

Characterization of the density and body size of a *Gammarus pulex* (Crustacea: Amphipoda) population in subsurface sediments reflects the sampling technique used

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Abstract The hyporheic zone and its invertebrate fauna play vital roles in the functioning of lotic ecosystems. However, although sampling invertebrates from subsurface sediments is recognized as challenging, few studies have quantified the effectiveness of common sampling techniques. We conducted laboratory experiments to compare two common, semi-quantitative pump-sampling techniques—Bou–Rouch and vacuum-pump sampling. We determined the proportion of a *Gammarus pulex* (Crustacea: Amphipoda) population sampled by each method in five sediment treatments comprising coarse (16–32 mm), medium (8–16 mm), and fine (4–8 mm) gravel and combinations thereof. We compared the body size of individuals sampled and not sampled by each technique to the population mean. Density estimates obtained using both methods were low: 33 ± 5 and $5 \pm 1\%$ of the population present for Bou–Rouch and vacuum-pump samples, respectively. Density estimates were significantly higher for Bou–Rouch than for vacuum-pump samples in four sediment

treatments, but were comparable in coarse gravel. The body size of organisms captured by the Bou–Rouch technique was comparable to the population mean, whereas those in vacuum-pump samples were smaller. With hyporheic invertebrates suggested as future biomonitors of ecosystem health, we recommend Bou–Rouch sampling as the more effective pump-sampling method for community characterization.

Keywords Hyporheos · Hyporheic refuge · Bioindicators · Sampling techniques · Sampling effectiveness · Mesocosm experiments

Introduction

The subsurface sediments of the hyporheic zone are inhabited by an abundant and diverse invertebrate fauna that includes macroinvertebrates and meiofauna, benthic taxa and obligate stygobites, and permanent and temporary inhabitants (Boulton, 2000). Temporary residents include predominantly benthic macroinvertebrate taxa, which may use the subsurface interstices as a refuge from both biotic interactions such as predation (McGrath et al., 2007), and to escape adverse abiotic conditions in the surface stream. For example, hyporheic refuge use by the predominantly benthic crustacean *Gammarus pulex* Linnaeus (Crustacea: Amphipoda) has been inferred from changes in its vertical distribution during both floods (Dole-Olivier et al., 1997) and low flows

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(Stubbington et al., 2011a, b), the latter refuge use being linked to increased biotic interactions in benthic sediments. In addition, Vander Vorste et al. (2016a) identified vertical migration from the hyporheic zone as the primary process promoting recovery of a benthic macroinvertebrate community after a drying event.

Research examining hyporheic invertebrate communities is increasing, including their provision of ecosystem services (Marshall & Hall, 2004; Boulton et al., 2008) and their responses to environmental change (Jones et al., 2015; Stubbington et al., 2015). In addition, hyporheic invertebrates are potential biomonitors of ecosystem health, for example in groundwater-dominated ecosystems (Malard et al., 1996; Tomlinson et al., 2007), and in temporary streams, where invertebrates may persist in hyporheic sediments after benthic communities are altered by surface water loss (Leigh et al., 2013; Boulton, 2014). Evaluation of the potential role of hyporheic invertebrate communities in biomonitoring programmes may become an increasing priority for national regulatory agencies, due to legal drivers such as the EU Water Framework Directive (2000/60/EC; European Commission, 2000; Reyjol et al., 2014).

Despite this increasing interest, sampling invertebrates from the hyporheic zone remains challenging due to the inaccessibility of subsurface sediments (Palmer, 1993). Quantitative methods such as freeze-coring, standpipe corers, and colonization chambers have been evaluated previously (e.g. Fraser & Williams, 1997; ISO, 2015; and see Stubbington et al., 2016), and due to their significant limitations, many studies of hyporheic invertebrate communities have instead used semi-quantitative methods. In particular, semi-quantitative pump-sampling methods are widely used (e.g. Dole-Olivier et al., 1997; Stubbington et al., 2011a, b; 2015; Datry, 2012; Vander Vorste et al., 2016a), their logistic advantages including ease of operation, rapid sample collection, modest financial outlay, and minimal ongoing sampling costs. In addition, because pump sampling causes only limited, localized disturbance to the sediments, these methods allow immediate sample collection (rather than requiring prior installation of equipment) and, if pipes are left in situ, allow repeated sample collection from specific points. Limitations of these methods include a potential bias towards the collection of smaller organisms, which are less able to resist the suction

forces exerted by pump sampling and which move more freely through interstices (Fraser & Williams, 1997; Boulton et al., 2004a; Tanaka et al., 2014).

