CHAPTER 4

Interstitial activity in sand-bed hyporheic zones - the effect of substrate size

4.1 Introduction

The exchange of water between the stream and its sediment pore spaces is essential for the proper functioning of many hyporheic and parafluvial processes (Brunke and Gonser 1997, Boulton 2000b, Hancock 2002). For example, surface water downwelling into the hyporheic zone is oxygen rich, and contains the nutrients required by aerobic interstitial bacteria and invertebrates. In an active hyporheic zone, transient water quickly loses oxygen and undergoes microbially induced chemical changes (Holmes *et al.* 1998, Chapter 3). Much of this transformed water exits the hyporheic zone at upwelling areas of the stream, and is washed downstream. Upwelling water can be rich in nutrient forms, such as nitrates and soluble reactive phosphorus (Chapter 1), which are readily assimilated by in-stream biota. Therefore, hydraulic exchange has important implications for whole-stream ecology (Dent *et al.* 2000, Chapter 3), particularly nutrient dynamics (Martí *et al.* 1997).

4.1.1 Exchange in sand-bed streams

Hydraulic exchange rates between a stream and its hyporheic zone depend largely on the stream's geomorphological properties. Topographic relief, sediment porosity, grain size distribution, the degree of packing, and the shear stress of the bed all determine the rate at which water enters and exits the hyporheic zone (Brunke and Gonser 1997). These factors in turn are affected by sediment particle size. Commonly, surface-subsurface exchange in gravel bed rivers occurs as a result of hydraulic gradients, with water entering at downwelling zones and exiting at upwelling zones (Chapter 1). Fine sandy sediments have a lower shear stress than coarser sediments and are more easily scoured by flow (Valett *et al.* 1990). This means that the sandy river has a smoother bedform and consequently fewer pool-riffle sequences (Raine 2000, Hancock *et al.* 2001) to induce downwelling or upwelling. Therefore, sand stream beds often rely less on larger-scale hydraulic gradients to drive exchange than gravel beds, and are likely to be physically and chemically different (Figure 4.1).
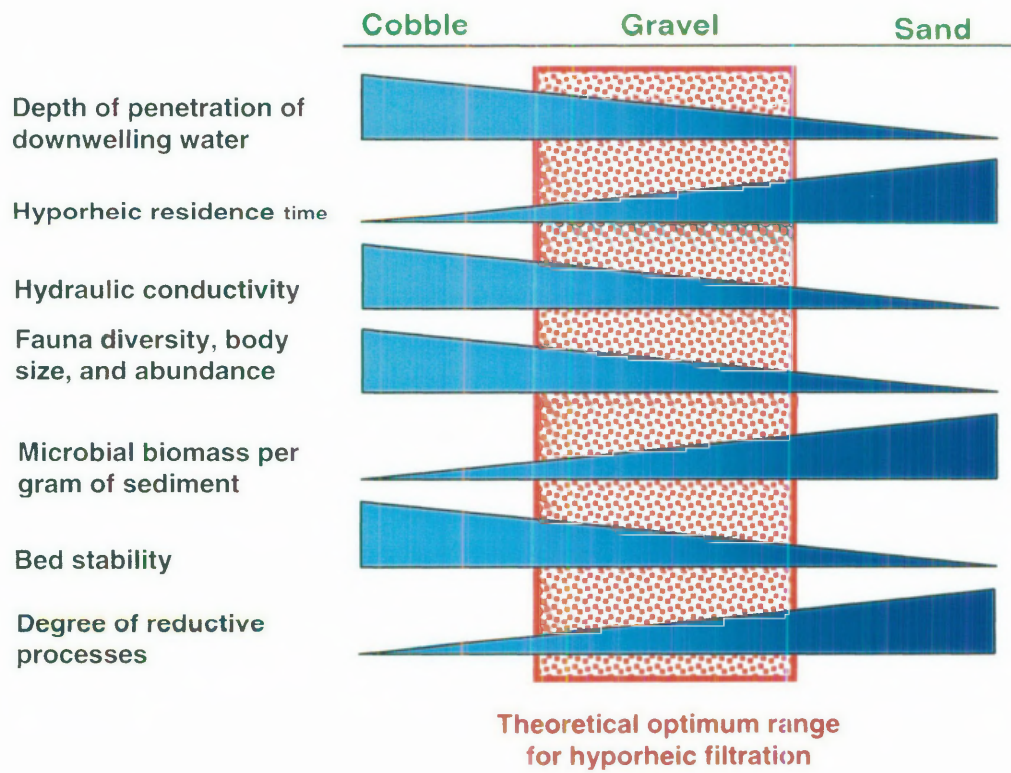
Figure 4.1. The predicted effect of different sediment size on some biological, hydrological, and physico-chemical parameters of the hyporheic zone (after Hancock *et al.* 2001).
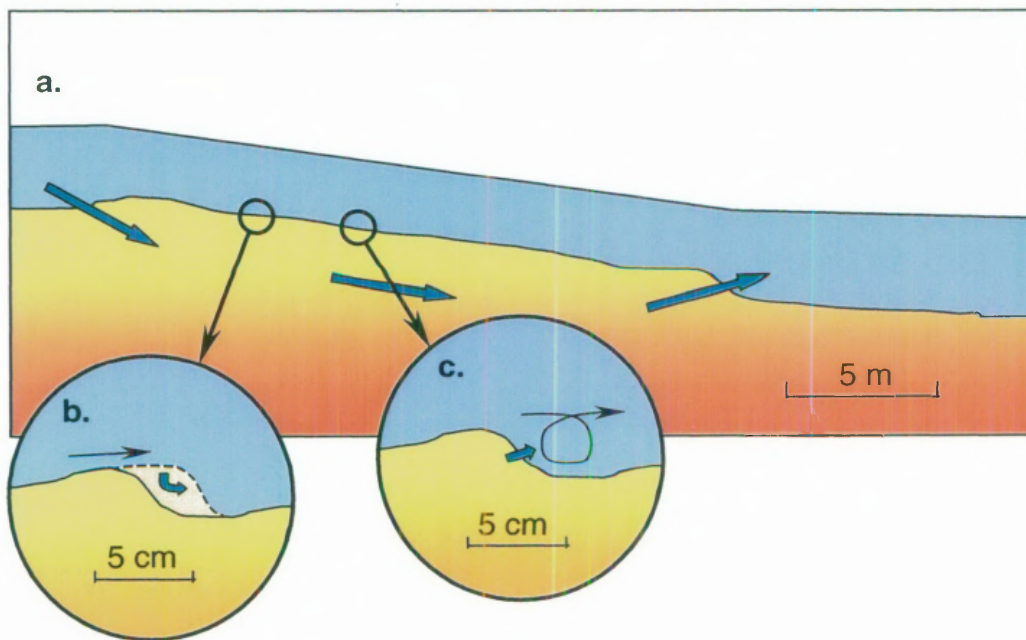

Figue 4.2. Three methods of hydrological exchange between a stream and its sand bed: a) exchange driven by hydraulic gradients, b) sediment turnover , and c) pumping. See text for details.

While this study only concentrates on medium-scale exchange (site scale) induced by hydraulic gradient, it is worth noting for completeness that there are two other mechanisms by which stream water exchanges with the sediments in a sand bed. Usually, both of these act on a relatively small scale and affect only the shallow sediments (Figure 4.2), but in floods or higher flows, they can affect deeper sediments. The first is called sediment turnover and results from the loose upper sediments being continuously picked up and deposited by flowing water, alternatively releasing and trapping pore-water (Figure 4.2b, Packman *et al.* 1997, Mutz and Rohde 2003). This method alternatively releases and traps pore-water and can have substantial consequences for some hyporheic processes. The advancing front of sediment sometime covers algae, woody debris, or leaves, which provides significant organic matter to the hyporheic zone. The migrating dune-like movement in the loose sediments of the River Spree, Germany, led to substantially higher bacterial activity there than in areas where the sediments were stationary (Fischer *et al.* 2003). The second method of hydraulic exchange is pumping. Spatial pressure variations are driven by local acceleration and seperartion of near-bed flow at protruding stream-bed structures (Thibodeaux and Boyle 1987, Mutz and Rohde 2003). Water is drawn out of the downstream end of sand ripples by these pressure gradients, which are caused by the turbulent flow at the downstream end of riffles (Figure 4.2c). The extent of pumping in natural streams is uncertain, since they experience much more complex flow patterns than are present in laboratory flumes (Mutz and Rohde 2003).

Influent groundwater may be a key factor in maintaining active exchange areas in the hyporheic zone (Chapter 3, Brunke 2002). As groundwater upwells into the hyporheic zone it displaces the water that is already there, causing it to circulate within the sediments. Eventually the groundwater will move upward into the 'active' part of the hyporheic zone and then into the stream. By exerting a pressure from below, the groundwater ensures that water in the deeper hyporheic sediments remains mobile, and eventually enters the stream (Figure 4.3a). However, unless occasional scouring occurs, groundwater that is rich in dissolved organic carbon or nutrients may stimulate microbial activity and hence lead to clogging at the interface of the frequent area of exchange and the area of less frequent exchange by bacterial biofilms. In the Ladberger Mühlenbach, a second order stream in Germany, a layer of sand, cemented
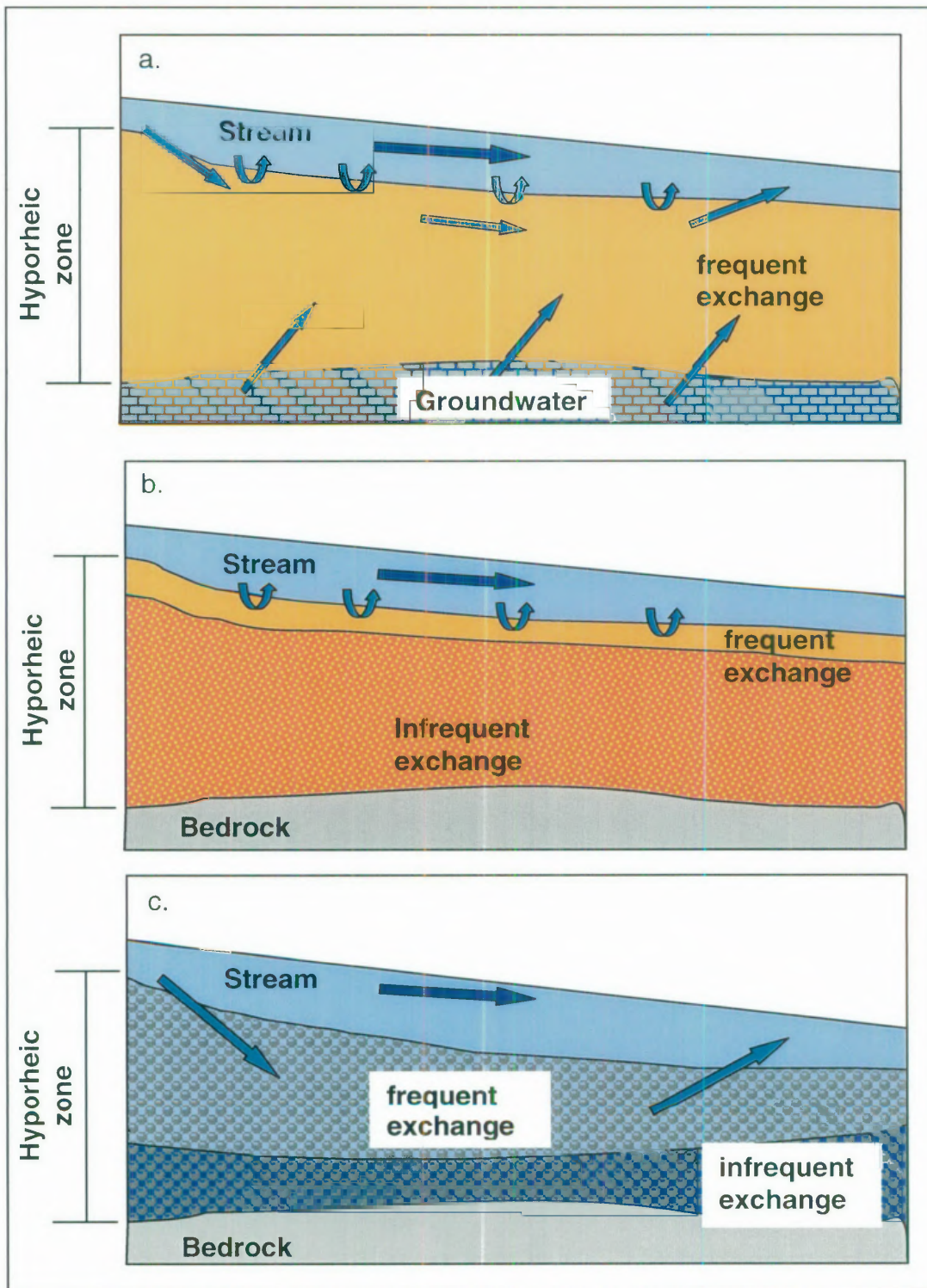
Figure 4.3. Vertical hydraulic exchange in three types of hyporheic zones. (a) In a sand bed with a strong groundwater influence, exchange with the surface stream will penetrate only a shallow distance but upwelling groundwater will prevent the storage of transformed water in deeper sediments. (b) Where groundwater upwelling has little or no influence on the hyporheic processes, or where it is nutrient-rich and stimulates biofilm clogging in the deeper sediments, the deeper sediments become a large storage area. (c) Exchange with the river in coarser sediments, such as gravel, is able to penetrate deeper, resulting in deeper active hyporheic areas.

by bacterial iron and manganese, limited the depth of the hyporheic zone and restricted exchange between surface and groundwater (Cleven and Meyer 2003).

