CHAPTER 5

The effects of an environmental flow release on the hyporheic zone of the Hunter River

5.1 Introduction

River regulation disrupts the natural flow regime of a river, depriving downstream areas of high flows in times when such flows would otherwise occur, and allowing high flow for irrigation at times where it would be minimal (Kingsford 2000). In an attempt to reduce the ecological effects of flow regulation, some managers have adopted the practice of releasing environmental flows (Ladson and Finlayson 2002). In 1994, the Council of Australian Governments agreed on a policy requiring the establishment of water entitlements for the environment (Cullen 1994). This resulted in all Australian governments outlining a set of 12 principles ensuring water specifically for the environment (Commonwealth of Australia 1996). Now, most of the major catchments with regulated rivers in the country have a set of flow management guidelines that allocate water specifically for environmental use.

Environmental flow releases potentially constitute a medium-level disturbance, and when coupled with more infrequent large floods may be a way of restoring natural river processes through simulating the pre-regulation regime. Essentially, environmental flows aim to restore natural flow variability in rivers and ensure that key chemical, ecological, and geomorphologic processes occur in the adjacent dependent ecosystems (Gippel 2001). They often rely on water released from a reservoir, but can also result from the cessation of abstraction for a specified period. In their conception and implementation, most environmental flows are designed to benefit in-stream ecological processes. Recently, in recognising the importance of groundwater dependent ecosystems, the hyporheic zone has gained specific consideration in river management (Department of Land and Water Conservation 2002).

In the Hunter River, a series of flow rules has been developed to maintain riverine ecosystems (Department of Land and Water Conservation 2000). Flow Rule 2 aims to re-introduce small to medium flows to the river. This rule specifies that the first 12 h of a high flow event (whether natural or released from Glenbawn Dam) be allowed to

pass without extraction. Following this, a maximum withdrawal of 50% of the high flow is permitted. Theoretically these two components of the rule allow environmental flows of varying magnitude for at least half a day.

The potential for river regulation to affect hyporheic zones stems mainly from the disruption caused to linkages between the river and hyporheic environments (Boulton 2000b, Hancock 2002). This disruption comes in the form of partial or complete separation of parafluvial habitats from the stream, the transformation of below-stream hyporheic zones to lateral parafluvial areas, and the weakening of fluxes between sediment and river through colmation and decreased hydraulic pressure (Chapter 1). A Swiss study concluded that links from the Brenno River to its floodplain were severely impaired by regulation, which reduced the baseflow to 27 % of natural, and made small to medium floods less frequent (Brunke 2002). It was subsequently recommended that annual releases of small to medium floods might re-establish links to the floodplain habitats.

Much of the overseas literature pertaining to the effects of river stage fluctuations on hyporheic ecology is summarised in Chapter 1. However, it is useful to present a conceptual model of how environmental flows may benefit the hyporheic zone. The current study is the first that I am aware of that investigates the effects of an environmental flow release specifically on hyporheic ecology.

5.1.1 A model of the effects of environmental flows on the hyporheic zone

Bank-breaking floods form a necessary re-structuring role for in-stream habitats, typically moving large sections of stream-bed. Medium flows can enhance linkages between the hyporheic zone and stream without causing such devastation. Strategically-timed pulses of water temporarily increase the river stage, covering a larger area of lateral bars and increasing the area available for hyporheic exchange (Figure 5.1). If the flow is of sufficient magnitude, fine particles will be flushed and sediment that has become compacted over time will be jostled loose, increasing the pore-space of the hyporheic zone. When this is coupled with the increased hydraulic pressure that comes with higher water levels, oxygen-rich surface water is able to travel further through the hyporheic zone and extend its oxidising margins both vertically and laterally. All of these processes will, in theory, enhance bed filtration through stimulating microbial processes such as nitrification, and help maintain faunal

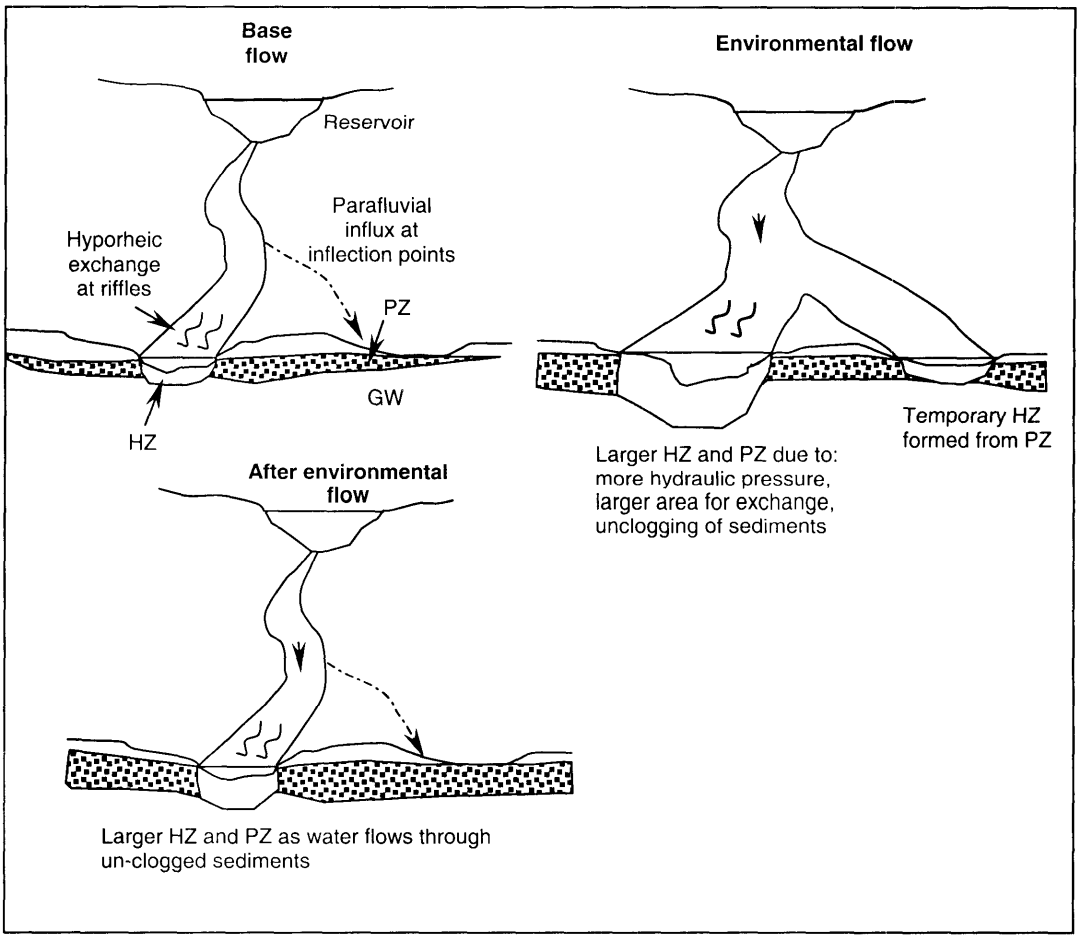


Figure 5.1. The potential effects of an environmental flow on hyporheic (HZ) and parafluvial (PZ) spatial dimensions. (a). At baseflow, hyporheic and parafluvial exchange are restricted. (b) Hydraulic pressure exerted by higher water levels extends the hyporheic and parafluvial boundaries. (c) The environmental flow potentially increases the porosity of the sediments by flushing silt and loosening sediments, thereby allowing more penetration of surface waters in the hyporheic and parafluvial zones.

diversity. However, it is important to note here that the water released for the flow probably needs to be of sufficient quality so as not to further impair hyporheic processes.

In this experiment I monitored a suite of physico-chemical, faunal, and sediment variables before and after an environmental flow release to test five hypotheses drawn from the model above. First, the porosity of the hyporheic and parafluvial zones will be higher after the release, especially in the upper sediments and at sites with smaller, more mobile substrata. The second hypothesis was that the proportion of fine sediments will be higher before the release, but the flushing effect of higher flows will remove some of the finer particles, especially in shallow sediments. Third, it was expected that interstitial nitrate-N concentrations will be higher after the release, indicating that there was a stimulation of microbially-driven nitrification. Fourth, there will be an increase in the magnitude of vertical hydraulic head (more negative at downwelling areas, and more positive at upwelling areas), indicating enhanced exchange between surface and hyporheic habitats. Finally, faunal communities following the release will have higher numbers of insects than non-insects, due to hyporheic conditions being more similar to surface conditions. This will be particularly evident in the upper sediments and will decline with depth.

5.2 Study sites

For this study three sites were sampled along the Hunter River - Aberdeen (ABER), Bowmans Crossing (BOWM), and Moses Crossing (MOSE). A description of each site is given in Chapter 2. Sites were selected because they had accessible riffles and lateral gravel bars, and contained a diverse fauna (Chapter 3). Sediment particle size was also considered during site selection, as it was thought that the larger sediment at ABER might provide a more stable environment during flow pulses.

5.3 Methods

Various sediment, faunal, and physicochemical characteristics (see below) were collected from ABER, BOWM, and MOSE prior to and following a flood mitigation release from Glenbawn Dam. Two different sampling methods were used: a freeze corer (Marchant and Lilywhite 1989), and a pump sampler (Boulton 1993).

5.3.1 Sampling schedule

Pre-release samples were collected from 9 - 11 September 2001 (Figure 5.2). Prior to any sampling, a spate occurred that possibly influenced the results of this manipulation (See section 5.5). This spate peaked at the Aberdeen gauge on 28 August, and at the Jerrys Plains gauge on 29 August, and flow had returned to pre-flow levels by 7 September (Figure 5.2). From 12 September to 14 September, a flood mitigation release of 5 000 ML per day was sent down the river (Figure 5.2). The released water had passed ABER by 19 September, and BOWM and MOSE by 20 September, allowing river stage to return to approximate pre-release levels. Subsequent sampling was then conducted on 27 – 29 September, 7 days after the release stopped, and 7 – 9 November, 49 days after. In between the second and third sampling occasions, river flow decreased substantially (Figure 5.2).

5.3.2 Field sampling

For each sampling occasion, triplicate pump samples of 6 L (Chapter 3) were collected from a depth of 40 cm from three habitats per site (Figure 5.3): downwelling riffle habitat (DW), upwelling riffle habitat (UW), and the leading edge of a lateral bar (Bar). To test for differences in water quality before and after the release, I measured electrical conductivity (TPS MC81 meter), dissolved oxygen, and temperature (TPS WP-824 dissolved oxygen meter with a YSI5739 probe) from the second 2 L of each sample. Samples were subsequently elutriated through a 125 μm sieve to separate the fauna. To assess differences in nutrient concentrations, 125 mL of water was filtered through Whatman GF/C filter papers into an acid-washed polyethylene bottle and frozen for analysis of nitrate and nitrite nitrogen (NOx) in the laboratory.

Vertical hydraulic head (HH) was measured with a purpose-built probe following the collection of nutrient and fauna samples. The probe consisted of a light emitting diode (LED) and charged (9 V) wires running down a graduated length of clear rigid tubing. This was lowered slowly into the well until the internal water level closed the circuit, causing the LED to illuminate. HH was determined as the difference between water levels in the well and the river.

The rise in river stage was measured with two rising stage samplers at each site. These were constructed from metal fencing posts driven vertically, part way into the stream bed. Each post had two offset columns of 70 mL jars fastened to them. A hole drilled

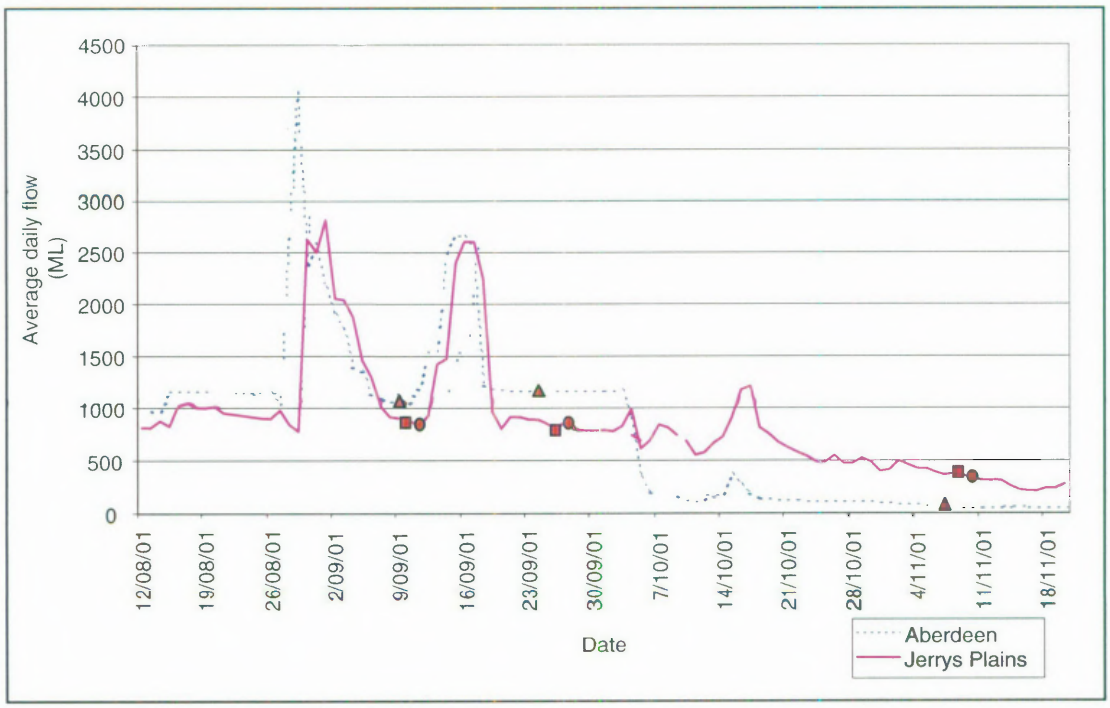


Figure 5.2. Average daily flow at Aberdeen and Jerrys Plains gauging stations (Department of Land and Water Conservation). Symbols indicate sampling dates: triangle = Aberdeen, square = Bowmans Crossing, circle = Moses Crossing.

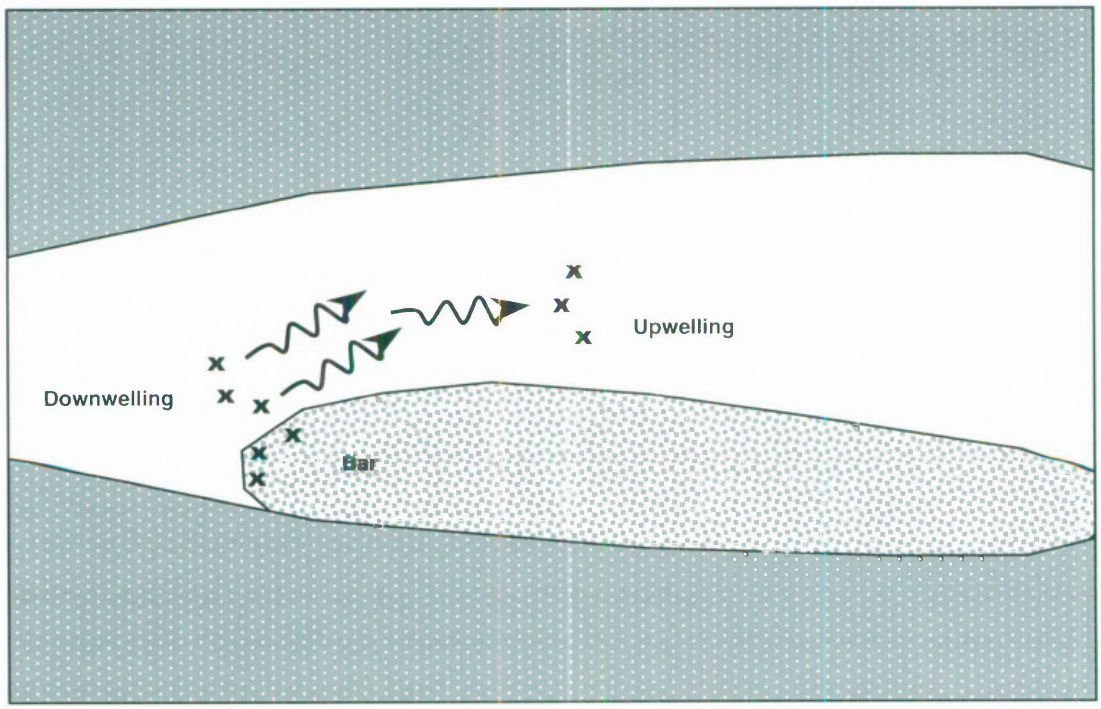


Figure 5.3. The location of downwelling, upwelling, and bar sample points in a theoretical site containing a riffle and a lateral bar.

in the wall of each jar allowed water to enter as the river rose. Once the river had subsided, the level to which it had risen could be measured by the height of the hole in the uppermost full jar. This method was accurate to 2.5 cm.

A freeze corer (Marchant and Lilywhite 1989) was used to collect triplicate cores from the three habitats. Metal stand-pipes (50 mm internal diameter) were inserted 50 cm upstream of the pump sample wells immediately following sample collection and well removal. This was to minimise any influence that the pump sample may have had in removing fine particles. Stand-pipes were driven into the bed to a depth of 50 cm below the level of consolidated sediment. They were then left to settle for 24 h to allow any fauna, which may have been disturbed by the introduction of the stand-pipes, to return. Although less than the five days used by Marchant (1995), a settling period of 24 h was greater than that of Olsen *et al.* (2001), who froze cores immediately and demonstrated that this resulted in only minor reductions in the density of some taxa in the upper 10 cm when compared with a 2-d settling period. Immediately prior to freezing, a flow deflection barrier was carefully placed around the stand-pipe to minimise warming caused by the flowing surface water. Sediment cores were frozen to the outside of each stand-pipe with 5-min injections of food-grade CO_2 (Figure 5.4). Cores were removed by hand at BOWM and MOSE, or with the use of a tripod and pulley at ABER. Each core was photographed (Figure 5.5) and cut into 10 cm sections, with each section being sealed in pre-weighed, doubled zip-lock bags and kept frozen for transportation to the laboratory.