The main two hyporheic invertebrate pump-sampling methods are Bou–Rouch sampling (Bou & Rouch, 1967) and vacuum-pump sampling (Boulton et al., 1992). These methods have been used to study regional biodiversity (Dole-Olivier et al., 2009); longitudinal (Malard et al., 2003) and sub-reach (Davy-Bowker et al., 2006) spatial variability in community composition; temporal changes in community composition in response to hydrological variability (Stubbington et al., 2011a); and the vertical distribution of fauna (Dole-Olivier & Marmonier, 1992; Dole-Olivier et al., 1997). Stubbington et al. (2016) compared the two pump-sampling methods in contrasting rivers across two biogeographical regions and recorded higher abundance and richness estimates in Bou–Rouch samples. In addition, Dole-Olivier et al. (2014) collected Bou–Rouch samples within the confines of a quantitative benthic sampler and found that this method captured 14.5% of the organisms present. However, no controlled experiments have been conducted to determine or compare the capture rates of pump-sampling methods, limiting interpretation of data collected by studies using these techniques.

We conducted experiments in laboratory-based mesocosms to compare the invertebrate abundance estimates obtained by Bou–Rouch and vacuum-pump sampling methods. Our first hypothesis was that abundance estimates would be higher in Bou–Rouch samples than in vacuum-pump samples. To test this hypothesis, we used each technique to sample a *G. pulex* population of known size and then calculated the proportion of the population sampled. Our second hypothesis was that both the methods would be biased towards the capture of smaller organisms. To test this hypothesis, we compared the body size of individuals that were captured and not captured to the population mean, for each method.

Methods

Test organisms

The freshwater amphipod *G. pulex* was selected as the test organism for laboratory experiments, because it is abundant in the surface and subsurface sediments of

many temperate-zone streams (Crane, 1994; Wood et al., 2010; Stubbington et al., 2011a, b), and populations can easily be maintained in laboratory conditions (Vadher et al., 2015; Vander Vorste et al., 2016b). *G. pulex* were collected from a small lowland stream (52.78 N, -1.39 E) where it was the only amphipod present and occurred at densities of >100 individuals m⁻². Collected organisms were kept at an ambient temperature of 13°C in tanks containing 8 l continuously aerated, dechlorinated tap water. Individuals of all sizes (and therefore both sexes) were retained to allow subsequent body size analysis. Tanks were subject to an 8:16 h light:dark regime. *G. pulex* were fed ad libitum on pre-conditioned native leaf litter dominated by beech (*Fagus sylvatica*) and ash (*Fraxinus excelsior*).

Pump-sampling equipment

Bou-Rouch sampling equipment was purchased from Uwittec (Mondsee, Austria) and used unmodified. The Bou-Rouch standpipe was 125 cm long with a 20-mm internal diameter and a perforated section that spanned 15 cm and comprised 35 5-mm diameter holes in 7 columns of 5 holes. Vacuum-pump sampling pipes each comprised a 25-cm length of PVC pipe with an internal diameter of 19 mm, with samples extracted using a manual bilge pump.

Sediment treatments

Five sediment treatments were used to represent a range of possible field conditions: (i) 100% coarse (16–32 mm) gravel, (ii) 100% medium (8–16 mm) gravel, (iii) coarse gravel with 10% interstitial volume filled with fine (4–8 mm) gravel, (iv) coarse gravel with 30% interstitial volume filled with fine gravel, and (v) 100% fine gravel. For treatments (iii) and (iv), the mean interstitial volume in the coarse gravel treatment was determined as 4.2 l per mesocosm, and the quantity of fine gravel required to displace 420 or 1260 ml of water (i.e. 10% or 30% of 4.2 l) was subsequently determined, and these sediment volumes were used in the respective treatments.