Sand-bed hyporheic and parafluvial zones have other characteristics that can influence stream metabolism. Fast moving water is likely to scour sand, so flow through relatively immobile sand beds is generally slower than coarser ones. Additionally, because of pool-riffle sequences with lower topographical relief, vertical exchange is less. This can result in the interstices beneath the area of active exchange becoming potentially vast storage areas for highly concentrated nutrients such as ammonium and soluble reactive phosphorus (Figure 4.3b, Chaper 3, Boulton *et al.* 2002a). This restricts the volume of sediments occupied by aerobic bacteria, but leaves substantial areas for anaerobic bacteria. In contrast to this, sediments with good exchange, such as porous gravels, have a continuous flow to flush transformed nutrients (Figure 4.3c).

4.1.2 Nutrient properties of sand bed hyporheic zones

The hyporheic zones of sand-bed streams can significantly influence whole-stream metabolism. For example, hyporheic and parafluvial metabolism contribute considerably to the nutrient dynamics of Sycamore Creek, a desert stream in Arizona (Jones *et al.* 1995, Martí *et al.* 1997). Their large surface area renders sandy sediments with a greater sediment/water interface for microbial activity. The microbial biofilms of a sandy stretch of the Riera Major in Spain were 14 – 34 times more efficient in their utilisation of polysaccharidic compounds than those of a rocky area (Romaní and Sabater 2001). Sandy sediments in a German stream (Breitenbach) had twice the bacterial carbon production (BCP) of coarser (gravel and cobble) sediments per area of bed (Marxsen 2001). However, when expressed as a per volume rate, sand BCP was half that of coarser sediments (Marxsen 2001). This is probably due to a more rapid decline in bacterial activity with depth in sandy sediments, as observed by a reduction in esterase activity at two sites in the Austrian stretch of the Danube River (Battin and Sengschmitt 1999). Further, clogging from the growth of heterotrophic bacteria in the surface sediments steepened the gradient of decline (Battin and Sengschmitt 1999). However, surface sediments in stream beds are relatively mobile, and abrasion probably prevents microbially induced colmation. Below these areas exchange can be impaired, leading to rapid declines in oxygen within a few centimetres of the surface. In Rocky River, a temperate sandbed stream in New South Wales, Australia, dissolved oxygen fell to 40 % of surface concentration at 10 cm depth, while at 50 cm the

dissolved oxygen was only 10 % (Boulton *et al.* 2002a). Buzzards Branch, Virginia, had sediments that were anoxic below depths of 10 cm (Strommer and Smock 1989).

4.1.3 The sandy stream hyporheos

As with coarser sediments, the distribution of invertebrates in the hyporheic zones of sand-bed streams is limited by the physical and chemical attributes of the environment. Steep gradients in physico-chemical properties can lead to gradients in the interstitial invertebrate community. Invertebrate abundance in the upper 5 cm of Buzzard Branch, Virginia, was four times higher than that in the 5 – 15 cm section of bed, corresponding to a significant decline in DO (Strommer and Smock 1989). Insect larvae and cyclopoids dominated the shallow hyporheic community of Rock Creek, Arizona, while deeper sediments contained high numbers of bathynellaceans, isopods, and harpacticoids (Clinton *et al.* 1996).

One of the primary factors determining distribution of invertebrates in the interstitial environment is the size of the sediments comprising the interstitial matrix (Boulton *et al.* 2002b). Sediment size not only limits hydraulic exchange, thus affecting the chemical environment of the hyporheic zone (Section 4.1.2), it also determines the amount of space available for organisms to inhabit (Swan and Palmer 2000). The pore spaces of sand are usually smaller than those in coarser substrata, so space is potentially a limiting factor in sand streams. In sand and gravel sites of the Ozange and Triouzoune Rivers, France, invertebrate densities correlated with porosity only in less porous areas (Maridet *et al.* 1996). There is often a strong correlation between the size of interstitial organisms and that of the particles in the surrounding matrix (Coineau 2000). Many interstitial crustaceans in coarse, well-sorted sediments are up to 3 mm long, whereas those occurring in fine sands are less than 1 mm (Coineau 2000).

Disturbance frequency is an important determinant of hyporheic faunal distribution, (Boulton *et al.* 2002b) and often, deeper sediments are more buffered than shallow ones (Dole-Olivier *et al.* 1997, Coineau 2000). Sandy sediments are more mobile than gravel sediments, so are more easily moved by flowing water. Scouring resulting from a storm in Buzzards Branch dramatically reduced invertebrate density and biomass (Strommer and Smock 1989). In Sycamore Creek, floods reduced the abundance of invertebrates in the upper hyporheic zone, but populations often recovered within 5 days (Boulton and Stanley 1995). Similarly, the invertebrate community in the

118

hyporheic zone of the Rhône River, France, consistently recovered from spates within 7 days (Dole-Olivier *et al.* 1997).

4.1.4   The Hunter River basin

Within the Hunter River catchment, there is a variety of sediment sizes actively involved in fluvial processes. Upstream of its confluence with the Goulburn River, the bed of the Hunter consists mostly of cobbles and coarse gravels. However, downstream, significant quantities of sand exist, making the substrate of these areas a mixed sand/gravel matrix. This has caused a marked decrease in the number of pool-riffle sequences in the river (Raine 2000). The two main sources of sand are the Goulburn River and Wollombi Brook (Figure 2.3). Both of these rivers are of a low gradient and have extensive sand beds. The sands in the Goulburn River are coarser than those of Wollombi Brook, and contain more pebbles.

This range of sediment, from cobble, through sand/gravel mix to sand provides an opportunity to compare fine-grain hyporheic zones to coarse-grained ones within the same river system. Four hypotheses were tested in this study. First, in finer sediments, the decline of oxygen was hypothesised to be rapid with depth and distance from downwelling. This would affect nitrate and SRP, which were also predicted to decline as oxygen becomes scarce and reductive processes dominate (Hypothesis 2). The third hypothesis was that the fauna would be dominated by taxa that are easily able to traverse the fine sediments, such as those that burrow or are small and elongate. Taxonomic diversity, body size, and abundance will also probably decline with depth and distance. Finally, the fourth hypothesis was that the rate of change in the chemistry of interstitial water, and the changes in the community composition in the sediments, occur more rapidly and over a shorter distance in sandy sediments than in coarser ones.

4.2   Study sites

Two sand-bed tributaries of the Hunter River were selected for this study. On the Goulburn River, which joins the Hunter River downstream of Denman, a site was selected at Sandy Hollow (SAHO). A bar and riffle upstream of Cockfighters Bridge at Warkworth (WARK) were sampled on Wollombi Brook. This stream enters the Hunter between Maison Dieu and Dights Crossing. These two sites, representing sand-bed hyporheic zones, were compared to Aberdeen (ABER), Denman (DENM), Bowmans

Crossing (BOWM), and Moses Crossing (MOSE). Figures and a more complete description of these sites are given in Chapter 2.

Sites were classified into three main substrate types: coarse gravel/cobble (ABER, DENM), gravel in coarse sand (BOWM, MOSE), and sand (SAHO, WARK). By measuring the b-axis of 100 sediment particles at 50 cm intervals, an average sediment size was obtained for each site at each time. This was done for all sites except WARK, where Erskine (1996) measured the average sediment particle size as 0.24 mm. At ABER and DENM, average particle size ranged between 35.94 and 46.32 mm, and at BOWM and MOSE these were between 32.64 and 33.88. Substrates at SAHO were coarser (3.44 ± 1.32 mm, mean ± SE) than those at WARK (0.24 mm − Erskine 1996).

4.3 Methods

4.3.1 Field sampling

For the non-sandy streams, data collected for May, August, and November 2000 (Chapter 3) were used. Triplicate samples were collected from 40 and 80 cm in the downwelling habitat at each riffle, and from 40 cm at the upwelling zone. Samples were taken from -1, 0, 1, 5, and 10 m along the flow path in the bar from 40 and 80 cm depths (Chapter 3). Duplicate surface water samples were also collected. The methods used for collecting physico-chemical, nutrient, and invertebrate samples were the same as those outlined in Section 3.3.1. However, because of the fine sand at WARK, water was extracted with a syringe through narrow rubber tubing from a mini piezometer. No faunal samples could be collected from this site because it was prohibitively slow to collect 6 L of water (e.g., > 2 h per sample). High water levels prevented the collection of upwelling samples from MOSE in August, and the 10 m location at WARK during November. In August, samples collected from -1 m at BOWM, −1, and 1 m at MOSE were contaminated, so are not included in this analysis.

4.3.2 Laboratory analysis

Laboratory analysis follows that outlined in Section 3.3.2.

4.3.3 Statistical analysis

Three different (see below) analysis of variance (ANOVA) models were used to compare mean temperature, conductivity, pH, DO, NOx, and SRP among sites of different substrate size. Prior to analysis the data were tested for normality using a Wilk-Shapiro test. Where required, non-normal data were transformed. Further transformations and re-analysis were done if *post hoc* residual and box-plots indicated that the data were heteroscedastic. Tukey's tests were used to assess the significance of pair-wise interactions. Pearson correlations were used to test for significant relationships among variables. Wilk-Shapiro and Pearson tests were done using Statistix, version 7.0 (2000, Analytical Software, Florida), while ANOVAs, Tukey's, and the post-hoc variance tests were done with SYSTAT, version 9.01 (1998, SPSS Incorporated, Illinois).

Mean surface physico-chemical and nutrient data were compared among sites of different substrate types using a three-factor mixed model ANOVA (Quinn and Keough 2002). As the main factor of Substrate and its interaction with Habitat/ Distance were major points of interest in testing the first hypothesis, Time was set as a fixed factor with three levels. Each site was classified by its dominant substrate, with ABER and DENM representing coarse gravel/cobble streams, BOWM and MOSE representing coarse sand with coarse gravel, and SAHO and WARK representing a sandy substrate. Substrate type, G, was treated as a fixed factor with 3 levels, while site nested within substrate, S(G), had two levels and was random. The expected mean square (EMS) of the T*S(G) interaction was used as the denominator to calculate the F-ratio for the T main effect and the T*G interaction. For the main effect of G, S(G) was used as the denominator. All other factors were tested over the error EMS (Quinn and Keough 2002).

Four-factor mixed ANOVA was used to compare means and test the riffle data for similarities among substrate types and spatial differences. The model for this included the three factors used for the surface analyses as well as a Habitat factor (H). This had three levels – downwelling at 40 cm, downwelling at 80 cm, and upwelling at 40 cm – and was fixed. For the sources of error present in this model that were also in the model for surface data, the EMSs used to calculate the F-ratio were the same. Additional F-ratios were calculated with H*S(G) as the denominator for H and G*H,

and T*H*S(G) as the denominator for T*H and T*H*G. All factors that contained the S(G) term were tested over the EMS of the error.