In all, 398 core sections were collected. Seven sections of core did not freeze adequately. At ABER, these included three from the pre-release samples (the 10 - 20 cm section from one bar core, and the 30 - 40 cm and 40 – 50 cm sections from two separate upwelling cores), two from the first post-release samples (the 10 – 20 cm and 20 – 30 cm sections from two separate bar cores), and one section from the second post-release sample (0 – 10 cm section from one downwelling core). At BOWM, the 40 – 50 cm section of one bar core did not freeze during the pre-release sampling.

For the second and third sampling occasions, care was taken to avoid placing stand-pipes and wells where cores taken on previous occasions might influence the samples. Moving each of the riffle sample locations (DW and UW) 1.5 to 2 m upstream with each time ensured this. For the bar habitat, sample locations were moved laterally

along the upstream edge of bar. Due to the large size of each habitat, movements of this scale were comparatively minor, and would not have compromised the spatial dimension of the replicates.

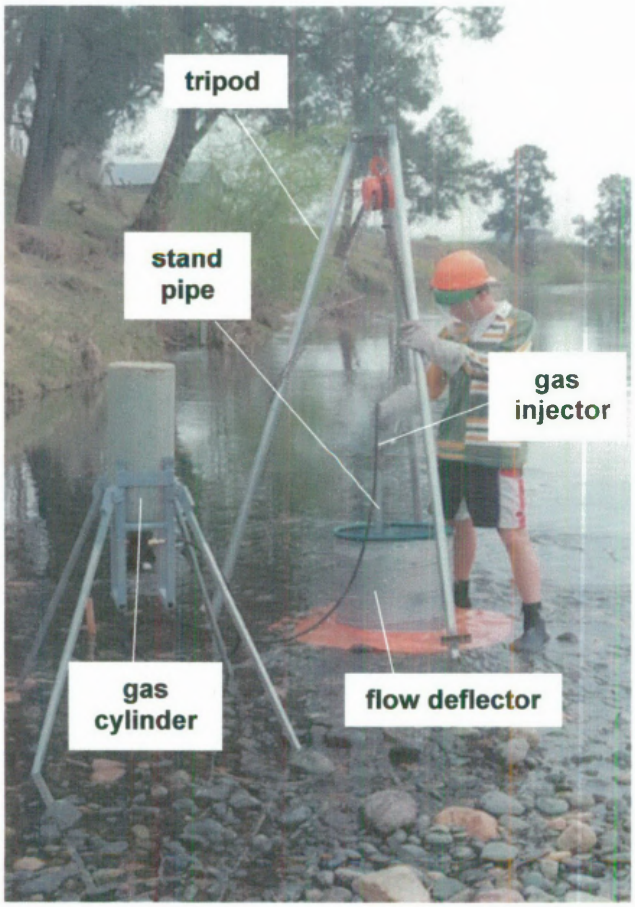


Figure 5.4. Operation of the freeze-corer at the downwelling habitat at Aberdeen.

Figure 5.5. A frozen core from the upwelling habitat at Aberdeen before cutting into 10 cm sections.

5.3.3 Laboratory processing

Water samples were thawed and analysed for nitrate- and nitrite-nitrogen (NOx) using the methods described in Chapter 3. To explore the linkages between surface water and the hyporheic zone, and to quantify the extent of change in water chemistry, NOx, dissolved oxygen, and conductivity are presented as a percentage of the corresponding surface value. For these variables, tables of the surface mean and standard errors are included with the figures to allow raw values to be calculated.

Faunal samples collected using the pump method were counted and identified to species level where possible under a dissecting microscope (10 – 40 x magnification). Because there is a non-linear relationship between sample volume and both taxonomic richness and invertebrate density in pump samples (Boulton *et al.* 2003a), these variables are expressed in 'densities per 6 L'.

Each section of frozen core was weighed before being thawed. Organic matter and invertebrates were elutriated through a 125 μm sieve. Invertebrates were counted and identified to the lowest possible taxonomic resolution with a dissecting microscope. Invertebrate counts are expressed as densities per 100mL for each core section. After the removal of invertebrates and organic matter, inorganic sediments were wet-sieved into Wentworth class sizes (Table 5.4). Larger particles were further separated into 32 mm and 64 mm classes following measurements of their longest and second longest axes. Each size-class was dried, and weighed using a PG 5002-S balance (Mettler, Toledo) accurate to 0.01 g.

To eliminate the bias caused by larger particles, the proportion of fine sediments was calculated from the pooled mass of the particles that passed through the 125 μm sieve using the following formula:

$$\text{Percent fines} = \frac{\text{Total dry mass of all sediments less than 125 μm} \times 100}{\text{Total dry mass of all sediments less than 16 mm}}$$

Sediment volume from the first sampling period was measured by recording the quantity of water displaced by complete immersion of each dried section of core. Volumes for the subsequent two sampling periods were derived from the resulting linear equations relating volume to dry weight (Table 5.1). The porosity of each core

section was determined from the percentage of free interstitial water to the total volume of each core section (Stocker and Williams 1972).

5.3.4 Data analysis

Multi-level analysis of variance (ANOVA) was used to compare the mean of physico-chemical and sediment data, and taxonomic richness and invertebrate abundance for each sampling occasion. Prior to analysis, all variables were tested for normality using a Wilk-Shapiro test in Statistix for Windows, version 7 (Analytical Software). Some traditional tests for homogeneity of variance are often more sensitive than the ANOVA tests themselves (McGuinness 2002) so homogeneity of variance was assessed graphically by analysis of residual plots and stem and leaf plots of residuals in SYSTAT. Where required, data were transformed to comply with the assumptions of ANOVA. All ANOVAs were done in SYSTAT for Windows, version 9.01 (SPSS Incorporated, Evanston, Illinois).

For data collected with the freeze-corer, a 4-factor mixed model ANOVA was used based on a split-plot design (Olsen *et al.* 2001, Quinn and Keough 2002). The factors Time (T), Site (S), and Habitat (H) were treated as fixed factors and were all crossed. Time was treated as fixed because it was measured relative to the flow release. The fourth factor, Depth nested within Habitat, D(H), was random. This was the sub-plot factor, with H being the whole-plot factor (Figure 5.6).

The general linear model (GLM) formula for deriving the expected mean squares, and the terms whose mean squares were used as the error when calculating F, can be found in Table 5.2. This model was used to analyse porosity, percent fines, invertebrate density, and taxonomic richness for each depth of core. Where core sections were absent (through incomplete freezing, see above) means, calculated from the remaining two replicates, were imputed.

As pump samples were collected from only one depth, a three-way crossed ANOVA was used to compare the means of temperature, conductivity (EC), nitrate concentration (NOx), dissolved-oxygen concentration (DO), total invertebrate numbers, and taxonomic richness among Sites, Times, and Habitats. Because of the low numbers of replicates taken for each variable at the lowest level of the models (n = 3), a significance level of 0.01 was used for interpreting the results of the ANOVAs.

Table 5.1. Equations for calculating sediment volume at each site - derived from the cores of the first sample occasion.

Site (no. core sections)	Equation	R^2
Aberdeen (42)	Vol = 0.3512(dry wt.) + 20.33	0.980
Bowmans Crossing (44)	Vol = 0.3931(dry wt.) + 3.1165	0.994
Moses Crossing (44)	Vol = 0.4062(dry wt.) – 3.9009	0.997

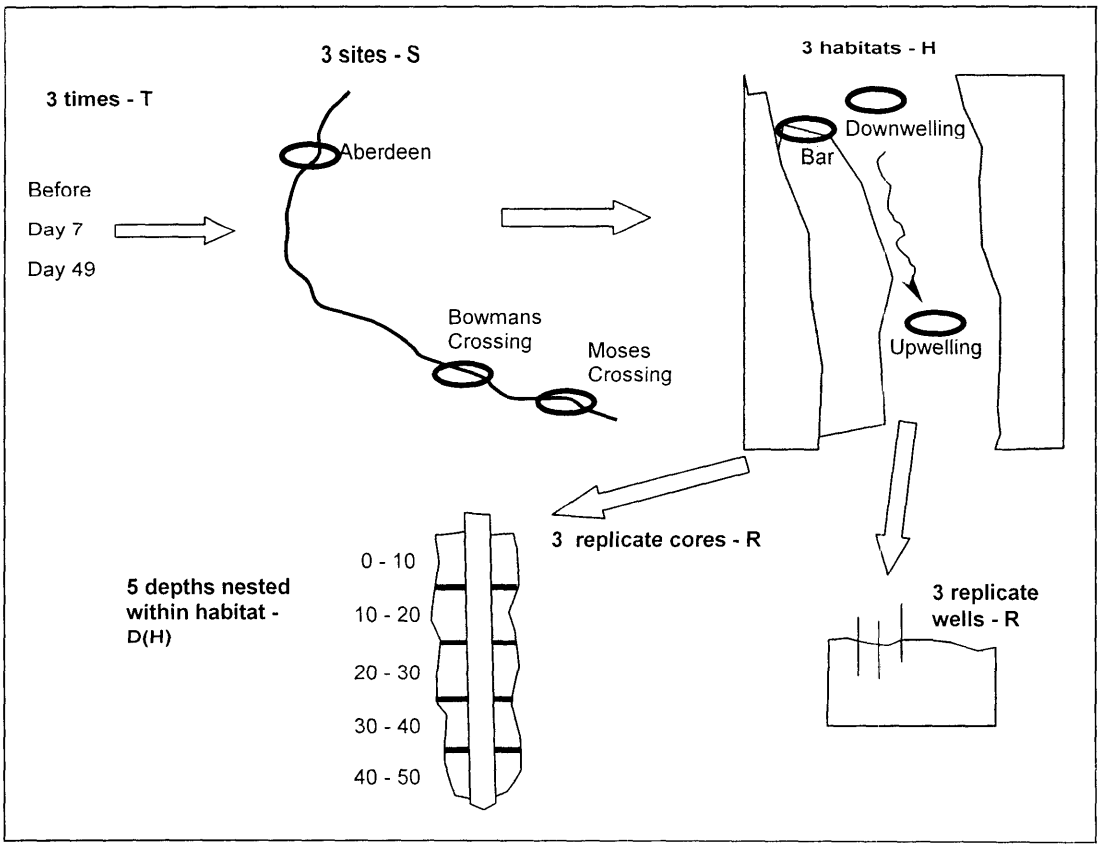


Figure 5.6. Pictorial representation of the sampling protocol showing the 3 times (T), 3 sites (S), 3 habitats (H), 3 replicate wells (R), and 5 depths nested within habitat D(H).

For both models, post-hoc multiple comparisons of means were conducted using Tukey's Honestly Significant Difference (HSD) test. This was done in the event of a significant difference occurring for main effects to detect differences between pairs. Because of the complexity involved in interpreting higher-level interactions, tests were restricted to single-factor variables. Pearson correlations were carried out to test for relationships between variables.

Variance components were calculated for each factor using the formulae from Underwood (1997). Formulae used in calculating the variance component for each factor of the mixed model are provided in Table 5.2. Despite Underwood's (1997) caution against using variance components, especially for fixed factors, Quinn and Keough (2002) recommend them as useful descriptors of explained variance. In this study I wanted to see which of the factors contributed most to any significant differences that occurred for each physico-chemical, nutrient, biotic, or sediment variable, so variance components were calculated.

All community composition data were analysed with the Primer statistical package (Version 5.2.9, Plymouth Marine Laboratories, Plymouth, UK) using non-metric multi-dimensional scaling (nMDS), analysis of similarities (ANOSIM), and similarity percentages (SIMPER) consecutively. See Chapter 3 for a more detailed account of these methods.

For fauna collected with the pump method, data are displayed on four separate nMDS plots – one containing all data to illustrate spatial variability among sites, and one for each Site to show how groupings changed over time. Community data collected with the freeze corer were plotted for each Site on an nMDS plot to distinguish changes among Times. A two-factor crossed ANOSIM was computed to examine differences between Site and Time for the pump data only (the large data set for the core samples resulted in an nMDS plot of a mass of indistinguishable and concurrent points). Separate analyses were then performed on data from each Site using Time and Habitat as the two crossed factors. Only 1000 permutations were possible for site-level ANOSIMs of pump data. A further Habitat by Time ANOSIM was done for the top two core sections. Comparison of community similarity in the third to fifth core

Table 5.2. Factors and interaction terms for analysis of variance, and the formulas used for deriving the estimated mean squares (EMS), error terms used to calculate the F ratio, and variance components. For the EMS, a = 3 (Habitats), b = 5 (Depth sections within Habitat), c = 3 (Sites), d = 3 (Times).

Source	Multipliers					Expected mean squares	df	F- ratio	Variance component
H Habitat	0	b	c	d	r	$\sigma_E^2 + cd\sigma_{D(H)}^2 + bcd\sigma_H^2$	2, 8	D(H)	$MS_H - MS_{D(H)}$/ bcdr
S Site	a	b	0	d	r	$\sigma_E^2 + d\sigma_{SD(H)}^2 + \sigma_S^2$	2 , 24	S*D(H)	$MS_S - MS_{SD(H)}$/ abdr
T Time	a	b	c	0	r	$\sigma_E^2 + c\sigma_{TD(H)}^2 + abc\sigma_T^2$	2 , 24	T*D(H)	$MS_T - MS_{TD(H)}$/ abcr
S*H Site x Time	0	b	0	d	r	$\sigma_E^2 + d\sigma_{SD(H)}^2 + bd\sigma_{SH}^2$	4 , 24	S*D(H)	$MS_{SH} - MS_{SD(H)}$ / bdr
T*H Time x Habitat	0	b	c	0	r	$\sigma_E^2 + c\sigma_{TD(H)}^2 + bc\sigma_{TH}^2$	4 , 24	T*D(H	$MS_{TH} - MS_{TD(H)}$/ bcr
T*S Time x Site	a	b	0	0	r	$\sigma_E^2 + r\sigma_{TSD(H)}^2 + ab\sigma_{TS}^2$	4 , 48	T*S*D(H)	$MS_{TS} - MS_{TSD(H)}$/ abr
T*S*H Time x Site x Habitat	0	b	0	0	r	$\sigma_E^2 + r\sigma_{TSD(H)}^2 + br\sigma_{TSH}^2$	8 , 48	T*S*D(H)	$MS_{TSH} - MS_{TSD(H)}$/ br
D(H) Depth nested in Habitat [sub-plot]	1	1	c	d	r	$\sigma_E^2 + cd\sigma_{D(H)}^2$	12 ,	E	$MS_{D(H)} - MS_E$/ cdr
S*D(H) sub-plot x Site	1	1	0	d	r	$\sigma_E^2 + d\sigma_{SD(H)}^2$	24 ,	E	$MS_{SD(H)} - MS_E$/ dr
T*D(H) sub-plot x Time	1	1	c	0	r	$\sigma_E^2 + c\sigma_{TD(H)}^2$	24 ,	E	$MS_{TD(H)} - MS_E$/ dr
T*S*D(H sub-plot x Time x Site	1	1	0	0	r	$\sigma_E^2 + r\sigma_{TSD(H)}^2$	48 ,	E	$MS_{TSD(H)} - M_{SE}$/ r
E Error	1	1	1	1	1	σ_E^2	270		

model = constant + H + S + T + S*H + T*H + T*S + T*S*H + D(H) + S*D(H) + T*D(H) + T*S*D(H) + E

sections were not analysed because the numbers of taxa in each section were too low. The significance level of 0.01 used in the ANOVA models was retained for the interpretation of ANOSIMs.

SIMPER analysis for each site was performed for core and pump fauna with the specified factor of Time to test which species dominated the assemblages before and after the release, and for Habitat to determine the species more common in bar, downwelling, and upwelling samples. All data were log(x+1) transformed prior to SIMPER analysis, and the cut-off level of contributing species was set to 90%.

5.4 Results

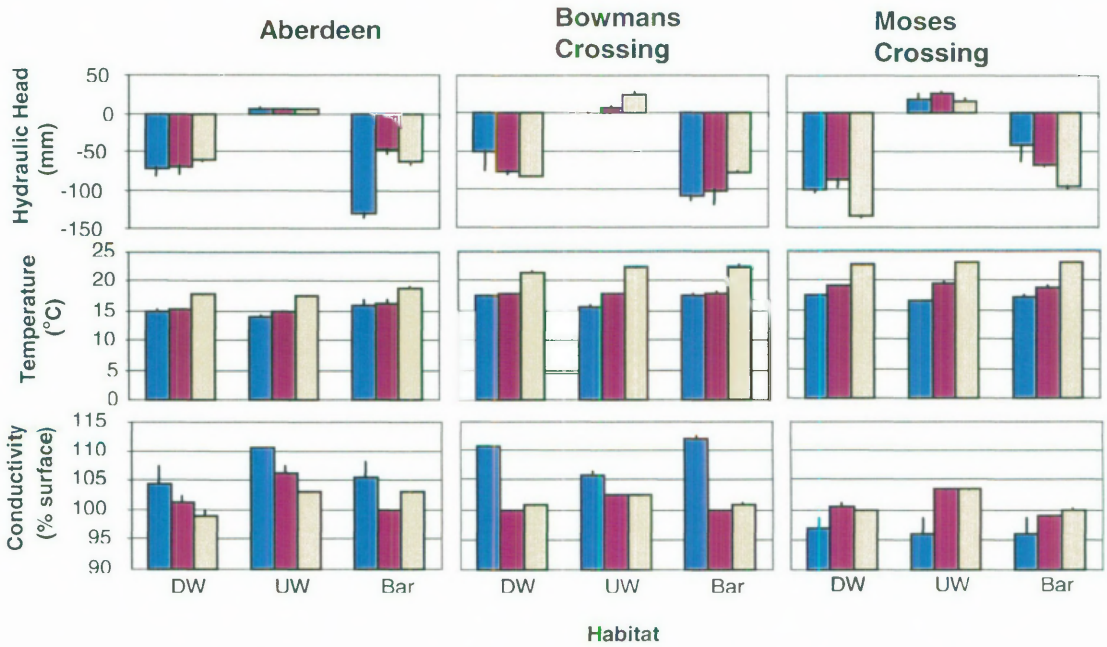
5.4.1 Hydrological data

Peak flow volume from the flow mitigation release was 2660 ML/day at ABER on 15/09/01, and 2602 ML/day at the Jerrys Plains gauging station on 16/09/01 (Figure 5.2). This equated to an increase in river stage of 38 ± 2.5 cm at ABER, 32 ± 2.5 cm at BOWM, and 27 ± 2.5 cm at MOSE despite abstraction. Since 1998, flows of this magnitude or larger have occurred 15 times in the Hunter River (DLWC river gauging data from ABER).