Mesocosm design

Each experimental mesocosm comprised an open-topped cylinder (46 cm height × 28 cm diameter)

constructed from 1-mm wire mesh and lined with <1-mm mesh muslin cloth (Fig. 1). Mesocosms were placed on a base of 16–32 mm gravel within a rigid polyethylene cylindrical container (74 cm height × 36 cm base internal diameter; Fig. 1).

For each treatment, sediment particles were added to the mesocosm to a depth of 15 cm (i.e. to the top of the perforated section of a Bou-Rouch standpipe), to ensure that all test organisms were within the sampled sediments during sample collection. One beech leaf and one ash leaf were broken into small (<5 mm diameter) pieces and evenly distributed across the sediment at 5 and 10 cm above the mesocosm base, to act as a food resource prior to the start of an experimental trial. Dechlorinated tap water was added to the container to a depth approximately 4 mm below the substrate surface, to ensure all *G. pulex* were within subsurface interstices during sample collection. Mesocosms were kept at an air temperature of 13°C.

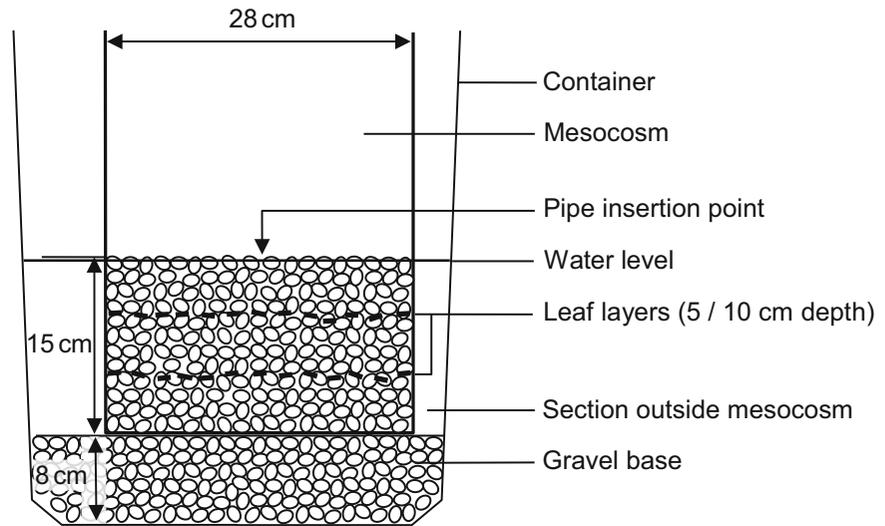
In Bou-Rouch experiments, prior to sediment addition, the standpipe tip was pushed through a hole in the mesocosm base into the gravel below; the muslin cloth then formed a tight seal around the standpipe and gravel covered the lower, non-perforated part of the standpipe. In vacuum-pump experiments, a sampling pipe was placed onto the end of a stainless steel T-bar, which was then inserted into the sediment-filled mesocosm to a depth of 7.5 cm (i.e. the mid-point of the depth range for Bou-Rouch sampling; Fig. 1).

Water temperature was maintained at $13.2 \pm 0.05^\circ\text{C}$, pH at 7.6 ± 0.07 , conductivity at $549 \pm 43 \mu\text{S cm}^{-1}$, and dissolved oxygen concentrations at $6.0 \pm 0.2 \text{ mg l}^{-1}$ during experiments. These parameters were measured using standard instrumentation (Hanna Instruments, Leighton Buzzard, UK) in the second 2-l aliquot of water extracted with vacuum-pump samples (see below).

Experimental procedure

Four replicate samples were collected for each sediment treatment and for both methods, totalling 40 experimental trials. Approximately 16 h before an experimental trial, 50 *G. pulex* were transferred from the tanks to a mesocosm in 75 ml of water, increasing the water depth by ≤ 1 mm to approximately 3 mm below the substrate surface. Individuals were introduced evenly across the substrate surface and left to

Fig. 1 Experimental mesocosms used to collect pump samples of a *Gammarus pulex* population from subsurface sediments. Either a Bou–Rouch standpipe or a vacuum-pump sampling pipe was inserted into the “pipe insertion point” prior to sample extraction. Not to scale



move into submerged interstices. During the 16 h pre-trial period, constant artificial light was used to encourage *G. pulex* (which are negatively phototactic; Bakker et al., 1997) to move into deeper sediments, rather than remaining near the substrate surface. Positioning of leaf layers at depths of 5 and 10 cm was also intended to encourage vertical migration of *G. pulex* into subsurface sediments.

