bars, pH (GLM, $F_{1, 68} = 32.4$, $P < 0.001$), dissolved oxygen ($F_{1, 68} = 24.375$, $P < 0.001$) and temperature ($F_{1, 68} = 63.914$, $P < 0.001$) decreased during the drying conditions whereas mean conductivity ($F_{1, 68} = 25.382$, $P < 0.001$) increased. Mean pH (GLM,

$F_{2,\,68}$ = 6.33, P = 0.003) and dissolved oxygen ($F_{1,\,68}$ = 6.569, P = 0.002) showed a mixed response to each duration in the gravel bars (Table 5.1).

5.4.2 The effects of change in abiotic parameters on the survivorship of G. pulex

Survivorship of *G. pulex* was not associated with changes in pH (GLM, $F_{1,\,134}$ = 0.37, P = 0.554), dissolved oxygen ($F_{1,\,134}$ = 2.001, P = 0.159) or water temperature ($F_{1,\,134}$ = 0.207, P = 0.650) recorded during control or drying experiments. However, survivorship of *G. pulex* was reduced during lower water levels (GLM, $F_{1,\,134}$ = 5.230, P = 0.024) and increasing conductivity ($F_{1,\,134}$ = 9.399, P = 0.008). In the temporary channel, conductivity remained stable over the 7, 14 and 21 day experiments for both control and drying conditions (Table 1). In contrast, the conductivity recorded in marginal gravel bars was higher during the drying experiments compared to control conditions (Table 5.1).

Table 5.1 Mean (± SE) pH, dissolved oxygen, water temperature, conductivity and water level after each experiment duration in the temporary channel and marginal gravel bars of the perennial channel during (a) control (surface water present) and (b) surface drying conditions.

	Temporary channel			Marginal gravel bars		
	Duration (days)			Duration (days)		
Mean parameter	7	14	21	7	14	21
(a) Control condition						
pH	7.6 ± 0.0	7.6 ± 0.0	7.6 ± 0.0	8.2 ± 0.0	8.3 ± 0.0	8.2 ± 0.0
Dissolved oxygen (mg l^{-1})	3.5 ± 0.2	2.8 ± 0.1	3.2 ± 0.2	7.7 ± 0.1	6.6 ± 0.2	7.6 ± 0.2
Water temperature (ºC)	11.3 ± 0.1	10.8 ± 0.0	11.3 ± 0.1	17.5 ± 0.0	16.8 ± 0.0	17.2 ± 0.0
Conductivity (µS cm^{-1})	843 ± 12.2	861 ± 2.7	863 ± 2.7	470 ± 0.7	485 ± 5.9	471 ± 0.5
Water level (mm)	35 ± 8.4	25 ± 3.9	18 ± 2.8	75 ± 5.7	47 ± 5.1	119 ± 11.8
Max. water level (mm)	110	50	40	110	65	170
(b) Surface drying condition						
pH	7.6 ± 0.0	7.8 ± 0.0	7.7 ± 0.0	7.8 ± 0.6	7.9 ± 0.4	7.8 ± 0.7
Dissolved oxygen (mg l^{-1})	4.6 ± 0.3	4.3 ± 0.3	4.9 ± 0.2	3.4 ± 0.7	4.6 ± 0.8	2.5 ± 0.4
Water temperature (ºC)	11 ± 0.0	10.9 ± 0.1	11 ± 0.0	11.7 ± 0.9	11.9 ± 0.8	14.3 ± 0.3
Conductivity (µS cm^{-1})	850 ± 1.7	852 ± 2.6	852 ± 1.8	711 ± 27.8	637 ± 32.4	666 ± 37.7
Water level (mm)	-19 ± 4.5	-8 ± 3.5	-24 ± 5.4	-29 ± 9.4	-33 ± 11.0	-24 ± 6.1
Min. water level (mm)	-50	-40	-50	-105	-95	-85

5.4.3 Effect of drying, drying duration and site on G. pulex *survivorship*

Surface drying reduced the survivorship of *G. pulex* in comparison to experiments in which surface water was present (Table 5.2; Fig. 5.3a). An increase in experiment duration reduced *G. pulex* survivorship during both control and drying conditions and in both the temporary channel and marginal gravel bars (Table 5.2; Fig. 5.3). *G. pulex* survivorship was higher in the temporary channel compared to the marginal gravel bars (Table 5.2; Fig. 5.3b). For each duration, survivorship was higher during control conditions compared to the drying conditions, in both the temporary channel (7 days, GLM, $F_{1, 22} = 16.298$, $P = 0.001$; 14 days, $F_{1, 22} = 19.366$, $P < 0.001$; 21 days, $F_{1, 22} = 18.140$, $P < 0.001$; Fig. 5.4) and in the gravel bars (7 days, GLM, $F_{1, 22} = 5.301$, $P = 0.031$; 14 days, $F_{1, 22} = 4.758$, $P = 0.040$; $F_{1, 22} = 7.152$, $P = 0.014$, Fig. 5.4).

Table 5.2 Full factorial 3-way general linear model (GLM) analysis for the effect of condition (flowing surface water / surface drying), duration (7, 14, 21 days) and site (temporary channel / marginal gravel bars) on *Gammarus pulex* survivorship. Significant values are emboldened.