The strength of the negative gradient at both the bar and downwelling habitats exceeded that of upwelling water (Figure 5.7). At the downwelling habitats, the mean hydraulic head (HH) ranged from –134 to – 51 mm. Bar habitats also showed a strong negative trend, ranging from a mean of –129 to – 43 mm. However, upwelling water showed only weak positive gradients throughout the study, with mean values from 4 to 26 mm. The interaction between Time, Site, and Habitat was highly significant for HH due to the strengh of the downwelling at the head of the riffle ($P < 0.001$, Table 5.3). However, no trend consistent with Time was observed over all sites (Figure 5.7). At the BOWM downwelling and upwelling habitats, an increase in HH was observed after the release (Figure 5.7). The marked decrease in HH in the bar at ABER after the release contrasted with the increase observed at the MOSE bar (Figure 5.7). Downwelling HH at MOSE was higher 49 days after the release.

Table 5.3. ANOVA results table for hydraulic head. Bold figures are significant at P = 0.01.

Source	SS	df	MS	F-Ratio	P	Var. comp.	% Var
T	1864.617	2	932.309	9.266	**0.000**	20.54	0.84
S	884.173	2	442.086	4.394	0.017	8.43	0.34
H	170982.543	2	85491.272	849.668	**0.000**	2108.41	86.01
T*S	7927.309	4	1981.827	19.697	**0.000**	92.90	3.79
T*H	3221.160	4	805.290	8.003	**0.000**	34.80	1.42
S*H	9921.827	4	2480.457	24.652	**0.000**	117.52	4.79
T*S*H	6366.025	8	795.753	7.909	**0.000**	68.66	2.80
Error	5433.333	54	100.617				



	Surface temperature (°C)			Surface conductivity (mS)		
	Before	Day 7	Day 49	Before	Day 7	Day 49
Aberdeen	13.35 (0.05)	14.25 (0.15)	16.85 (0.15)	0.34 (0.01)	0.27 (0.00)	0.34 (0.00)
Bowmans	18.65 (0.35)	17.65 (0.05)	21.85 (0.05)	0.46 (0.00)	0.41 (0.00)	0.59 (0.01)
Moses	18.7 (0.00)	20.00 (0.10)	22.70 (0.10)	0.56 (0.00)	0.42 (0.01)	0.63 (0.03)

Figure 5.7. Mean (+ SE) hydraulic head, temperature, and electrical conductivity for the three sites before and after the flow release. Mean and standard error of surface parameters are given in the table.

5.4.2 Sediment characteristics

Sediment size class distribution

The dominant sediment class at ABER was gravel (2 to 32 mm, -1 to -6 ϕ), which averaged between 42 and 85 % but remained fairly stable despite the release at all depths (Table 5.4). Of the three sites sampled, ABER had the highest proportion of silt (<63 μm, > 4 ϕ), which was higher than 3 % in some areas of bar below 20 cm, but which was mostly below 2 % in both riffle habitats (Figure 5.8). Sand (63 μm to 2 mm, 4 to –1 ϕ) was common at ABER, especially below 20 cm (Figure 5.8). Cobbles (> 64 mm, < -6 ϕ) occurred in higher proportions at ABER than BOWM and MOSE, making 21.12 %, 8.53 %, and 9.43 % of the sediment respectively (Figures 5.8 – 5.10).

Gravel was the dominant sediment class at BOWM and MOSE, in the main constituting 60 – 80 % of the sediments (Figures 5.9 and 5.10). The proportion of gravel remained relatively unaffected by the flow release (Figures 5.9 and 5.10). Silt concentration was < 1% at BOWM on all occasions and habitats except for in the upper 10 cm of the bar on day 49 (Figure 5.9a). This was also the time of highest silt concentration in the MOSE bar (Figure 5.10a).

Porosity

The average volume of each 10 cm section of core was 485 ± 20 cm^3 (mean ± SE) at ABER, 302 ± 13 cm^3 at BOWM, and 317 ± 15 cm^3 at MOSE. Interstitial water volumes per section of core averaged 60 mL (range = 9.5 – 280 mL) over all times and sites. Mean porosity differed among Depths within Habitats ($P < 0.001$, Table 5.5, Figure 5.11). At ABER, mean porosity varied with time and depth. Overall mean porosity (± SE) was 14.8 ± 0.2% at the downwelling habitat, 15.2 ± 0.3% at the upwelling habitat, and 16.3 ± 0.2% at the bar habitat averaged over all times and depths. Mean porosity at BOWM averaged 21.7 ± 0.2 % at the downwelling zone, and was higher a week after the release (Figure 5.11). BOWM upwelling zone had an overall average porosity of 19.3 ± 0.3 %. Here, there was an increase in porosity of the top 20 cm of sediment after the release (Figure 5.11). The bar averaged 21.7 ± 0.3 % porosity, and its response to the release varied.

At MOSE, mean porosities were 19.8 ± 0.2 %, 19.4 ± 0.3 %, and 16.6 ± 0.2 % at each of the downwelling, upwelling, and bar habitats (Figure 5.11). MOSE bar and

Table 5.4. Average proportion of each sediment Wentworth size-class at each site. Standard errors are given in brackets.

Class	mm	ϕ	ABER	BOWM	MOSE
Silt and clay	< 0.063	> 4	0.83 (0.05)	0.18 (0.02)	0.17 (0.02)
Very fine sand	0.125 to 0.063	3 to 4	0.56 (0.04)	0.04 (0.01)	0.07 (0.01)
Fine sand	0.25 to 0.125	2 to 3	0.95 (0.07)	0.19 (0.01)	0.24 (0.03)
Medium sand	0.5 to 0.25	1 to 2	1.75 (0.11)	3.39 (0.20)	2.03 (0.14)
Coarse sand	1 to 0.5	0 to 1	2.09 (0.13)	6.23 (0.30)	7.65 (0.49)
Very coarse sand	2 to 1	-1 to 0	2.97 (0.23)	10.88 (0.52)	10.26 (0.48)
Very fine gravel	4 to 2	-1 to -2	4.23 (0.28)	10.01 (0.47)	8.33 (0.39)
Fine gravel	8 to 4	-3 to -2	6.74 (0.35)	11.30 (0.45)	9.66 (0.44)
Medium gravel	16 to 8	-4 to -3	12.12 (0.63)	15.36 (0.60)	14.08 (0.63)
Coarse gravel	32 to 16	-5 to -4	16.73 (0.84)	16.30 (0.88)	15.55 (0.79)
Very coarse gravel	64 to 32	-6 to -5	30.57 (1.66)	17.68 (1.45)	22.54 (1.66)
Cobbles and boulders	>64	< -6	21.12 (2.18)	8.53 (1.63)	9.43 (1.74)

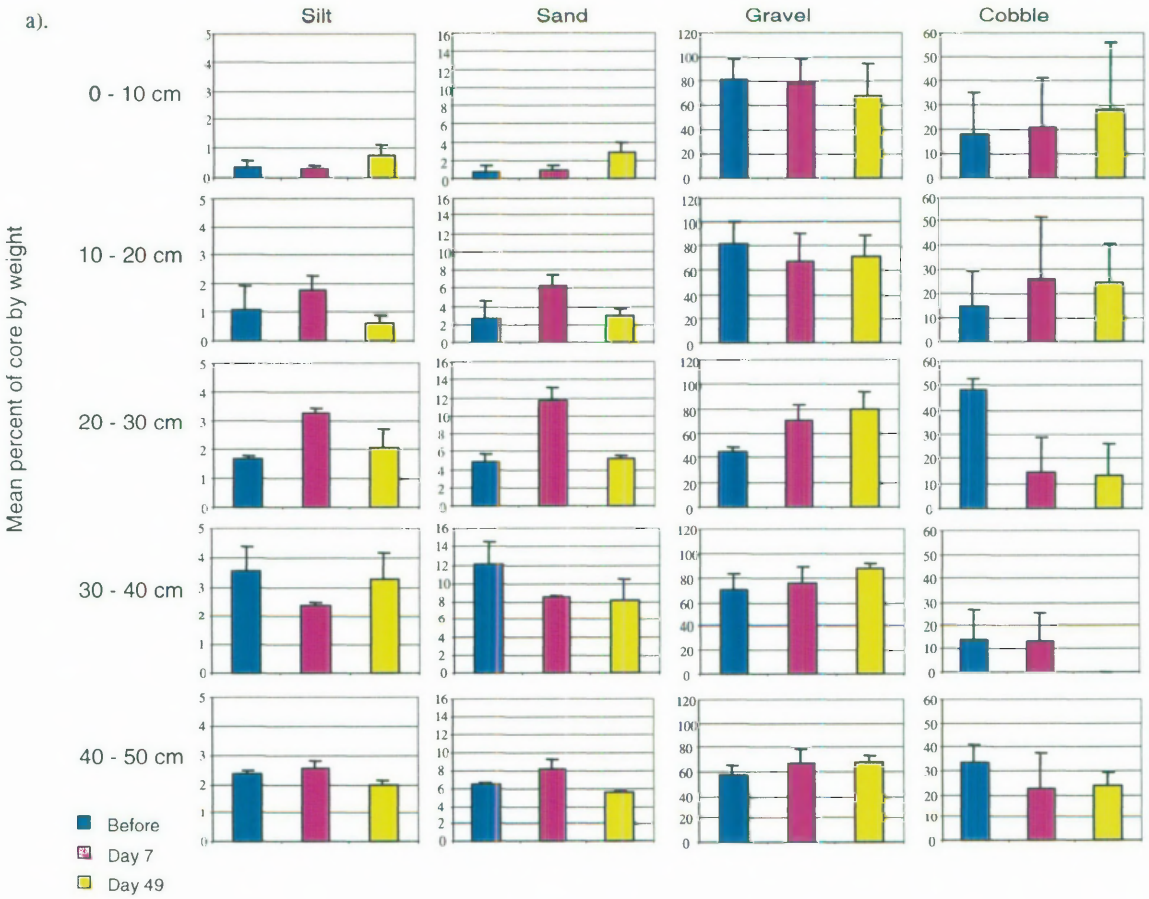


Figure 5.8. Mean sediment particle size distribution (+SE) for a) the bar, b) downwelling zone, and c) upwelling zone at Aberdeen. Blue = before release, red = Day 7, yellow = Day 49.

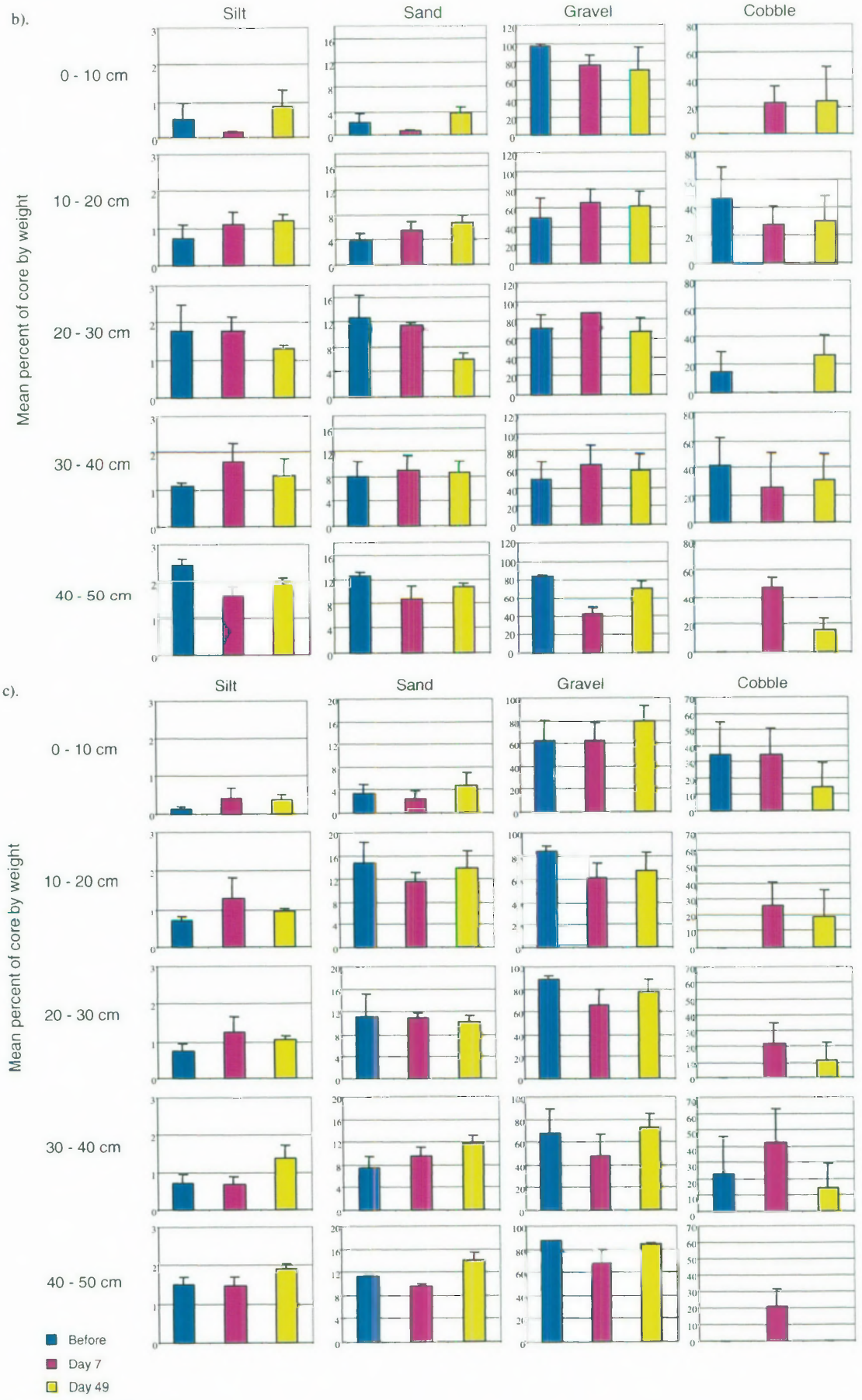


Figure 5.8. continued

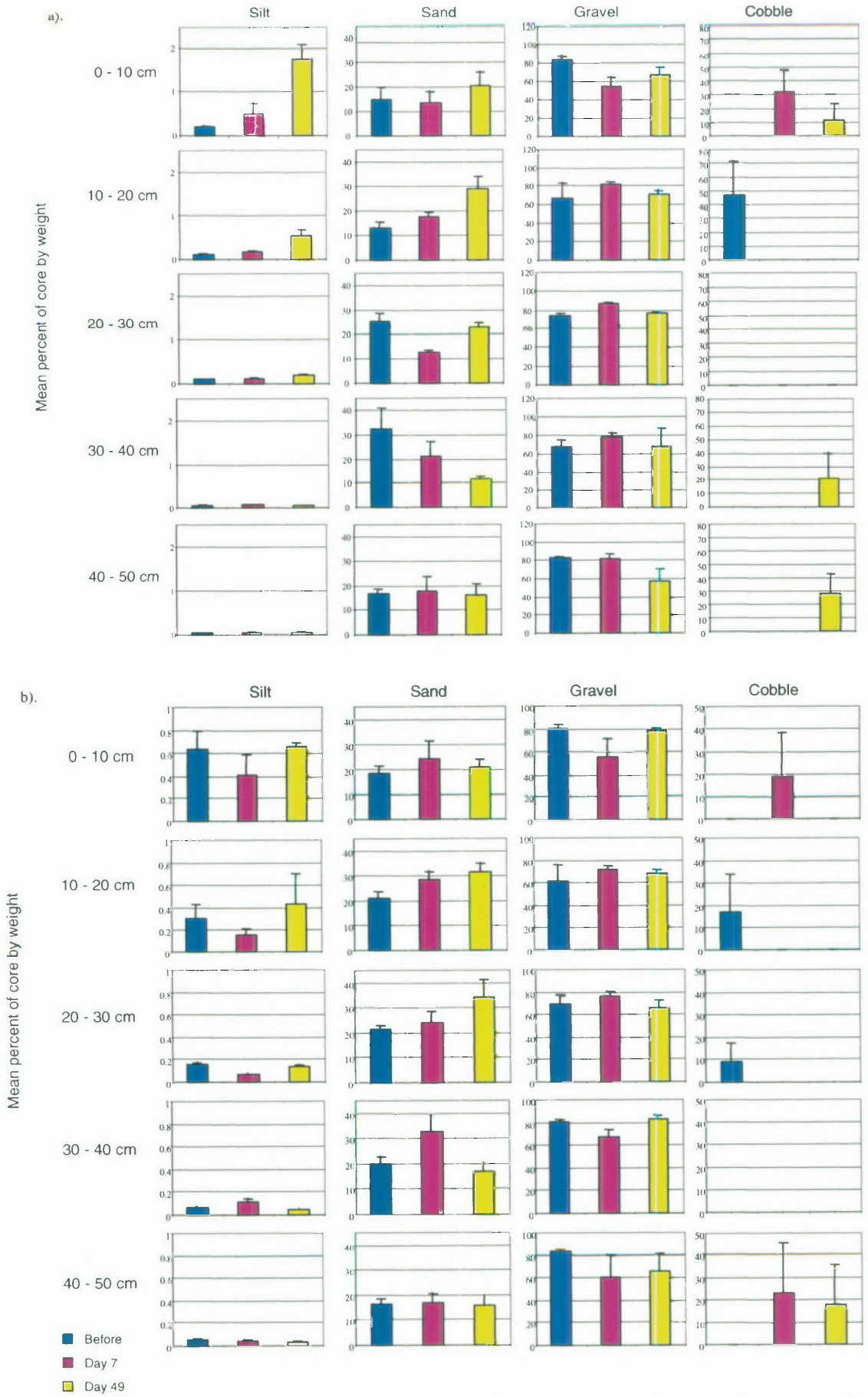


Figure 5.9. Mean sediment particle size distribution (+ SE) for a) the bar, b) downwelling zone, and c) upwelling zone at Bowmans Crossing. Blue = before release, red = Day 7, yellow = Day 49.