A 6-l sample volume was collected, as this is typical of hyporheic macroinvertebrate studies and hence facilitated comparison with previous work (Boulton et al., 1992; Scarsbrook & Halliday, 2002; Boulton et al., 2004b; Wood et al., 2010; Stubbington et al., 2011a, b; Datry, 2012). At the start of Bou–Rouch sample collection, a 500-ml volume of dechlorinated tap water was used to prime the apparatus. The Bou–Rouch pump was then manually operated to extract a 6.5-l sample (a 6-l invertebrate sample and 500 ml priming water). To collect a vacuum-pump sample, a length of hose was inserted into the pipe and the bilge pump operated manually to extract three 2-l aliquots (i.e. one 6-l sample) from the pipe base. During sample collection, water was continuously added to the space external to the mesocosm (Fig. 1) to prevent a decline in the water depth. Pumps were operated at the fastest rate possible, determined as 0.43 l s^{-1} for the Bou–Rouch pump ($n = 6$) and 0.17 l s^{-1} for the vacuum pump ($n = 6$). Although standardization of pumping rate is desirable *within* each method (Hunt & Stanley, 2000), using a consistent rate *between* methods would

have been impractical and would have resulted in ineffective operation of the Bou–Rouch pump.

Extracted samples were passed through a 1-mm mesh net to retain all captured amphipods. Mesocosms were then deconstructed and the contents rinsed through sieves to locate *G. pulex* that had not been sampled. Sampled and non-sampled amphipods were preserved in 70% industrial methylated spirits and stored at 13°C for later analysis.

Measurement of test organisms

The body size of all sampled and non-sampled *G. pulex* was measured, with the exception of individuals that were damaged during passage through the Bou–Rouch apparatus or during mesocosm deconstruction (7% of sampled individuals and 27% of non-sampled individuals). Body size was measured from the base of antenna 1 to the posterior margin of the final urosome segment, using Motic Image Plus software version 2.0 (Motic, Hong Kong).

Data analysis

Analyses were conducted in IBM SPSS Statistics Version 22 (IBM Corporation, New York, USA), with proportional data $\arcsin(\sqrt{X})$ -transformed prior to analysis. Results are stated as mean ± 1 standard error (SE).

Estimation of population size

To test our first hypothesis, we examined differences in the proportion of the *G. pulex* population captured by the two methods in the five sediment treatments using a two-way ANOVA test, with method (Bou–Rouch; vacuum pump) and sediment treatment (coarse; medium; 10% fine; 30% fine; fine) as fixed factors, and with the proportion captured as the dependent variable. A significant interaction between sediment treatment and method was found (see below) and this initial test was therefore supplemented by: one-way ANOVA tests, to determine differences in capture rates between methods for each individual sediment treatment; one-way ANOVA tests with post hoc Tukey's tests, to examine differences in capture rates between treatments for each individual method.

Characterization of body size

To test our second hypothesis, we examined differences in the body size of *G. pulex* sampled by the two methods and differences in the size of sampled and non-sampled individuals. This was achieved using a three-way ANOVA test with method, sediment treatment, and sampling outcome (sampled; not sampled) as fixed factors and with mean organism size (calculated from 4 replicates) as the dependent variable. Where significant interactions between fixed factors were identified, one-way ANOVA tests were conducted to test for differences between groups. A separate one-sample *t* test was used to compare the body size of organisms sampled by each method with the known population mean.