Fixed Factors	***df***	***F***	***P***
Main effects			
Condition (wet / dry)	1	53.510	< **0.001**
Duration (7, 14, 21 days)	2	5.907	**0.003**
Site (channel / gravel bar)	1	19.102	< **0.001**
2-way			
Condition*Duration	2	0.159	0.853
Condition*Site	1	0.104	0.748
Duration*Site	2	1.640	0.198
3-way			
Duration*Site*Condition	2	0.701	0.498

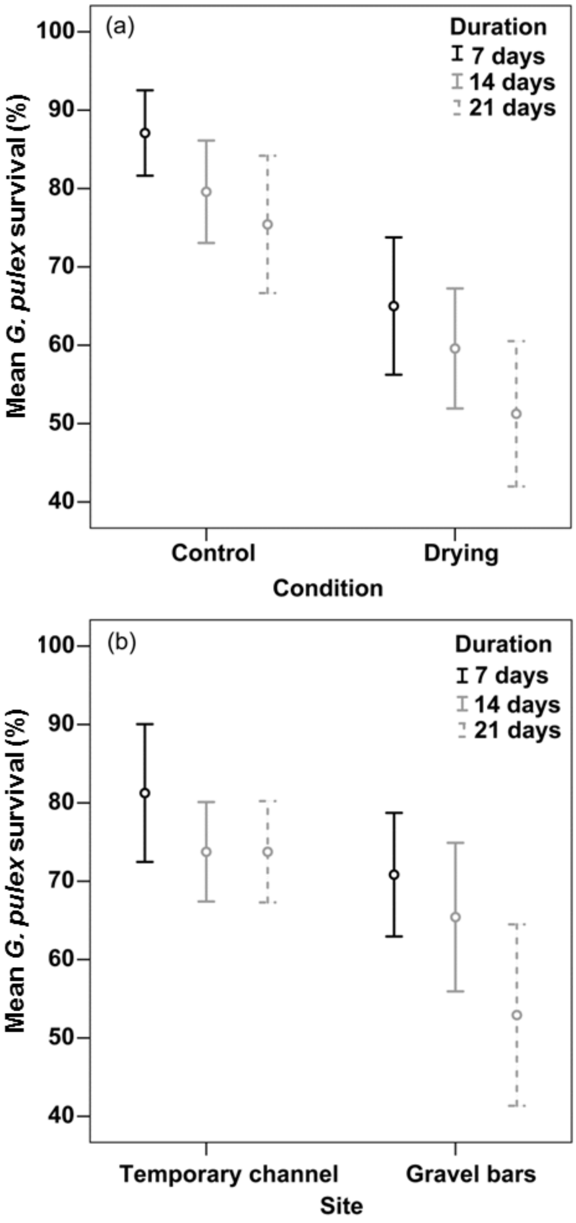

Fig. 5.3 Mean ± 2 SE percentage survival of *Gammarus pulex* in each experiment duration (7, 14 and 21 days) in (a) flowing (control) and surface drying experimental conditions; and (b) sites in a temporary channel and in exposed gravel bars of a perennial channel. *Y* axes start at 40%.

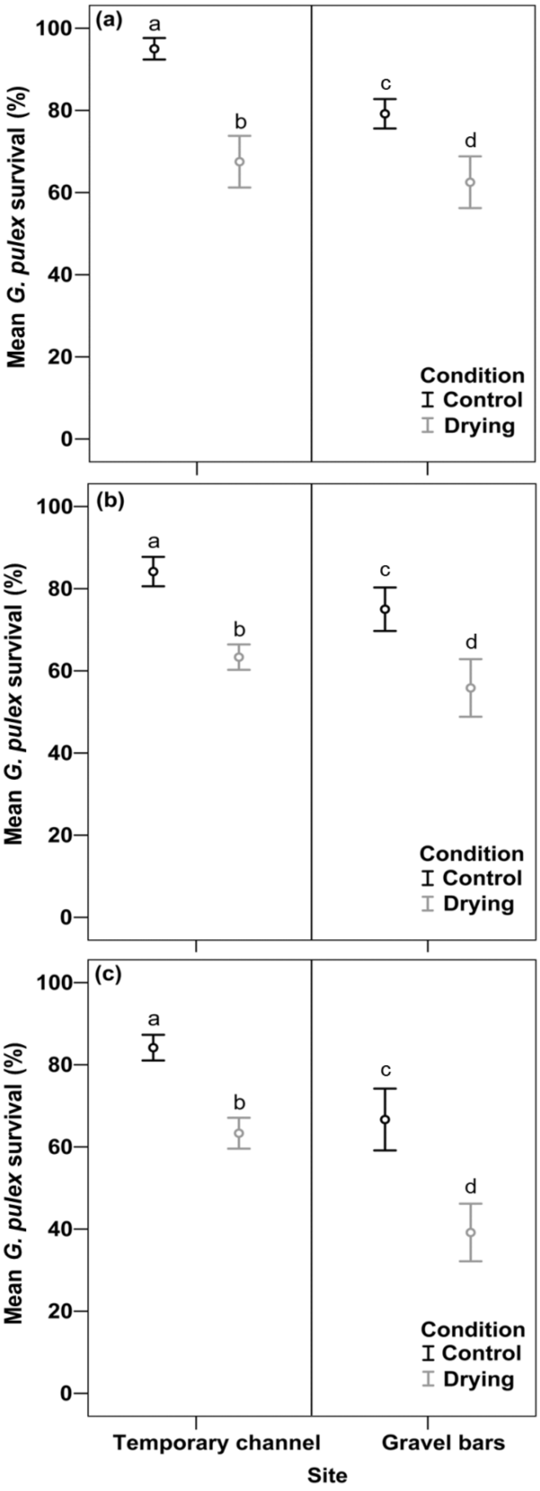

Fig. 5.4 Mean (± 1 SE) percentage survival of *Gammarus pulex* in each duration for control and drying experiments within each site (temporary channel and exposed gravel bars): (a) 7 days, (b) 14 days, (c) 21 days. Letters ‘a’ to ‘d’ represent values

that are significantly different within the temporary channel and gravel bars (GLM, $P < 0.05$).