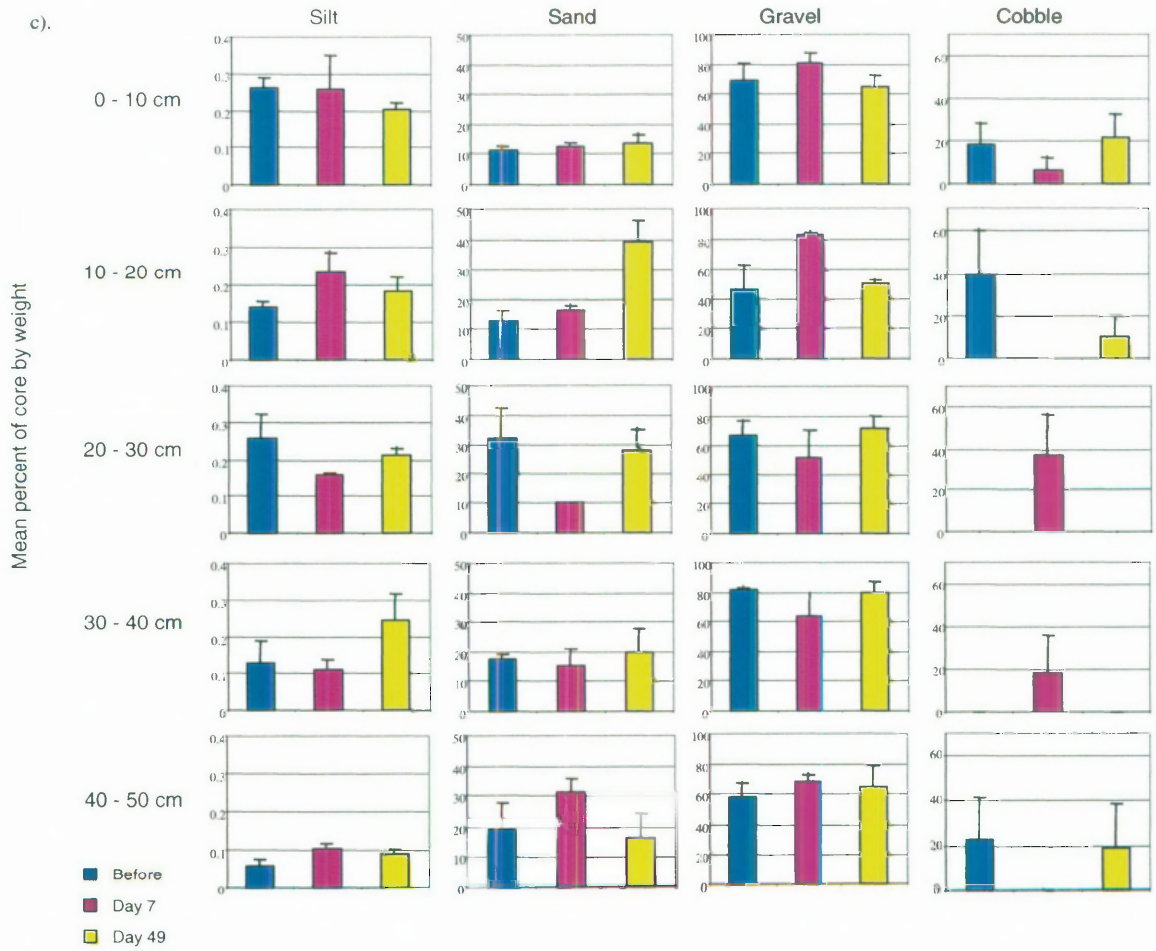


Figure 5.9. continued

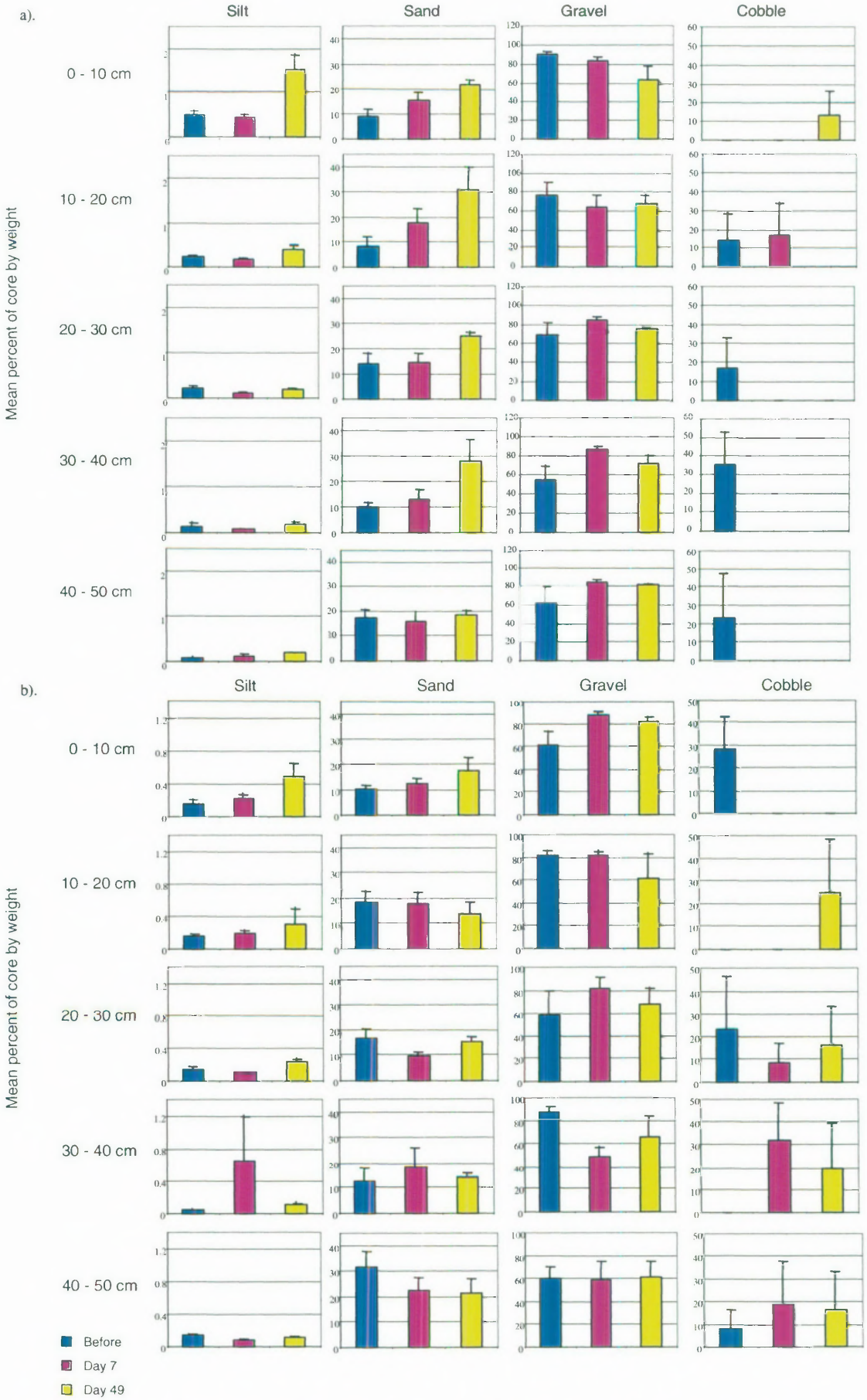


Figure 5.10. Mean sediment particle size distribution (+ SE) for a) the bar, b) downwelling zone, and c) upwelling zone at Moses Crossing. Blue = before release, red = Day 7, yellow = Day 49.

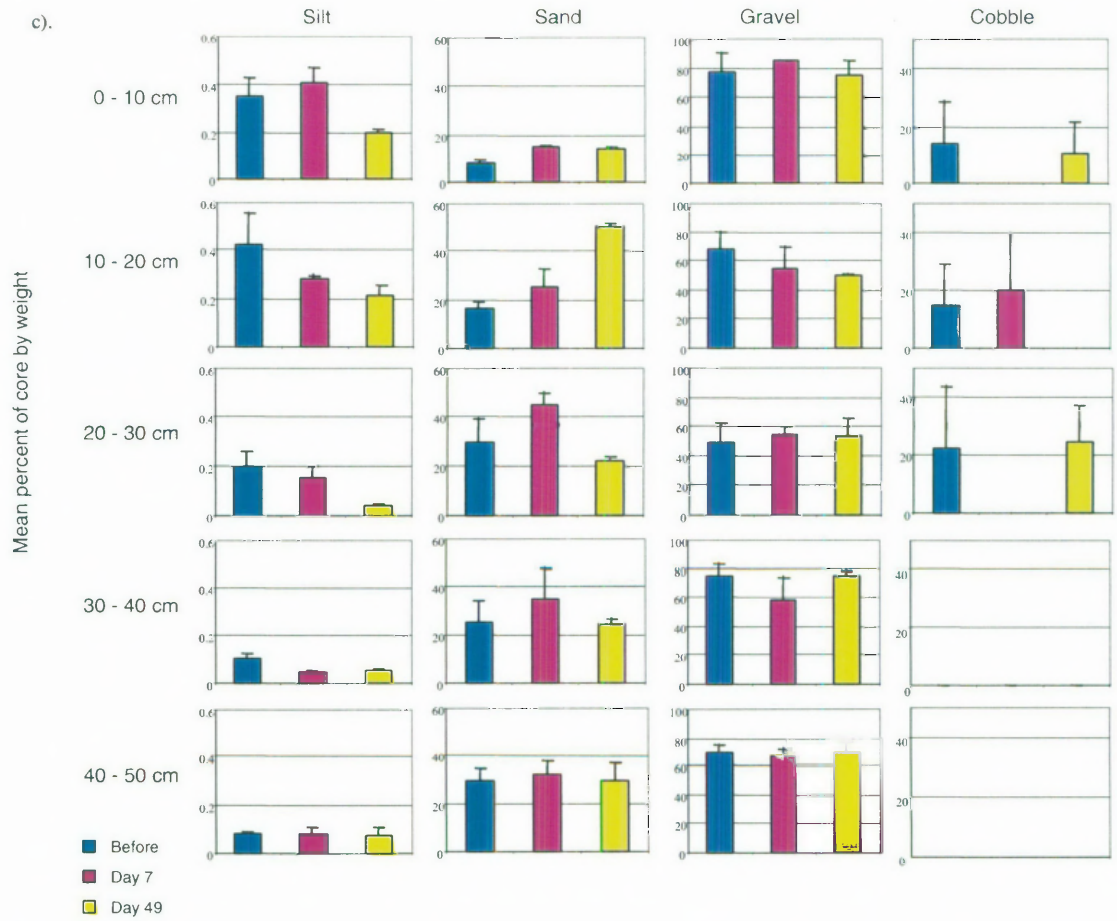


Figure 5.10. continued

Table 5.5. ANOVA results table for sediment porosity. Bold figures are significant at P = 0.01.

Source	SS	df	MS	F-Ratio	P	Var. comp.	% Var
H	46.332	2	23.166	0.260	0.775	0.00	0.00
S	2083.358	2	1041.679	32.366	**0.000**	7.48	36.38
T	924.980	2	462.490	16.023	**0.000**	5.35	26.04
S*H	465.761	4	116.440	3.618	0.019	1.87	9.11
T*H	63.469	4	15.867	0.550	0.701	0.00	0.00
T*S	321.291	4	80.323	2.582	0.049	1.82	8.87
T*S*H	188.045	8	23.506	0.756	0.643	0.00	0.00
D(H)	1069.078	12	89.090	3.475	**0.000**	1.41	6.86
S*D(H)	772.424	24	32.184	1.255	0.195	0.44	2.12
T*D(H)	692.733	24	28.864	1.126	0.314	0.36	1.75
T*S*D(H)	1493.265	48	31.110	1.214	0.173	1.83	8.88
Error	6921.501	270	25.635				

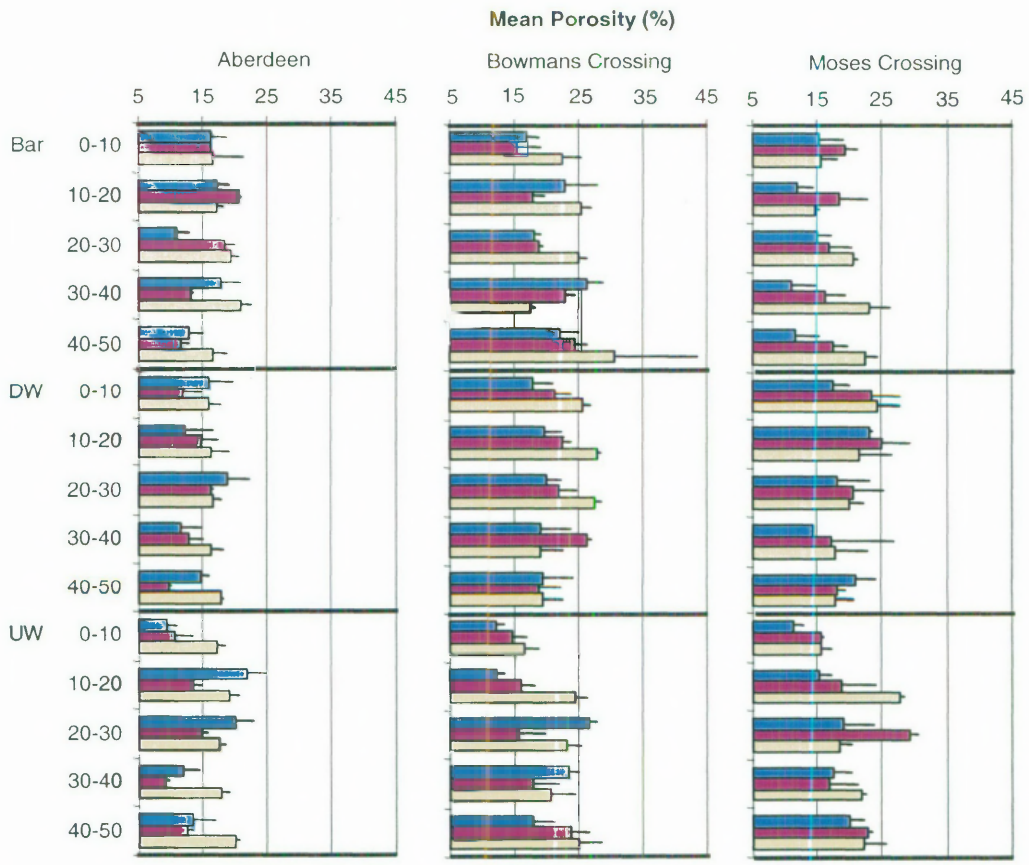


Figure 5.11. Mean porosity (+ SE), for each depth section at each site. Blue = before release, red = Day 7, yellow = Day 49.

downwelling habitats increased in porosity between the pre- and initial post-release samplings for the first 40 cm, but the increase in porosity only occurred in the upper 20 cm at the upwelling zone (Figure 5.11). Time was highly significant ($P < 0.001$, Table 5.5), and overall porosity for the first two Times (16.9 ± 0.3% and 17.7 ± 0.2 %) were similar to each other ($P = 0.45$) but different to Time 3 (20.2 ± 0.2 %). Sites were also significantly different ($P < 0.001$, Table 5.5), with BOWM Habitats being more porous than the other two.

Fine sediments

Fine sediments (< 125 μm by dry mass; mean ± SE) at ABER made up an average of 4.21 ± 0.14 % of each core section in the bar habitat, 3.00 ± 0.07% in the downwelling zone, and 2.00 ± 0.007 in the upwelling zone (Figure 5.12). Sections of core from BOWM contained 0.40 ± 0.04% (bar), 0.28 ± 0.02 % (downwelling), and 0.29 ± 0.01 % (upwelling) of fines. At MOSE, proportions of fines were 0.51 ± 0.04 %, 0.26 ± 0.01 %, and 0.29 ± 0.02 % respectively for the three habitats. Overall, ABER core sections averaged 3.07 ± 0.09 % fines which was higher than the other two Sites ($P < 0.001$, Table 5.6). This contributed 75.7% to the variance analysed in this ANOVA. The content of fines at BOWM and MOSE were similar ($P = 0.74$) with fines making up 0.32 ± 0.02 % and 0.35 ± 0.02 % of the sediment matrix respectively.

There was a strong interaction for Depth nested in Habitat at each Site ($P < 0.001$, Table 5.6), with fines being more common in the upper 10 cm for BOWM and MOSE, whereas fines at ABER occurred deeper in the bed. Significant temporal interactions were present between site and habitat ($P < 0.001$, Table 5.6) with bar habitats having more fines than the other two. This was especially the case 49 d after the release at MOSE and BOWM. The Time-Depth interaction is not significant at $P = 0.01$ ($P = 0.015$, Table 5.6), probably because the relatively high proportion of fines at ABER outweighed interactions with time and depth in the other two sites, or the high proportion of fines for Day 49 at BOWM and MOSE obscured any interaction.

The amount of fines in the sediments of ABER was higher after the release for all depths except the upper 10 cm in the downwelling zone, where fines appeared to be flushed (Figure 5.12). Both riffle habitats at BOWM experienced a decrease in the amount of fines present in the upper 20 cm whereas fines increased in the top 20 cm of

Table 5.6. ANOVA results table for the proportion of fine sediments. Bold figures are significant at P = 0.01.