A similar four-factor mixed ANOVA model was used to test for differences along the bar, between depths, and among times in pH, conductivity, dissolved oxygen, temperature, NOx, and SRP. For these analyses Habitat was replaced with Distance (D), a fixed factor with 5 levels. To determine any differences that occurred with depth, two-factor (Time, Depth) crossed Analyses of Covariance (ANCOVA), with Depth as the covariate, were conducted at each site. Vertical differences for the non-sandy streams are discussed in Chapter 3. To test for gradients in each variable along the bar, both depths (40 and 80 cm) were treated as duplicate samples for the analysis.

Faunal communities from SAHO were compared with one site upstream of the confluence of the Goulburn River with the Hunter River (DENM), and one site downstream (BOWM). Separate analyses were done for each time to determine if spatially-proximate community assemblages were similar to each other in composition. A Bray-Curtis similarity matrix was calculated using log (x+1) data. Non-metric multi-dimensional scaling (MDS), followed by analysis of similarities (ANOSIM, Clarke and Warwick 2001), were used to establish whether differences between sites occurred. Two separate 2-way ANOSIMs with 10 000 permutations were conducted on Site x Habitat, and Site x Depth interactions. These analyses were followed by similarity percentage (SIMPER) analysis of log (x+1) data to determine which taxa characterised the community of each site.

4.4 Results

4.4.1 Physico-chemical variables

At the coarse-bed sites and at SAHO, surface pH ranged between 8.05 ± 0.01 and 8.75 ± 0.06 (mean ± SE, Figure 4.4). At WARK pH was between 6.60 ± 0.10 and 7.30 ± 0.03, contributing to a difference between surface pH among sites within each substrate classification (P < 0.001, Table 4.1).

In the hyporheic zone below the riffle, sites differed among each other within substrate class at each time (P < 0.001, Table 4.1, Figure 4.4). The pH at WARK in August and November were higher than any other of the sites (pH > 100 % surface in most cases). The pH of the downwelling habitat at ABER varied less than 60 % of surface

concentrations for all three times, contributing to the significant T*H*S(G) interaction (P = 0.011, Table 4.1).

Interactions for the bar matched those of the riffle. Within substrates, pH of sites differed with time (P < 0.001, Table 4.1, Figure 4.4). The biggest difference here was between the two sandy sites, with pH at WARK being higher than that at SAHO (Figure 4.4), and this was most noticeable in August. In contrast with the expectations of hypothesis 2, there was no difference in pH with distance along the bar, indicating that pH is not significantly influenced by interstitial residence time.

Surface water temperature was highest in November (19.6 ± 1.6 to 27.5 ± 0.5 °C, Figure 4.5), especially at SAHO (27.5 ± 0.5 °C, P = 0.028, Table 4.2). The temperatures of sites within substrate groupings differed from each other (P < 0.001, Table 4.2). All interactions for riffle samples containing the S(G) term were significant, since sites within each substrate type differed in their temperatures (Table 4.2). For the cobble/ coarse gravel sites, the temperature of interstitial water at DENM was generally more similar to surface than that at ABER (Figure 4.5, P < 0.001). This was particularly the case for the downwelling habitat at 80 cm, where interstitial water temperature was consistently at least 44 % less than of the surface water temperature (Figure 4.5). Temperature beneath the riffle at SAHO was between 90 and 100 % that of the surface water in May and August, but was less than 83 % for all habitats at this site in November. WARK hyporheic temperatures were consistently higher than surface temperatures for all habitats and times (Figure 4.5).

Table 4.1. Mixed-model ANOVA results table for pH in the surface, hyporheic (riffle), and parafluvial (bar) habitats. T = Time, G = Substrate, S(G) = Site nested within Substrate, H = Habitat, D = Distance. Bold numbers are significant at P = 0.05

Source	SS	df	MS	F-ratio	P
Surface - rank ph					
T	512.542	2	256.271	1.570	0.283
G	546.000	2	273.000	0.593	0.607
T*G	259.583	4	64.896	0.398	0.804
S(G)	1381.667	3	460.556	40.989	**0.000**
T*S(G)	979.458	6	163.243	14.528	**0.000**
Error	202.250	18	11.236		
Riffle - rank pH					
T	7947.926	2	3973.963	0.539	0.609
G	58385.949	2	29192.975	1.469	0.359
H	12646.713	2	6323.356	11.740	0.008
T*G	29486.537	4	7371.634	1.000	0.475
T*H	4493.926	3	1497.975	1.673	0.225
G*H	2418.423	4	604.606	1.123	0.427
T*H*G	16003.500	8	2000.437	2.234	0.101
S(G)	59630.690	3	19876.897	51.474	**0.000**
T*S(G)	44211.003	6	7368.500	19.082	**0.000**
H*S(G)	3231.600	6	538.600	1.395	0.223
T*H*S(G)	10743.866	12	895.322	2.319	**0.011**
Error	40932.333	106	386.154		
Bar - untransformed pH					
T	2.437	2	1.219	0.012	0.988
G	353.693	2	176.846	0.854	0.509
D	79.158	4	19.790	2.686	0.088
T*G	48.494	4	12.123	0.123	0.969
T*D	64.679	8	8.085	0.734	0.661
G*D	103.859	7	14.837	2.014	0.144
T*D*G	149.674	16	9.355	0.849	0.626
S(G)	621.193	3	207.064	38.607	**0.000**
T*S(G)	590.435	6	98.406	18.348	**0.000**
D*S(G)	81.034	11	7.367	1.374	0.200
T*D*S(G)	242.293	22	11.013	2.053	**0.010**
Error	461.250	86	5.363		

May

August

November

Riffle habitat

Distance along bar

40 cm

80 cm
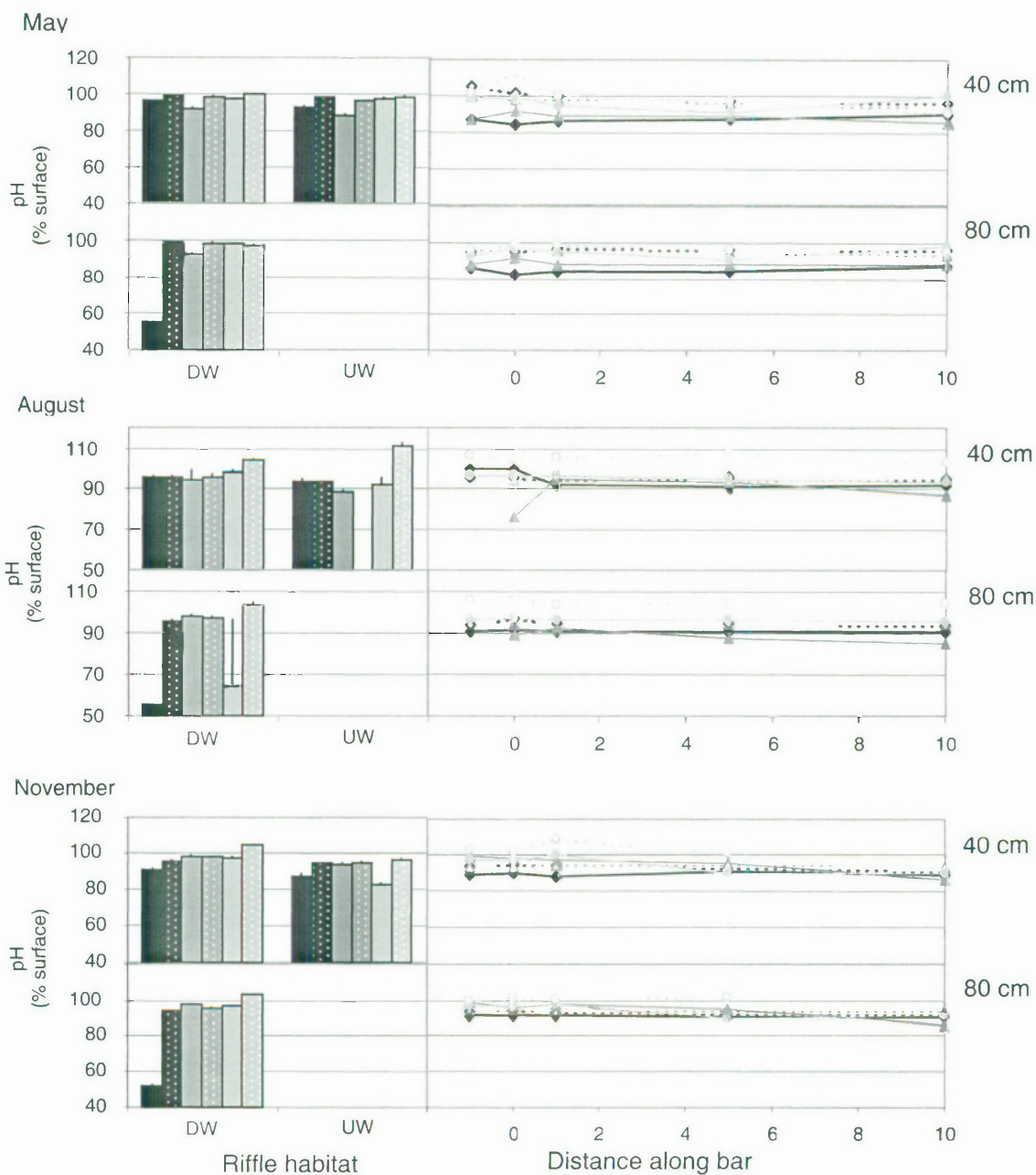
Surface pH	ABER ■ ─◆─	DENM ▦ ··◇··	BOWM ▨ ─▲─	MOSE ▨ ··△··	SAHO □ ─●─	WARK □ ··□··
May	8.12 ± 0.02	8.05 ± 0.00	8.67 ± 0.02	8.15 ± 0.15	8.06 ± 0.06	7.30 ± 0.03
August	8.28 ± 0.06	8.50 ± 0.01	8.30 ± 0.05	8.41 ± 0.14	8.62 ± 0.00	6.60 ± 0.10
November	8.75 ± 0.06	8.24 ± 0.05	8.55 ± 0.06	8.54 ± 0.00	8.62 ± 0.03	7.00 ± 0.34

Figure 4.4. Hyporheic (bar graphs + standard error, on left) and parafluvial (line graphs) pH for six sites and three times. Black represents the coarser cobble sites, dark-grey represents mixed substrate sites, and light-grey represents sandy sites. Mean (± SE) surface values are given in the table below.