5.5 Discussion

5.5.1 Surface water loss and increasing drying duration reduced G. pulex *survivorship within the subsurface sediments*

This study examined channels that regularly experience a reduction in surface flow which facilitated an experimental approach to examine the effect of drying and duration of drying in the field. The findings of this study show that *G. pulex* survivorship within subsurface mesocosms was lower when channel drying occurred compared to locations at which surface water persisted. The findings also show that increasing drying duration reduced survivorship within subsurface sediments. Previous research has reported reduced survivorship of common benthic invertebrates such as gammarids due to surface water loss (Poznańska et al., 2013; Vander Vorste et al., 2016b), and field investigations have reported reduced abundance of individuals with increasing intermittence (Clarke et al., 2010; Datry et al., 2014b) and duration of drying events (Storey, 2016). The majority of *G. pulex* individuals survived within the subsurface for periods of surface water loss < 21 days, indicating that subsurface sediments can facilitate population persistence during short-term drying events. These experimental observations support field studies (predominantly based on hyporheic sampling) which indicate that subsurface sediments form an important refuge for macroinvertebrates during streambed drying events (Hose et al., 2005; Fenoglio et al., 2006; Vander Vorste et al., 2016a).

It has been widely acknowledged that the increasing duration of dry events severely affects lotic ecosystem communities (Lake, 2003; Datry, 2012). Unless precipitation sustains sediment moisture, increasing drying duration typically reduces moisture content and decreases biotic persistence within subsurface sediments (Stubbington et al., 2009; Stubbington & Datry, 2013). The results presented in this study support previous observations that increased dry event duration reduces benthic and hyporheic invertebrate density (Arscott et al., 2010; Datry, 2012; Datry et al., 2014b). Fritz & Dodds (2004) reported a 50% reduction in macroinvertebrate density following a (2-month) drying period compared to an 86% reduction following a longer (9-month) dry period at intermittent sites over 2-year study. The study sites had comparable subsurface sediments (gravels and cobbles) but the shallow bedrock and packed clay in the subsurface resulted in a hyporheic zone that completely dried (Fritz & Dodds, 2004). Given the inherent heterogeneity of streambed sediments, the wider application of mesocosms in field experiments may be particularly useful for quantifying taxon-specific responses to drying by controlling for spatial heterogeneity of sedimentary characteristics and via their deployment over standard time periods.

5.5.2 Declining water level and variable conductivity reduced G. pulex *survivorship within the subsurface sediments*

The retention of water in subsurface sediments is a key determinant of macroinvertebrate survivorship in channels subject to surface drying (Hose et al., 2005; Chester & Robson, 2011). In the current study, water level never declined below the base of the mesocosms and shows fully saturated interstices (retention of free water) can support longer periods of macroinvertebrate persistence, whereas moist interstices (reduced free water) have been shown to facilitate persistence of *G. pulex* for short durations (Stubbington et al., 2009). In addition, the temporary channel had higher organic matter and fine sediment contents compared to the gravel bars, potentially reducing interstitial flow (Greig et al., 2005) and facilitating moisture retention within benthic sediments (Strachan et al., 2014). Field observations from this study indicate that saturated conditions in the subsurface sediments of the temporary channel (where water level declined by < 50 mm on average) resulted in more stable abiotic conditions even during channel drying compared to the abiotic variability recorded within the marginal exposed gravel bars. These results suggest a positive relationship between reduced variability in subsurface habitat conditions (e.g. water level) and enhanced survivorship of *G. pulex*.

Channel surface drying reduced the survivorship of *G. pulex* in subsurface sediments, supporting the findings of other studies which found stranding of individuals in the subsurface as water level declined (Poznańska et al., 2013; Vadher et al., 2015; Vander Vorste et al., 2016b). Chapter 2 of this thesis demonstrated the importance of sediment porosity on the ability of *G. pulex* to move vertically through sediments. Considering the gravel particles used in the present experiment (medium gravel—10 – 20 mm in diameter), most *G. pulex* should have been able to move vertically and avoid stranding, suggesting experiment duration and abiotic parameters affects survivorship.

Conductivity increased significantly in the gravel bars during all drying experiments, reflecting the increased residence time of water and increased contribution of solutes from groundwater due to reduced dilution by surface water as water level in the stream declined (Caruso, 2002; Acuña et al., 2005; Sprague, 2005). Mathers et al., (2017) reported comparable conductivity values on Black Brook to those recorded in control experiments on gravel bars in this study, indicating values were elevated in the temporary channel, and during drying in both channels during this study. Both the reduction in water level and increase in conductivity may have reduced *G. pulex* survivorship compared to the temporary channel, which experienced a reduced magnitude of change in water level and conductivity. Previous research has reported elevated conductivity during drying events when examining the effects of water quality changes on macroinvertebrate communities (Caruso, 2002; Ferreira et al., 2014; Verdonschot et al., 2015); however, the direct effect of increasing conductivity during drying events on the survival of macroinvertebrates is poorly understood.

5.5.3 Conclusions and future directions

Drying events are likely to increase in frequency and duration in some regions of the globe as a result of climate change (Forzieri et al., 2014; Ledger & Milner, 2015; Pyne & Poff, 2017) and increasing pressures on water resources (Arroita et al., 2017). This study highlights the effect of surface water loss and increasing dry period duration on the survivorship of the common benthic macroinvertebrate *G. pulex* within the subsurface sediments. This research also highlights the need for effective management to maintain subsurface sediments that provide a refuge and promote population persistence during short periods of drying (Vander Vorste et al., 2016a), particularly in near-perennial temperate zone streams with perennial communities exposed to day-to-week drying events. Future research should determine the characteristics of sediments with a high potential to serve as a refuge during drying and use field-based mesocosm experiments to improve understanding of the effect of longer drying durations in streams from individual and population to community levels. Such experiments should encompass the recovery of aquatic fauna after surface water returns to further understanding into drying persistence and recolonization processes.