Percent fines - log transformed

Source	SS	df	MS	F-Ratio	P	Var. comp.	% Var
H	1.377	2	0.688	1.511	0.260	0.00	0.39
S	92.577	2	46.289	42.379	**0.000**	0.33	75.73
T	0.559	2	0.280	2.803	0.081	0.00	0.50
S*H	2.418	4	0.604	0.553	0.698	0.00	0.00
T*H	0.715	4	0.179	1.790	0.164	0.00	0.66
T*S	1.093	4	0.273	5.528	**0.001**	0.01	1.88
T*S*H	2.392	8	0.299	6.049	**0.000**	0.01	2.09
D(H)	5.467	12	0.456	8.188	**0.000**	0.01	2.01
S*D(H)	26.214	24	1.092	19.631	**0.000**	0.07	15.62
T*D(H)	2.395	24	0.100	1.794	0.015	0.00	1.11
T*S*D(H)	2.372	48	0.049	0.888	0.682	0.00	0.00
Error	15.022	270	0.056				

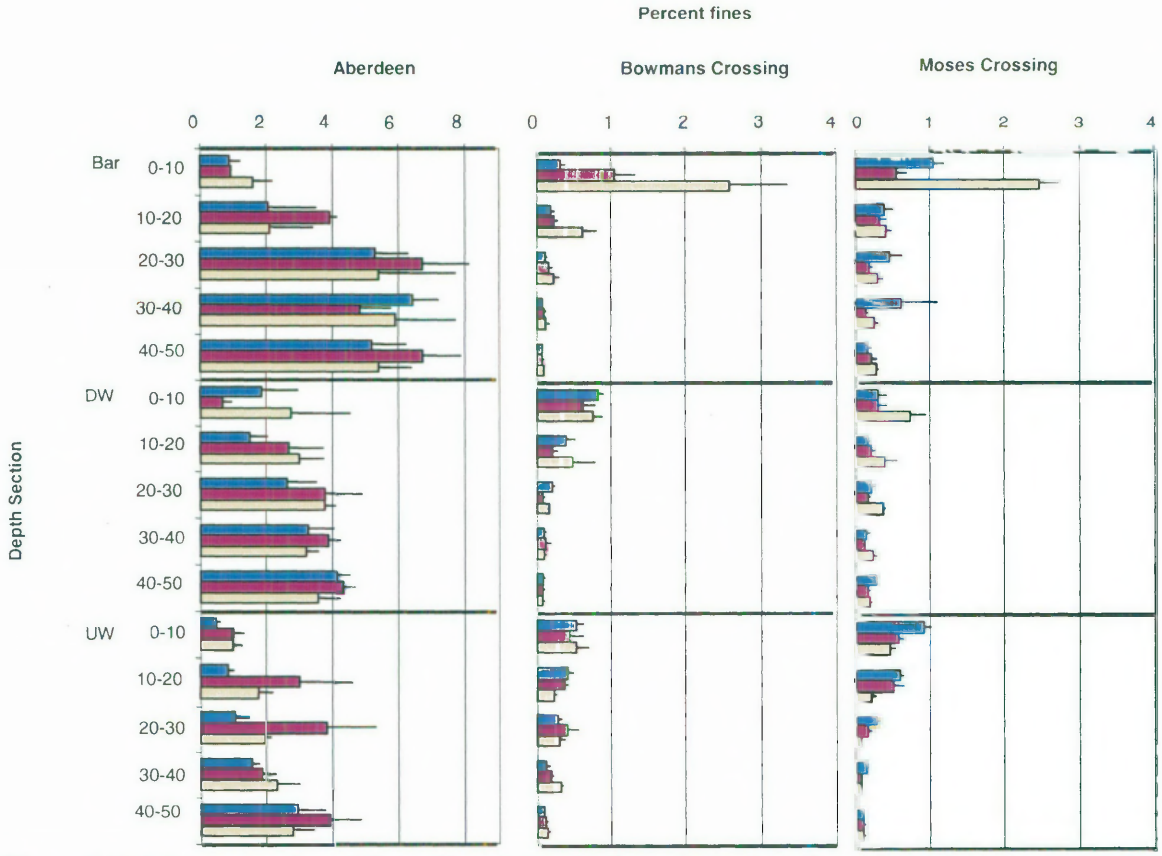


Figure 5.12. Mean proportion of fine sediments (+ SE), for each depth section at each site. Blue = before release, red = Day 7, yellow = Day 49.

Table 5.7. ANOVA results table for temperature. Bold figures are significant at P = 0.01.

Temperature (rank transformed)

Source	SS	df	MS	F-Ratio	P	Var. comp.	% Var
T	21476.963	2	10738.481	169.135	**0.000**	263.58	54.52
S	14834.463	2	7417.231	116.824	**0.000**	181.57	37.56
H	1067.185	2	533.593	8.404	**0.001**	11.61	2.40
T*S	1711.241	4	427.810	6.738	**0.000**	17.99	3.72
T*H	558.185	4	139.546	2.198	0.081	3.76	0.78
S*H	776.519	4	194.130	3.058	0.024	6.45	1.33
T*S*H	382.944	8	47.868	0.754	0.644	-1.54	-0.32
Error	3428.500	54	63.491				

Table 5.8. ANOVA results table for the electrical conductivity. Bold figures are significant at P = 0.01.

Conductivity % surface

Source	SS	df	MS	F-Ratio	P	Var. comp.	% Var
T	137.088	2	68.544	16.479	**0.000**	1.59	10.59
S	337.844	2	168.922	40.611	**0.000**	4.07	27.10
H	75.615	2	37.808	9.089	**0.000**	0.83	5.53
T*S	556.195	4	139.049	33.429	**0.000**	6.66	44.36
T*H	64.372	4	16.093	3.869	**0.008**	0.59	3.92
S*H	77.954	4	19.488	4.685	**0.003**	0.76	5.04
T*S*H	75.271	8	9.409	2.262	0.036	0.52	3.45
Error	224.615	54	4.160				

the bar (Figure 5.12). At MOSE, there was a decrease in fines between pre-release and initial post-release samples in most core sections, with the exception of the upper two downwelling sections. For most core sections at BOWM and MOSE, there was a large input of fines by 49 d post-release. As would be expected, there was a moderate negative correlation between porosity and the proportion of fines (r_{390}= -0.396, $P < 0.001$).

5.4.3 Physico-chemical variables

Temperature

Across all sites, interstitial water temperature ranged from 14 – 17 °C before the flow release, 15 – 22 °C seven days after, and 17 – 23 °C forty-nine days later. Temperature was higher 49 days after the flow release than it was 7 days after and prior to the release ($P < 0.001$, Table 5.7, Figure 5.7). Temperature increased with each time at all sites ($P < 0.001$, Table 5.7), with temperature being higher at downstream sites. The main factor interaction of temperature among Time ($P < 0.001$, Table 5.7) contributed 55 % of the variance in this analysis, indicating a strong temporal influence. Similar temperatures occurred in bar and downwelling habitats ($P = 0.21$), and downwelling and upwelling habitats ($P = 0.054$).

Electrical conductivity

Mean interstitial EC at all sites ranged from 0.35 – 0.51 mS/cm before the release to 0.27-0.42 mS/cm and 0.35 – 0.62 mS/cm for the two times post-release. As a percent of surface conductivity, interstitial EC ranged from 96 - 112 % throughout the study (Figure 5.7). The Time-Site interaction was highly significant ($P <0.001$, Table 5.8), and contributed most to the total variance (44.36 %, Table 5.8). EC at ABER and BOWM was lower for both sampling occasions after the flow release than it was before (Figure 5.7). At these sites, relative EC exceeded 100 % of the surface EC, indicating some mixing with groundwater. Conductivity at MOSE showed a converse trend, being higher at both times after the release. Across all sites, EC at both times after the release were similar ($P = 0.988$), but different to conductivity before. EC at ABER was similar to that at BOWM ($P = 0.88$), but was higher than at MOSE ($P < 0.001$). Downwelling zones had similar EC to bar habitats ($P = 0.952$) but not upwelling zones ($P = 0.003$).

Dissolved oxygen

Dissolved oxygen (DO) differed at each time for all habitats in the three sites ($P < 0.001$, Table 5.9). At downwelling habitats, DO % saturation averaged (± SE) 87.3 ± 7.1, 80.9 ± 3.3, and 66.3 ± 12.3 before the flow release and for the two consecutive times after. Bars had DO concentrations of 82.8 ± 7.8, 87.2 ± 7.8, and 40.2 ± 6.7 % saturation for pre-, initial post-, and 49 day post-release respectively. DO saturations at upwelling zones were consistently lower than those of the other two habitats, being 36.2 ± 6.6, 24.9 ± 5.4, and 20.6 ± 5.3 % saturation for the three times respectively. There was a highly significant interaction between Habitat, Site, and Time ($P < 0.001$, Table 5.9), suggesting that DO differed with time at each of the sites' habitats. DO in the bar habitats of ABER and MOSE increased from pre- to initial post-release sampling (Figure 5.9). At BOWM, DO in all three habitats decreased over time (Figure 5.13). However this did not hold true for the habitats at the other sites, or for the overall Time-Habitat interaction ($P = 0.001$, Table 5.9). The among-Habitat main factor ($P < 0.001$) strongly contributed to variance for DO (60.01 %) with shallow downwelling water having higher DO than deep downwelling and upwelling habitats ($P < 0.001$, Figure 5.13). Post-release DO saturations were similar to each other ($P = 0.531$).

Nitrate and nitrite concentrations

Interstitial nitrate and nitrite concentrations (NOx) ranged from 0.01 – 0.20 mgL^{-1} throughout the study, which equated to a range of 56 – 664 % of surface concentrations (Figure 5.13). At downwelling zones, mean NOx concentrations (± SE) were 0.12 ± 0.01 mg/L before the release, 0.08 ± 0.01mg/L at Day 7, and 0.09 ± 0.01 mg/L at Day 49 after the release. Upwelling habitats had mean NOx concentrations of 0.11 ± 0.01, 0.11 ± 0.00, and 0.12 ± 0.03 mg/L respectively for the three times. Bar NOx concentrations were 0.10 ± 0.01, 0.07 ± 0.02, and 0.10 ± 0.01 mg/L respectively for the three times.

As interest lies in the extent of nitrification in the hyporheic zone promoted by the release, rather than the actual NOx concentration, interstitial NOx concentration is expressed as a percent of surface concentration. Interactions between all crossed and main factors were significant (Table 5.10). There was a significant interaction between Time, Site, and Habitat ($P = 0.003$, Table 5.10). As there was also a strong and significant interaction between site and habitat ($P < 0.001$, contributing 34.86% to the

Table 5.9. ANOVA results table for dissolved oxygen. Bold figures are significant at P = 0.01.

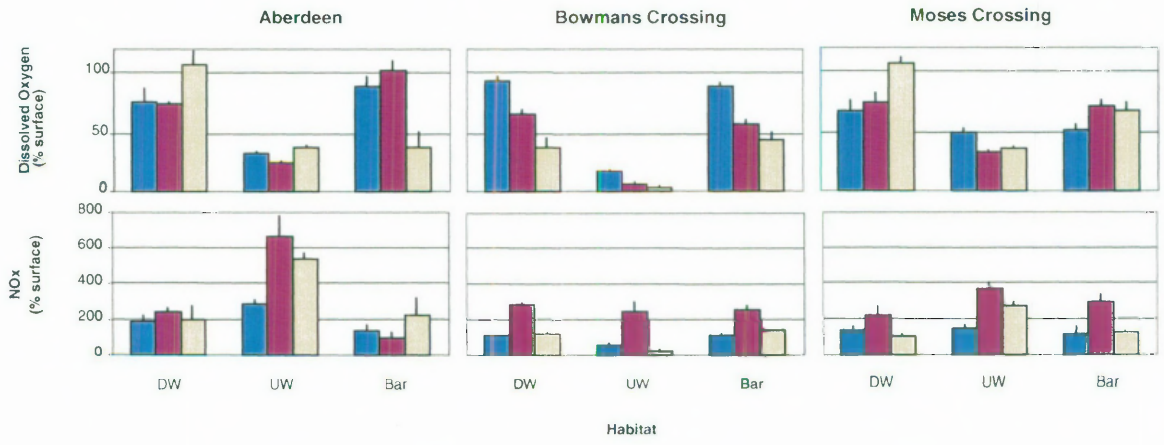
DO %surface

Source	SS	df	MS	F-Ratio	P	Var. comp.	% Var
T	1183.278	2	591.639	4.985	**0.010**	11.68	1.46
S	5124.747	2	2562.374	21.591	**0.000**	60.34	7.52
H	39248.982	2	19624.491	165.361	**0.000**	481.63	60.01
T*S	6366.956	4	1591.739	13.412	**0.000**	72.74	9.06
T*H	4373.682	4	1093.421	9.213	**0.000**	48.14	6.00
S*H	2337.896	4	584.474	4.925	**0.002**	23.00	2.87
T*S*H	9456.061	8	1182.008	9.960	**0.000**	105.02	13.09
Error	6408.545	54	118.677				

Table 5.10. ANOVA results table for nitrate and nitrite nitrogen. Bold figures are significant at P = 0.01.

NOx -N% surface - Log(x)

Source	SS	df	MS	F-Ratio	P	Var. comp.	% Var
T	1.474	2	0.737	29.415	**0.000**	0.02	22.52
S	1.148	2	0.574	22.911	**0.000**	0.01	17.37
H	0.243	2	0.121	4.841	0.012	0.00	3.04
T*S	0.834	4	0.209	8.324	**0.000**	0.01	11.64
T*H	0.287	4	0.072	2.867	0.032	0.00	2.97
S*H	2.304	4	0.576	22.997	**0.000**	0.03	34.86
T*S*H	0.681	8	0.085	3.396	**0.003**	0.01	7.59
Error	1.353	54	0.025				



	Surface dissolved oxygen (% saturation)			SurfaceNOx (mg/L)		
	Before	Day 7	Day 49	Before	Day 7	Day 49
Aberdeen	97.35 (0.75)	107.95 (2.55)	93.50 (1.10)	0.04 (0.01)	0.02 (0.01)	0.02 (0.00)
Bowmans	118.45 (0.45)	110.60 (0.30)	104.15 (0.75)	0.13 (0.02)	0.04 (0.01)	0.09 (0.01)
Moses	115.75 (0.35)	124.05 (0.65)	129.95 (0.55)	0.10 (0.00)	0.03 (0.00)	0.03 (0.01)

Figure 5.13. Mean (+ SE) dissolved oxygen, and nitrate and nitrite nitrogen for the three sites before and after the flow release. Mean and standard error of surface parameters are given in the table.

variance, Table 5.10) some difference can be attributed to time. NOx at all sites was higher 7 days after the release than it was pre-release for all Habitats, except for the bar at ABER (Figure 5.13). Additionally, with the exception of the ABER bar, all NOx concentrations were lower 49 days after the release than they were at 7 days. A strong Time-Site interaction was present ($P < 0.001$, Table 5.10). No correlation was found between NOx and dissolved oxygen ($r_{79} = -0.100$, $P = 0.390$).

5.4 .4 Total invertebrate numbers and taxonomic richness

In all 46 taxa were collected from the hyporheic and parafluvial areas of the Hunter River with the pump sampler. There was a positive interaction among Time, Site, and Habitat ($P = 0.002$, Table 5.11), with ABER having more taxa than the other two sites (Figure 5.14). Taxonomic richness declined after the release at the two riffle habitats at ABER and at the upwelling habitat at BOWM. Other habitats at the three sites increased in richness (Figure 5.14). Taxonomic richness differed among sites ($P < 0.001$, Table 5.11), contributing to 46.6 % of the total variance. Among habitats, there was a difference ($P < 0.001$, Table 5.11) in taxonomic richness, with downwelling usually having the highest number of taxa. Taxonomic richness was positively correlated with DO ($r_{79} = 0.399$, $P < 0.001$).

Invertebrate abundance averaged (± SE) 64 ± 10 animals per 6L sample at ABER, 145 ± 24 animals at BOWM, and 80 ± 11 animals at MOSE. The number of invertebrates differed at each habitat among sites ($P < 0.001$, Table 5.12), and also at each habitat among times ($P < 0.001$, Table 5.12). Upwelling habitats at each site averaged fewer invertebrates than downwelling and bar habitats (Figure 5.14). Invertebrates became more abundant in all three bars following the release, but less abundant in all riffle habitats except the downwelling at BOWM and upwelling at MOSE (Figure 5.14). Invertebrate abundance also correlated strongly with dissolved oxygen ($r_{79} = 0.546$, $P < 0.001$).

Freeze-core samples yielded more taxa than the pump samples (60 taxa over all sites and habitats). However, 36 of these were taxa found only in the upper 20 cm of bed and would not have been sampled by the pump sampler at 40 cm depth. All interactions containing the sub-plot variable (Depth nested within Habitat) were significant ($P < 0.01$, Table 5.13), indicating that the number of taxa per 10-cm core section differed (generally decreased)

with Depth (Figure 5.15). The significant Time – Depth interaction ($P = 0.002$, Table 5.13) is most likely due to the increase in invertebrate numbers at all depths of the BOWM bar following the release, and the increase in taxonomic richness at 49 d at the ABER habitats (Figure 5.15). At MOSE there were more taxa in the 0 – 10 cm core sections at all three times (mean = 5 taxa) than in deeper sections (mean = 1 taxon; Figure 5.15). This was also the case among times at ABER and BOWM (Figure 5.15). Following the release, richness was lower in the top 0 – 10 cm but higher in 10 – 20 cm in riffle habitats at BOWM.

Each section of core at ABER averaged 12 animals, with BOWM and MOSE averaging 20 and 12 animals respectively. As with taxonomic richness, invertebrate abundances had significant interactions for all factors containing the sub-plot variable Depth ($P < 0.01$, Table 5.14). At most Times and Sites, there were more invertebrates in the upper 20 cm of sediment at each habitat. Depth nested within Habitat made the highest contribution to variance in the analysis (35.7 %). There was no correlation between invertebrate numbers and either porosity or percent fines. Seven days post-release, invertebrate numbers at most depths at BOWM were greater than they were before the release whereas MOSE habitats experienced a drop in numbers after the release, but only at 0 – 10 cm depth (Figure 5.16).