In the parafluvial zone beneath the bars, temperature for sites in substrate groupings differed from each other with time (P < 0.001, Table 4.2) and with distance (P = 0.046, Table 4.2). The difference in temperature among the two sand sites, and among the cobble sites were probably the greatest contributors to these within-substrate differences, as BOWM and MOSE were relatively similar to each other at most times except May (Figure 4.5). Temperature increased with distance along the bar for the coarse sand/gravel sites, and the coarse gravel/cobble sites (Figure 4.5). This was also the case for WARK in May, which otherwise, like SAHO, displayed little thermal change with distance along the flowpath.
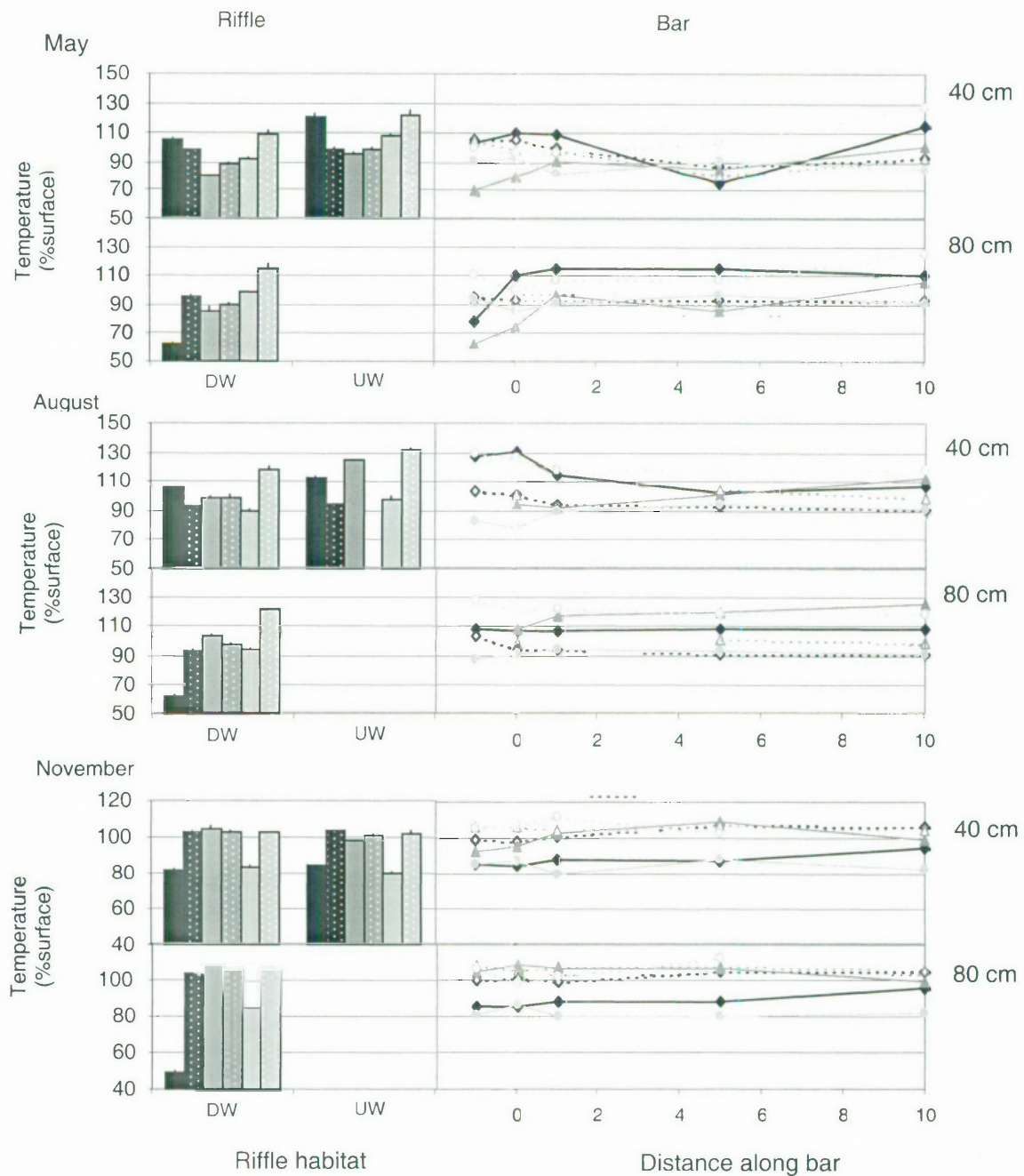
Surface electrical conductivity (EC) was higher at the sand sites than for other sites with levels as high as 3.46 ± 0.07 mS/cm (P = 0.007, Table 4.3, Figure 4.6). Other sites had EC between 0.17 ± 0.01 and 0.91 ± 0.01 mS/cm. Conductivity in November was higher than in May and August at all sites (P < 0.001, Table 4.3, Figure 4.6).

At the upwelling zones in May and November, the difference between the EC at WARK and SAHO was at least 200 % of surface water (P < 0.001, Table 4.3). Upwelling habitats had higher EC than both of the downwelling habitats (P = 0.011, Table 4.3, Figure 4.6), possibly indicating that there were inputs of groundwater into the system. Among cobble/gravel sites, EC at the deep downwelling site was less than the same habitat at DENM. This, and the difference between WARK and SAHO, contributed to the significant S(G) main factor (P < 0.001, Table 4.3, Figure 4.6), indicating that there were differences in EC for the different substrate classes. The difference between conductivity at WARK and SAHO was greatest in November, while that between ABER and DENM was greatest in May (P < 0.001, Table 4.3, Figure 4.6).

Surface DO concentrations were low at WARK during August (39.00 ± 2.00 % saturation) and November (50.25 ± 1.25 % saturation), contributing to the significant T*S(G) interaction (P < 0.001, Table 4.4, Figure 4.7). The DO of the two sandy sites were also different to each other in May, although at this time the difference was due to supersaturated surface DO at WARK (132.75 ± 0.49 % saturation) rather than low interstitial concentrations. DO at WARK was the only variable that differed with depth at the sandy sites ($F_{1,21} = 10.13$, P = 0.005).

Table 4.2. Mixed-model ANOVA results table for temperature in the surface, hyporheic (riffle), and parafluvial (bar) habitats. T = Time, G = Substrate, S(G) = Site nested within Substrate, H = Habitat, D = Distance. Bold numbers are significant at P = 0.05.

Source	SS	df	MS	F-ratio	P
Surface - log (x + 1) temperature					
T	0.323	2	0.162	101.632	**0.000**
G	0.003	2	0.001	0.106	0.903
T*G	0.009	4	0.002	1.436	0.329
S(G)	0.036	3	0.012	23.346	**0.000**
T*S(G)	0.010	6	0.002	3.125	**0.028**
Error	0.009	18	0.001		
Riffle - untransformed temperature					
T	757.524	2	378.762	0.549	0.604
G	410.510	2	205.255	0.091	0.915
H	419.009	2	209.504	5.610	**0.042**
T*G	4490.937	4	1122.734	1.628	0.282
T*H	940.425	4	235.106	12.185	**0.000**
G*H	60.320	3	20.107	0.538	0.673
T*H*G	167.515	8	20.939	1.085	0.433
S(G)	6755.207	3	2251.736	415.783	**0.000**
T*S(G)	4138.308	6	689.718	127.356	**0.000**
H*S(G)	224.081	6	37.347	6.896	**0.000**
T*H*S(G)	231.529	12	19.294	3.563	**0.000**
Error	574.059	106	5.416		
Bar - untransformed temperature					
T	1485.712	2	742.856	1.475	0.301
G	4.121	2	2.061	0.006	0.994
D	66.453	4	16.613	0.222	0.921
T*G	2029.400	3	676.467	1.343	0.346
T*D	531.741	7	75.963	1.188	0.348
G*D	512.761	8	64.095	0.857	0.574
T*D*G	957.767	15	63.851	0.998	0.488
S(G)	1097.425	3	365.808	9.257	**0.000**
T*S(G)	3022.004	6	503.667	12.746	**0.000**
D*S(G)	897.201	12	74.767	1.892	**0.046**
T*D*S(G)	1470.872	23	63.951	1.618	0.058
Error	3398.326	86	39.515		

Figure 4.5. Hyporheic (bar graphs + standard error, on left) and parafluvial (line graphs) temperature for six sites and three times. Black represents the coarser cobble sites, dark-grey represents mixed substrate sites, and light-grey represents sandy sites. Mean (± SE) surface values are given in the table below.

Surface Temperature (°C)	ABER	DENM	BOWM	MOSE	SAHO	WARK
May	14.0 ± 0.0	16.0 ± 0.0	17.7 ± 1.50	17.3 ± 0.8	16.5 ± 0.5	14.0 ± 0.0
August	12.2 ± 0.7	13.8 ± 0.0	12.0 ± 1.0	13.4 ± 0.2	14.3 ± 0.1	11.0 ± 0.1
November	22.6 ± 0.0	20.7 ± 0.2	21.4 ± 0.1	22.8 ± 0.3	27.5 ± 0.4	19.6 ± 1.6

Table 4.3. Mixed-model ANOVA results table for conductivity in the surface, hyporheic (riffle), and parafluvial (bar) habitats. T = Time, G = Substrate, S(G) = Site nested within Substrate, H = Habitat, D = Distance. Bold numbers are significant at P = 0.05

Source	SS	df	MS	F-ratio	P
Surface - rank conductivity					
T	2542.042	2	1271.021	1271.021	**0.000**
G	811.792	2	405.896	39.546	**0.007**
T*G	128.167	4	32.042	1.404	0.338
S(G)	30.792	3	10.264	0.795	0.512
T*S(G)	136.958	6	22.826	1.769	0.162
Error	232.250	18	12.903		
Riffle - rank conductivity					
T	38647.704	2	19323.852	3.704	0.090
G	30805.333	2	15402.667	1.909	0.292
H	17456.824	2	8728.412	10.446	**0.011**
T*G	18531.407	3	6177.136	1.184	0.392
T*H	2155.822	4	538.956	0.135	0.966
G*H	6083.720	4	1520.930	1.820	0.244
T*H*G	5090.046	8	636.256	0.159	0.993
S(G)	24209.852	3	8069.951	10.898	**0.000**
T*S(G)	31305.615	6	5217.602	7.046	**0.000**
H*S(G)	5013.580	6	835.597	1.128	0.351
T*H*S(G)	47937.48.	12	3994.790	5.395	**0.000**
Error	78489.333	106	740.465		
Bar - untransformed conductivity					
T	2320.065	2	1160.032	0.339	0.725
G	5014.692	2	2507.346	1.179	0.419
D	456.701	3	152.234	0.326	0.807
T*G	11332.727	4	2833.182	0.828	0.553
T*D	2613.857	8	326.732	0.940	0.505
G*D	3024.731	8	378.091	0.810	0.607
T*D*G	4280.346	15	285.356	0.821	0.647
S(G)	6381.971	3	2127.324	6.543	**0.000**
T*S(G)	20532.337	6	3422.056	10.526	**0.000**
D*S(G)	5602.152	12	466.846	1.436	0.166
T*D*S(G)	7647.427	22	347.610	1.069	0.395
Error	27960.209	86	325.119		

Figure 4.6. Hyporheic (bar graphs + standard error, on left) and parafluvial (line graphs) conductivity for six sites and three times. Black represents the coarser cobble sites, dark-grey represents mixed substrate sites, and light-grey represents sandy sites. Mean (± SE) surface values are given in the table below.
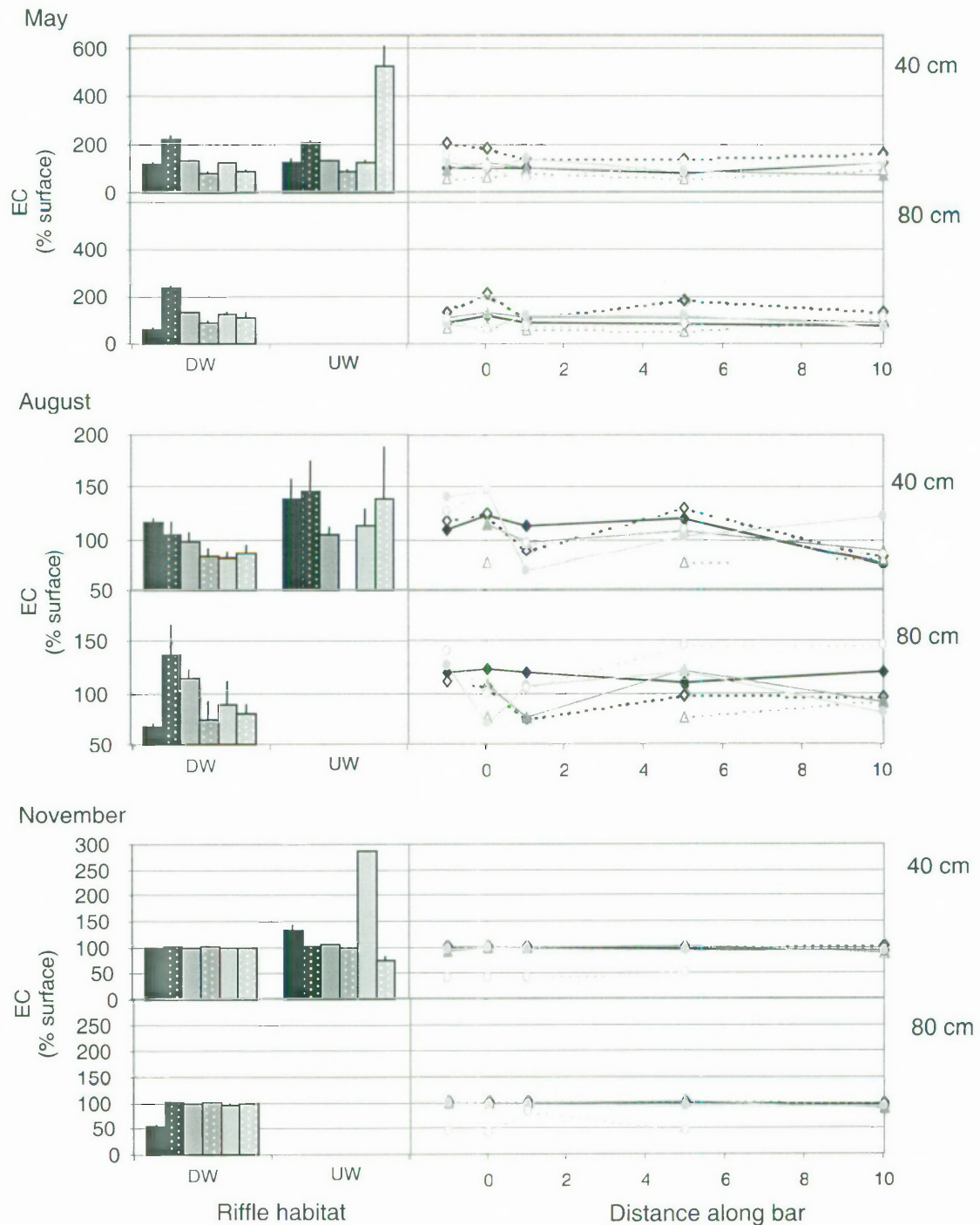
Surface EC	ABER ■	DENM ▣	BOWM ▣	MOSE ▣	SAHO ▢	WARK ▢
	──◆──	···◇···	──▲──	···△···	···	···□···
May	0.30 ± 0.02	0.17 ± 0.01	0.43 ± 0.03	0.44 ± 0.02	0.40 ± 0.05	0.34 ± 0.06
August	0.29 ± 0.05	0.27 ± 0.06	0.47 ± 0.12	0.47 ± 0.01	0.40 ± 0.03	0.50 ± 0.02
November	0.52 ± 0.01	0.65 ± 0.01	0.86 ± 0.00	0.91 ± 0.01	1.02 ± 0.02	3.46 ± 0.07