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Chapter 6 Synthesis

6.1 Thesis overview

Anthropogenic driven climate change and pressures on freshwater resources are increasing the frequency and duration of drying events in streams (Ledger & Milner, 2015; Arroita et al., 2017; Pyne & Poff, 2017; see section 1.1). It is, therefore, important to understand how aquatic organisms respond to these pressures in order to promote effective river management strategies that support faunal resistance to drying. In this thesis, the response to water level drawdown, sediment characteristics and dry period duration of individuals of different benthic macroinvertebrate species and populations of one species (the amphipod, *Gammarus pulex*), was examined in both laboratory and field mesocosm experiments. Direct observation through transparent sediments of the vertical movement of macroinvertebrates in response to water level reduction and drying provided clear evidence of active movement by individuals into the subsurface by five species of aquatic macroinvertebrates (Chapter 2 Exp. 1; Chapter 3 Exp. 2). Sediment characteristics and reduced sediment porosity significantly reduced the ability of the different benthic macroinvertebrate species (*Asellus aquaticus*; *Gammarus pulex*; *Heptagenia sulphurea*; *Hydropsyche siltalai* and *Nemoura cambrica*) to move vertically (Chapter 2 Exp. 1). This response was particularly pronounced where fine sediments bridged interstitial pathways and clogged the substrate (Chapter 3 Exp. 2). Increasing dry period duration reduced *G. pulex* survival within subsurface sediments (Chapter 4 Exp. 3), particularly when exposed to the highly variable water quality conditions experienced under natural field conditions (Chapter 5 Exp. 4).

Understanding these small-scale responses at the scale of individual organisms and populations (Chapter 2 Exp. 1; Chapter 3 Exp. 2) can potentially enhance the understanding of community and ecosystem responses to stream drying. This thesis has highlighted and found evidence to address some of the potential differences in faunal responses reported in previous studies which have sought to test the hyporheic refuge hypothesis (Williams & Hynes, 1974; Palmer et al., 1992; Chapter 1 Table 2). This hypothesis predicts that subsurface sediments and the hyporheic zone can become a refuge from adverse surface conditions (i.e., loss of surface water) for the benthic macroinvertebrates that can temporarily inhabit them (Williams & Hynes, 1974). The four experiments outlined and presented in this thesis provide evidence to support these predictions and so the hyporheic refuge hypothesis by providing direct observation of active vertical movements by benthic macroinvertebrates into the subsurface in response to drying (Chapter 2 Exp. 1; Chapter 3 Exp. 2). The research presented in both laboratory and field experiments, that *G. pulex* survivorship declines linearly as dry period duration increases, suggests that subsurface sediments function as a temporally limited refuge (Chapter 4 Exp. 3; Chapter 5 Exp. 4).

6.2 Fulfilment of thesis aims and objectives

This thesis has specifically examined the effect of sedimentological characteristics on the use of subsurface sediments by specific benthic macroinvertebrate taxa during experimental and natural drying on riverine substrates. To achieve this two overarching research question were considered:

1: To what extent do sedimentological characteristics affect the use of subsurface sediments by benthic macroinvertebrate individuals in direct response to a reduction in water level?

2: How does the duration of drying events affect benthic macroinvertebrate survivorship within the subsurface sediments?

6.2.1 Fulfilment of research question 1

Thesis question 1 was addressed in Chapter 2 (Exp. 1) and Chapter 3 (Exp. 2) of the thesis. The results of both studies indicated that sediment characteristics affected the vertical movement of benthic macroinvertebrates into subsurface sediments. The findings from these experiments suggest that sedimentological characteristics are one of the primary determinants of subsurface sediment use by benthic fauna in response to drying. In particular, these experiments highlighted the effect of sediment porosity (i.e., volume of free-water space between sediment clasts) on the subsurface refuge potential which can be conceptualised simply (Fig. 6.1). Sediments which produce an open framework (increasing interstitial volume) for benthic macroinvertebrates to move through have the highest potential as a refuge in response to drying as long as free water persists / is present at depth (Chapter 2 Exp. 1). With the exception of *Nemoura cambrica* (which did not strand above the waterline in any sediment treatment), the active vertical movement of species primarily associated with the benthic sediments of streams (*Hydropsyche siltalai* and *Heptagenia sulphurea*) were strongly affected by sedimentological characteristics (Chapter 2 Exp. 1). Consequently, the degradation of the sediment surface by fine sediment infiltration severely reduces the potential of the subsurface to function as a refuge as interstitial gaps become blocked (Chapter 3 Exp. 1; Navel et al., 2010;

Descloux et al., 2013; Vadher et al., 2015).

Fig. 6.1 Conceptual diagram showing the effect of sediment porosity on subsurface sediment refuge potential based on Chapter 2 (Experiment 1) and Chapter 3 (Experiment 2).

Fine sediment potentially reduces subsurface porosity; however, it can also increase the subsurface water retention capacity of streambed sediments, which also promoted macroinvertebrate persistence during the field experimental study undertaken in this thesis (Chapter 5 Exp. 4). The temporary stream in the field experiment had an increased fine sediment and organic matter content in the streambed compared to the gravel bars at the margin of the perennial channel. This increased water retention and prevented the subsurface from drying in the intermittent flowing channel (Chapter 5 Exp. 4). This finding indicates that in some situations fine sediment may be beneficial in the subsurface as it enhances the moisture retention capacity of streambeds and has been highlighted as a key determinant of macroinvertebrate survival within subsurface sediments during surface water loss (Stubbington & Datry, 2013). Therefore, the removal of fine sediments from streams which dry, or are likely to become dry, in temperate regions should not necessarily be recommended, or undertaken without prior knowledge, given that fine sediments play a role in the maintenance of subsurface moisture / moisture retention which is likely to increase survivorship of fauna (Chapter 5 Exp. 4).