Table 5.11. ANOVA results table for taxonomic richness for samples collected with the pump sampler. Bold figures are significant at P = 0.01.

Taxonomic diversity

Source	SS	df	MS	F-Ratio	P	Var. comp.	% Var
T	6.722	2	3.361	1.175	0.317	0.01	0.27
S	181.241	2	90.620	31.673	**0.000**	2.17	46.60
H	104.389	2	52.194	18.243	**0.000**	1.22	26.19
T*S	27.704	4	6.926	2.421	0.059	0.20	4.32
T*H	40.889	4	10.222	3.573	0.012	0.36	7.82
S*H	7.926	4	1.981	0.693	0.600	-0.04	-0.93
T*S*H	82.185	8	10.273	3.591	**0.002**	0.73	15.74
Error	154.500	54	2.861				

Table 5.12. ANOVA results table for invertebrate abundance for samples collected with the pump sampler. Bold figures are significant at P = 0.01.

Invertebrate abundance - Log(x+1) transformed

Source	SS	df	MS	F-Ratio	P	Var. comp.	% Var
T	0.367	2	0.183	3.151	0.051	0.00	1.93
S	0.434	2	0.217	3.726	0.030	0.00	2.46
H	6.904	2	3.452	59.281	**0.000**	0.08	52.44
T*S	0.759	4	0.190	3.259	0.018	0.01	4.08
T*H	1.409	4	0.352	6.050	**0.000**	0.01	9.09
S*H	3.524	4	0.881	15.131	**0.000**	0.04	25.43
T*S*H	1.057	8	0.132	2.269	0.036	0.01	4.57
Error	3.144	54	0.058				

Table 5.13. ANOVA results table for taxonomic richness for samples collected with the freeze-core sampler. Bold figures are significant at P = 0.01.

Taxonomic richness - Log(x+1) transformed

Source	SS	df	MS	F-Ratio	P	Var. comp.	% Var
H	0.626	2	0.313	0.272	0.766	0.00	0.00
S	0.903	2	0.451	4.624	0.020	0.00	3.40
T	1.518	2	0.759	10.850	**0.000**	0.01	11.08
S*H	0.316	4	0.079	0.809	0.532	0.00	-0.55
T*H	0.051	4	0.128	1.826	0.157	0.00	2.80
T*S	1.968	4	0.492	7.620	**0.000**	0.02	20.59
T*S*H	1.431	8	0.179	2.770	0.013	0.00	5.50
D(H)	13.786	12	1.149	35.115	**0.000**	0.02	32.29
S*D(H)	2.343	24	0.098	2.984	**0.000**	0.00	5.64
T*D(H)	1.679	24	0.070	2.138	**0.002**	0.00	5.35
T*S*D(H)	3.100	48	0.065	1.974	**0.000**	0.01	13.89
Error	8.834	270	0.033				

Table 5.14. ANOVA results table for invertebrate abundance for samples collected with the freeze-core sampler. Bold figures are significant at P = 0.01.

Invertebrate numbers - Log(x+1) transformed

Source	SS	df	MS	F-Ratio	P	Var. comp.	% Var
H	3.987	2	1.994	0.354	0.709	0.00	0.00
S	4.215	2	2.108	4.998	**0.015**	0.01	3.63
T	2.173	2	1.087	5.173	**0.014**	0.01	3.15
S*H	1.061	4	0.265	0.629	0.647	0.00	0.00
T*H	2.443	4	0.611	2.606	0.043	0.01	4.32
T*S	9.519	4	2.380	10.977	**0.000**	0.08	23.29
T*S*H	8.814	8	1.102	5.082	**0.000**	0.03	9.53
D(H)	67.572	12	5.631	53.826	**0.000**	0.12	35.70
S*D(H)	10.121	24	0.422	4.031	**0.000**	0.02	6.14
T*D(H)	5.040	24	0.210	2.008	**0.004**	0.01	3.39
T*S*D(H)	10.406	48	0.217	2.072	**0.000**	0.04	10.85
Error	28.246	270	0.105				

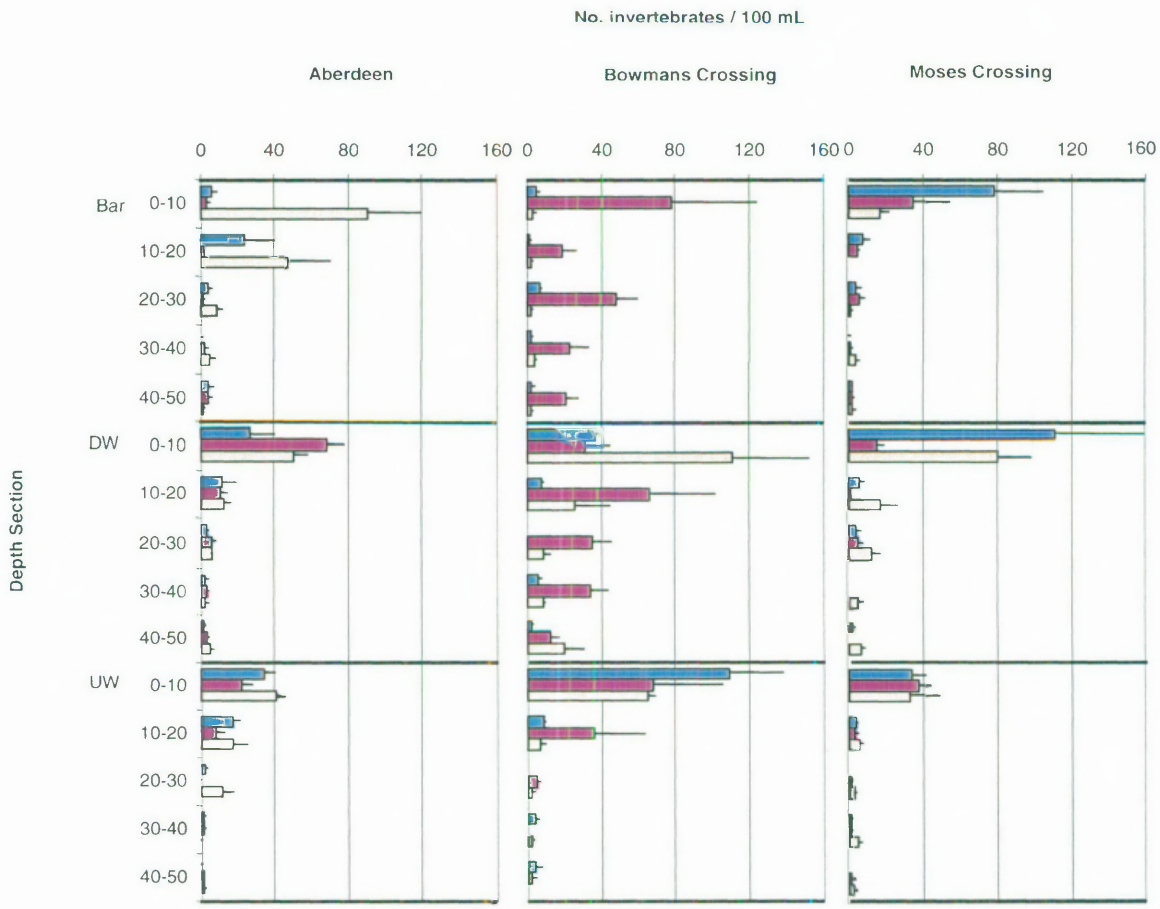


Figure 5.16. Mean invertebrate abundance (+ SE) per 100 mL of interstitial water for each depth section at each site. Blue = before release, red = Day 7, yellow = Day 49.

Table 5.14. ANOVA results table for invertebrate abundance for samples collected with the freeze-core sampler. Bold figures are significant at P = 0.01.

Invertebrate numbers - Log(x+1) transformed

Source	SS	df	MS	F-Ratio	P	Var. comp.	% Var
H	3.987	2	1.994	0.354	0.709	0.00	0.00
S	4.215	2	2.108	4.998	**0.015**	0.01	3.63
T	2.173	2	1.087	5.173	**0.014**	0.01	3.15
S*H	1.061	4	0.265	0.629	0.647	0.00	0.00
T*H	2.443	4	0.611	2.606	0.043	0.01	4.32
T*S	9.519	4	2.380	10.977	**0.000**	0.08	23.29
T*S*H	8.814	8	1.102	5.082	**0.000**	0.03	9.53
D(H)	67.572	12	5.631	53.826	**0.000**	0.12	35.70
S*D(H)	10.121	24	0.422	4.031	**0.000**	0.02	6.14
T*D(H)	5.040	24	0.210	2.008	**0.004**	0.01	3.39
T*S*D(H)	10.406	48	0.217	2.072	**0.000**	0.04	10.85
Error	28.246	270	0.105				

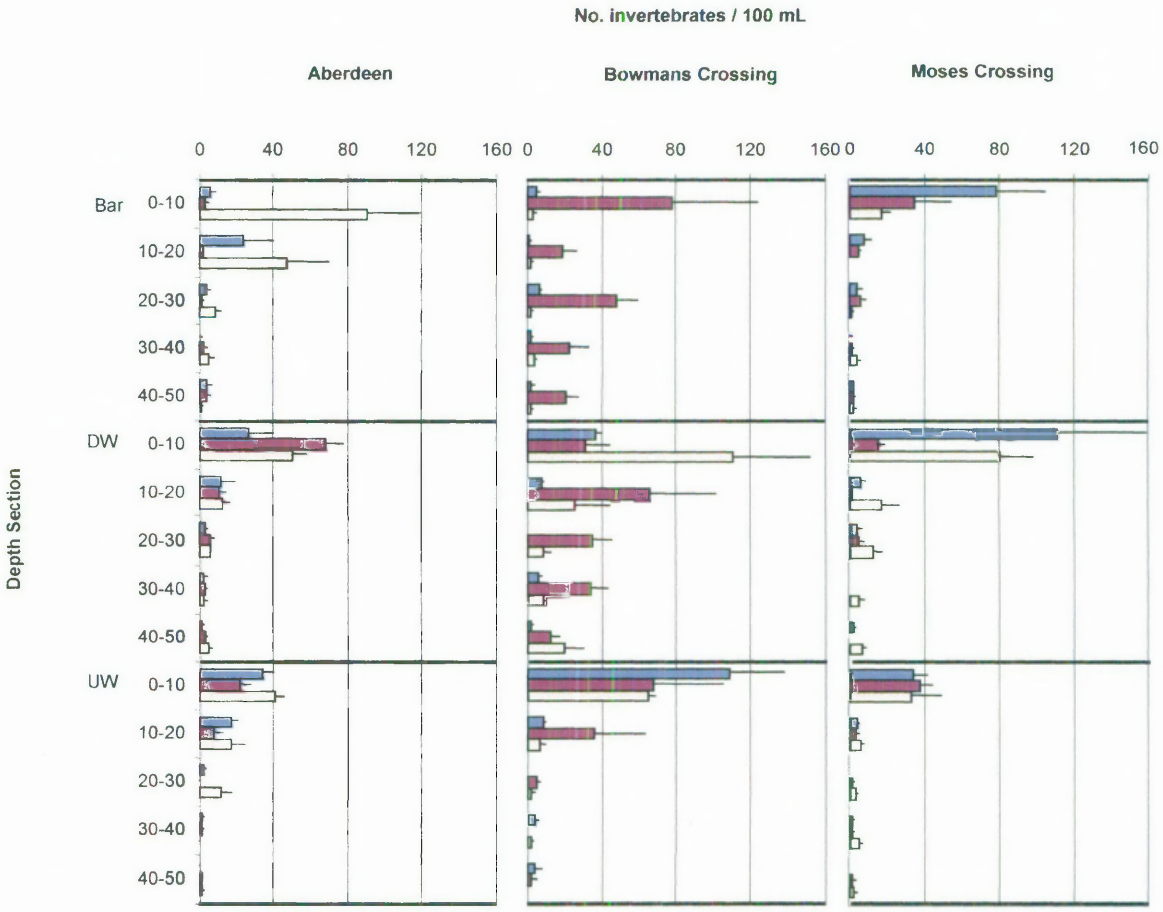


Figure 5.16. Mean invertebrate abundance (+ SE) per 100 mL of interstitial water for each depth section at each site. Blue = before release, red = Day 7, yellow = Day 49.

5.4.5 Community dynamics

Spatial analysis of pump data

The nMDS plots of the interstitial fauna collected from a depth of 40 cm with the pump sampler showed distinct groupings (Figure 5.17). Pairwise ANOSIM revealed that community structure at BOWM and MOSE were similar (P = 0.024, Table 5.15), but both differed from that at ABER. Fauna in each habitat was different for each site, with the community composition at ABER bar being similar to its upwelling zone (P = 0.017, Table 5.15), and the bar and downwelling area at BOWM being similar (P = 0.018, Table 5.15).

Four common taxa (Oligochaeta, Cyclopoida, Parastenocaridae, and Microturbellaria) contributed 90 % of the dissimilarity among samples at BOWM and MOSE. Together these taxa contributed 74 % of dissimilarity at ABER, with Paramelitidae (Amphipoda) contributing a further 9 %. All habitats at ABER were dominated by cyclopoids and oligochaete worms (Table 5.16), with harpacticoids and microturbellarian flatworms making significant contributions to the fauna at downwelling and upwelling zones respectively (Table 5.16).

Oligochaete worms (13 % contribution to dissimilarity), cyclopoids (13 %), parastenocarids (13 %), and paramelitid amphipods (8 %) contributed to the difference between ABER and BOWM. At BOWM, oligochaetes, cyclopoids, and parastenocarids comprised the majority of the fauna at bar and downwelling habitats, while at the upwelling zone microturbellarians replaced cyclopoids as the second dominant taxon (Table 5.16).

MOSE was distinguished from ABER with contributions of 21 %, 16 %, 15 %, and 8% to dissimilarity by the cyclopoids, oligochaetes, parastenocarids, and paramelitids respectively. A fauna similar to that of BOWM dominated the habitats at MOSE (Table 5.16) with the exception of the downwelling zone where cyclopoids, oligochaetes, and microturbellarians prevailed. The four main taxa common to BOWM and MOSE are

Cyclopoida (20 %), parastenocarids (16 %), Oligochaeta (15 %), and microturbellarians (8%).

Temporal analysis of pump data

Across all sites, Time was not a significant factor influencing community structure (P = 0.02, Table 5.15, Figure 5.18). However, Time did have influence at the Site level (Table 5.15). Oligochaeta and Cyclopoida dominated community structure for all Times at ABER and MOSE, but the other dominant taxa differed for each sampling occasion (Table 5.16). The ABER community changed from one where paramelitids and harpacticoids dominate, to one where ostracods and nematodes were common. By day 49, paramelitids and partidomomonid mites were the most common taxa (Table 5.16). At MOSE, Eutardigrada and microturbellarians were replaced by parastenocarids and copepod nauplii as dominating taxa 7 d after the release. 49 d later, microturbellarians and parastenocarids dominated.

Table 5.15. ANOSIM results from pump samples. Where parameter is: Time, 1 = before, 2 = day 7, 3 = day 49; Site, 1 = Aberdeen, 2 = Bowmans Crossing, 3 = Moses Crossing; Habitat, 1 = bar, 2 = downwelling, 3 = upwelling. * denotes that only 1 000 permutations were possible.

Pump fauna

Parameter	ANOSIM global tests		ANOSIM pairwise tests 1 v 2		1 v 3		2 v 3	
	R	P	R	P	R	P	R	P
Time (all sites)	0.073	0.020	0.036	0.193	0.082	0.044	0.094	0.035
Site (all times)	0.255	**<0.001**	0.394	**<0.001**	0.325	**<0.001**	0.094	**0.024**
Time (Aberdeen only)	0.462	**<0.001**	0.420	**0.008***	0.432	**0.009***	0.531	**0.007***
Habitat (Aberdeen only)	0.657	**<0.001**	0.778	**0.001***	0.481	0.017*	0.568	**0.006***
Time (Bowmans only)	0.476	**<0.001**	0.333	0.018*	0.617	**0.001***	0.639	**0.002***
Habitat (Bowmans only)	0.570	**<0.001**	0.296	0.032*	0.749	**0.001***	0.903	**0.001***
Time (Moses only)	0.591	**<0.001**	0.687	**0.001***	0.494	**0.003***	0.709	**0.002***
Habitat (Moses only)	0.755	**<0.001**	0.508	**0.007***	0.875	**0.001***	0.926	**0.001***

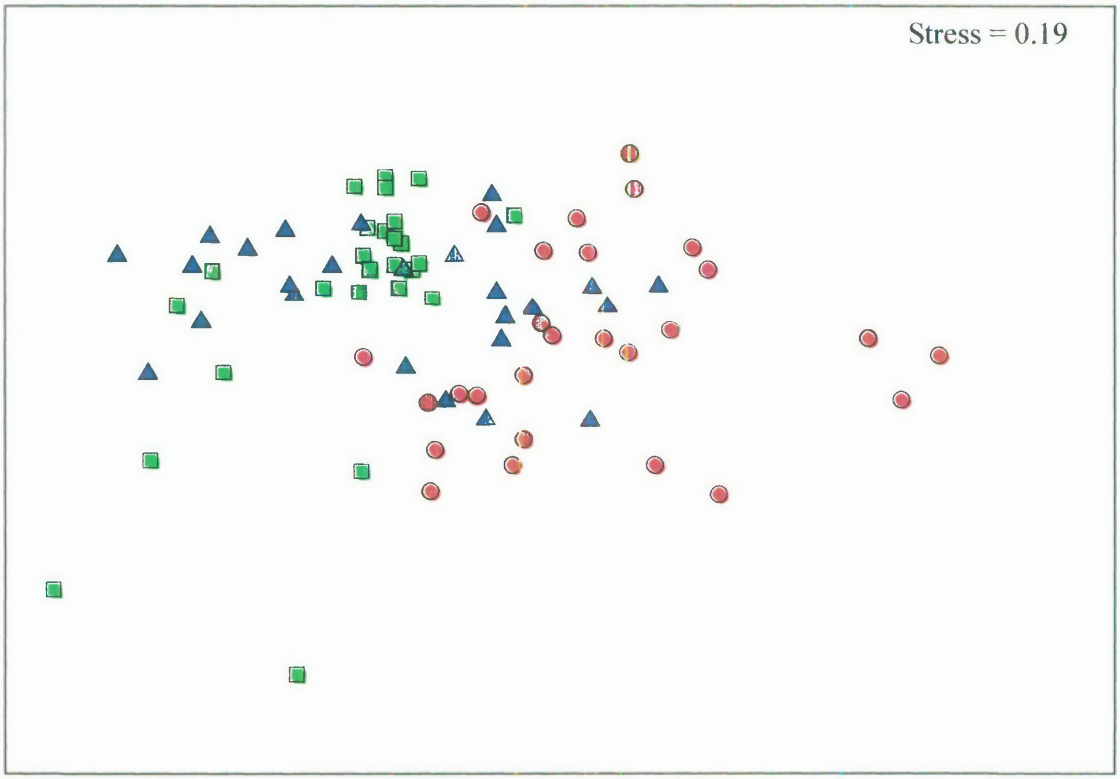


Figure 5.17. Non-metric multidimensional scaling plot for invertebrate communities at three sites pooled for all times. Circle = Aberdeen, square = Bowmans Crossing, and triangle = Moses Crossing.