Table 4.4. Mixed-model ANOVA results table for dissolved oxygen in the surface, hyporheic (riffle), and parafluvial (bar) habitats. T = Time, G = Substrate, S(G) = Site nested within Substrate, H = Habitat, D = Distance. Bold numbers are significant at P = 0.05.

Source	SS	df	MS	F-ratio	P
Surface - square root DO					
T	20.391	2	10.195	2.333	0.178
G	7.840	2	3.920	1.023	0.458
T*G	1.942	4	0.485	0.110	0.974
S(G)	11.490	3	3.830	29.996	**0.000**
T*S(G)	26.225	6	4.371	34.233	**0.000**
Error	2.298	18	0.128		
Riffle - untransformed DO					
T	4192.014	2	2096.007	7.404	**0.024**
G	1806.670	2	903.335	0.282	0.772
H	4274.124	2	2137.062	1.762	0.250
T*G	598.012	4	149.503	0.528	0.721
T*H	62.992	4	15.748	0.127	0.970
G*H	1291.334	3	430.445	0.355	0.788
T*H*G	3289.136	8	411.142	3.316	**0.030**
S(G)	9615.067	3	3205.022	25.402	**0.000**
T*S(G)	1698.438	6	283.073	2.244	**0.045**
H*S(G)	7276.435	6	1212.739	9.612	**0.000**
T*H*S(G)	1488.067	12	124.006	0.983	0.470
Error	13374.035	106			
Bar - untransformed DO					
T	2370.773	2	1185.386	0.376	0.704
G	362.767	2	181.384	0.023	0.977
D	14795.871	4	3698.968	12.211	**0.000**
T*G	3046.674	4	761.669	0.242	0.903
T*D	2496.011	8	312.001	0.913	0.523
G*D	3038.131	6	506.355	1.672	0.211
T*D*G	7496.785	15	499.786	1.463	0.197
S(G)	23599.781	3	7866.594	25.853	**0.000**
T*S(G)	15753.939	5	3150.788	10.355	**0.000**
D*S(G)	3634.916	12	302.910	0.995	0.460
T*D*S(G)	8201.228	24	341.718	1.123	0.338
Error	26168.302	86	304.283		

Figure 4.7. Hyporheic (bar graphs + standard error, on left) and parafluvial (line graphs) dissolved oxygen for six sites and three times. Black represents the coarser cobble sites, dark-grey represents mixed substrate sites, and light-grey represents sandy sites. Mean (± SE) surface values are given in the table below.
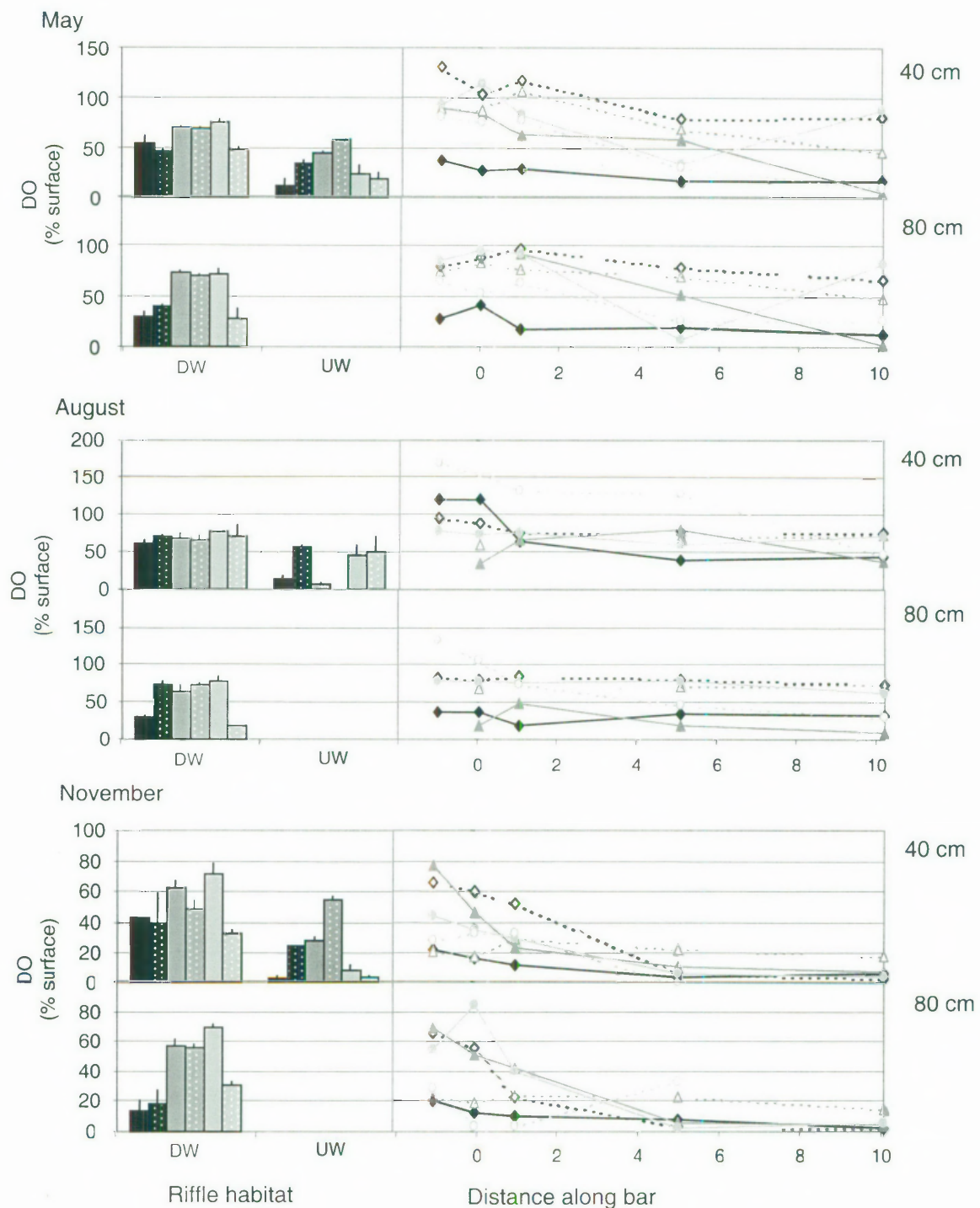
Surface DO (mg/L)	ABER ■	DENM ⬚	BOWM ▢	MOSE ▢	SAHO ▢	WARK ▢
May	93.03 ± 3.96	159.65 ± 4.09	111.74 ± 14.52	114.63 ± 11.24	92.06 ± 0.14	132.75 ± 0.49
August	88.95 ± 3.25	103.70 ± 2.40	88.10 ± 2.1	100.72 ± 4.88	107.70 ± 2.80	39.00 ± 2.00
November	97.90 ± 1.70	88.85 ± 1.25	85.00 ± 3.50	85.55 ± 4.05	93.85 ± 5.06	50.25 ± 1.25

Deep downwelling riffle habitats had less oxygen at ABER than at DENM. WARK consistently had less oxygen than SAHO at both downwelling depths (P < 0.001, Table 4.4, Figure 4.7). There was, at all times, a decline in DO with depth at the cobble/gravel sites, and at the sandy sites (Figure 4.7). Downwelling water at SAHO had higher DO concentrations than downwelling water at other sites (Figure 4.7). The differences among sites of similar substrates were greatest in November (P = 0.045, Table 4.4, Figure 4.7). In the hyporheic zone, DO concentrations did not differ significantly among substrates (P = 0.772, Table 4.4).

Overall, DO concentration decreased with distance along the bar (P < 0.001, Table 4.4). With the exception of SAHO in May, where DO at 10 m was higher than at 5 m, and the deep parafluvial zone at WARK in November (Figure 4.7), DO patterns in all other sites supported the expectations of hypothesis one. The difference among sandy sites was greatest for the shallow sediments during August. There was also some longitudinal disparity in DO among cobble/gravel sites at both depths in May and November (P < 0.001, Table 4.4, Figure 4.7). There was no difference between the DO of sandy parafluvial habitats and the DO of bars with coarser substrate (P = 0.977, Table 4.4). The rate of DO consumption in sandy sediments does not appear to to be significantly greater than that of coarser sediments, contrasting with the expectations of hypothesis 2.

4.4.2 Nutrient patterns

WARK had the lowest surface SRP concentrations of all sites (0.004 ± 0.001 to 0.015 ± 0.001 mg/L), and the difference between this site and SAHO (average 0.053 ± 0.005 mg/L) contributed to the variability among sites within substrate class (P < 0.001, Table 4.5, Figure 4.8). This dissimilarity was particularly marked in May (P = 0.001, Table 4.5, Figure 4.8). Over all sites, the highest SRP concentrations occurred in May (P = 0.018, Table 4.5). Interstitial SRP concentrations at sandy sites generally did not differ to those of other sites, contradicting the expectations of my second hypothesis (Section 4.1.4).