6.2.2 Fulfilment of research question 2

Thesis question 2 specifically considered and examined the effect of the duration of drying events on the survival of a model benthic macroinvertebrate taxon (*Gammarus pulex* – Crustacea: Amphipoda) within subsurface sediments. This research question was addressed using a dual approach of both laboratory flume (Chapter 4 Exp. 3) and a field experiment (Chapter 5 Exp. 4). The results of both experimental studies indicated that increasing drying duration reduced the survivorship of the test organism (*Gammarus pulex*) within subsurface sediments. Survivorship in the field experiment was lower when compared to survivorship in the flume experiments, probably due to the dynamic natural variability of hydrological parameters in the natural environment e.g., temperature, conductivity, pH and dissolved oxygen (Chapter 5 Exp. 4).

Temporary stream research has been centred on semi-arid and Mediterranean locations which suggest the response of water quality to drying in streams typically includes an increase in both temperature and conductivity and a reduction of pH and dissolved oxygen (Lake, 2003; Boulton & Lake, 2008; Bogan et al., 2015). However, these changes, typical of drier and more arid regions, were not experienced in the field experiment presented in this thesis. Other field observations from previous studies and investigation of temporary streams in temperate regions have also reported limited (non-significant) changes in abiotic parameters during flow cessation and drying (e.g., Stubbington et al., 2009; Verdonschot et al., 2015). Therefore, caution is required when transferring or comparing the ‘typical’ responses to changes in abiotic parameters (temperature, conductivity, pH and dissolved oxygen) from one region (arid and semi-arid e.g., Bogan et al., 2015) to another (Chapter 5 Exp. 4). In addition, generalisations do not consider the short term variability associated with summer rainfall events and the relative rise and fall in water level during the experiment (Chapter 5 Exp. 4).

The greater variability of water quality parameters to drying in the field study may partially reflect the frequency of observations (week intervals), between which discharge may have increased due to precipitation and changes in the outflow of Blackbrook reservoir upstream. Both field sites were shaded by deciduous trees which have been reported to mitigate the effects of rising stream temperatures and reduce temperature fluctuations (Kristensen et al., 2013; Dohet et al., 2015). A conceptual model, based on the observations of abiotic parameters recorded from the stream parameters during the drying event in the temperate stream studied in this thesis is presented in Figure. 6.2. As flow declined and remnant pools formed in the channel, porous sediments promoted macroinvertebrate survival due to sedimentological characteristics (moisture retention) increasing the subsurface and hyporheic refuge potential (Fig. 6.2). However, in some circumstances reduced sediment porosity may limit vertical penetration and movement by macroinvertebrates through the subsurface, resulting in reduced macroinvertebrate survival during drying events (Fig. 6.2). During the process of surface water loss abiotic parameters fluctuate which, depending on the magnitude and frequency of the changes experienced, may influence macroinvertebrate survival and persistence (Fig. 6.2).