Results

Estimation of population size

Considering all 40 experimental trials (i.e. both methods and all sediment treatments), $18.9 \pm 3.1\%$ (range 0–48%) of the 50-individual *G. pulex* population was retrieved from mesocosms. Considering all sediment treatments, a higher proportion of the population was captured by Bou–Rouch sampling than by vacuum-pump sampling (Table 1; Fig. 2). The interaction between method and sediment treatment was significant (two-way ANOVA, $P = 0.005$): the

Bou–Rouch method captured more individuals than vacuum-pump sampling in medium, fine, and mixed sediment treatments but there was no difference in the proportion of individuals captured in the 100% coarse gravel treatment (Table 1; Fig. 2). The comparable proportion captured by the two methods in the coarse gravel treatment reflected the lowest proportion sampled by the Bou–Rouch method (Tukey's test, $P = 0.040$ compared to coarse gravel with 30% fine gravel) and the highest proportion collected by the vacuum pump ($P = 0.028$ compared to 100% fine gravel; Table 1; Fig. 2).

Characterization of body size

The mean body size of *G. pulex* individuals was 9.0 ± 0.2 mm (range 2.0–18.4 mm, $n = 1623$). Considering both methods, captured individuals were smaller (8.3 ± 0.3 mm, $n = 378$) than those which were not captured (9.5 ± 0.2 mm, $n = 1252$; three-way ANOVA, $P = 0.001$). The interaction between sampling method and sampling outcome was significant (three-way ANOVA, $P = 0.015$): sampled *G. pulex* were smaller than non-sampled individuals in vacuum-pump samples (one-way ANOVA, $P < 0.001$), but there was no significant difference in the body size of organisms sampled and not sampled using the Bou–Rouch method ($P = 0.291$; Fig. 3). At 9.2 ± 0.4 mm ($n = 324$), the size of *G. pulex* in Bou–Rouch samples was comparable to the population mean (one-sample *t* test, $P = 0.542$); at 7.0 ± 0.2 mm, individuals in vacuum-pump samples were on average 1.9 mm smaller than the population mean ($P = 0.003$; Fig. 3). There was no difference in the number of individuals sampled in the five sediment treatments (three-way ANOVA, $P = 0.169$) or any significant interaction between treatment and either other fixed factor (method; sampling outcome, $P \geq 0.310$).

Discussion

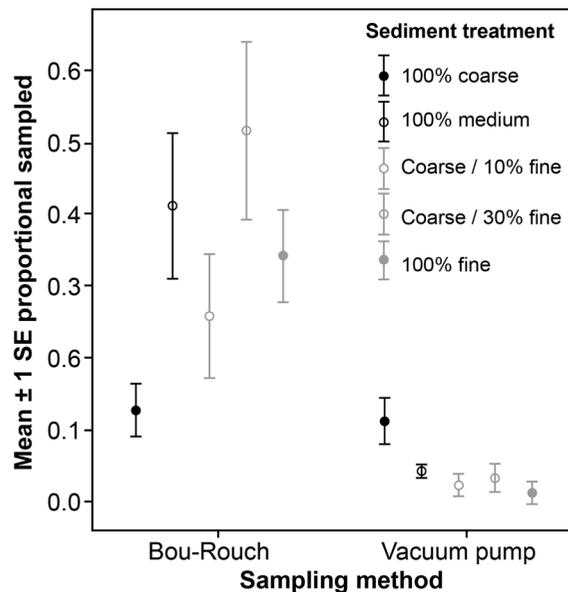
Bou–Rouch sampling captured more invertebrates than vacuum-pump sampling

In four of five sediment treatments, our first hypothesis was supported: Bou–Rouch sampling captured a higher proportion of an invertebrate population than

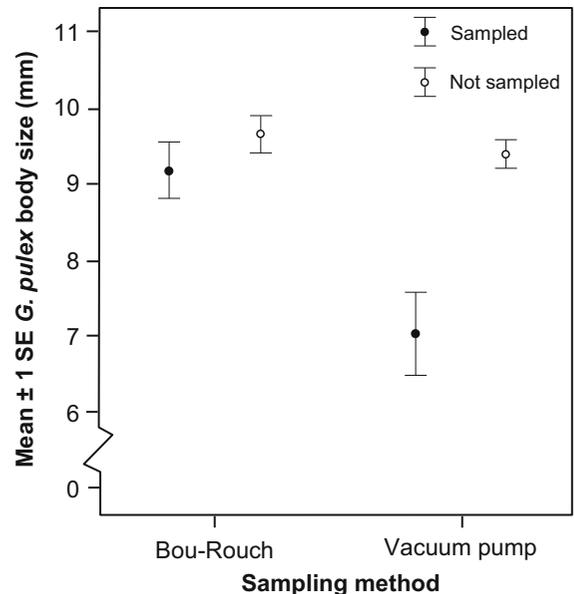
Table 1 Mean \pm 1 SE percentage of a known-size population of *Gammarus pulex* captured by Bou–Rouch and vacuum-pump sampling techniques in five sediment treatments