For each time, one site within each substrate type had the highest SRP concentration (P < 0.001, Table 4.5, Figure 4.8). Specifically, in May, DENM SRP concentration was more than 250 % of the surface concentration (Figure 4.8). SRP was also high in the ABER riffle sediments. BOWM had the highest SRP in the hyporheic zone during

August, where concentrations ranged between 330 and 1382 % of surface, indicating that the sediments are significant areas of SRP storage or transformation. In November, SRP concentrations in the deep downwelling and upwelling habitats at WARK were higher than in the other sites. Although there was a difference in SRP concentrations among sites of similar substrate with habitat and time (P = 0.006, Table 4.5), there was no consistent pattern (Figure 4.8). In the riffle, SRP did not correlate with DO (r_{158} = -0.010, P = 0.904).
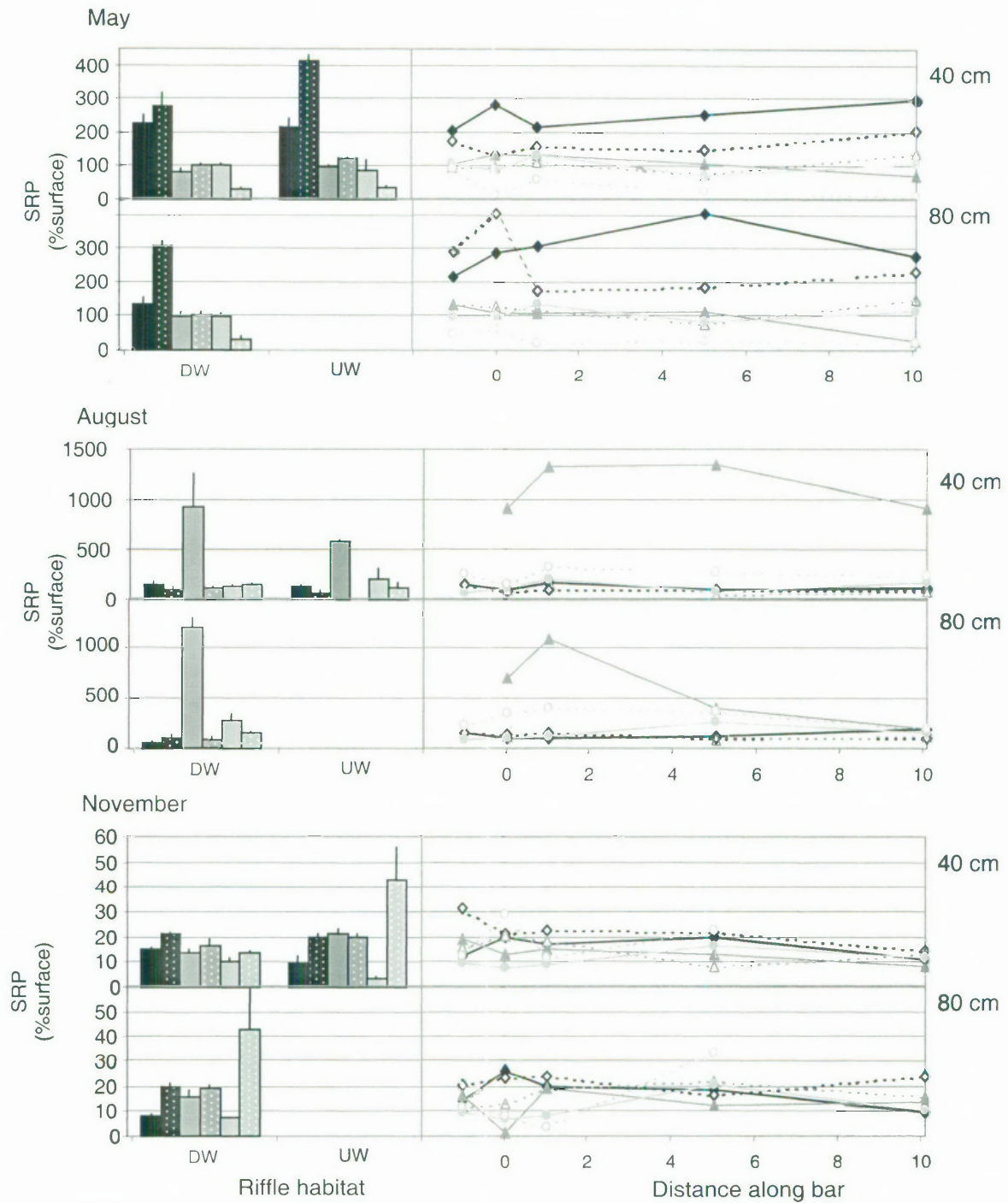
August SRP concentrations in the bar at BOWM averaged 860.68 ± 148.29 % surface, while SRP at MOSE averaged 92.48 ± 12.13 % surface (Figure 4.8). The magnitude of difference between these two coarse sand/gravel sites, and the difference among gravel/cobble sites in May changed with time (P < 0.001, Table 4.5, Figure 4.8). SRP concentrations of sites within substrate classes generally became more similar with distance along the bar (P = 0.042, Table 4.5, Figure 4.8). SRP concentrations in each substrate class changed with distance along the bar with each time (P = 0.007, Table 4.5). In the first 3 locations along the bar, concentrations in May were highest in the cobble/gravel sites, then in the coarse sand/gravel sites, and the sandy sites. In August, the mixed coarse sand/gravel sites had higher SRP in the bar, followed by the sandy sites, and gravel/cobble sites (Figure 4.8). SRP concentrations in the bar did not correlate with DO (r_{170} = 0.019, P = 0.808) but did with pH (r_{170} = -0.180, P = 0.018).

Surface NOx concentrations at the sand sites averaged 0.025 ± 0.010 mg/L, while that of the other sites averaged 0.075 ± 0.011 mg/L (Figure 4.9). Sites within each substrate class differed from each other (P = 0.001, Table 4.6). The magnitude of this difference changed with time (P < 0.001, Table 4.6) and was greatest among the coarse sand/gravel sites in August (Figure 4.9).

High NOx concentrations characterised the WARK riffle habitats in May and November, with the upwelling habitat in May being between 4300 and 6500 % of surface concentrations (Figure 4.9). Overall, variability among sites of similar substrates was highest for the sandy sites (P < 0.001, Table 4.6, Figure 4.9). Variability within the coarse sand/gravel sites was high in August and May at the downwelling habitats (P < 0.001, Table 4.6, Figure 4.9). There was no correlation of riffle NOx with DO (r_{158} = 0.076, P = 0.342).

Table 4.5. Mixed-model ANOVA results table for soluble reactive phosphorus in the surface, hyporheic (riffle), and parafluvial (bar) habitats. T = Time, G = Substrate, S(G) = Site nested within Substrate, H = Habitat, D = Distance. Bold numbers are significant at P = 0.05

Source	SS	df	MS	F-ratio	P
Surface - fourth root SRP					
T	0.056	2	0.028	8.389	**0.018**
G	0.011	2	0.005	0.174	0.848
T*G	0.062	4	0.015	4.668	**0.047**
S(G)	0.093	3	0.031	61.763	**0.000**
T*S(G)	0.020	6	0.003	6.635	**0.001**
Error	0.009	18	0.001		
Riffle - Log(x+1) SRP					
T	0.552	2	0.276	0.452	0.657
G	1.416	2	0.708	9.381	0.051
H	0.046	2	0.023	0.548	0.605
T*G	3.762	4	0.941	1.540	0.303
T*H	0.041	4	0.010	0.122	0.972
G*H	0.112	4	0.028	0.670	0.636
T*H*G	0.131	8	0.016	0.196	0.986
S(G)	0.226	3	0.075	2.337	0.078
T*S(G)	3.665	6	0.611	18.916	**0.000**
H*S(G)	0.250	6	0.042	1.291	0.268
T*H*S(G)	0.919	11	0.084	2.588	**0.006**
Error	3.423	106	0.032		
Bar - Log(x+1) SRP					
T	0.834	2	0.417	0.943	0.440
G	0.785	2	0.392	1.841	0.352
D	0.025	4	0.006	0.145	0.962
T*G	3.241	4	0.810	1.832	0.242
T*D	0.202	7	0.029	1.794	0.137
G*D	0.200	8	0.025	0.574	0.781
T*D*G	0.753	15	0.050	3.119	**0.007**
S(G)	0.426	2	0.213	9.417	**0.000**
T*S(G)	2.653	6	0.442	19.536	**0.000**
D*S(G)	0.524	12	0.044	1.928	**0.042**
T*D*S(G)	0.370	23	0.016	0.711	0.822
Error	1.924	85	0.023		

Figure 4.8. Hyporheic (bar graphs + standard error, on left) and parafluvial (line graphs) soluble reactive phosphorus concentration for six sites and three times. Black represents the coarser cobble sites, dark-grey represents mixed substrate sites, and light-grey represents sandy sites. Mean (± SE) surface values are given in the table below.

Surface SRP	ABER	DENM	BOWM	MOSE	SAHO	WARK
May	0.016 ± 0.000	0.013 ± 0.003	0.066 ± 0.005	0.076 ± 0.000	0.094 ± .009	0.015 ± 0.001
August	0.032 ± 0.000	0.016 ± 0.002	0.006 ± 0.001	0.025 ± 0.004	0.017 ± .003	0.004 ± 0.001
November	0.018 ± 0.006	0.024 ± 0.004	0.022 ± 0.002	0.019 ± 0.005	0.047 ± .002	0.006 ± 0.002

136

The extent to which bar habitats at sites of the same substrate class differed from each other, changed with time (P = 0.002, Table 4.6). Variability among the mixed coarse sand/gravel sites was high in August and November (Figure 4.9). During November, NOx concentrations in both sandy sites fell nearly to zero after 5 m (Figure 4.9), while concentrations at both of these sites became less similar with distance in May and August (Figure 4.9). NOx in the bar did not correlate with DO (r_{170} = -0.042, P = 0.586).

The high NOx concentrations in the riffle hyporheic zone at WARK and SAHO during May and November indicate that there was significant nitrification in the sandy sediments. However, low concentrations at these sites along subsurface flowpaths of the bar suggest that there was significant denitrification. These observations support my first and second hypothesis.
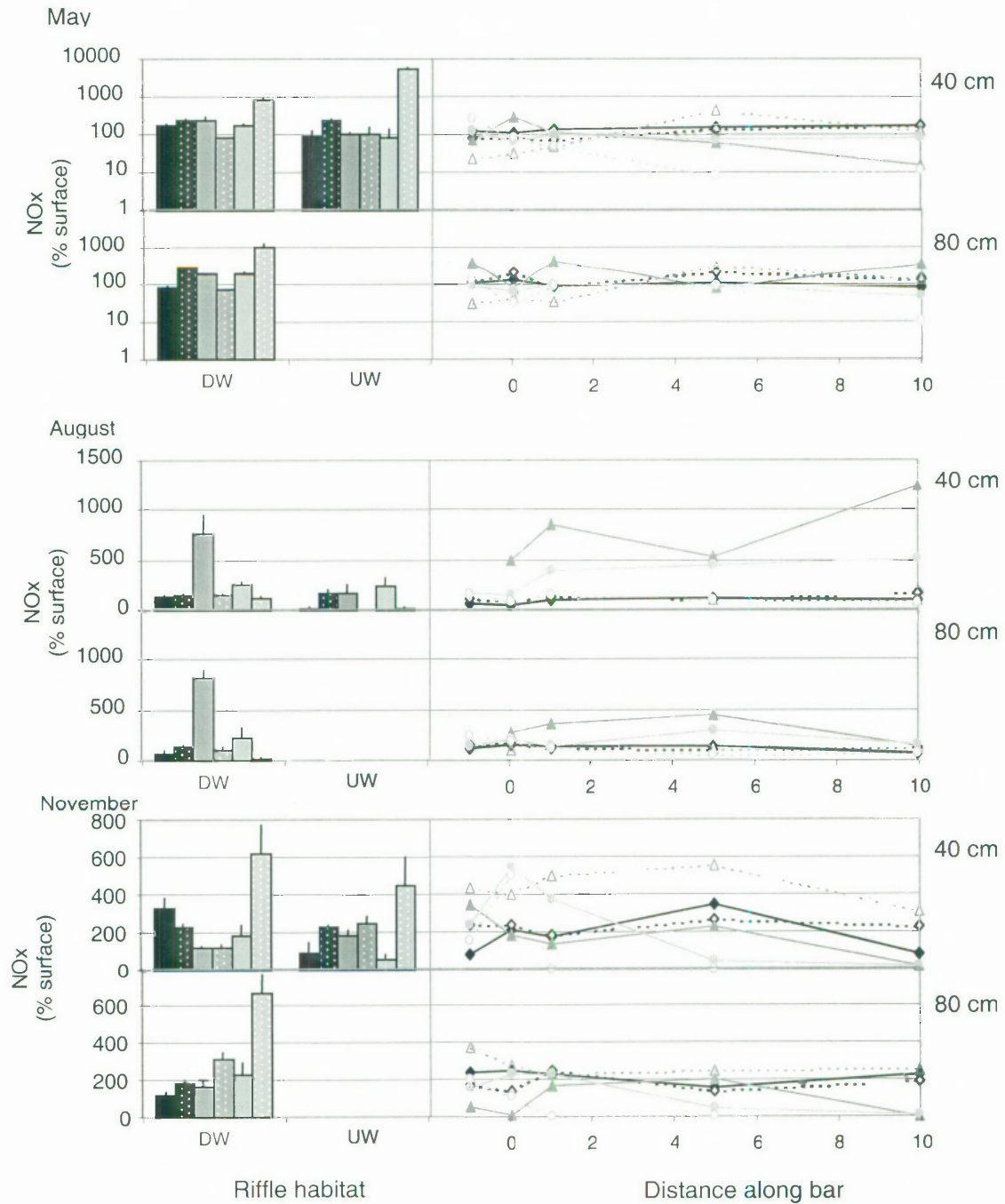
4.4.3 Faunal communities

Over all dates, the cobble site at DENM had the hghest species richness with 44 taxa collected from the hyporheic and parafluvial zones. BOWM interstitial habitats contained 25 taxa, while those at SAHO harboured 20.

