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LIMNOLOGICA
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Does groundwater influence the sediment fauna beneath a small, sandy stream?

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Received 9 May 2006; received in revised form 17 November 2006; accepted 19 December 2006

Abstract

Gradients in the sediment fauna comprising groundwater (GW) and hyporheic taxa were investigated in the sand/silt-bottomed Marbling Brook in Western Australia. The structure of sediment invertebrate assemblages from Marbling Brook sediments and the adjacent GW were studied at five sites over 1 year and hydrological interactions were characterized using a suite of abiotic factors. Although all five stream sites were upwelling, the sites differed in the degree of hydrological interactions between GW and surface water. Sediment fauna taxa abundances were not correlated with any of the abiotic factors investigated and did not change gradually with depth. Faunal assemblages in the stream sediments were distinct from faunal assemblages in alluvial GW. While water exchanged between alluvial GW and sediment water, as shown by abiotic factors, the distinct differences in faunal assemblages indicated an unpredicted complexity in the catchment with fundamentally different hydrogeological situations on the decimetre scale. Sampling in sandy sediments needs to take this small-scale variability into account.

Keywords: Groundwater fauna; Sediment fauna; Groundwater/surface water interactions; Hydrology; Catchment

Introduction

Gradients of faunal assemblages have been found along the transition from surface to subsurface water (e.g. the upper Danube: Danielopol et al. 2000; the River Rhône: Marmonier 1988; review by Malard, Tockner, Dole-Olivier, & Ward 2002; the Kye Burn: Olsen & Townsend 2005). Groundwater (GW) fauna (hypogean taxa; stygobites; also called phreatobites) predominate at the GW end of the gradient, and stream fauna (epigean taxa) predominates close to the surface (e.g. Brunke & Gonser, 1997; Ward & Palmer 1994). E.g., Brunke and Gonser (1999) showed that in the gravel-bed Toess River, phreatobites dominated at the deeper strata of below 50 cm in the GW exfiltrating zone, while surface water (SW) fauna characterized shallower strata and stream water-characterized zones. Usually, the interaction zone between GW and SW forms a spatially

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doi:10.1016/j.limno.2006.12.002

Please cite this article as: Schmidt, S. I., et al. Does groundwater influence the sediment fauna beneath a small, sandy stream? *Limnologica,* (2007), doi:10.1016/j.limno.2006.12.002

and temporally highly variable ecotone underneath the sediment surface with intense exchange of faunal assemblages and water bodies (e.g. Fraser & Williams 1998). While these gradients are well-studied in large and small rivers with highly conducive sediments, sandy-bottomed rivers have rarely been investigated so far. Three sandy sediment fauna investigations sampled streams that were flowing on top of a clay layer or iron pan (Cleven & Meyer 2003; Strommer & Smock 1989; Whitman & Clark 1984). Thus, these streams did not interact with underlying groundwater. Hahn (1996) found stream sediment water down to a depth of 50 cm and groundwater to be distinct from each other. He did not sample the groundwater itself. In the present study, our aim was to test whether the SW/groundwater gradients known from gravel-bed rivers can be observed – at smaller scales (Boulton, Hakenkamp, Palmer, & Strayer 2002) – in sandy, low hydraulic conductivity systems. The ecotone where the exchange between groundwater and SW is most intense was expected to be closer to the surface than in coarser sediment rivers, therefore, high resolution sampling was performed within the first 40 cm of stream depth. Another aim was to verify that it was indeed groundwater sampled in the deep layers of the stream sediments. This was done by comparing samples from alluvial groundwater wells up-slope, within the groundwater/SW gradient, with the sediment water samples. Since stream water was expected to flush into the alluvial aquifer at high discharge events, the groundwater was sampled at different points with increasing distances from the stream. The scale for this inflow was expected to be in the range of 10 m (Hill, Devito, Campagnolo, & Sanmugadas 2000).

In wells with demonstrated episodical inflow from the brook – and subsequent input of nutrients, carbon, dissolved oxygen (Marmonier & Creuzé des Châtelliers 1991) – we expected invasions of stream fauna into the aquifer. However, stream fauna was not believed to be able to build stable populations but would recede as soon as subsurface conditions were to become less favourable again (Dole-Olivier, Marmonier, & Beffy 1997). At the same time, groundwater fauna was expected to proceed further into the stream sediments because with increasing groundwater influence these assemblages would be able to out-compete stream fauna in harsher conditions (Brunke & Gonser 1999).

We expected that the hydrological patterns leading to enrichment of aquifer zones and impoverishment afterwards would shape faunal assemblages to a higher degree than the individual sampling site situation in terms of geology, elevation, distance from spring, and to a higher degree than differences in water chemical gradients alone (Hakenkamp & Palmer 2000).

Our first hypothesis was that the groundwater/SW gradient, both in terms of faunal patterns and abiotic conditions, in the sand/silt Marbling Brook should form within smaller ranges than in gravel-bed rivers, i.e. within tens of centimetres beneath the stream bed. We further tested (2) whether the subterranean water at the groundwater end of the gradient reflected the alluvial groundwater sampled up slope, and (3) whether fauna would reflect these gradients or rather physical and chemical features. The underlying assumption was that the alluvial groundwater would be homogeneous.

Materials and methods

The study was conducted in the Marbling Brook catchment, which has one of the few permanently flowing brooks in the mediterranean climate southwest of Western Australia. In this part of the country, permanent flow is usually directly related to groundwater upwelling, especially groundwater from shallow aquifers (not deeper than about 15 m) in the alluvial valley. The topography of the 30 km^2 Marbling Brook catchment shows a high (highest point of the catchment >260 m above sea level; confluence at 80 m above sea level) and well-defined relief. Because of the well-defined relief of our study site, we expected that only one local flow system with a clear gradient would evolve, whose boundaries would be located under the hill chains (Tóth 1963). Sampling, monitoring and recording took place between May 2001 and April 2002 in the Marbling Brook catchment in Western Australia (easting: 0414602, and northing: 6506513).

The catchment

Marbling Brook is situated 60 km from Perth, Western Australia and has a catchment area of 30 km^2. Median flow measured between April 2001 and March 2002 was 132 L s^{-1}. The region experiences a mediterranean climate with dry, hot summers and cool, rainy winters (Bridgewater 1987). In Perth, only 5% of the average annual rainfall occurs during summer (December, January, and February). The average yearly rainfall in Perth is 869 mm.

The second order Marbling Brook is a tributary of Brockman River, which feeds into the Swan River. The catchment is situated in the Darling Ranges, in a mountainous area. Geology is heterogeneous, comprising quartz-feldspar-biotite granofels in the northern part, quartz-micas schist and augengneiss in the confluence area of the catchment, and alluvial/colluvial, sandy material, in the central part. These structures are overlain by laterite, an iron-rich crust (Fig. 1).

Five study sites were investigated, each including one or more groundwater sampling wells as well as a stream sediment sampling site. At each site, at least three stream