Table 5.16. Results of SIMPER analysis for pump samples. Parameters of analysis are shown in the left column. Numbers indicate the contribution of each taxon to the dissimilarity between samples within a parameter (as a percent). Only the contribution of the four most common taxa are shown for each parameter.

	Nematoda	Microturbellaria	Oligochaeta	Eutardigrada	Cyclopoida	Harpacticoida	Copepod nauplii	Parastenocaridae	Paramelitidae	Ostracoda	*Partidomomonia*
Spatial analysis											
Aberdeen											
Bar			12.39		38.62				17.88		7.69
Downwelling			32.32		26.78	13.27				7.01	
Upwelling		7.96	41.71		35.87				3.65		
Bowmans											
Bar		8.95	41.7		31.41			16.71			
Downwelling		5.89	38.73		31.02			17.34			
Upwelling		16.41	66.27					11.97			
Moses											
Bar			40.59		46.75			4.53			
Downwelling		4.8	33.27		51.33					1.93	
Upwelling		10.17			58.51			21.77			
Temporal analysis											
Aberdeen											
Before			29.88		35.38	5.66			9.16		
Day 7	5.18		36.08		31.6					5.83	
Day 49			21.78		37.43				10.52		8.36
Bowmans											
Before		11.44	50.7		20.16			16.95			
Day 7		7.26	43.31		25.85			19.38			
Day 49		10.56	44.31		20.08			13.44			
Moses											
Before		3.4	57.3	4.37	28.08						
Day 7		34.72	38				4.88	11.1			
Day 49		12.02	46.95		17.95			12.82			

Figure 5.18. MDS plots of hyporheic invertebrate communities collected with the pump sampler from 40 cm depth at a. Aberdeen, b. Bowmans Crossing, and c. Moses Crossing. Different symbols represent the different sampling occasions.

● = Before release, ▲ = first post-release samples, and ■ = second post release samples

Spatial analysis of freeze core data

Communities at each of the three sites differed in composition from each other in both the whole core (Global R = 0.263, P < 0.001, Table 5.17) and the upper 20 cm (Global R = 0.336, P < 0.001, Table 5.18). This was due to the numerical dominance of insect larvae in the top 20 cm of sediment over other taxa at all sites (Table 5.19). The grouping of all depth sections in the analysis led to a dominance of taxa from the upper two layers, since the invertebrate populations were far more numerous here than in lower sections. Because of this dominance, and the low invertebrate numbers collected from deeper sections, subsequent analyses focus on the upper 20 cm of the cores.

Although leptophlebiid mayflies dominated the upper 20 cm samples at both riffle habitats (Table 5.19), differences between the populations of less dominant taxa in the upper 20 cm (Chironomidae, Caenidae, and the leptophebiid mayfly genus *Jappa*) caused a difference in habitat community structure in the upper 20 cm of ABER (Global R = 0.254, P < 0.001, Table 5.18).

Composition of the bar and downwelling communities at BOWM were marginally different when analysed over the whole core (P = 0.050, Table 5.17), but similar for the top 20 cm (P = 0.092, Table 5.18). Samples in the top 20 cm were dominated by caenids, cyclopoids and chironomids (Table 5.19). The community in the upwelling habitat comprised caenids, chironomids, the snail *Physa acuta,* and oligochaete worms (Table 5.19). For the top 20 cm of core, downwelling and upwelling habitats were deemed similar (P = 0.081, Table 5.18), but this was not the case for whole core (P = 0.001, Table 5.17) due to the overall dominance of cyclopoids (> 37%) and oligochaetes (>34%) in the cores of both sections.

At MOSE, communities differed among all three habitats (Global P< 0.001 for the whole core and top 20 cm, Table 5.17, Table 5.18). Downwelling communities were dominated by baetids, chironomids, and caenids; bar communities were dominated by oligochaetes, chironomids, and cyclopoids; and upwelling communities were dominated by *Physa acuta*, chironomids, and caenids (Table 5.19).

Table 5.17. ANOSIM results from freeze-core samples. All depths are pooled. Where parameter is: Time, 1 = before, 2 = day 7, 3 = day 49; Site, 1 = Aberdeen, 2 = Bowmans Crossing, 3 = Moses Crossing; Habitat, 1 = bar, 2 = downwelling, 3 = upwelling. * denotes that only 1 000 permutations were possible.

Freeze-core

Parameter	ANOSIM global tests		ANOSIM pairwise tests 1 v 2		1 v 3		2 v 3	
	R	P	R	P	R	P	R	P
Time (all sites)	0.160	**<0.001**	0.114	**<0.001**	0.188	**<0.001**	0.189	**<0.001**
Site (all times)	0.263	**<0.001**	0.381	**<0.001**	0.325	**<0.001**	0.080	**<0.001**
Time (Aberdeen only)	0.263	**<0.001**	-0.022	0.759	0.424	**<0.001**	0.415	**<0.001**
Habitat (Aberdeen only)	0.128	**<0.001**	0.171	**<0.001**	0.039	0.15	0.143	**0.003**
Time (Bowmans only)	0.187	**<0.001**	0.284	**<0.001**	0.065	0.055	0.236	**<0.001**
Habitat (Bowmans only)	0.126	**<0.001**	0.054	0.05	0.221	**<0.001**	0.155	**0.002**
Time (Moses only)	0.24	**<0.001**	0.121	0.025	0.333	**<0.001**	0.216	**0.001**
Habitat (Moses only)	0.227	**<0.001**	0.214	**<0.001**	0.199	**<0.001**	0.264	**<0.001**

Table 5.18. ANOSIM results from the pooled top two sections of freeze-core samples. Parameter numbers correspond to those in Table 5.17.

Freeze-core top 20 cm

Parameter	ANOSIM global tests		ANOSIM pairwise tests 1 v 2		1 v 3		2 v 3	
	R	P	R	P	R	P	R	P
Time (all sites)	0.206	**<0.001**	0.068	0.011	0.341	<0.001	0.213	**<0.001**
Site (all times)	0.336	**<0.001**	0.471	**<0.001**	0.482	**<0.001**	0.036	0.109
Time (Aberdeen only)	0.474	**<0.001**	0.064	0.183	0.742	**<0.001**	0.578	**<0.001**
Habitat (Aberdeen only)	0.254	**<0.001**	0.293	**0.004**	0.232	**0.006**	0.241	**0.001**
Time (Bowmans only)	0.139	0.013	0.090	0.131	0.096	0.112	0.238	**0.005**
Habitat (Bowmans only)	0.152	**0.006**	0.119	0.092	0.288	**0.002**	0.099	0.081
Time (Moses only)	0.291	**<0.001**	0.210	0.013	0.350	**0.001**	0.331	**0.003**
Habitat (Moses only)	0.362	**<0.001**	0.605	**<0.001**	0.326	**<0.001**	0.241	**0.009**

Table 5.19. Results of SIMPER spatial and temporal analysis for the upper two sections of core samples. Parameters of analysis are shown in the left column. Figures indicate the contribution of each taxon to the dissimilarity between samples within a parameter (as a percent). Only the contributions of the four most common taxa are shown for each parameter.

	Physa acuta	Cyclopoida	Oligochaeta	Baetidae	Leptophlebiidae	*Jappa sp.*	Caenidae	Chironomidae	Simuliidae	*Cheumatopsyche sp.*
Spatial analysis										
Aberdeen										
Bar			27.46			10.81	25.18	16.2		
Downwelling					80.95	5.19				4.71
Upwelling					40.86	15.66	16.15	17.24		
Bowmans										
Bar		26.6					6.77	52.86		
Downwelling		27.71		14.16			18.17	52.12		
Upwelling	15.53		15.24				33.76	26.05		
Moses										
Bar		15.13	56.17					18.74		
Downwelling				21.84			19.48	20.38		11.69
Upwelling	29.47		10.4				27.96	28.26		
Temporal analysis										
Aberdeen										
Before					91.51					
Day 7	8.11				71.07	7.4	14.02			
Day 49					3.02	15.5	20.45	44.6		
Bowmans										
Before	9.3	12.43					8.38	55.04		
Day 7		35.39	6.97				20.36	25.78		
Day 49			13.59	11.6			39.39	15.72		
Moses										
Before			3.54				33.58	50.64	3.54	
Day 7		14.09	41.7				14.88	18.62		
Day 49	15.35		29.97	9.78			11.34			

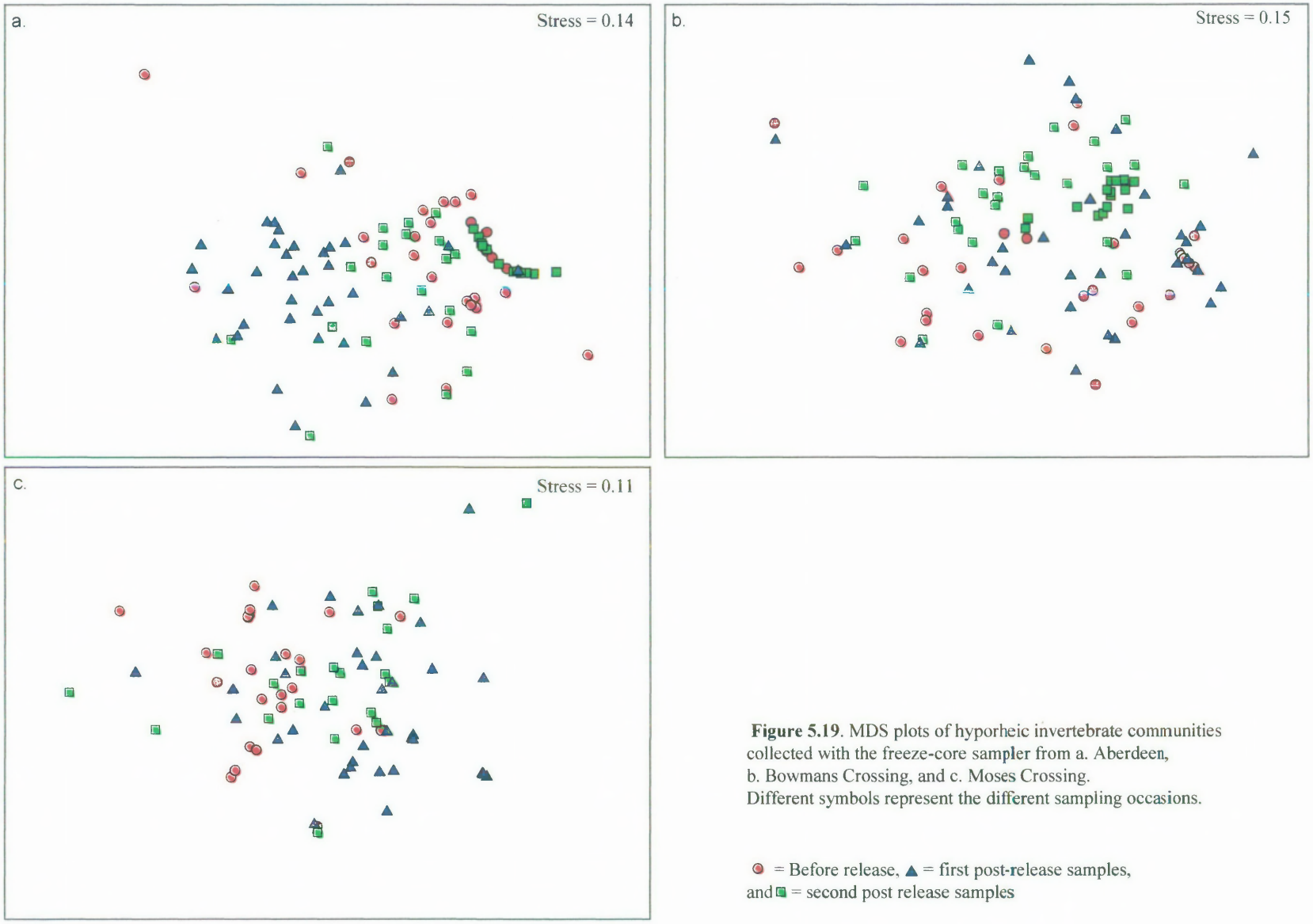


Figure 5.19. MDS plots of hyporheic invertebrate communities collected with the freeze-core sampler from a. Aberdeen, b. Bowmans Crossing, and c. Moses Crossing. Different symbols represent the different sampling occasions.

● = Before release, ▲ = first post-release samples, and ■ = second post release samples

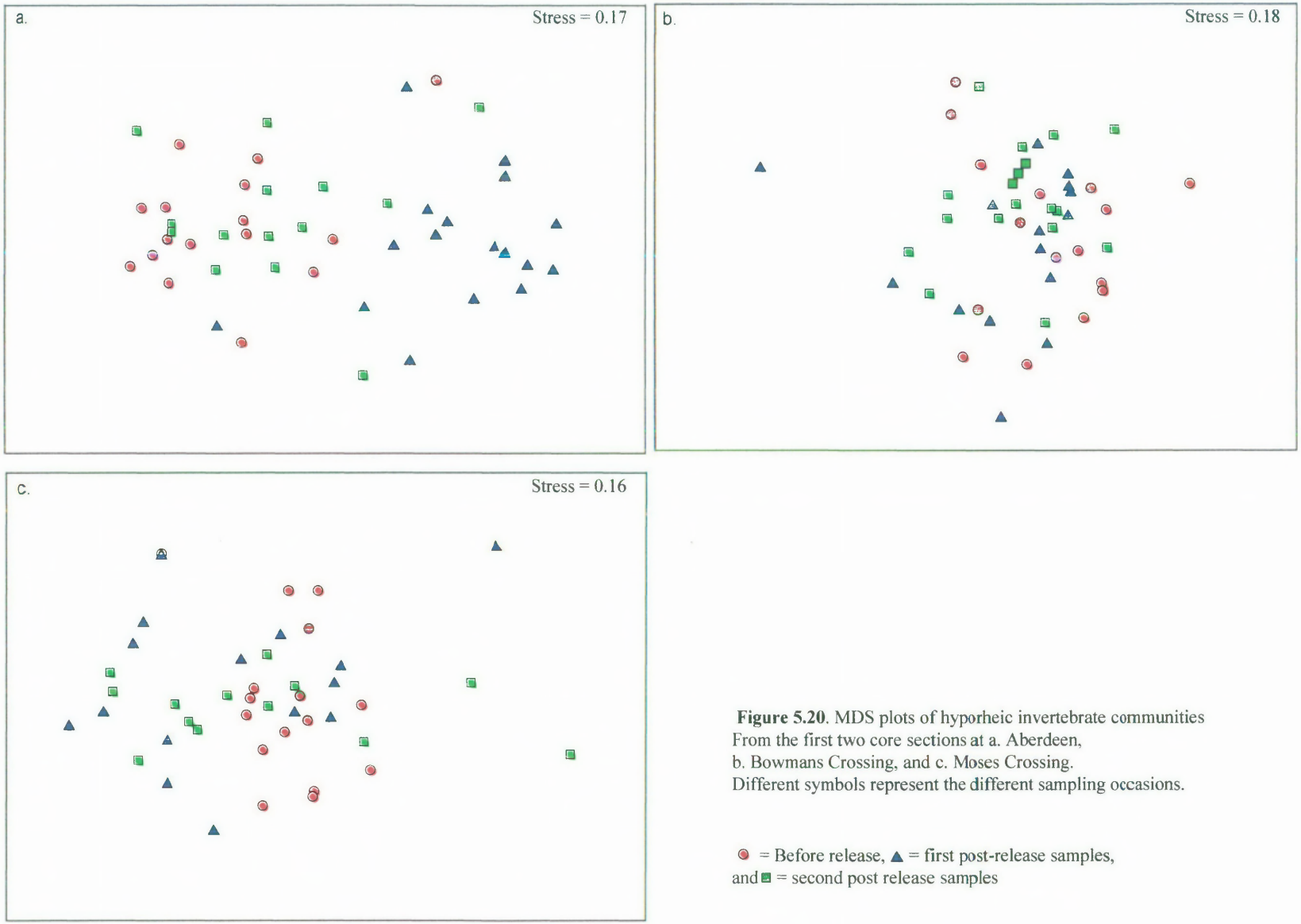


Figure 5.20. MDS plots of hyporheic invertebrate communities From the first two core sections at a. Aberdeen, b. Bowmans Crossing, and c. Moses Crossing. Different symbols represent the different sampling occasions.