The assemblage composition of hyporheic invertebrates formed groups by Site for May samples (Global R = 0.615, P < 0.001). During May, the composition of the BOWM and SAHO samples overlapped on the MDS (Figure 4.10), but were deemed distinct groups by the pairwise ANOSIM test (R = 0.304, P <0.001). The assemblage composition of DENM samples, containing high numbers of cylopoids, oligochaetes, and ostracods, was significantly different from both SAHO (R = 0.890, P < 0.001) and BOWM (R = 0.744, P < 0.001). BOWM samples were dominated by oligochaetes, microturbellarian flatworms, and parastenocarid harpacticoids. At SAHO, oligochaetes, ceratopogonid midge larvae, and parastenocarids were the principal taxa. Invertebrate communities differed among habitats within sites (Global R = 0.387). All pairwise P-values for interactions of the downwelling habitat (where parastenocarids, oligochaete worms, and ostracods dominated) with distances along the bar were < 0.05, indicating that the fauna of these two habitats differed. There was no significant differentiation of communities with depth (Global R = 0.043, P = 0.168).

Table 4.6. Mixed-model ANOVA results table for NOx in the surface, hyporheic (riffle), and parafluvial (bar) habitats. T = Time, G = Substrate, S(G) = Site nested within Substrate, H = Habitat, D = Distance. Bold numbers are significant at P = 0.05

Source	SS	df	MS	F-ratio	P
Surface - square root NOx					
T	0.087	2	0.044	4.487	0.064
G	0.120	2	0.060	6.789	0.077
T*G	0.031	4	0.008	0.804	0.565
S(G)	0.027	3	0.009	7.876	**0.001**
T*S(G)	0.058	6	0.010	8.631	**0.000**
Error	0.020	18	0.001		
Riffle - Log(x+1) NOx					
T	0.115	2	0.058	0.033	0.967
G	0.611	2	0.305	0.372	0.717
H	0.154	2	0.077	0.224	0.806
T*G	1.554	4	0.389	0.224	0.916
T*H	0.534	4	0.134	0.745	0.580
G*H	0.221	4	0.055	0.161	0.950
T*H*G	1.157	7	0.165	0.922	0.523
S(G)	2.463	3	0.821	19.212	**0.000**
T*S(G)	10.426	6	1.738	40.663	**0.000**
H*S(G)	2.054	6	0.342	8.011	**0.000**
T*H*S(G)	2.152	12	0.179	4.196	**0.000**
Error	4.530	106	0.043		
Bar - square root NOx					
T	203.295	2	101.648	1.840	0.252
G	100.136	2	50.068	1.846	0.300
D	32.333	4	8.083	0.380	0.818
T*G	180.419	4	45.105	0.816	0.566
T*D	144.963	8	18.120	1.167	0.360
G*D	213.514	8	26.689	1.256	0.348
T*D*G	292.233	14	20.874	1.344	0.257
S(G)	81.351	4	27.117	1.986	0.122
T*S(G)	276.220	5	55.244	4.047	**0.002**
D*S(G)	255.053	12	21.254	1.557	0.120
T*D*S(G)	357.172	23	15.529	1.137	0.325
Error	1160.436	85	13.652		

Figure 4.9. Hyporheic (bar graphs + standard error, on left) and parafluvial (line graphs) nitrate and nitrite nitrogen concentration for six sites and three times. Black represents the coarser cobble sites, dark-grey represents mixed substrate sites, and light-grey represents sandy sites. Mean (± SE) surface values are given in the table below.

Surface NOx (mg/L)	ABER	DENM	BOWM	MOSE	SAHO	WARK
May	0.082 ± 0.006	0.057 ± 0.000	0.107 ± 0.015	0.108 ± 0.017	0.078 ± 0.001	0.012 ± 0.003
August	0.123 ± 0.008	0.122 ± 0.027	0.027 ± 0.013	0.122 ± 0.004	0.019 ± 0.003	0.023 ± 0.002
November	0.028 ± 0.004	0.067 ± 0.030	0.036 ± 0.000	0.020 ± 0.008	0.015 ± 0.003	0.002 ± 0.002

In August, despite some overlap in the MDS (Figure 4.10), samples formed groups by site (Global R = 0.771, P < 0.001). SAHO (pairwise R = 0.860, P < 0.001) and DENM (pairwise R = 0.719, P < 0.001) formed groupings that differed from the BOWM groups and each other (pairwise R = 0.777, P < 0.001). At SAHO, community groupings were determined by the small syncarid Parabathynellidae, oligochaetes, and parastenocarids. Cyclopoids, oligochaetes and *Heterias* sp. isopods dominated at DENM, and oligochaetes, parastenocarids, and cyclopoids dominated the BOWM community. Communities in the downwelling habitats contained high numbers of oligochaetes, parastenocarids and cyclopoids and differed from those of upwelling zones (oligochaetes and parastenocarids) and bars (oligochaetes, cyclopoids, and *Heterias* sp.). Communities at 80 cm were similar to those at 40 cm (Global R = 0.045, P = 0.181).

In November, the community composition at all sites differed from each other (Global R = 0.533, P < 0.001). BOWM samples grouped tightly away from the other overlapping groups (Figure 4.10). At SAHO, only oligochaetes were found in significant numbers, while oligochaetes and parastenocarids dominated at BOWM. Oligochaetes, ostracods, and *Heterias* sp.were common in the samples at DENM. Community composition in each habitat differed from each other during November (Global R = 0.215, P = 0.011), but it was the upwelling rather than the downwelling community that was most dissimilar to the bar fauna. All habitats were dominated by oligochaetes and parastenocarids, but low numbers of each taxon in the downwelling zone (18 and 7 per sample respectively) distinguished it from the other habitats, where populations of each taxa exceeded 40 and 20 individuals respectively. Communities at both depths were similar (Global R = -0.027, P = 0.674).
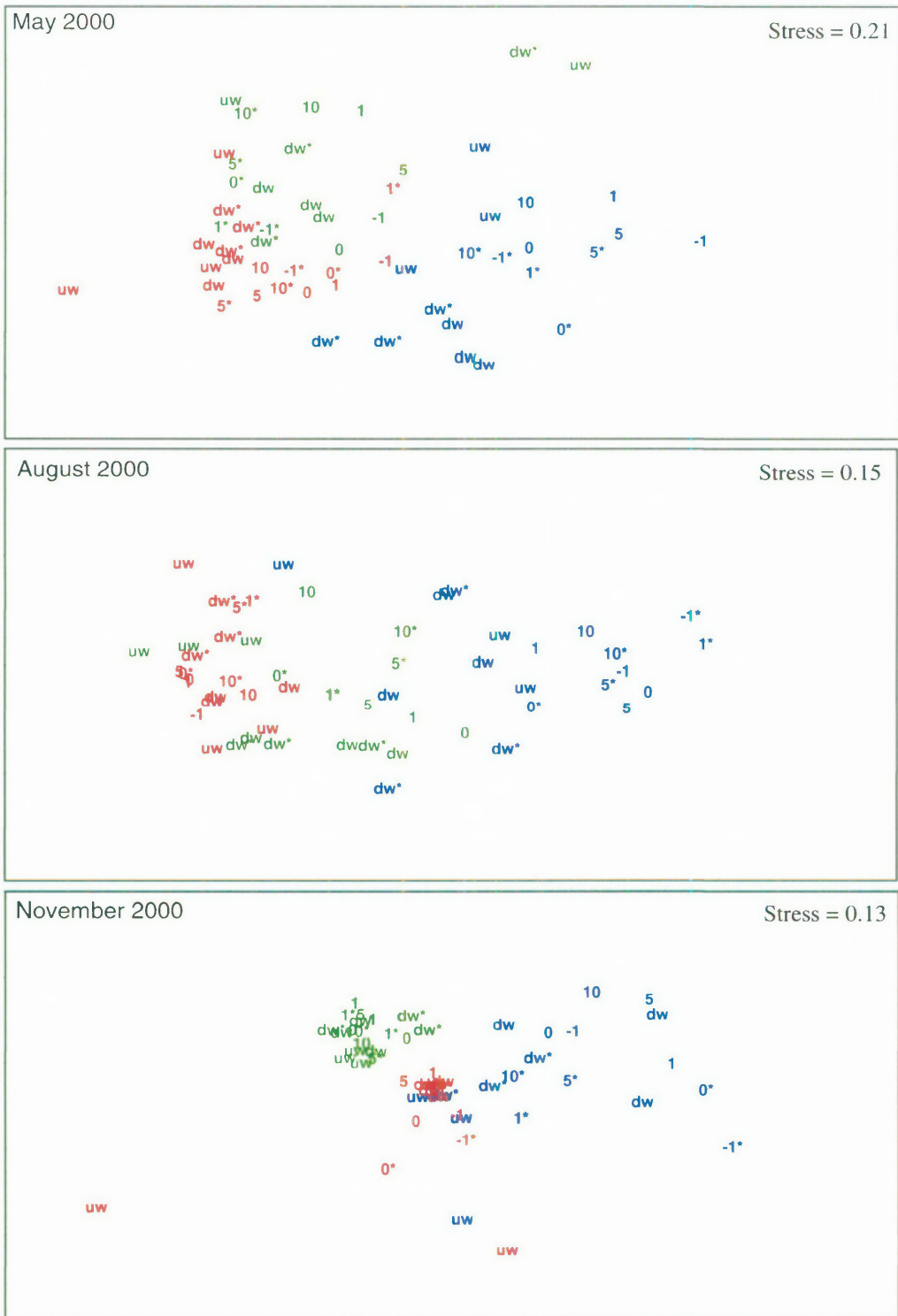
Figure 4.10. Non-metric multi-dimensional scaling plots for invertebrate community assemblages during May, August, and November 2000. dw = downwelling hyporheic habiat. uw = upwelling hyporheic habitat. Numbers represent parafluvial distance from the leading edge of the bar. * denotes samples collected from 80 cm. All other samples were from 40 cm. Blue = Denman, green = Bowmans Crossing, red = Sandy Hollow.

4.5 Discussion

4.5.1 Physico-chemical patterns

Interstitial pH at both sand-bed sites closely resembled that of the stream. These findings contrast with those of Hendricks and White (1991), who found hyporheic pH in the surface water of a sandy Michigan River to be 0.5 - 1 pH unit higher than that of hyporheic water. In general, there was little variation in the pH with distance, both along the bar or riffle at WARK or SAHO. Hendricks and White (1991) also found no difference in interstitial pH with distance along flow paths.

In May and August electrical conductivity in the riffle increased at least three-fold between downwelling and upwelling zones at WARK. In the Rhône River, the electrical conductivity was higher in the upwelling zone than in the downwelling zone (Mermillod-Blondin *et al.* 2000) and similar patterns were found in the Maple River, Michigan (Hendricks and White 1991). However, such an increase was not apparent in the bar at WARK at any time, or at the riffle during November. Since the groundwater around Wollombi Brook is generally brackish (Lamontagne *et al.* 2003), this could indicate localised groundwater influx into the hyporheic zone at WARK. The absence of high EC in November may further indicate that groundwater influx varies seasonally, with upwellings occurring in May and August, but not November. Conductivity patterns at SAHO contrasted with this, being highest in the upwelling zones during November, then August, which are probably the times when groundwater recharge in this river is most influential.