| Sediment treatments | % Captured by method | | <i>F</i> | <i>P</i> |
|--|----------------------|----------------|----------|----------|
| | Bou–Rouch | Vacuum-pump | | |
| All | 32.6 \pm 4.5 | 5.1 \pm 1.1 | 69.84 | 0.001 |
| 100% Coarse gravel | 14.0 \pm 3.2 | 12.0 \pm 2.6 | 0.66 | 0.806 |
| 100% Medium gravel | 41.5 \pm 10.2 | 4.5 \pm 1.0 | 20.06 | 0.004 |
| Coarse gravel + 10% volume fine gravel | 26.0 \pm 8.6 | 2.5 \pm 1.5 | 11.18 | 0.016 |
| Coarse gravel + 30% volume fine gravel | 52.0 \pm 12.6 | 3.5 \pm 2.2 | 19.08 | 0.005 |
| 100% Fine gravel | 34.5 \pm 6.4 | 1.5 \pm 1.5 | 34.70 | 0.001 |

Sediment particle sizes are stated in the text. Significance was determined using two-way (all treatments) and one-way (individual treatments) ANOVA tests

**Fig. 2** Mean \pm 1 SE proportion of a 50-individual *Gammarus pulex* population captured from subsurface sediments using two pump-sampling methods (Bou–Rouch; vacuum pump) in five sediment treatments (100% coarse gravel, 100% medium gravel, coarse gravel with 10% interstitial volume filled with fine gravel, coarse gravel with 30% interstitial volume filled with fine gravel, 100% fine gravel); $n = 4$ for each treatment

vacuum-pump sampling. These results are consistent with our previous field-based comparison of these pump-sampling methods, in which we recorded higher abundance estimates in Bou–Rouch samples for all common taxa in six streams across two bioregions (Stubington et al., 2016). We suggest that these higher abundance estimates reflect the stronger

**Fig. 3** Mean \pm 1 SE body size (mm) of *Gammarus pulex* individuals captured and not captured by Bou–Rouch and vacuum-pump sampling methods. Body size was measured from the base of antenna 1 to the posterior margin of the final urosome segment; $n = 324$ for Bou–Rouch, sampled, $n = 526$ for Bou–Rouch, not sampled, $n = 54$ for vacuum pump, sampled, and $n = 726$ for vacuum pump, not sampled

suction forces generated by the Bou–Rouch pump, which allow more rapid sample extraction than the manual bilge pump typically used in vacuum-pump sampling (Boulton et al., 1992). Similarly, Hunt & Stanley (2000) compared different modes of operating the Bou–Rouch pump and found that faster pumping rates increased population density estimates.

Pump sampling exposes invertebrates in sampled sediments to a sudden increase in flow velocity. When such increases occur naturally, for example at the onset of a spate, macroinvertebrates including *G. pulex* may respond by moving through interstitial pathways into sediments with lower velocities (Dole-Olivier et al., 1997). As such, stronger swimming ability may help amphipods and other invertebrates to avoid both displacement by increasing flow velocities during floods and entrainment in faster-moving water during pump sampling (Boulton et al., 2004a).

The relationship between sample extraction rates and population densities observed by Hunt & Stanley (2000), and the results of this study, might suggest that pump-sampling techniques capture invertebrates most effectively in coarse sediments, in which wide interstitial pathways facilitate rapid sample extraction. However, in our 100% coarse gravel treatment, the proportion of the population captured was comparable between techniques, being particularly low in Bou–Rouch samples and particularly high in vacuum-pump samples. The higher capture rates achieved by the vacuum pump in coarse gravel may reflect reductions in both interstitial pathway blockages by finer sediment particles and in organisms trapped between particles. In contrast, the reduced effectiveness of the Bou–Rouch technique in coarse gravel may be due to the greater hydraulic conductivity (Datry et al., 2015) and therefore reduced suction forces associated with larger interstices: in finer and more heterogeneous sediment treatments, the lower interstitial pore volume would have increased suction forces.