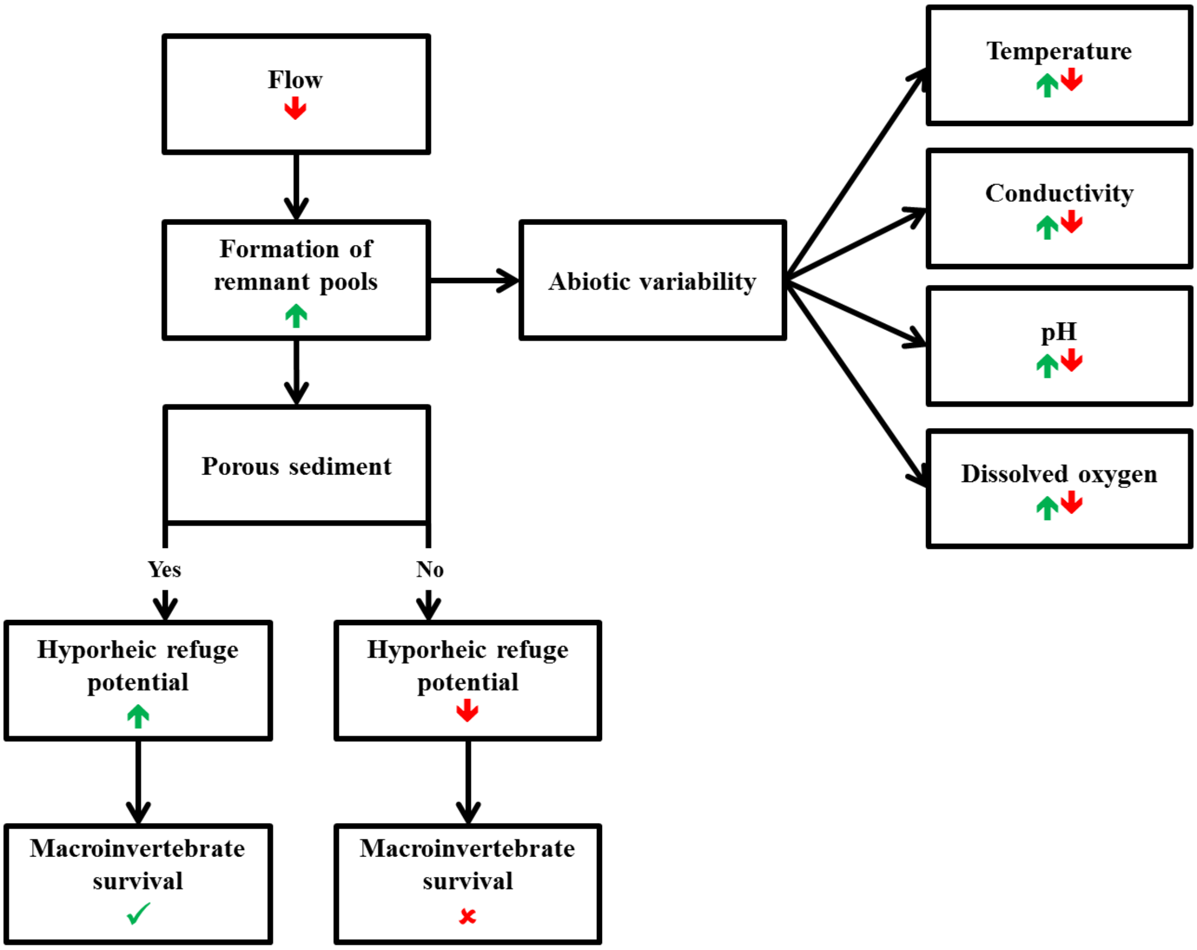

Fig. 6.2 Conceptual diagram highlighting the variability of water quality parameters during drying in temperate streams and the influence of sediment porosity on macroinvertebrate survival in subsurface sediments. Green and red arrows represent the increase, decrease and variability of factors.

6.3 Scale and realism of laboratory mesocosm experiments

The laboratory experiments outlined in this thesis facilitated direct observation of the vertical movements of species in response to drying at an individual and population scale in transparent laboratory mesocosms (Chapter 2 Exp. 1; Chapter 3 Exp. 2). The experiments examined the persistence of *G. pulex* within the subsurface sediments during drying events at the population and reach scale within flume and field mesocosms (Chapter 4 Exp. 3; Chapter 5 Exp. 4). The small scale laboratory experiments (Chapter 2 Exp. 1; Chapter 3 Exp. 2) supported field studies (see Chapter 1, Table 2) which have reported macroinvertebrates within the subsurface in response to drying (Vander Vorste et al., 2016a; Maazouzi et al., 2017). However, the experiments reported in this thesis represent the first direct observation of active movements by benthic macroinvertebrates into subsurface sediments. Previously, active movements could only be inferred (e.g., see Chapter 1, Table 2) as direct observations within natural streambeds are inherently difficult. Although small tightly controlled experiments such as these undertaken in this thesis are at a small spatial scale, they are a vital tool in providing greater mechanistic and ecosystem-wide understanding (Fig 6.3; Stewart et al., 2013).

The flume (Chapter 4) and field (Chapter 5) mesocosm experiments presented in this thesis reflect field conditions (e.g., flow, abiotic variability) relatively realistically compared to the highly controlled laboratory experiments in Chapter 2 and 3. As a result, flume and field scale experiments are therefore important for bridging the gap between the highly controlled laboratory experiments and the biological complexity of natural systems (Fig. 6.3; Stewart et al., 2013). Furthermore, mesocosm experiments are not limited by the lack of replicability of field surveys, and reduced realism of laboratory experiments (Stewart et al., 2013). With regards to other hybrid approaches to ecological science, if large datasets from both mathematical models (Fig. 6.3: Models) and field surveys can be synthesised, phenomenological models may be developed to infer future scenarios (Fig. 6.3; Stewart et al., 2013). Mechanistic models may also be employed to understand, for example, how biotic interactions influence ecological responses to drying in streams (Fig. 6.3; Stewart et al., 2013). These different approaches each have their strengths and weakness (e.g., realism, scale and lack of predictive power / replicability) which means a combined multi-scale approach should be considered wherever appropriate (Fig. 6.3: Integrative approach). Understanding and predicting the ecological effects of drying in streams ultimately necessitates the use of complementary and integrative approaches, which include mesocosm experiments, to allow insights into the faunal responses and the physical and biological processes driving them (Fig 6.3; Stewart et al., 2013).