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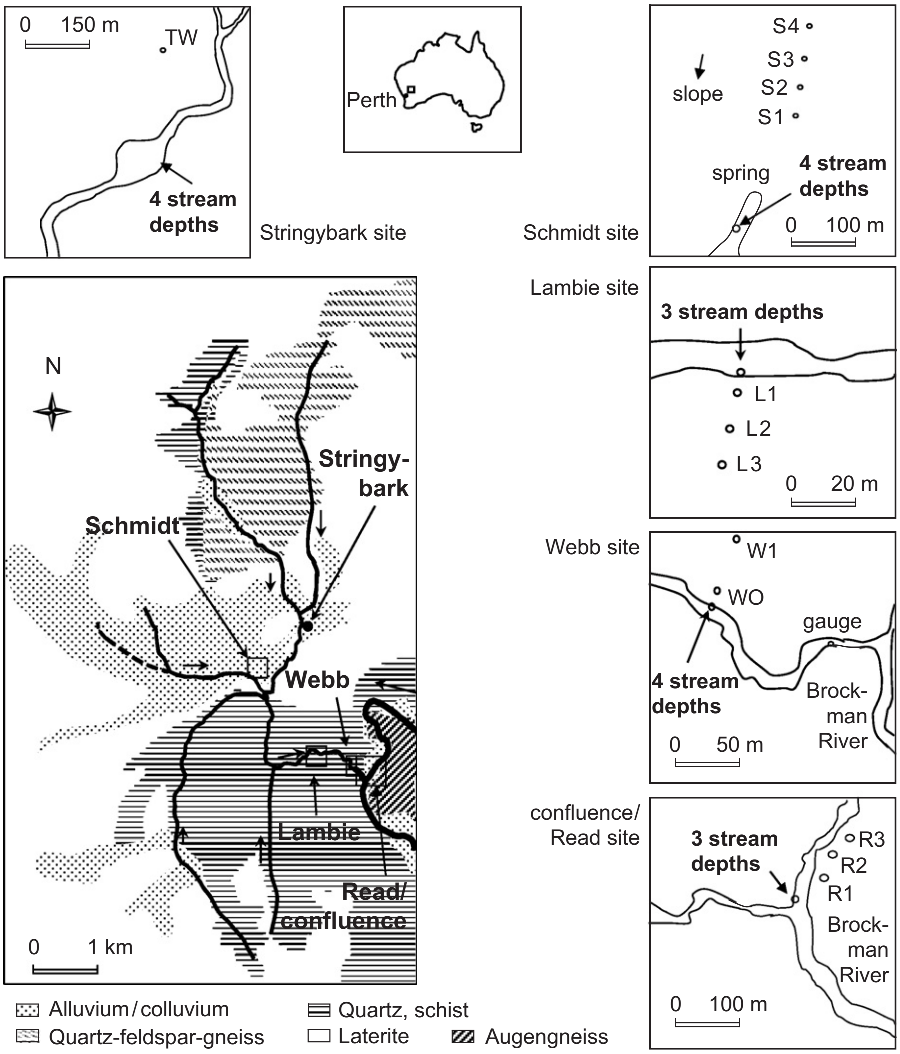

Fig. 1. Catchment outline, shaded according to the underlying geology, a map of Australia and magnified sketches of the investigated sites along the Marbling Brook. Stream widths not to scale. The thick line in the outline represents a section of the Brockman River, of which Marbling Brook is a tributary. Circles represent the position of the groundwater wells in the catchment map.

sediment depth layers, as well as the SW, were sampled for fauna and water quality (Fig. 1). Prior knowledge about the hydrological exchange processes was not available. The current study was therefore intended as a preliminary study of groundwater influence on stream sediment fauna in Western Australian sandy streams.

The sampling sites

Groundwater and stream sampling sites were chosen to complement existing monitoring wells. The groundwater wells that had been drilled prior to the study (WO at the Webb site and the Stringybark well) deviate in construction from those that were drilled for the present study, having different diameters of the well, casing, and slotted lengths. All other wells were drilled in March 2001 and were constructed of white PVC (50 mm diameter) with horizontal slots of 1 mm width. At the Read/confluence site it was not possible to do a transect because of the gradient of the slope. Here, three wells were drilled next to each other at the same distance from the brook to serve as parallels. The sampling site at the Schmidt area was a hand-dug gully used to conduct the shallow groundwater towards the Marbling Brook, and is included with the stream sites. Stream sampling sites were investigated with four nested tubes (Schmidt, Hahn, Hatton, Watson, & Woodbury 2004), installed a week prior to the first sampling. The stream tubes were constructed in a similar way to the groundwater sampling sites: PVC or acrylic pipes reaching into the sediment were slotted in the sediment depth layer to be sampled. At each site one tube for each sediment depth layer was sampled. The stream sediment tubes were slotted at 0–5 cm for the shallowest sediment depth

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layer, then at 5–10, 10–20 and 20–30 cm. These tubes were located approximately 40 cm apart. At the Lambie site the deepest tube could not be installed due to the prevailing sediment properties. This was also the case for the tube second from the top at the confluence/Read site.

Groundwater levels, vertical hydraulic gradient (VHG) and stream discharge

In order to characterize groundwater/SW interactions we described hydrological exchange along the hydrological pathway in the sediments from distant groundwater to SW in the Western Australian Marbling Brook catchment by physical and chemical factors. Lamontagne, Leaney, and Herczeg (2002, 2005) and Westbrook et al. (2005) have used a similar suite of factors to map the distribution of water types and transition zones in the subsurface and the discharge for fresh groundwater towards the sediment/water interface, especially for the upper strata of hyporheic interstices (Brunke & Gonser 1999). E.g., a trend of increasing salinity along the groundwater flow path was demonstrated for catchments in the Western Australian Wheat Belt due to the combination of evaporation and rock dissolution in conjunction with travel time in the aquifer (Salama, Bartle, & Farrington 1994).

Groundwater levels were recorded manually with a dipper in the wells fortnightly. In June 2001, five of the wells (WO, W1, L1, L3, S2; Fig. 1) were equipped with water level data loggers (Capacitive Water Level Probe, Dataflow Systems Pty, Christchurch, New Zealand). Vertical hydraulic gradient in the stream sediments was estimated by measuring the difference between the water level in the stream tube and the water level of the SW from the top of the tube in question to the nearest mm. The VHG is then calculated by dividing this distance by the depth of the sampling tube (Freeze & Cherry 1979; Pepin & Hauer 2002).

In April 2001, a stream gauge (Unidata Starflow UDI, Model 6526B; Unidata Australia, O'Connor, Australia) was installed at the Webb site, measuring the flow velocity. Measurements were taken of the flow each minute and were averaged for every 15 min to calculate discharge. Precipitation was measured with a tipping bucket rain gauge (Rimco RIM 7499, 0.2 mm resolution; McVan Instruments, Mulgrave, Australia) at the Schmidt site. Daily precipitation sums were calculated.

Sampling

Approximately 1.5 L, according to the wells' ability to recover, were pumped from wells and stream tubes with a 12 V electric peristaltic vacuum pump (flow rate 0.03 L s^{-1}; design by CSIRO, Floreat, Australia). We did not purge the wells with several well volumes, because of the resulting effects on the aquifer sediments. Also, some wells did not recover immediately after removing the standing water. As a consequence, the recharging and subsequent increased exchange with the atmosphere would have altered the water quality considerably. Instead we used a micro-purging method (Puls & Barcelona 1995) in all wells. Electrical conductance (EC), dissolved oxygen, temperature, and pH were measured using WTW probes and instruments (WTW, Weinheim, Germany). Since it was known that the groundwater was of a different quality than the stream water (Hahn 2002), we used EC as the principal tracer for groundwater/SW interactions (Salama, Farrington, Bartle, & Watson 1993; Stanford, Ward, & Ellis 1994).

Hydrogen carbonate concentrations were measured using the auto-titrator TTT 85 with auto-burette ABU 80 and titration unit TTA 80 (Radiometer, Copenhagen, Denmark) within 10 h of sampling. Subsamples were filtered using a Millipore EP filtration unit with MF membrane filters pore size 0.45 μm, and pre filter AP 15 (Millipore, Billerica, USA) and analysed for dissolved iron. Calcium, magnesium, sodium, potassium, and total iron analyses were performed from the acidified sample. These samples and the filtered iron sample were analysed with a Varian 600 flame atomic absorption spectrophotometer (Varian NMR, Palo Alto, USA). Total organic carbon (TOC) was measured with a TOC Analyser CA 10 (Skalar, De Breda, The Netherlands).

Stream sediment cores were taken with a 50 mm corer, pushed about 15 cm into the sediment within 1 m from the stream tubes. Minimum distance to the nearest stream tube was at least 30 cm from the next one. For grain size analysis, about 350 g of stream sediment cores (taken monthly) or soil sample from drilling the groundwater wells, were dried at 110 °C and then ground up. Sediments were sieved for 10 min on a shaker, using a set of sieves with the mesh sizes 0.063, 0.09, 0.125, 0.25, 0.355, 0.5, 1, and 2 mm. After sieving, the fractions <1 mm and >1 mm were burnt at 550 °C for 4 h in a furnace. The weight loss after burning (loss on ignition) was noted and used as a measure for FPOM (fine particular organic matter; particles <1 mm) and CPOM (coarse particulate organic matter; particles >1 mm).