Pump sampling underestimated population densities

The field study of Stubbington et al. (2016) also demonstrated that Bou–Rouch sampling provides higher invertebrate abundance estimates than vacuum-pump sampling. However, although such field studies allow comparisons, for example, between bioregions and/or streams, they do not indicate the proportion of a population that is sampled. In our controlled mesocosm study, both pump-sampling techniques consistently captured <50% of the amphipod population present. In a related study, Dole-Olivier et al. (2014) operated a Bou–Rouch pump within a quantitative Hess sampler to determine the proportion of an invertebrate assemblage collected by

the pump in a stream with cobble to coarse-sand-sized sediments. On average, Dole-Olivier et al. (2014) found that the Bou–Rouch pump collected 14.5% of the invertebrates present in Hess samples, lower than our 33% mean Bou–Rouch capture rate for *G. pulex*, which may reflect differences in environmental parameters such as substrate composition. This experimental identification of low capture rates informs interpretation of biotic data collected using pump-sampling techniques. However, further research is needed to investigate capture rates for other taxa and to compare rates for a range of environmental conditions and invertebrate traits.

Density estimates decrease as sample volume increases, and therefore, to maximize the accuracy of hyporheic population size estimates obtained using a pump-sampling technique, a small sample volume (Hunt & Stanley, 2000; Boulton et al., 2003) and sufficient replicates (Kibichii et al., 2009) should be collected. Conversely, where a study aims to characterize taxa richness, a larger sample volume may be required; accordingly, several previous studies have collected 10-l pump samples (e.g. Malard et al., 2003; Boon et al., 2016). However, where accurate population density estimates are required, we do not recommend pump-sampling methods as appropriate. Instead, fully quantitative methods such as freeze-coring with electro-positioning (Bretschko & Klemens, 1986; Marchant, 1995) or colonization devices (Gibbins et al., 2016, but see Fraser & Williams, 1997) should be considered.

Bou–Rouch but not vacuum-pump sampling accurately characterized body size

Our second hypothesis, that both methods would be more likely to capture smaller individuals, was based on the size bias associated with pump sampling (Fraser & Williams, 1997; Mauclair et al., 1998; Boulton et al., 2004a) being common to both techniques. Our results lead us to reject this hypothesis: although organisms captured in vacuum-pump samples were smaller than the population mean, the body size of amphipods in Bou–Rouch samples reflected the population present. The smaller body size of amphipods in vacuum-pump samples indicates that larger individuals could escape the weaker suction forces exerted by this technique, while individuals from across the size range present were drawn into the Bou–Rouch

standpipe. Larger organisms' greater ability to resist capture by weaker suction forces may reflect enhanced friction forces between organisms and sediment particles (Fraser & Williams, 1997; Scarsbrook & Halliday, 2002); the stronger swimming ability of adults compared to juveniles (Conlan, 1994); and/or the greater activity of larger males (Peeters et al., 2009), all of which facilitate the innate positive rheotaxis of *G. pulex* (Hughes, 1970).

The vacuum-pump sampling bias towards the capture of smaller individuals, which we observed across sediment treatments, is consistent with previous field studies. In particular, Scarsbrook & Halliday (2002) compared vacuum-pump sampling to colonization pots and freeze-coring and found this method to overestimate the relative abundance of Ostracoda and Copepoda (i.e. very small taxa) compared to other methods and to underestimate the occurrence of mayfly nymphs, stonefly nymphs, and caddisfly larvae (i.e. larger organisms). This size bias, and its implicit exclusion of both individual organisms and potentially whole taxa, may limit the accuracy with which vacuum-pump sampling characterizes invertebrate community composition. Alongside the lower capture rates of this method, the size bias quantified by this experimental study may partly explain the finding of Stubbington et al. (2016) that vacuum-pump sampling characterizes differences in community composition among streams less effectively than Bou–Rouch sampling.