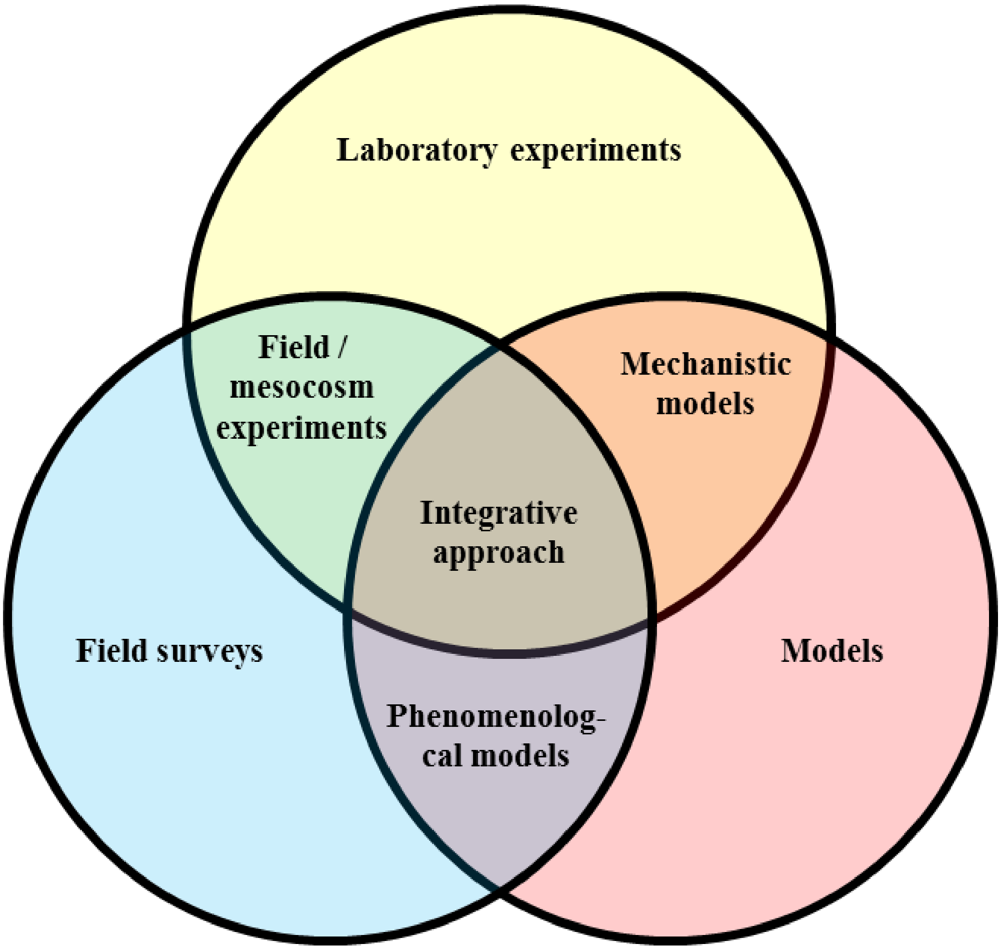

Fig. 6.3 Complementary approaches used to investigate ecological processes using laboratory experiments, field surveys and models. Adapted from Stewart et al. (2013).

It is important to recognise the laboratory mesocosm (Chapter 2 Exp. 1; Chapter 3 Exp. 2) and flume (Chapter 4 Exp. 3) experiments used in this thesis did not fully simulate the natural environmental conditions associated with stream drying (see Boulton & Lake, 2008). This could potentially have resulted in different responses (vertical movement and survivorship) by macroinvertebrates to drying, sediment characteristics and dry period duration compared to natural drying events and river worked sediments. However, the results of the flume (Chapter 4 Exp. 3) and field (Chapter 5 Exp. 4) mesocosm experiments were relatively comparable and suggest that the responses of *G. pulex* to dry period duration under laboratory conditions are similar to patterns reported from field studies of natural drying events (Arscott et al., 2010; Datry, 2012). Although *G. pulex* survivorship was higher overall in the laboratory flume, presumably due to stable water quality parameters (Chapter 4; Exp 3), the reduction of *G. pulex* survivorship to increasing dry period duration exhibited a linear relationship in both the flume and field experiments (see Chapter 4 Exp. 3 and Chapter 5 Exp. 4). This consistent pattern between the two experiments provides compelling evidence for the use of laboratory mesocosm experiments to examine the effect of dry period duration on *G. pulex* survivorship.

A previous study of the field site described in Chapter 5 (Exp. 4; Black Brook, Loughborough, UK) sampled the naturally occurring macroinvertebrate community within the subsurface sediments using mesocosms similar to those utilised in Chapter 5 (Mathers et al., 2017). *G. pulex* occurred frequently in the subsurface colonization devices at this site (Mathers et al., 2017) and therefore provided evidence to support the use of *G. pulex* in the subsurface experiments in this thesis and support the realism of subsurface sediment use of the field mesocosm experiment. A previous study examined the replicability of using channel-mesocosms alongside a stream and found mesocosms closer to the natural source were more replicable and therefore reliable (Harris et al., 2007). This study supports the use of mesocosms within Black Brook (Chapter 5; Exp. 4) as they were embedded in the stream system and therefore can be considered as replicable and reliable as they were exposed to natural conditions (Harris et al., 2007). Utilising mesocosm experiments in temporary stream ecological research is therefore a useful tool to facilitate understanding of ecological responses to drying (e.g., Poznańska et al., 2013; Vander Vorste et al., 2016b; Vadher et al., 2015).

6.4 Contribution to theoretical understanding

The hyporheic refuge hypothesis proposes that benthic macroinvertebrates move into the hyporheic zone in response to adverse hydrological surface conditions (flooding and drying) (Williams & Hynes, 1974; Palmer et al., 1992; Stubbington, 2012). However, evidence from research investigating the use of the hyporheic zone and subsurface sediments as a refuge from flooding and drying has been equivocal, with studies reporting evidence both for and against this hypothesis (see Chapter 1 Table 1 and 2). One of the primary issues associated with testing this hypothesis is distinguishing between active or passive movement into a subsurface refuge by benthic fauna. This is important because the two different responses can play an important role in determining differences in community persistence (Stubbington, 2012). Studies have often supported the hyporheic refuge hypothesis by reporting the presence of benthic organisms in the hyporheic zone (e.g., Imhof & Harrison 1981; Fenoglio et al., 2006) and the persistence of benthic fauna in the hyporheic zone during drying (e.g., Griffith & Perry, 1993; del Rosario & Resh, 2000). However, the mechanism used by benthic macroinvertebrates (i.e., active shelter-seeking behaviour vs passive refuge inhabitation) has only been inferred due to the inherent problems associated with direct observation (Stubbington, 2012). In this thesis, Chapter 2 (Exp. 1) provides the first definitive evidence of active movements by a variety of benthic macroinvertebrate species into the subsurface sediments through the use of transparent mesocosms. This same study also provides the first directly observed evidence of the behaviour of benthic macroinvertebrates as they move vertically through the subsurface sediments in response to water level reduction to avoid stranding and desiccation (Chapter 2 Exp. 1).

A key determinant of the hyporheic refuge is sediment composition which has been reported to directly affect macroinvertebrate entry and movement through the subsurface, and therefore the refuge potential of subsurface / hyporheic sediments (see Chapter 1 Section 1.6.2). This thesis highlights the potentially negative effect of reduced sediment porosity (Chapter 2 Exp. 1) and fine sediment deposition (Chapter 3 Exp. 2) on the vertical movements by benthic macroinvertebrates through subsurface sediments.