The fauna was sampled with 45 mm diameter plankton net with 125 μm mesh, whenever water quality measurements were taken, i.e. twice a month in stream sites and monthly in groundwater. After water had been pumped for chemical and physical analyses, the narrow plankton net was lowered down the well. The net was drawn up and lowered down as described in Bou (1974). Dumas and Fontanini (2001) demonstrated the efficiency of sampling by nets to be similar to sampling with pumps. Raising and lowering the net was repeated 14 times in the stream sediment tubes because initial tests had shown that no fauna was retrieved after the 12th draw. In some groundwater wells the net was always clogged up after two draws, so for consistency, all groundwater wells were sampled by lowering and pulling up the net twice. Thus, the standard was the sample of 14 draws in the short, 50 mm diameter × 50 mm or 100 mm vertical section for stream wells, and two draws in the deep 50 mm diameter × 2 m vertical section in groundwater wells. This type of sampling, although not entirely quantitative, allowed a regular and effective monitoring of all wells which would not have been possible by time-consuming and sediment-disrupting pumping. Since the sampling, especially in groundwater, was semi-quantitative, calculations were not performed on the abundances of taxa, but on the frequencies. The faunal elements were sorted alive under 40 × magnification and identified microscopically at a magnification of 400 × .

Statistics

None of the values (abiotic or biotic) were normally distributed, and the variances were not homogeneous, even after transformation. Therefore, only nonparametric tests were performed. Mann–Whitney–Wilcox tests were used for pair-wise comparisons between variables from streams and groundwater, and the Wilcoxon signed rank test was used to test whether the VHG values per sediment depth layer were significantly larger or smaller than zero. The Behrens–Fisher test with the Satterthwaite t-approximation was employed for nonparametric multiple comparisons (Munzel & Hothorn 2001; R Development Core Team 2004). Scatter plots were drawn to visualise potential relationships between abundances of the total fauna, or of single taxa with any one of the investigated factors. In the case of a visible relationship, the correlation was tested with the Spearman rank correlation.

The frequencies of the taxa occurring at the different depths were analysed with a reciprocal averaging analysis (RA; Hill 1973; algorithm implemented by B. König, BfG Koblenz, Germany, unpublished). The taxa and sites were ordinated according gradients in relative abundance of taxa. The exploratory data analyses also included Nonmetric Multi Dimensional Scaling (referred to as NMDS from here on; Primer 5, Plymouth Laboratories, Plymouth, UK) performed on the faunal data matrix, calculated using the Bray Curtis similarity measure of the presence/absence-transformed data for each site and depth. Groups of faunal assemblages per site, per hydrological characteristics and per hydrological unit were compared using the ANOSIM (analysis of similarities) procedure (Clarke & Green 1988; Primer 5, Plymouth Laboratories, Plymouth, UK). ANOSIM is a nonparametric and multivariate equivalent to ANOVA and tests the differences among groups (Clarke 1993), in this case among groups of faunal assemblages at sites.

Relationships between faunal assemblages and physical and chemical factors were tested with the BIO-ENV routine (Primer 5, Plymouth Laboratories, Plymouth, UK), a multivariate correlation test. This procedure does a stepwise search for the combination of environmental variables to determine the best match (in this case using the normalised Euclidean distance) between the biotic and the abiotic configuration (Clarke & Ainsworth 1993). For this procedure, abiotic values were $\log(x+1)$ transformed and standardized. Faunal data were presence/absence-transformed.

Results

Hydrogeological patterns

According to VHG, all the sites were significantly upwelling in at least one sediment layer ($p<0.05$; Fig. 2a). The sites differed markedly though in the intensity of exchange. In Figs. 2(b) and (c), groundwater wells were treated as consecutive sediment depth layers with distance from the brook (see Introduction). The sediments were mostly well oxygenated. Dissolved oxygen in the sediments was significantly lower than at the surface of all sites (Mann–Whitney $W=24{,}039$, $p<0.001$). The patterns of VHG, EC and dissolved oxygen were not gradational along the presumed flow paths and these factors did not vary in a consistent way across the sampled sites (Table 1, Fig. 2).

Water level data further indicated groundwater flow towards the brook at all sites; groundwater level above sea level decreased with decreasing distance to the brook (Fig. 3). Gradational changes in relative ion compositions were found for L3–L1, S4–S2 and W1–WO (Fig. 3).

The groundwater well at the Stringybark site had a very constant head and its water was significantly fresher than that of the brook. At the Schmidt site the gradient of increasing EC with increasing groundwater flow distance downhill did not include the lowest well S1, which was more similar in EC and relative ion compositions to the well S3. Stream water was significantly fresher than the groundwater. At the Webb site, water levels in the well W1 were lower in summer than those in the well WO (Figs. 3e, f). At the confluence/Read site, where the three wells had been intended as parallels for the same aquifer, very different patterns in seasonal water level and EC variations were observed.

The distribution of organic matter, hydraulic conductivity and grain sizes was heterogeneous in space and time. Percentage of FPOM in the organic fraction of the stream sediments ranged from 8.6 to 98.8, varying

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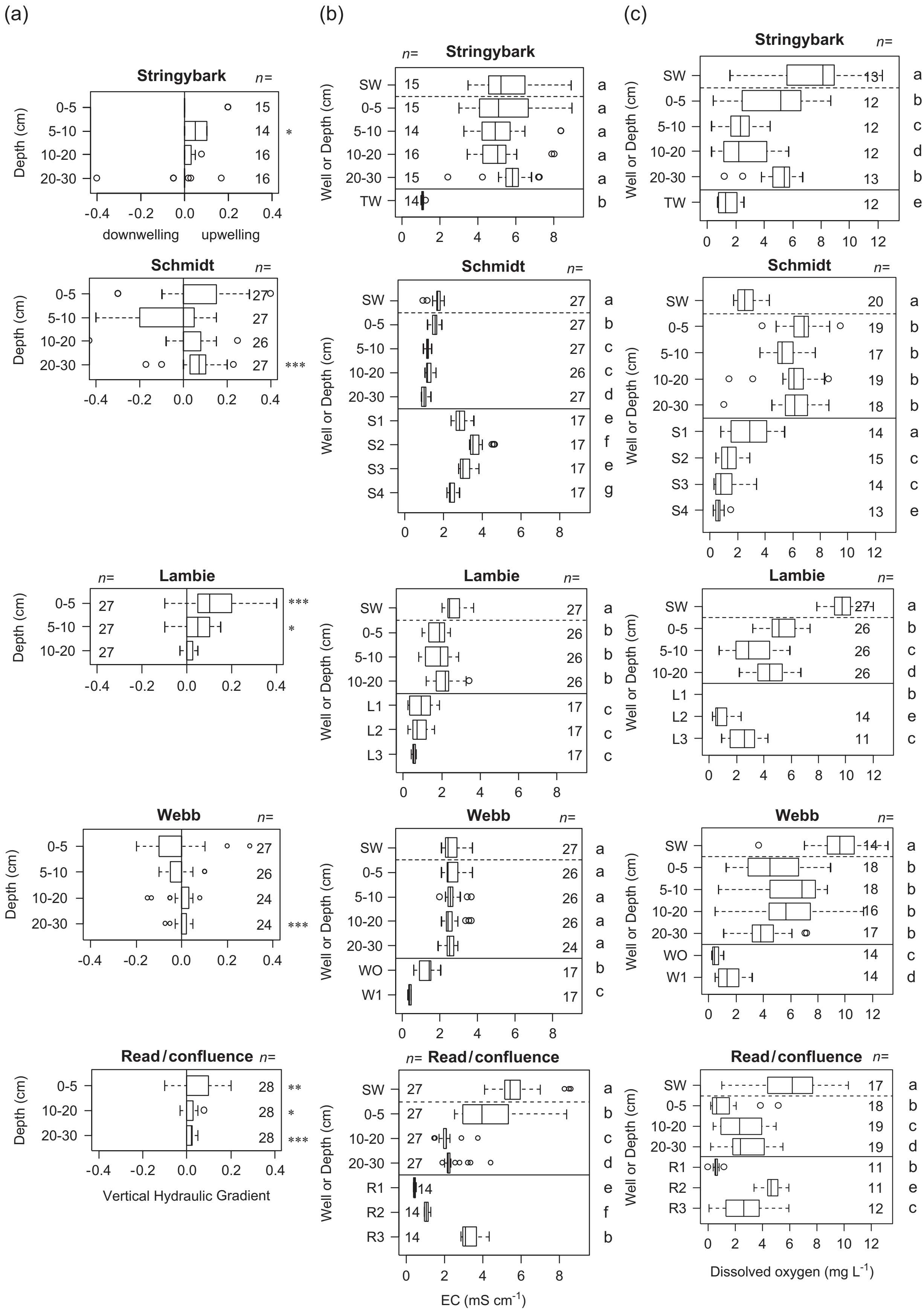
(a)
(b)
(c)
Stringybark
n=
Depth (cm)
0-5
5-10
10-20
20-30
15
14
16
16
*
-0.4
-0.2
0.0
0.2
0.4
downwelling
upwelling
Well or Depth (cm)
SW
TW
15
14
a
b
0
2
4
6
8
10
12
13
12
c
d
e
Schmidt
27
26
***
S1
S2
S3
S4
17
f
g
20
19
18
Lambie
***
L1
L2
L3
11
Webb
24
WO
W1
Read/confluence
28
**
R1
R2
R3
Vertical Hydraulic Gradient
EC (mS cm⁻¹)
Dissolved oxygen (mg L⁻¹)