The absence of a size bias in Bou–Rouch experiments partly contrasts with Fraser & Williams (1997), who noted that at a depth of 20 cm (but not 40 or 60 cm) chironomid (Diptera) larvae collected from one riffle using a Bou–Rouch pump analogue were smaller than those in quantitative freeze-coring and standpipe corer samples. Although direct comparison of our results with Fraser & Williams (1997) is limited by contrasting methodological and taxonomic details, the absence of a size bias in Bou–Rouch samples in our study could reflect stronger suction forces generated in experiments, which were conducted in sediments with minimal organic matrix and therefore unclogged interstitial pathways. In contrast, the size bias we observed in vacuum-pump samples supports Fraser & Williams' (1997) suggestion that larger organisms have a greater capacity to resist entrainment by moving water. However, the presence of a size bias in vacuum-pump but not Bou–Rouch samples in our

study suggests that the size bias is overcome as suction forces increase.

Given that the body size of *G. pulex* in Bou–Rouch samples reflected the population mean, we suggest that this method is appropriate for studies seeking to characterize both absolute and relative organism size, for example in relation to environmental characteristics or population densities (Schmid et al., 2000). However, further research is required to determine whether the lack of size bias we observed for the amphipod *G. pulex* is representative of other taxa. In addition, despite organism size in Bou–Rouch samples reflecting the population mean across sediment treatments with differing interstitial fine gravel content, comparison of these results (i.e. the lack of size bias in Bou–Rouch samples) with the size bias observed both in our vacuum-pump samples and by Fraser & Williams (1997) highlights that environmental characteristics and taxon traits interact to determine individual responses to sample extraction (Stubbington, 2012). In particular, Scarsbrook & Halliday (2002) caution that comparisons of organism size should only be conducted among sites with similar hydraulic conductivity, due to its influence on the tenacity of invertebrates exposed to suction forces.

Characterization of hyporheic invertebrate communities: future challenges

The subsurface sediments of the hyporheic zone provide a habitat for a diverse invertebrate fauna. In addition, the role of these sediments as a refuge during instream disturbances (Dole-Olivier, 2011; Stubbington, 2012) may mean that they become an increasingly important ecosystem component where extreme hydrological conditions become more common in a changing climate (Ledger & Milner, 2015). Understanding the limitations of current sampling methods is therefore important to inform interpretation of field sampling data. Pumping methods are widely used and their use may further increase if hyporheic communities become new biomonitors of ecosystem health (Leigh et al., 2013; Boulton, 2014) under the requirements of international legislation. Our study contributes to recent work seeking to inform both interpretation of research data and development of biomonitoring programmes by quantifying the limitations of such hyporheic invertebrate sampling methods (Dole-Olivier et al., 2014; Stubbington et al., 2016).

We demonstrated that vacuum-pump sampling collects a particularly small proportion (mean $5.1 \pm 1.1\%$) of the population present and that the individuals captured have a body size less than the population mean. This low proportion and size bias may both restrict vacuum-pump sampling's characterization of community composition, with larger, more adherent, more robust, and more active taxa and individuals potentially less likely to be sampled. Such limitations may prevent standard vacuum-pump sampling from distinguishing between contrasting invertebrate communities (e.g. Scarsbrook & Halliday, 2002; Stubbington et al., 2016), although the method has previously proved capable of identifying both spatial and temporal changes in community composition (Boulton et al., 1992; Stubbington et al., 2011a, b). Adaptation of standard vacuum-pump sampling, for example using a higher flow-rate manual bilge pump or an electric pump (Tanaka et al., 2014), may improve the method's performance by increasing sample extraction rates. In contrast, although Bou-Rouch pumping also extracted a limited proportion of the population present (mean $32.6 \pm 4.5\%$; also see Dole-Olivier et al., 2014), this technique sampled organisms from the entire population size distribution.

A recognized limitation of all pump-sampling methods is the unknown position of the sampled sediments (Roy & Danielescu, 2014). Our study describes a simple mesocosm set-up that could be adapted to address such research gaps. We recommend that further experimental work be conducted to determine where sampled invertebrates originate from, in terms of both their vertical distribution and their proximity to the sampling pipe. In addition, our study examined a single taxon, and further research is required to examine the effectiveness of pump sampling for capturing a wide range of taxa, including both macroinvertebrates and meiofauna.

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