● = Before release, ▲ = first post-release samples, and ■ = second post release samples

Temporal analysis of freeze-core data

Analysis of the whole-core data indicated that invertebrate community composition differed for each of the sampling occasions (Global $P < 0.001$, Table 5.17). This was mirrored in the upper 20 cm of sediment (Global $P < 0.001$, Table 5.18). At ABER, communities were similar in the top 50 cm ($P = 0.759$, Table 5.17, Figure 5.19) and top 20 cm ($P = 0.183$, Table 5.18, Figure 5.20) of the sediment before and after the release, indicating no change in communities due immediately to the release. In fact, there were no significant differences in freeze-core communities between pre- and initial post-release samples for the upper 20 cm of any site (Table 5.18, Figure 5.20). The majority of change in community structure reflected in the global P values appears to be due to differences between the two post-release occasions, where dominance at ABER shifted from Leptophlebiidae (71 %) to Chironomidae (45 %), and dominance at BOWM shifted from Cyclopoida (36 %) to Caenidae (39 %). At MOSE, Oligochaetes always contributed most to community structure, but their involvement decreased from 42 % to 30 % with an increase in the *Physa acuta* population (Table 5.19).

Insects, cyclopoids, and oligochaetes dominated the fauna in the upper 20 cm at all times (Table 5.19). At ABER, leptophlebiid mayfly nymphs contributed significantly to most samples collected before and 7 days after the release (Table 5.19). Chironomid larvae and caenid nymphs dominated at 49 days after the release. The BOWM community comprised mostly chironomids, cyclopoids, and caenids for the first two sampling occasions, but included baetids and oligochaetes in the final sampling (Table 5.19). Chironomids and caenids contributed to a large proportion of the similarity between samples at MOSE for the first 2 times. By 49 days after the release, oligochaetes and *Physa acuta* were the most common taxa (Table 5.19).

At BOWM, invertebrate community composition was similar before and following the flow release in the upper 20 cm ($P < 0.001$), but different to both of these at 49 days after the release ($P = 0.005$, Table 5.18). At all times the four highest contributing taxa were oligochaetes, cyclopoids, parastenocarids, and Microturbellaria. However, a slight decrease in the supremacy of parastenocarids and microturbellarians at 49 d changed the structure of the community (Table 5.19). Faunal community structure at BOWM in the entire upper 50 cm did change substantially following the flow release ($P < 0.001$, Table

5.17) with the contribution of cyclopoids increasing from 5.8 % to 59.6 %, causing the increase in invertebrate numbers following the release (Figure 5.16).

5.5 Discussion

Hyporheic hydrological and physico-chemical dynamics

There was a difference in the strength of hydraulic exchange between downwelling and upwelling areas in the Hunter River, with much stronger negative exchange than positive. In two rivers in Montana, negative hydraulic head was also of a much higher magnitude than positive hydraulic head (Pepin and Hauer 2002). Hydraulic head in both riffle habitats at BOWM, and the upwelling and bar habitat at MOSE increased following the release, although some of these increases were marginal. The magnitude of hydraulic head for these habitats was higher again 49 days following the release, so it is possible that the increase in porosity and decrease in the percentage of fines continued to influence the rate of exchange for at least this length of time. However, at ABER, where the substrate is less mobile, the release caused no change in hydraulic head in the riffle, but a marked decrease in the magnitude of exchange in the bar. The reason for this is uncertain, but may be due to the lower porosity at 30-50 cm restricting water entrance into the piezometer. The results of this study indicate that an environmental flow of this magnitude is more likely to increase hydraulic exchange in mobile beds than it is in consolidated beds.

Increases in hydraulic exchange allow more nitrogen to be transported into the sediments and removes accumulated interstitial nitrate to the stream, thereby enhancing microbial activity (Mulholland *et al.* 1997). The stimulation of microbial activity is an essential component of the biological mechanism in gravel-bed filtration. Higher interstitial nitrate concentrations 7 days following the release indicate that nitrification at the three Hunter River sites was stimulated by this release. These findings support those of Wondzell and Swanson (1996), who also found that fluctuations in stream stage increased hyporheic nitrogen fluxes. However, it may be that nitrate concentration in the Hunter River sediments had already fallen by day 7, since Stanley and Boulton (1995) reported hyporheic nitrate concentrations of Sycamore Creek dropped substantially 8 days after a flood. Contrasting with my results, Baker and Vervier (2004) observed increased de-nitrification during a high flow in the Garonne River, France, that raised the water table by

35 cm. This was probably due in part to concurrent inputs of low molecular weight organic acids from soil organic matter (Baker and Vervier 2004).

Dissolved oxygen concentrations are often closely linked to nitrate concentration as it is consumed during the nitrification process (Duff and Triska 2000). At BOWM, this trend was apparent, with high nitrate concentrations immediately after the release coinciding with a decrease in dissolved oxygen. However, this did not hold true at the other sites. The absence of a significant correlation between dissolved oxygen and nitrate concentration may be because of the effect of a spate that preceded the environmental flow (see later). Microbial nitrification may be impaired due to bacteria being abraded from the sediment surfaces as observed by Holmes *et al.* (1998), and dissolved oxygen concentrations being in excess (> 75% saturation in downwelling habitats for the first two times) of what was required for nitrification.

In the Hunter valley, some coalmines take advantage of higher than normal flows to release water from on-site storage dams (Allan Raine *pers. comm.*). This water can be saline, resulting from ground-water intrusion in the mines. The two main power stations also have large volumes of saline water in their storage dams and release water during flushes. By releasing at high flows, dilution minimises the impact of this water on downstream users and the environment. It is likely that such releases took place during this experimental release. Surface water conductivity was lower at all three sites on Day 7, after the environmental flow. It is possible that a discharge of saline water may have occurred, but this was diluted but the increased flow. In contrast to the other two sites, interstitial EC at MOSE is higher on Day 7 than it was before the release. This indicates that there may have been an increase in groundwater influx at this site.

Sediment characteristics

Freeze-coring is the most useful method for quantitatively measuring sediment characteristics (Fraser and Williams 1996, Olsen *et al.* 2002). It captures vertical profiles, especially of fine sediments, of river-beds with minimal disturbance. Although the porosity of gravel beds can be extremely variable (Gayraud and Phillippe 2003), porosity of the Hunter River sites (15.4 % at ABER, 20.9 % at BOWM, and 18.6 % at MOSE) resembles that of other rivers studied in Australia and overseas. Using similar methods to

those used in this study, Marchant (1995) measured porosities of 19.5 % and 18.0 % in the top 30 cm of two sites along the Acheron River in Victoria. A study examining the sediment characteristics of 99 frozen cores collected from 15 French streams found porosity to range from 2.05 % to 24.16 % (mean ± SD = 15.28 ± 4.51 %, Gayraud and Philippe 2003).

Porosity increased in the hyporheic riffle habitats at BOWM and MOSE in the upper 40 cm at both downwelling zones, and the upper 20 cm at the upwelling zones following the flow release. The porosity of the MOSE bar also increased in the upper 40 cm. Since a given volume of water will more easily move small loose particles than larger ones, sites with small particle size may experience a greater level of disturbance than sites with coarser substrate (Townsend *et al.* 1997). Substrata at BOWM and MOSE consist of a large proportion of coarse to medium sand interspersed with cobbles and gravel (Chapter 2 – Study sites). Although ABER had a larger percent of fine sediments than both MOSE and BOWM, these were not as accessible to disturbance by flow, being more tightly packed and containing more clay. In addition to this, a significant proportion of sediment at ABER was small to large cobbles. The proportionate dominance of mobile sand in the riffles at both BOWM and MOSE, and not ABER could explain why porosity at these two former sites was more affected by the release.

The strong negative correlation between the proportion of fines and the porosity was as hypothesised. However, habitat-by-habitat comparisons did not display such clear patterns. There was a decrease in the amount of fines collected from top 20 cm of three riffle habitats at BOWM and MOSE following the release. The amount of fines was subsequently higher 49 days later in all habitats. Over time at a constant flow, fine sediments can build up in the gravel-bed of rivers, clogging pore spaces and impairing exchange (Amoros and Bornette 2002, Osmundson *et al.* 2002). The clogging of interstitial spaces can impair hyporheic microbial and faunal activity by reducing interstitial flow, and thus dissolved oxygen circulation (Maridet *et al.* 1996, Brunke and Gonser 1999). It has been proposed that flushing with strategically-timed flow releases may mitigate the build up of fine sediments in regulated rivers and consequently stimulate microbial filtration of water by the hyporheic zone (Boulton 2000b, Hancock 2002).

Interstitial faunal communities

The hyporheic zone has often been proposed as a refuge for surface-dwelling insects during disturbance (Panek 1991, Gayraud *et al.* 2000). Whether the invertebrates actively move into the sediment or are washed in remains the subject of debate (Matthai and Townsend 2000, Palmer *et al.* 1992). Fauna in the upper 30 cm of sediments of the Acheron River appeared to penetrate deeper into the stream bed during periods of high flow (Marchant 1995). Similar observations were made of fauna in the Rhône River (Marmonier and Creuzé des Châtelliers 1991). However, with the exception of BOWM this was not observed in this study for freeze core samples. The top 20 cm of sediment consistently hosted the most abundant and diverse invertebrate assemblages. At BOWM, the decrease in invertebrate numbers in the upper 10 cm of the riffle habitat 7 days after the release, when taken in view of the increased abundance in the 10 - 20 cm section, could indicate active migration. ABER and MOSE communities showed no change in either the number of taxa, or the number of invertebrates between pre- and immediately post-release. It is possible that refuge temporarily was sought by some taxa, but they were able to re-colonise the benthos before the second sampling occasion. Insects, especially mayfly nymphs and chironomid larvae, dominated the ABER fauna at Day 49, so perhaps the increased diversity at most habitats and depths is due to epigean invertebrates taking refuge from the ever-diminishing stream flow. This was the case in the Rhône River, where surface fauna increasingly dominated the downwelling community as flow decreased (Marmonier and Creuzé des Châtelliers 1991).

The dominance of non-insect taxa in the lower depth sections of the freeze-core samples signals that surface fauna were limited in their penetration of the sediments to the upper 20 cm of bed. The environmental flow temporarily shifted the dominance at ABER from cyclopoid copepods to oligochaete worms which, being larger, were better able to resist flushing. A similar pattern was observed at MOSE where, although oligochaetes remained dominant, cyclopoids disappeared after the release. Boulton *et al.* (2003b) speculate that rapid throughflow of water in coarse sediments may be too strong for the persistence of small-bodied meiofauna. Between depths of 30 and 50 cm, invertebrate abundance and taxonomic richness correlated strongly with dissolved oxygen, highlighting the importance of maintaining a constant flow of oxygenated water through the sediments. However, at BOWM the cyclopoid population increased after the release, despite the

decrease in dissolved oxygen, indicating that this taxon may not be particularly sensitive to decreases in oxygen. Dissolved oxygen is often not a limiting factor in the distribution of groundwater fauna (Strayer 1994, Galassi *et al.*2002), with some taxa frequently occurring in waters with DO concentrations less than 1 mg/L (Malard and Hervant 1999).

Effect of preceding spate

Unfortunately a spate occurred two days prior to the planned date of the environmental flow release (Figure 5.2) and this will have influenced some of the results discussed above. A spate that occurred 35 days before sampling of the hyporheic zone in Rocky River, New South Wales, buried particulate organic matter, the decomposition of which was thought to contribute to strong vertical trends in dissolved phosphorus (Claret and Boulton 2003). It is unlikely that significant decomposition of organic matter would have occurred before the first sampling took place for the current study. However, the decomposition of any organic matter buried by the spate in the Hunter River could have influenced the nutrient concentrations of Days 7 and 49.

It is possible that the spate may have flushed nitrate from the sediment, so that concentrations recorded before the flow release were lower than they would normally have been. Alternatively, the spate could have stimulated nitrate production so that concentrations were artificially high. Stanley and Boulton (1995) found that hyporheic nitrate concentrations of Sycamore Creek increased within two days after a spate. Since two days had lapsed in the present study between the end of the spate and the commencement of sampling, some degree of stimulated nitrification probably occurred. However, this is likely to have been of little consequence, since the environmental flow probably removed most of the residual nitrogen-rich water resulting from spate-stimulated nitrification. Martí *et al.* (2000) reported that there was also a flushing of nitrates from the sediments by a spate in Sycamore Creek. It is highly plausible that the increase in nitrates observed in this study was therefore due to nitrification stimulated by the experimental flow release.

The occurrence of a spate of a similar magnitude to the environmental flow a few days prior to initial sampling may dampen some of the effects of such a release. Potential microbial stimulation in the sediments by the spate contributed to uncertainty in

interpretation when no significant difference could be attributable to the release. The spate may have also influenced the faunal composition of the pre-release samples. For example, the absence of a significant difference in invertebrate numbers in the upper 20 cm of bar habitat at ABER may have been due to: resilience or rapid recovery by the fauna, the flow release having no real effect, or because smaller taxa were washed away by the preceding spate.

It is likely that the spate reduced the importance of the environmental flow release on bed porosity and proportion of fine sediments. For example, fines that would have been removed by the environmental flow release may have already been removed by the spate. Similarly, the recorded changes in porosity may have been different if the spate had not occurred a few days before.

Contrasting sampling techniques

The advantage of using more than one sampling technique is that limitations in one method may be overcome by the adoption of another (Dumas and Fontanini 2001). The low numbers of invertebrates collected in the lower sections of core section limited the certainty of any claims that can be made from these data. It is possible that a longer settling period and lack of electro-positioning, as used in other studies (Bretschko 1992, Gayraud and Philippe 2003) prior to freezing contributed to this, but settling period has been found not to affect invertebrate densities in core sections below 10 cm (Olsen *et al.* 2002).

Freeze-coring is a useful method for obtaining quantitative sediment and invertebrate data from stream beds. However, in relatively sparsely populated hyporheic zones, such as those in the Hunter River, the use of a freeze-corer might be best applied to collecting sediment data only. Although variable in its efficiency and prone to a 'filtration effect' (Fraser and Williams 1997), the pump sampler can be used to collect semi-quantitative faunal samples if pump velocity (Hunt and Stanley 2000) and sample volume (Boulton *et al.* 2003b) are kept constant. It can also be used to collect nutrient and water chemistry samples. Pump samples contained consistently higher numbers of hyporheic invertebrates than the freeze samples, allowing a clearer picture of hyporheic community dynamics. By

combining the two methods, an accurate assessment could be made of hyporheic sediment, faunal, and physico-chemical conditions.

Freezing with CO_2 is much quicker than with liquid nitrogen, taking only 5 minutes freezing time (rather than 15 minutes – Bretschko 1992, Gayraud and Philippe 2001, Olsen *et al.* 2002, Olsen and Townsend 2003) to produce a core. The rapid advance of the freezing front further minimises the invertebrates' chances of escape. Freezing with CO_2 produces cores of a smaller volume than freezing with nitrogen (compare mean volumes of 10 cm sections from the Hunter River sites with the mean of 1065 cm^3 measured by Olsen *et al.* 2002), but it has several features that commend its use in streams. First, faster freezing rates mean that more replicate cores can be extracted from an area of bed per sampling effort. This allows a more representative sampling of bed-sediments. If a larger volume of sediment is required for analysis, then replicates can be pooled. Second, smaller cores are easier to extract. In the absence of large embedded cobbles, two people were able to hand-extract the cores collected from BOWM and MOSE. This has implications for working in hard-to-access sites, since it reduces the amount of heavy equipment required. Finally, it is safer. The CO_2 is stored in easily manageable gas cylinders, limiting the risk of spillage that is involved in working with liquid nitrogen stored in Dewar flasks. Since the CO_2 is injected into the stand-pipes rather than poured, the risk of spillage is further reduced. CO_2 is warmer than liquid nitrogen, and it can be quickly brushed off the skin, reducing the risk of damage from cold burns.

Implications for management of the Hunter River

In the Hunter River, managed environmental flows come in two main forms: releases from Glenbawn Dam, and restrictions on pumping resulting from Flow Rule 2. Although it was the former that was studied here, both of these types of flows are expected to influence the functioning of the river's hyporheic zone. As Flow Rule 2 does not specify a volume or duration for an environmental flow, it allows a large degree of variability in river discharge. Environmental flows of different magnitudes could affect sites differently, depending largely on the level of bed consolidation and substrate size. While not drastically impacting faunal dynamics, a release of the magnitude studied here (5 000ML for three days) stimulated bed-filtration of nitrogen at all sites and increased porosity at BOWM and MOSE. It is difficult to say from this one event how different sized

environmental releases will affect hypotheic activity. Speculatively, a release larger than this could increase porosity, flushing of fines, and consequently hydraulic exchange in the bed at ABER. At BOWM and MOSE, the impact could be more severe, potentially re-shaping the river bed and lateral bars.

In view of the findings of this report it is strongly recommended that the environmental flows resulting from Flow Rule 2 be maintained as part of the management regime of the Hunter River. The environmental flows resulting from Flow Rule 2 may not necessarily be of the same size (magnitude or duration) as the release studied here, but heterogeneity of flow is an important factor in maintaining vertical connectivity (Amoros and Bornette 2002). Monitoring different-sized releases will give a better understanding of the impacts environmental flows can have on the hyporheic zone of the Hunter River.

5.6 Conclusions

This study tested the influence of an environmental flow release (5 000 ML for three days) on hyporheic nutrient and faunal patterns. Although no substantial impacts on the faunal community were observed, increases in porosity and the flushing of fine sediments from the upper 20 cm at BOWM and MOSE enhanced the hyporheic filtration potential of the bed. Nitrification was stimulated at all sites following the flow release, with nitrate concentrations at ABER being up to twice the initial concentrations. The findings of this study indicate that environmental flow releases can encourage the hyporheic filtration of nutrients and are useful tools in maintaining the health of the hyporheic zone.

The flow release studied above was similar to one that would result from Flow Rule 2, with pumping being allowed after 12 h of high flow. This medium-level flow did not have the severe effect on the hyporheic zone that resulted from the November flood (Chapter 4). This may have been due to the spate that occurred prior to the environmental flow release, which would have influenced the results found in the first sampling occasion. The results of this survey should therefore be interpreted with this in mind.

At the temporal resolution of this study it was not possible to discern whether the 12 hour ban on pumping, resulting from Flow Rule 2 had any impact on the hyporheic zone. By preventing pumping in the first 12 h of a large flow event, there would effectively be a

temporary increase in water level. In the next chapter, a 12-hour increase in flow was simulated, and the effects of this on nutrient and microbial activity are investigated.