without obvious patterns in space and time. Hydraulic conductivity, estimated from grain size distribution based on Beyer (1964), was low in the stream sediments (minimum: 3×10^{-6} at the Schmidt site; maximum: $0.02\,\mathrm{m\,s^{-1}}$ at the Lambie site; Fig. 4). In groundwater, sediment samples could only be taken once during drilling. Samples were taken from the depth where the well casing was slotted. The sampled water and fauna were derived from this area. The values are given as diamonds in Fig. 4.

Groundwater and stream sediment fauna

Well fauna was distinctly different from stream sediment fauna (Table 2). Only two species occurred in both groundwater and SW: *Parapseudoleptomesochra* sp. 1 and *Australocamptus similis* Karanovic, 2004. Of the 5792 individuals in 65 taxa observed in all samples from groundwater and SW sediments, *A. similis* was found with five individuals in samples of the well S1. One individual was found in the shallowest sediment depth layer of the Stringybark site. About 678 individuals of *Parapseudoleptomesochra* sp. 1 were found in stream sediments at the Webb, confluence/Read and Lambie sites, and two in the well WO. While 4026 individuals belonged to the crustacea, only 164 individuals were insects. However, numbers in groundwater and stream samples cannot be directly compared because of the different sample sizes.

RA site scores showed that faunal compositions within stream depths were much more similar among each other than the groundwater well groups were (by distance from the brook). The sequence of the stream sediment layers was not consistent with the sequence that was implied by the RA site scores (Table 2).

All correlations between single physical, chemical or hydrological factors and faunal abundances in total or per species were not strong (Spearman r_S not stronger than the absolute value of 0.26; scatter plots not presented). The ANOSIM test resulted in a significant difference between the groundwater and stream sediment assemblages ($R = 0.543$; $p = 0.001$; Fig. 5a). The taxa composition of the stream sites by itself (Fig. 5b) did not exhibit a gradient in faunal composition with increasing numbers of groundwater taxa with depth. A gradient in faunal composition from SW to deeper sediment layers was absent. The assemblages of the sediment depth layers were not different from each other (Fig. 5b).

The BIO-ENV procedure to find the combination of environmental variables that correlated best with the faunal matrix, was conducted with the variable suite of hydraulic conductivity, pH, temperature, EC, dissolved oxygen, nitrate, iron, calcium, and magnesia values. For the groundwater matrix, additionally distance to the brook, sodium, potassium, sulphate, and silica were included in the test. For the groundwater samples an abiotic variables' matrix consisting of distance to the brook, hydrogen carbonate, nitrate, and total organic carbon concentrations resulted in the best correlation (Spearman $r_s = 0.23$, $n = 13$). For the stream samples sediment depth, landuse intensity, coarse particulate organic matter, and dissolved iron concentrations correlated best with the faunal assemblage (Spearman $r_s = 0.28$, $n = 18$). A significance value is not calculated here because the BIO-ENV is an exploratory tool (Clarke & Warwick 1994, pp. 11–10).

Discussion

This study showed that small sediment grain size and the subsequently reduced exchange along the groundwater/SW flow path resulted in interstitial invertebrate communities to be largely unconnected. The underlying assumption that deep stream sediment water would reflect alluvial groundwater proved wrong. Deep sediment fauna was not similar to alluvial groundwater fauna. As expected, faunal patterns were also not related to variability in chemical and physical factors.

The expected small-scale groundwater/SW gradient was not detected, neither in terms of faunal patterns, nor abiotic conditions. Vertical hydraulic gradient, relative water level, EC, and relative ion composition did not show a consistent pattern (compare Brunke, Hoehn, & Gonser 2003) of hydrological interactions at all sites of Marbling Brook. EC and dissolved oxygen were significantly lower in the stream sediments than at the surface, but the clear gradients with depth known from other studies (e.g. Boulton, Findlay, Marmonier, Stanley, & Valett 1998; Strommer & Smock 1989; Whitman & Clark 1984) were missing.

Fauna sampled in the stream sediments contained almost none of the taxa characteristic for alluvial groundwater and faunal composition was significantly

Fig. 2. Boxplots of (a) VHG (vertical hydraulic gradient), (b) EC (electrical conductance) and (c) dissolved oxygen. Solid horizontal lines differentiate among groundwater wells and sediment layers, whereas dashed lines show the interface between sediment and surface water (SW). Difference from VHG = 0 is *statistically significant at the $\alpha = 0.05$ level; **statistically significant at the $\alpha = 0.01$ level; ***statistically significant at the $\alpha = 0.001$ level (Wilcoxon signed rank test; with continuity correction). The sediment depth layer 20–30 cm at the Stringybark site was significantly downwelling. In (b) and (c) boxplots that share the same letter represent mean values that were not significantly different (Behrens–Fisher test; significance level $p = 0.05$). Note that the wells at the Read site were all close to the river, but are drawn next to each other here to show their different character.

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Table 1. Behrens-Fisher statistic and two-sided significance value for the pairwise comparison of electrical conductance