Habitats which promote biotic resistance (ability to withstand) and resilience (ability to recover) during biophysical disturbances are considered refuges (Sedell et al., 1990). It has been recognised that the ability of subsurface sediments to function as a refuge may be spatially limited by the direction of vertical hydrological exchange (Dole-Olivier et al., 1997), sediment characteristics (Richards & Bacon, 1994; Vadher et al., 2015) and water quality (Lake, 2003). This thesis provides evidence that the subsurface refuge is strongly temporally limited as increasing duration of drying events decreased macroinvertebrate survivorship (Chapter 4 Exp. 3; Chapter 5 Exp. 4). Broader community level studies examining temporary streams have demonstrated that the duration of drying represents a primary control on the structure and diversity of communities (e.g., Feminella, 1996; Datry et al., 2014;

Storey, 2016). This thesis makes a direct contribution to this field of study, demonstrating that the duration of short-term (1 – 3 weeks) drying can change community structure as a result of temporally limited refuges.

6.5 Managing the subsurface refuge for benthic macroinvertebrates in temporary streams

This thesis has highlighted the impact that sediment porosity has on the potential of the subsurface refuge during drying (Chapter 2 Exp. 1; Chapter 3 Exp. 2). The subsurface sediments of the hyporheic zone is one of the most important refuges available to aquatic fauna during drying and therefore needs to be managed effectively (e.g., Boulton et al., 2010; Leigh et al., 2015). In particular, fine sediment can have a detrimental effect on subsurface sediments use by benthic macroinvertebrates, as demonstrated by *G. pulex* in this thesis (Chapter 3 Exp. 2). Although fine sediment occurs naturally in streams due to erosion and deposition processes (Lawler et al., 1999; Smith et al., 2003), fine sediment inputs have increased globally as a result of anthropogenic activities including agricultural practices (Lamba *et al*., 2015), channel management (Dunbar *et al*., 2010) and urbanisation (Taylor & Owens, 2009) and may potentially reduce subsurface sediment porosity (Richards & Bacon, 1994; Vadher et al., 2015). However, fine sediment also influences the moisture retention capacity of sediments, which has been clearly shown to be important for the subsurface persistence of organisms during stream drying (Chapter 5 Exp. 4; Stubbington & Datry, 2013). Therefore, the total removal of fines from the subsurface sediments to increase sediment porosity in temporary streams may not necessarily be beneficial for the functioning of the subsurface refuge of temporary streams. Although a reduction in the volume of fine sediment may increase access to the subsurface, it may also reduce the vital moisture retention properties required to support the persistence of many macroinvertebrate taxa (Chapter 5 Exp. 4 and see Stubbington & Datry, 2013). River management strategies therefore need to balance the potential positive and negative effects of reducing fine sediment loads in streams beds.

Fine sediment management techniques have been described in this thesis (Chapter 3; Exp. 2), and management of fine sediment in riverbeds has traditionally included sediment restoration through either cleaning or replenishing depleted gravels (Merz & Setka, 2004; Meyer et al., 2008; Pulg et al., 2013). These techniques have been reported to reduce fine sediment and improve the hyporheic water quality (Merz & Setka, 2004; Meyer et al., 2008; Pulg et al., 2013). However, in these studies, the length of time that restored conditions were maintained varied from 5 months to 5 years (Naden et al., 2016). This is likely to be due to fine sediment continuously being delivered into the system which would suggest river management strategies need to be implemented at the catchment-scale to reduce fine sediment inputs from external sources into streams (Greig et al., 2005; Boon & Raven, 2012; Naden et al., 2016). Reducing external anthropogenic fine sediment inputs into streams will protect subsurface sediments effectively functioning as a refuge (Vadher et al., 2015). Catchment-scale management should encourage streams to recover naturally by allowing space for natural fluvial processes to restore the system (Boon & Raven, 2012). Streams with flushing flows are effective at mobilising fine sediment and therefore should be allowed to self-cleanse wherever possible (Naden et al., 2016).

6.6 Future directions

This thesis has primarily focussed on the influence of sediment characteristics on macroinvertebrate use of subsurface sediments in response to dewatering, and the effect of drying duration on macroinvertebrate persistence. Firstly, this work could be extended further by experimental work examining macroinvertebrate recovery following the return of surface water. This thesis demonstrated macroinvertebrates move into the subsurface in response to water loss, however, vertical movements into streambed sediments will only be beneficial to macroinvertebrate populations and their persistence if recovery is able to occur following the resumption of flowing conditions (in this instance the rewetting of sediments and the resumption of flowing conditions). Field studies have examined the recovery of macroinvertebrates from the hyporheic zone following drying (e.g., Fritz & Dodds, 2004; Acuña et al., 2005; Vander Vorste et al., 2016a), although, the mechanics of upward vertical movements and factors affecting surface recolonization has not been directly examined. Therefore, examining factors controlling vertical movements from the hyporheic zone / subsurface sediments, using similar approaches used in this thesis, would complement this work.

Secondly, further field mesocosm experiments to complement those described in Chapter 5 (Exp. 4), using a wider variety of species (i.e., those used in Chapter 2 Exp. 1) to examine subsurface survival following the loss of surface water would facilitate community-wide understanding of macroinvertebrate resistance to drying in streams. Experiments such as these would inform the effective management of subsurface sediments once their capacity to maintain community survival / persistence has been determined. Thirdly, using a sediment composition which represents a natural streambed within subsurface mesocosms, rather than a uniform size class (Chapter 5 Exp. 4), would assist in bridging the level of realism between mesocosm experiments and the natural world. Future field studies could also consider the elimination of species within the subsurface over time during dry phases by excavating sediments of the hyporheic zone in streams at multiple time intervals.

Ultimately, research using novel techniques to understand processes in areas inherently difficult to observe, such as subsurface sediments, should be encouraged as processes that occur within them, such as macroinvertebrate resistance to drying, are vital to ecosystem functioning.

6.7 References

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