Warmer water in the upwelling zones at both sites in May and August illustrate the buffering effect that the hyporheic zone can have on water temperature. Indications of diel patterns of buffering were also observed in Rocky River (Claret and Boulton 2003). Interstitial warming possibly results from either radiant warming from sun-warmed sediments, or from the influence of more temperature-stable upwelling groundwater. Despite the bar at WARK having more vegetation, and the riffle site being better shaded, the interstitial temperature here was consistently higher than the interstitial temperature of SAHO. This may be a result of temperature-stable groundwater upwelling at the site at WARK, and this was supported by high EC at this site.

4.5.2 Dissolved oxygen

Dissolved oxygen (DO) at WARK supported the predictions of hypothesis 1 of the model, with concentrations declining rapidly with distance from the stream. Generally, bacterial activity is higher in the upstream end of bars (Fisher *et al.* 1998), resulting in a rapid decline of oxygen with distance along the bar. Therefore, the decline of DO to less than 50 % of surface concentration after only 10 m of travelling in the bar at WARK is not surprising and lends support to my first hypothesis. Lamontagne *et al.* (2003) also found low concentrations of dissolved oxygen in the interstitial waters of Wollombi Brook. However, parafluvial DO at SAHO did not decline in May or August with distance along the bar. A possible reason for this could be the presence of shallow spear-point pumping in the bar. Spear-point pumping is a method of extracting river water or near-river groundwater via a series of connected pipes sunk vertically into gravel bars. Pipes for spear-point pumps were previously observed in this bar approximately 20 m south (towards the bank) of the 10 m location on the flow path. While no pumping was observed during sampling, it is possible that pumping prior to sample collection could have drawn stream water into the parafluvial zone. As well as withdrawing water, pumps could clear interstices by removing silt and other clogging material. This could effectively create subsurface channels where flow is relatively unrestricted. While increasing the amount of oxygen available for aerobic bacteria, the residence time of the water is shortened, and the filtration capacity of the bar may be reduced (Hancock 2002). Pumping from an alluvial aquifer of the Rhône River in France reduced the amount of time needed for stream water to influence the aquifer from two days to two hours (Mauclaire and Gibert 1998). While this pumping rate (200 m^3/hr for 3 hr) is of a far greater scale than that at the SAHO bar, the implications are the same. It is even possible that, if the spear-points are far enough from the stream and the pumping rate not too great, bar filtration can be enhanced. Another French study in the alluvial sediments of the Lot River found that microbial activity effectively filtered zinc from the water in the first 10 – 15 m of sediments (Bourg and Bertin 1993). This led the authors to suggest that bores could be strategically located to take advantage of this filtration.

Rapid vertical declines in DO concentrations of sandy stream interstitial areas are commonly reported in the literature (e.g., Sycamore Creek – Valett *et al.* 1990, Buzzards Branch – Strommer and Smock 1989). Most of the DO in the sand-bed

Rocky River was consumed within the upper 25 cm of sediments, with DO at 10 cm being nearly three times less than surface concentration (Boulton *et al.* 2002a). DO in the hyporheic zones of WARK and SAHO also declined with depth, but most of the consumption occurred within the upper 40 cm. Between 40 cm and 80 cm, there was often no significant difference in concentration observed, especially at SAHO, indicating no significant consumption beyond 40 cm. This supports the findings of Battin and Sengschmitt (1999) that the majority of microbial activity occurs in the upper sediments.

4.5.3 Nutrient patterns

It was hypothesised that nutrient transformations in sandy sediments would be more rapid, and occur over a shorter distance than in coarser sediments. In general, the hypothesised patterns of NOx and dissolved oxygen were observed in the sediments at WARK: both of these variables were consumed or created much more rapidly here than in coarser substrates. However, NOx and dissolved oxygen at SAHO showed rates of consumption/transformation comparable to the rates in both the cobble and mixed-sediment sites The hyporheic and parafluvial zones of SAHO were probably similar in behaviour to other sites because the sediments were coarser than those at WARK and allowed a better infiltration of surface water. The substrates at ABER and DENM, although dominated by large cobbles, also contained substantial amounts of fine particles (Chapters 2 and 5). It is possible that the fine sediments at these sites provided a surface area for microbial adhesion similar to that provided by the sand at the other sites, while the coarser particles facilitated rapid infiltration of water.

The consumption of dissolved oxygen in the bar at WARK but not at SAHO is reflected in patterns of subsurface NOx concentrations. At WARK, NOx concentrations decreased substantially after just 1 m, consistent with my second hypothesis. In November, NOx was virtually undetectable at distances greater than a metre. Lamontagne *et al.* (2003) also found very low NOx concentrations in the interstitial sediments near Wollombi Brook, hypothesising that this was due to anoxic sediments. These patterns are consistent with the dominance of denitrification and ammonification processes, which are likely to have become dominant when oxygen became scarce (Duff and Triska 1990, Hendricks and White 1991). NOx concentrations in a Sycamore Creek parafluvial zone displayed different patterns to those at WARK, with NOx concentrations increasing with distance along the flow path

(Holmes *et al.* 1994a, b). Given the persistence of dissolved oxygen along the bar at SAHO, it is not surprising that NOx concentrations changed little. If spear-point pumping in the bar was drawing water rapidly through the sediments before sampling in May and August, it appeared to have increased the length of bar required for nitrification. In contrast to this, November NOx concentrations were nearly 6 times the surface concentration, before declining rapidly as NOx was denitrified, mineralised, or transformed to ammonium.

Mostly, NOx concentrations below the riffles declined with longitudinal distance, as would be expected if the distance of subsurface travel was long enough to substantially decrease the available oxygen. However, during May, NOx in the hyporheic zone at WARK increased during its subsurface flow. That the hyporheic zone of Sycamore Creek is a NOx source is well known (e.g., Jones *et al.* 1995, Fisher *et al.* 1998) but the results from WARK indicate that the role of the hyporheic zone in sandy streams can change with time from a source to a sink. While the sediment volume in the channel of these sandy sites is large, it is likely that only a small amount of the pore water frequently exchanges with the stream. The interstitial habitats of Rocky River in New South Wales, also had higher activity in the upper sediments (<20 cm, Boulton *et al.* 2002a, Claret and Boulton 2003) with a large area of infrequently exchanged water below.

In August and November, the SRP concentration exceeded surface concentrations in the parafluvial and hyporheic zones. SRP gradients at WARK in November were much steeper than those of SAHO. These findings partly agree with the observations of Boulton *et al.* (2002a), who reported enhanced SRP concentrations in Rocky River hyporheic zone, but no subsequent increase with depth. In the riffle at WARK, SRP concentrations during November increased four-fold with longitudinal distance, and depth. Extensive iron flocculations coated the benthic sediments during November and Lamontagne *et al.* (2003) reported high concentrations of Fe^{2+} from the alluvial groundwater at Warworth. The sorption of phosphate by iron partly determines the amount of free phosphate in interstitial water (Hendricks and White 2000), but no correlations were found between Fe^{2+} and SRP at Warkworth (Lamontagne *et al.* 2003). The presence of significant quantities of iron did not seem to limit the availability of SRP in the interstitial environments of Wollombi Brook.

4.5.4 Invertebrate community

Invertebrate communities in each substrate type differed from each other and strongly support my third hypothesis: that the fauna of sandy hyporheic zones are dominated by taxa adapted to living in fine sediments. Generally, there was a higher proportion of stygobiontic animals in the sandy hyporheic zones. Groundwater invertebrates are typically uncorrelated with dissolved oxygen concentrations (Strayer 1994), but this appears not to be the case for temporary interstitial invertebrates in the Hunter River.

Oxygen limitation may only be one factor restricting the penetration of epigean taxa into the hyporheic zone. Another is pore space. In many interstitial environments, particle size correlates with animal body size (Coineau 2000). Sand particles often have smaller interstices, which may be too narrow to accommodate larger taxa such as insect larvae, but may be favoured by small crustacean species due to the absence of larger predators (Boulton et al. 2002b). In addition to small size, invertebrates that inhabit the interstices of fine sediments have a flexible body form and are streamlined (Gayraud and Philippe 2001). Most of the insect larvae that were found in the SAHO hyporheic zone were elongate forms such as Ceratopogonidae and Chironomidae, which are relatively flexible. Conversely, in the coarser sites, where particles are separated by larger interstices, there is more room for larger species (Boulton et al. 2002b). It is probably for this reason that cyclopoids, *Heterias* sp., ostracods, and small insect larvae dominated the sediments at DENM, while parastenocarids and parabathynellids dominated at SAHO. At BOWM, there were high numbers of insects, cyclopoids and parastenocarids, and this may be because this site is downstream of both DENM and SAHO, and accumulates their sediment and fauna as predicted by the hyporheic corridor concept (Stanford and Ward 1993).

The invertebrate fauna of SAHO averaged 20 invertebrate taxa over the three sampling periods, which is significantly fewer than the 71 taxa found in the Hunter River hyporheic zone (Chapter 3), supporting the hypothesis that sand-bed sediments contain fewer species than coarser sediments. However, the hyporheic zone of SAHO contained more invertebrate taxa than Buzzards Branch, Virginia, which had only 15 taxa at depths of 30-40 cm (Strommer and Smock 1989). Accounts of the number of taxa in Sycamore Creek interstitial habitats vary from 17 (Boulton *et al.* 1991), to 31 (Boulton and Stanley 1995), and 55 taxa (Boulton *et al.* 1992). In the Triouzoune,

France, 48 taxa were found (Maridet *et al.* 1996). In Rock Creek, a tributary of Sycamore Creek with sand and small gravel substrate, 20 taxa were collected (Clinton *et al.* 1996).

A combination of epigean insect larvae and small, blind stygobiontic crustaceans indicated that the hyporheic zone at SAHO was linked to both surface and groundwater environments. However, the majority of the taxa at 40 cm were obligate groundwater species, and as such gave an important indication of the degree to which the hyporheic zone here is connected to the aquifer. Members of the syncarid family Parabathynellidae were common in many of the samples, mostly from the parafluvial zone. Syncarids (Bathynellidae) and harpacticoids dominated the deeper hyporheos of Sycamore Creek (Clinton *et al.* 1996). These, along with similar taxa are thought to play an important role in maintaining interstitial porosity and stimulating microbial activity (Mermillod-Blondin *et al.* 2003).

4.6   Conclusions

Both of the sandy streams sampled in this study displayed contrasting hyporheic activity. The fine particles at WARK restricted most of this activity to the sediments less than 40 cm deep, with the sediments below this area of active exchange becoming storage areas at base flow. At this site, deeper sediments probably exchange nutrients less frequently with the surface, facilitated by upwelling groundwater and floods. The hyporheic and parafluvial habitats at WARK supported my hypotheses about sand-bed streams. However, this was not the case with the interstitial habitats at SAHO. At most times the physical, chemical, and nutrient dynamics at SAHO resembled those of the other sites with coarser sediments, contrasting with the expectations of my second hypothesis. Possible reasons for this are the loose nature and coarseness of the sand particles, and the strength of groundwater connection. Another could be the existence of spear-point pumps in the bar at SAHO. Water entering these will move relatively rapidly through the sediments, minimising its contact time with transforming bacteria. However, faunal communities did differ between substrates with small obligate groundwater species being more common at SAHO, and insect larvae, as well as groundwater species penetrating deeper into the coarser sediments at DENM, supporting the third hypothesis.

The main implication of these findings is that generalisations about the conditions in the hyporheic zone of sand-bed streams cannot always be made. Subtle differences in sediment size, coupled with pumping and flow regimes, can invoke differences with important repercussions for surface and groundwater management.

Chapters 3 and 4 highlighted the significance of the hyporheic and parafluvial zones in the Hunter River and two of its tributaries. In Chapter 3, the occurrence of the November flood indicated the importance of high flows in resetting or stimulating many hyporheic activities. In regulated rivers, such large-scale events can be rare. More common are controlled 'environmental flow' releases, designed to simulate flushes for the benefit of surface stream ecosystems. The benefits, of environmental flows to hyporheic ecology have not yet been investigated and this is the aim of the next chapter of this thesis.