	Read/confluence		Webb		Lambie		Schmidt		Stringybark	
	Behrens-Fisher	Two-sided significance value	Behrens-Fisher	Two-sided significance value	Behrens-Fisher	Two-sided significance value	Behrens-Fisher	Two-sided significance value	Behrens-Fisher	Two-sided significance value
VHG										
A-B	0.384	0.329	0.552	0.923	0.265	0.009	0.330	0.115	0.673	0.424
A-C	0.452	0.857	0.595	0.622	0.181	0.000	0.416	0.743	0.497	1.000
A-D			0.607	0.543			0.529	0.994	0.423	0.885
B-C	0.533	0.927	0.570	0.812	0.303	0.044	0.630	0.358	0.250	0.063
B-D			0.584	0.719			0.758	0.003	0.208	0.017
C-D			0.481	0.998			0.675	0.123	0.416	0.847
EC										
S-A	0.223	0.008	0.558	1.000	0.095	0.000	0.207	0.010	0.444	1.000
S-B	0.000	0.000	0.580	1.000	0.166	0.000	0.055	0.000	0.390	0.976
S-C	0.003	0.000	0.489	1.000	0.228	0.013	0.061	0.000	0.383	0.953
S-D			0.484	1.000			0.025	0.000	0.575	0.999
S-GW1	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000
S-GW2	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000		
S-GW3	0.005	0.000	0.000	0.000	0.000	0.000	1.000	0.000		
S-GW4							1.000	0.000		
A-B	0.022	0.000	0.533	1.000	0.516	1.000	0.047	0.000	0.471	1.000
A-C	0.054	0.000	0.442	1.000	0.668	0.425	0.080	0.000	0.467	1.000
A-D			0.466	1.000			0.007	0.000	0.600	0.992
A-GW1	0.000	0.000	0.000	0.000	0.143	0.000	1.000	0.000	0.000	0.000
A-GW2	0.000	0.000	0.000	0.000	0.102	0.000	1.000	0.000		
A-GW3	0.337	0.666	0.000	0.000	0.000	0.000	1.000	0.000		
A-GW4							1.000	0.000		
B-C	0.822	0.000	0.401	0.993	0.624	0.837	0.551	1.000	0.511	1.000
B-D			0.454	1.000			0.129	0.000	0.701	0.588
B-GW1	0.000	0.000	0.002	0.000	0.183	0.002	1.000	0.000	0.000	0.000
B-GW2	0.000	0.000	0.000	0.000	0.140	0.000	1.000	0.000		
B-GW3	0.963	0.000	0.000	0.000	0.000	0.000	1.000	0.000		
B-GW4							1.000	0.000		
C-D			0.545	1.000			0.093	0.000	0.717	0.385
C-GW1	0.000	0.000	0.000	0.000	0.072	0.000	1.000	0.000	0.000	0.000
C-GW2	0.000	0.000	0.000	0.000	0.029	0.000	1.000	0.000		
C-GW3	0.915	0.000	0.000	0.000	0.000	0.000	1.000	0.000		
C-GW4							1.000	0.000		
D-GW1			0.007	0.000			1.000	0.000	0.000	0.000
D-GW2			0.000	0.000			1.000	0.000		
D-GW3			0.000	0.000			1.000	0.000		
D-GW4							1.000	0.000		
GW1-GW2	1.000	0.000	0.000	0.000	0.476	1.000	0.950	0.000		
GW1-GW3	1.000	0.000	0.000	0.000	0.422	1.000	0.680	0.840		
GW1-GW4							0.104	0.000		
GW2-GW3	1.000	0.000	0.478	1.000	0.339	0.861	0.137	0.001		
GW2-GW4							0.000	0.000		
GW3-GW4							0.014	0.000		
DO										
S-A	0.049	0.000	0.067	0.000	0.000	0.000	0.997	0.000	0.224	0.111
S-B	0.133	0.001	0.110	0.000	0.000	0.000	0.991	0.000	0.077	0.000
S-C	0.138	0.002	0.137	0.002	0.000	0.000	0.933	0.000	0.099	0.000
S-D			0.037	0.000			0.944	0.000	0.222	0.138
S-GW1	0.005	0.000	0.000	0.000			0.525	1.000	0.032	0.000
S-GW2	0.310	0.675	0.000	0.000	0.000	0.000	0.143	0.005		
S-GW3	0.142	0.005	0.023	0.000	0.000	0.000	0.148	0.020		
S-GW4							0.000	0.000		
A-B	0.756	0.115	0.654	0.851	0.148	0.001	0.249	0.185	0.243	0.267
A-C	0.795	0.044	0.611	0.989	0.299	0.293	0.363	0.949	0.264	0.334

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Table 1. (*continued*)

	Read/confluence		Webb		Lambie		Schmidt		Stringybark	
	Behrens-Fisher	Two-sided significance value	Behrens-Fisher	Two-sided significance value	Behrens-Fisher	Two-sided significance value	Behrens-Fisher	Two-sided significance value	Behrens-Fisher	Two-sided significance value
A-D			0.404	0.996			0.392	0.995	0.506	1.000
A-GW1	0.449	1.000	0.000	0.000			0.024	0.000	0.184	0.167
A-GW2	0.949	0.000	0.093	0.000	0.000	0.000	0.000	0.000		
A-GW3	0.722	0.543	0.325	0.767	0.028	0.000	0.000	0.000		
A-GW4							0.000	0.000		
B-C	0.551	1.000	0.422	1.000	0.742	0.116	0.656	0.892	0.521	1.000
B-D			0.243	0.154			0.663	0.839	0.901	0.001
B-GW1	0.160	0.011	0.014	0.000			0.069	0.000	0.285	0.553
B-GW2	0.868	0.002	0.069	0.000	0.074	0.000	0.000	0.000		
B-GW3	0.493	1.000	0.169	0.020	0.400	0.987	0.000	0.000		
B-GW4							0.000	0.000		
C-D			0.274	0.344			0.497	1.000	0.830	0.024
C-GW1	0.096	0.001	0.027	0.000			0.070	0.000	0.347	0.859
C-GW2	0.849	0.006	0.058	0.000	0.005	0.000	0.023	0.000		
C-GW3	0.467	1.000	0.152	0.007	0.111	0.000	0.019	0.000		
C-GW4							0.004	0.000		
D-GW1			0.004	0.000			0.062	0.000	0.058	0.000
D-GW2			0.105	0.000			0.039	0.000		
D-GW3			0.391	0.994			0.016	0.000		
D-GW4							0.006	0.000		
GW1-GW2	1.000	0.000	0.872	0.001			0.200	0.067		
GW1-GW3	0.841	0.085	1.000	0.000			0.153	0.008		
GW1-GW4							0.027	0.000		
GW2-GW3	0.129	0.015	0.918	0.000	0.903	0.000	0.348	0.968		
GW2-GW4							0.154	0.009		
GW3-GW4							0.332	0.915		

S = surface water; A = 0–50; B = 50–100; C = 100–200; D = 200–300 mm. GW1 = groundwater well closest to the stream; GW2–4 = groundwater wells with increasing distance from the stream.

different. This is in contrast to Hahn's (2002) preliminary study during which he found e.g. *Atopobathynella* sp. in both stream sediments and groundwater, whereas this taxon was restricted to groundwater in the present study. Most recent studies based on samples from more than two sediment depths revealed gradients with increasing groundwater/decreasing stream water properties (e.g. Dole-Olivier, Creuzé des Châtelliers, & Marmonier 1993; Fraser, Williams, & Howard 1996; Malard et al. 2002; Marmonier 1988). Even where gradual changes were not present, patches represented different degrees of influence from the groundwater and could be characterized within the groundwater/SW gradient (Brunke et al. 2003; Dole-Olivier 1998; Dole-Olivier et al. 1997). Dole-Olivier (1998) predicts gradual changes on the large scale and patchy situations on the reach scale. While our intention was to sample five sites within the catchment, apparently the 3–4 sediment tubes at each site represented the reach, not site scale in the Marbling Brook system.

Additionally, the difference between stream sediment and groundwater fauna in this study, which was based on a larger data set than Hahn's (2002) raises the question whether the groundwater fauna found in other studies in the sediments and the riparian zone (e.g. Ward & Palmer 1994) is actually typical of the surrounding groundwater aquifer, or rather of that part within the ecotonal hyporheic zone that is least stream water-influenced.

The VHG indicated net groundwater flow towards the brook but this was not reflected in the gradients of decreasing EC with increasing depth. A reason for this might be that the changes within the sediment were complex, similar to what Morrice, Valett, Dahm, and Campana (1997) described in three alluvial montane systems, to what Malcolm & Soulsby (2001) found for wetland dunes, and to what Hoehn and Cirpka (2006) modelled in two Alpine floodplains. Vertical hydraulic gradients varied without the seasonal pattern shown in Malard et al. (2001).

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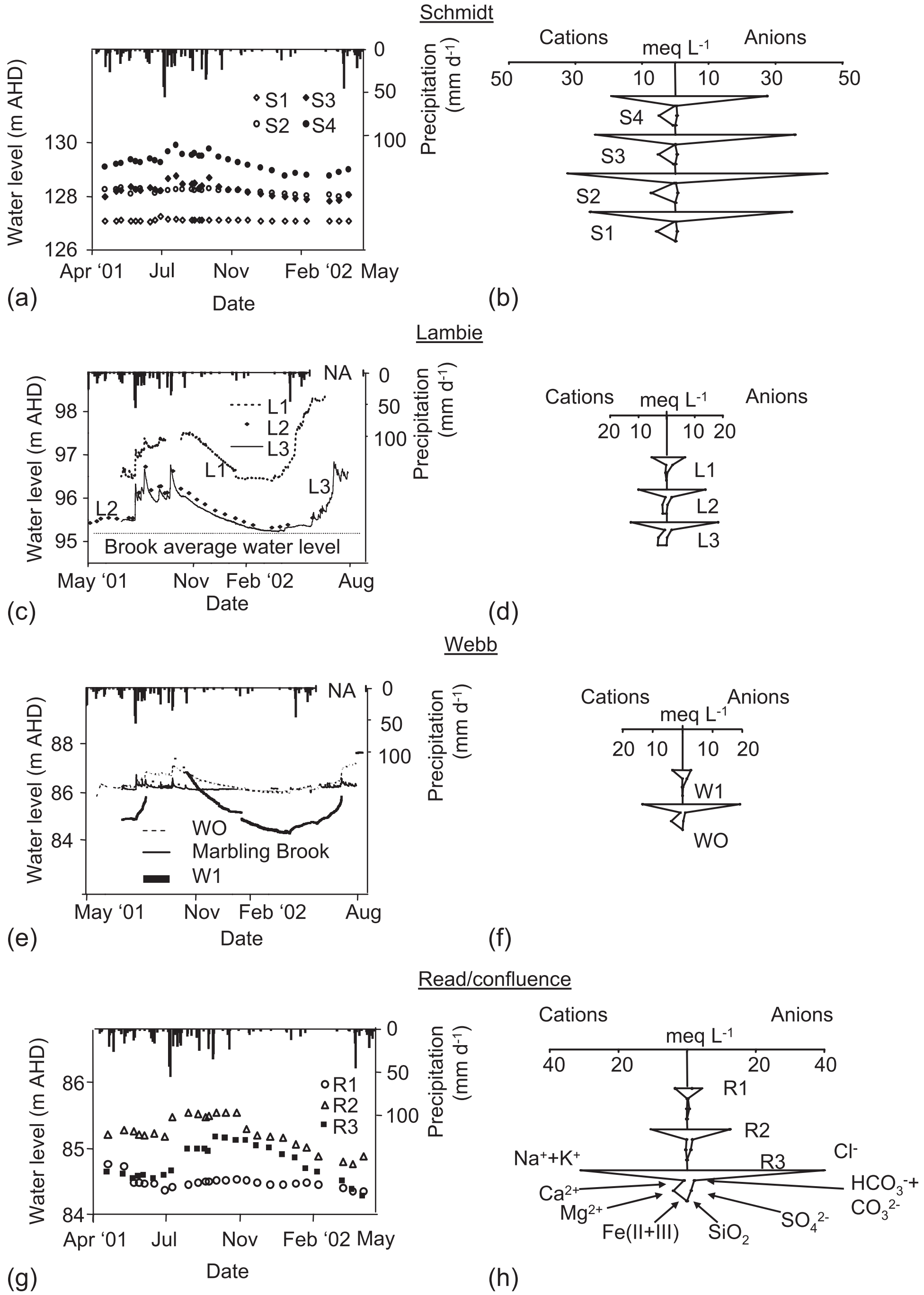

Fig. 3. Water levels (a, c, e, g) and relative ion composition of groundwater (b, d, f, h), represented as Stiff diagrams. (a, b) Schmidt site. (c, d) Lambie site. (e, f) Webb site. (g, h) Read/confluence site. NA = no measurements. AHD = Australian Height Datum, elevation over sea. Legend for the Stiff diagrams in (h). See Fig. 1 for sampling site details.

Alluvial groundwater sampled in the different wells was not homogeneous. Although the 25 km^2 area in Bangladesh studied by Cheng, Vangeen, Seddique, and Ahmed (2005) was probably of a similar geomorphic and geologic heterogeneity as the Marbling Brook valley, the author found much less temporal and spatial heterogeneity over the course of 3 years in 20 wells. Martin et al. (2004) found heterogeneity in groundwater nitrate levels to be related to altitude, well depth, and to stream water level in the stream-near wells. Jankowski and Beck (2000) described in large detail four hydrogeological units in a New South Wales aquifer, but these units were stratified in a stable way and on the longitudinal axis variability was low.

Differences among Marbling Brook groundwater wells were generally larger than those among stream

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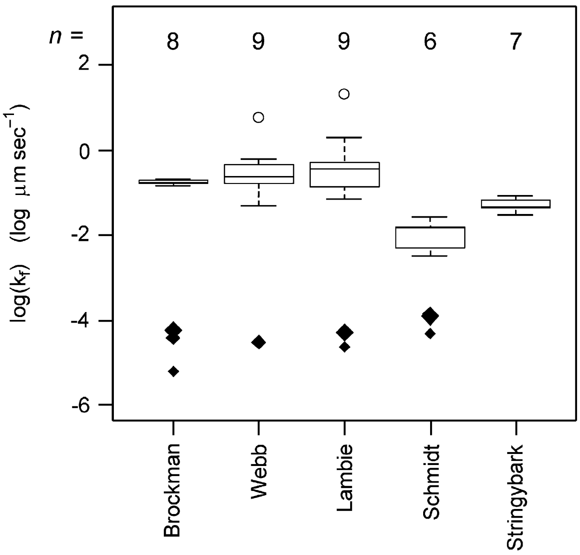

Fig. 4. Sediment characteristics: boxplots of hydraulic conductivity averaged over *n* sampling events for the stream sediments and diamonds for the sediment of the corresponding groundwater wells – sediment was obtained once from the depth where the wells were slotted, when installing the wells. The size of the diamond corresponds to the distance from the stream. The well at the Stringybark was pre-existing, so no sediment sample was taken.

sediment strata. Thus, while the stream sediments are still considered a highly active zone, steeper gradients (maybe even ecotones) seemed to occur within the groundwater aquifer (Schmidt; unpublished data). While some variability in the alluvial aquifer had been hypothesised as a consequence of seasonal inflow of SW into the alluvial aquifer, the actual variability rather indicated fundamental differences in hydrogeological patterns throughout the catchment. Most studies of groundwater spatial and temporal variability at the 1–50 km^2 scale though show consistent variation of chemical and physical factors within the catchment (e.g. Muñoz-Carpena, Ritter, & Li 2005).

Hydrological patterns on the site scale

In detail, at the Stringybark site the groundwater well showed a very constant head and its water was significantly fresher than that of the brook. A hydrological connection between the groundwater sampled in this well and the summer-dry, saline stream reach at this site is thus unlikely. The brook was probably fed by runoff and interception, carrying a high salt load (compare Salama, Hatton, & Dawes 1999).

At the Schmidt site the continuum of increasing EC along the groundwater pathway (Salama et al. 1999) followed the transect from S4 down to S2, but the aquifer at S1 was influenced by another source of ions and was more similar in EC and relative ion composition to the well S3. The upwelling water in the stream site was fresher than the sampled groundwater, indicating yet another source of water. The groundwater body sampled by S4–S3–S2 obviously took another path than S1. This means that the groundwater wells at 20 m distance did not reliably capture groundwater flow.

At the Webb site, water levels in the well W1 were lower in summer than those in the well WO, but if there had been a hydrologic connection, EC in W1 would have had to increase with input from the higher EC WO water. Therefore, it appears there is a hydrologic barrier between the wells W1 and WO, which were only 10 m apart from each other.

At the confluence/Read site, where the three wells had been intended as parallels to sample the same hydrogeologic situation, very different patterns in seasonal water level and EC variations were observed. Studies on a similar small scale mostly reveal much less heterogeneity within an alluvial channel (e.g. Katz, Chelette, & Pratt 2004, study of the Woodville karst plane; the Victorian Murray River bank studied by Lamontagne et al. 2002, 2005). However, Lee, Choi, Kim, and Lee (2005) differentiated four types of aquifer situations according to groundwater level variation, but these four types reflected different groundwater extraction practices, not different hydrogeological units. At the Read site the three wells were drilled down to bedrock, assuming that the same unit would prevail on top of bedrock within 30 m. However, the three wells were drilled to different depths. Jankowski and Beck (2000) have demonstrated high variability with depth within a seemingly uniform aquifer, and apparently the three wells at the Read site sampled a similar variety of subunits (compare water levels in various rock formations in Fig. 8 in Reddy, Raju1, Reddy, & Reddy 2000).

All stream sites were upwelling, and thus groundwater discharge was expected, in accordance with Pepin and Hauer (2002), Lamontagne et al. (2002, 2005), and Hunt, Strand, and Walker (2006). However, this groundwater discharge was only traced reliably at one of the five sites: the Lambie site. The middle alluvial well at the Lambie site might sample an old meander of the Marbling Brook, judging from the especially coarse material extracted when drilling (own observations). In the present study fauna in all the alluvial wells was significantly different from hypogean, upwelling stations in the active channel. Dole and Chessel (1986) found along a gradient from active channel via side arm to old, 'never'-flooded meander that the hypogean fauna did not vary to a high degree between side-arm and old meander, but was markedly different from the surface-water fauna characterized active channel samples. However, the direction of subsurface flow had not been estimated in the cited study and it cannot be excluded that the active channel stations were actually downwelling. This is all the more likely since Ward and

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Table 2. Reciprocal averaging analysis of the distribution of the taxa (frequency based on abundances averaged for the five sites and over time) in the sediment depths and groundwater wells closest to Marbling Brook (GW1) and nearby (GW2)

Species	Species score	10–20 cm	5–10 cm	20–30 cm	0–5 cm	GW1	GW2
		$n_{\text{sites}} = 5$	4	4	5	4	3
	Site score	0	0.1	0.5	2.3	96.4	100
Eucyclops sp. 3	0	100					
Nitocrella sp. 1	0	100					
Nitocra sp. 1	0	100					
Onychocamptus bengalensis (Sewell, 1934)	0	100					
Paracyclops sp. 5 (cf. McRae, unpublished)	0.06		100				
Paracyclops sp. 8 (cf. McRae, unpublished)	0.06		100				
Cypridae sp. 1	0.06		100				
Candonidae sp. 6	0.12	30.5	52.2	17.3			
Eucyclops australiensis, Morton, 1990	0.36	83.9			16.1		
Fierscyclops fiersi Karanovic, 2004	0.42	50.9	11.6	25.5	12.0		
Mesocyclops brooksi Pesce, De Laurentiis, & Humphries, 1996	0.50	47.1	32.3		20.7		
Candonidae sp. 5	0.53	64.5	12.4		23.0		
Nitocra sp. 4 (cf. McRae, unpublished)	0.56	16.9	60.5		22.6		
Nitocra sp. 5 (cf. McRae, unpublished)	0.60	11.2	64.1		24.7		
Paracyclops sp. 1 (cf. McRae, unpublished)	0.66	21.3	19.6	40.2	19.0		
Candonopsis cf. *tenuis* (Brady) 1886	0.66	38.3	7.6	33.3	20.8		
Austrochiltonia subtenuis (Sayce, 1901)	0.68	70.1			29.9		
Paracyclops chiltoni (Thomson, 1882)	0.87	27.4	13.8	27.5	31.4		
Macrocyclops albidus (Jurine, 1820)	0.95	34.2	24.7		41.1		
Candonidae sp. 1	0.99	19.8	37.7		42.6		
Candonidae sp. 2	1.00	22.8	27.2	8.7	41.3		
Candonocypris novaezelandiae (Baird, 1843)	1.01	42.3	6.7	8.7	42.3		
Australocyclops australis (Sars, 1896)	1.18	48.4			51.6		
Metacyclops arnaudi (Sars, 1908)	1.47	28.7	6.6		64.7		
Cypridae sp. 2	1.50	33.5			66.5		
Calamoecia tasmanica subattenuata (Fairbridge, 1945)	1.70		26.3		73.7		
Candonidae sp. 4	1.85		18.9		81.1		
Metacyclops mortoni, Pesce, De Laurentiis, & Humphries, 1996	2.28				100		
Nitocra sp. 2 (cf. McRae, unpublished)	2.28				100		
Parapseudoleptomesochra sp. 1	6.23	10.9	13.5	12.9	57.2	5.4	
Australocamptus similis, Karanovic, 2004	84.70				13.0	87.0	
Canthocamptus sp. 3	97.02					100	
Bathynella sp. 1	97.02					100	
Protocrangonyx fontinalis, Nicholls, 1926	97.02					100	

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Table 2. (*continued*)

Species	Species score	10–20 cm	5–10 cm	20–30 cm	0–5 cm	GW1	GW2
		$n_{\text{sites}} = 5$	4	4	5	4	3
	Site score	0	0.1	0.5	2.3	96.4	100
Diacyclops sp. 1	97.06					98.8	1.2
Candonidae sp. 3	97.60					83.7	16.3
Canthocamptus sp. 1	97.75					80.3	19.7
Canthocamptus sp. 2	98.44					61.0	39.0
Metacyclops sp. 1	99.09					42.8	57.2
Hurleya sp. 1	99.60					28.8	71.2
Atopobathynella sp. 1	100					18.4	81.6

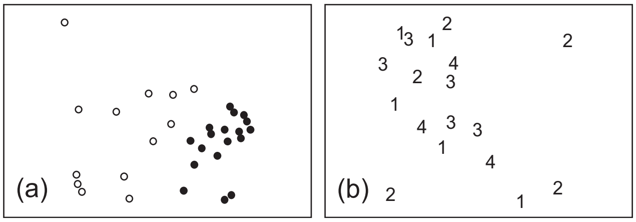

Fig. 5. Two-dimensional NMDS plot of the faunal assemblages of the different hyporheic depth layers and groundwater wells (only stream depth layers in (b)), averaged over the study: (a) ANOSIM $R = 0.58$, $p = 0.001$; (b) ANOSIM $R = -0.03$; $p = 0.59$. In (b) sampling sites are labelled according to the stream sediment depth layers, sampled in depths of $1 = 0$–5 cm, $2 = 5$–10 cm, $3 = 10$–20 cm, $4 = 20$–30 cm. Stress of (a): 0.12; stress of (b): 0.16. Solid circles: stream sediment water; open circles: groundwater.

Palmer (1994) conclude that the side arm was a mixing zone between groundwater and SW.

Although at all sites at least one sediment layer was upwelling, the sites differed markedly and unexpectedly in the type of interaction observed, and to a higher degree than e.g. detailed by Hill, Labadia, and Sanmugadas (1998) or Hill and Lymburner (1998). The patterns of vertical hydraulic gradient, EC, and dissolved oxygen were not gradational along the presumed flow paths, probably because there was more than one flow path. These factors did not vary in a consistent way across the sampled sites, so the expected conformity in groundwater flow across all the upwelling sites was not observed. However, Ward, and Voelz (1990) have shown that interstitial riffle i.e. upwelling assemblages did not differ between riffles along a 2000 m altitudinal gradient.

One reason for this higher-than-expected hydrological complexity may lie in the low hydraulic conductivity. Marbling Brook sediments' median k_f at the Lambie site was $0.01\,\mathrm{m\,s^{-1}}$ and lower at the other sites; gravels by comparison have an average k_f of 0.1 m s (Huggenberger, Hoehn, Beschta, & Woessner 1998). In Schultz and Ruppel (2002) study of sediments along groundwater/SW transect the Beyer estimates of the k_f revealed stream sediments to be largely similar with the groundwater values, except for very shallow stream depths which had a lower k_f. In Marbling Brook sediments we found the inverse: stream sediments were considerably coarser than groundwater sediment. The low k_f probably led to complex exchange processes that are highly variable. In contrast to this, dissolved oxygen did not fall below a level of $1\,\mathrm{mg\,L^{-1}}$ although one might have assumed depletion of oxygen due to stagnation. Similar oxygenation in a sandy aquifer was observed in the Mill Creek and might be due to thermal convection (Whitman et al., 1984).

In summary, at the Stringybark, Schmidt, and Webb sites the groundwater feeding into the stream was not the same as that sampled in the alluvial wells. At the confluence site the three wells that had been intended as replicates sampled three different situations. Methods such as natural isotopes (Lamontagne et al. 2002, 2005) would help clarify which of the wells was hydrologically connected to the Brockman River. The shallow groundwater was not always in hydrological contact with the brook where we expected it, highlighting the unpredictable nature of groundwater exchange in sandy streams.

Fauna and the abiotic environment

Faunal distribution in the Marbling Brook catchment did not correlate to a high degree to the variation in physical and chemical variables in general, contrary to results by e.g. Pospisil, Danielopol, and Dreher (1994), and Datry, Malard, and Gibert (2005), but in accordance with Marmonier, Dole-Olivier, and Creuzé des Châtelliers (1992) and Hakenkamp, Palmer, and James (1994). Sediment depth was among those factors describing faunal distribution best, similar to what Maridet, Wasson, and Philippe (1992) found. However, no combination of factors described more than a quarter of faunal distribution variation. Gradients of

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competition for resources from the surface, as described by Brunke and Gonser (1999), were thus not confirmed. Nor did fauna relate to the VHG, as opposed to what Pepin and Hauer (2002) described for benthic fauna.

Marmonier and Creuzé des Châtelliers (1991) and Datry et al. (2005) have shown that fauna was especially rich in the carbon-rich recharge zones ('eu-alimonic' zones according to Hahn 2006). We assume that the weak relationship between fauna and carbon in the Marbling Brook catchment was due mainly to two factors. Firstly, carbon concentrations might have sustained fauna at some sites, while at other sites similar concentrations might have led to clogging of the interstices and thus created unfavourable conditions (Brunke & Gonser 1997). Thus, increasing concentrations could have led to different phenomena at the different sites. This was probably due to the catchment's high spatial heterogeneity – while at some sites carbon might be a limiting factor, at other sites, due to completely different hydrogeological characteristics, other factors such as nutrients and subsequent microbial populations would be more critical to fauna. Carbon in particular is known to be distributed in an extremely patchy way (Kaplan & Newbold 2000; Marmonier 1988). Secondly, temporal heterogeneity blurs the effects of chemical concentrations; a peak in dissolved nutrients and/or carbon over days – which might be missed in our sampling regime – might induce microbial growth which in turn might provide food for fauna for weeks and months. In those instances, dissolved nutrient concentrations may tell nothing about the alimentary state of the groundwater zone. Poole, Stanford, Running, and Frissell (2006) highlight the importance of temporal and spatial heterogenic groundwater/SW interactions for hyporheic assemblages. However, the above-said will mainly be true for impoverished groundwater zones common in older aquifers of weathered hydrogeological settings.

In conclusion, hydrological exchange (and related features such as depth of sediment, input and output of nutrients and organics) is one of the factors best describing faunal occurrence. This was expressed recently in the development of the groundwater-fauna-index (Hahn 2006) based on the relationship between the hydrological exchange and fauna. However, since this index also relies on relative amount of detritus in the sample, it cannot be applied for the current data.

Methodology and scales

It is astonishing that the increasing groundwater characteristics with increasing sediment depths as described by Hahn (2002) were not found at other sites in the present study of the same catchment. One possible explanation is that the sampling method by Hahn (2002) was based on stacked traps, while in the present study distinct tubes at a distance of about 40 cm from each other were used to sample disparate sediment depth layers. Thus, e.g. the stream well sampling the average depth layer at the Lambie site probably captured the zone of the most intense groundwater input into the stream, while the other two wells sampled zones not along this vertical gradient, but adjacent zones with lower groundwater flow.

Movement underground

Stream fauna has been found at several kilometres from the 8th order Flathead River (Stanford et al. 1994). Valett, Fisher, and Stanley (1990) showed that four times as much water flows underground than on the surface. Hill et al. (1998) found water in 80 cm depth to be characterized by stream water by more than 90% in the Brougham Creek, which had half as much flow as the Marbling Brook. They found the tracer to move up to 5 m laterally into the bank. The lateral extent of the riparian zone is controlled by riverine floods as well as by the interstitial flow patterns (Brunke & Gonser 1997).

Factors indicating hydrological exchange

With hydraulic connectivity having been low, one might argue that interstices were too small for fauna to live or even wander in the sediment. However, the sediment pores were large enough to contain fauna in Marbling Brook catchment, and fauna was found in all of the studied wells, even if in small numbers. Fauna was not necessarily small, in contrast to the study by Hakenkamp et al. (1994) who only found the smaller copepods in the sandy aquifer they had studied. Coineau (2000) relates sediment taxa to prevailing grain size. In Marbling Brook catchment, the amphipod *Protocrangonyx fontinalis* Nicholls, 1926 (size between 2.4 and 3.6 mm) was found in two wells; in the well WO this species was observed at almost every sampling occasion and in regularly high numbers, even after a pumping test during which the well volume had been extracted several times. The fact that numbers were not decreasing even after extracting high volumes means that the fauna sampled in the well WO was representative of the fauna in the surrounding aquifer and was not just living in the well itself. Dumas (2004) found faunal assemblages to be similar in wells which were pumped to different degrees, while Rouch, Pitzalis, and Descouens (1993) had found fauna at a pumped site to be lastingly depopulated. While grain size data are not available for the well WO, generally, grain sizes were small in the catchment (e.g. diamonds in Fig. 4), yet not related to faunal occurrences, as shown by BIO-ENV. In fact, the largest groundwater taxa, amphipods, were found in the well with the second smallest k_f of 6.2×10^{-6}, as well as in the well with the largest k_f of 5.3×10^{-4}. We believe that

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fauna have modified porosity in these aquifers by bioturbation (e.g. Nogaro et al. 2006).

Conclusion – recommendations for studies beneath sandy streams

Groundwater input into the stream influences spatial and temporal variation in the physical sediment habitat. However, in the sandy-bottomed system of Marbling Brook this influence was restricted to water exchange, while faunal assemblages were largely distinct between alluvial groundwater and sediment water. While the steep-gradient ecotone had been expected to occur in the upper layers of the stream sediments, in fact the gradients within the alluvial groundwater aquifer were more pronounced. This indicates an unpredicted complexity in the catchment with fundamentally different hydrogeological situations. Due to high unexpected lateral variability on the decimetre scale the sampling design with stream tubes at 20 cm distance did not capture the expected vertical variability in a representative way. However, unexpected variability also occurred within the alluvial aquifer on a smaller scale than the 10 m scale described in Hill et al. (2000). In systems such as Marbling Brook catchment it will be extremely difficult to determine the zones of intense interaction between groundwater and SW (Brunke & Gonser 1997). The interaction zones might be extremely narrow and variable on small scales, falling through the sampling grid applied in the present study. It follows that in systems like Marbling Brook it is not sensible to use disparate wells to sample different stream sediment layers. Instead, the use of stacked systems, as in Hahn (2002), is recommended. This would e.g. enable to single out vertical zones of preferred groundwater recharge to the stream. Nutrients and carbon resources also need to be sampled at a narrower spatial and temporal scale and including particulate as well as dissolved compartments. At every assumed exchange situation, several replicates have to be installed, for stream as well as for groundwater (Hakenkamp et al. 1994), and the groundwater wells need to sample different depths to capture aquifer stratification. It might be necessary to develop new fauna sampling techniques that consider the centimetre scale rather than the decimetre scale. However, such approaches will increase the problem of small sample size for fauna. One option could be sub-organism techniques from molecular biology (e.g. Read, Sheppard, Bruford, Glen, & Symondson 2006).

Acknowledgements

Ulrich-Neumann-Stiftung, Essen, and FAZIT-Stiftung, Frankfurt, funded S. Schmidt. We are indebted to all people at CSIRO, especially to Robert J. Woodbury, Gerald D. Watson, and Tuyen Pham. Jan Dunzweiler, University of Kaiserslautern, developed hydrological concepts. Stuart Halse, CALM, identified the ostracods and Peter Serov, UNE, identified the syncarids. Jane McRae, CALM, identified the calanoids and checked some identifications of cyclopoids, harpacticoids and amphipods. Discussions with Andrew Boulton, UNE Armidale, and Helmut Fischer, BfG Koblenz, as well as the anonymous reviews provided many thoughts that helped improving the manuscript.

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