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Effects of a diesel spill on freshwater macroinvertebrates in two urban watercourses, Wiltshire, UK

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Abstract

The impacts of a spill of approximately 9800 L of diesel on a small stream and the River Ray (near Swindon, Wiltshire, UK) were examined using kick-net sampling of freshwater macroinvertebrate families at impacted and reference sites. Initial impacts (10 days after the spill) 50 m downstream of the spill were severe, with only 9% survival of individuals (excluding oligochaete worms) and 56% survival of invertebrate families. The percentage survival of macroinvertebrates increased progressing downstream from the spill, with no detectable impacts beyond approximately 4 km downstream. The crustacean families Asellidae and Gammaridae were particularly sensitive to the diesel spill. The recovery of the macroinvertebrate community was assessed 13.5 months after the spill. At this time, recovery was almost complete, with only minor impacts at the sites closest to the spill. The use of live laboratory sorting of samples from impacted sites provided essential information on the impacts of the diesel spill.

Introduction

A large number of domestic, commercial and industrial sites store diesel in above-ground or below-ground tanks. Diesel is also widely used in transport and spills from vehicles are the main source of small diesel spills in England and Wales. In 2003 the Environment Agency recorded 1386 incidents involving diesel spills in England and Wales. Of these, 65 were serious incidents (Categories 1 and 2) (Lee & Fitzsimmons 2005). In 2007, diesel was an identified pollutant in 10% (seven out of 70) of the most serious (Category 1) water pollution incidents in the United Kingdom (R. Strickland, pers. comm., Environment Agency).

The literature on the impacts of diesel spills on streams and rivers is rather limited, with most being from the United States of America (e.g. Bury 1972; Lytle & Peckarsky 2001). The impacts of a spill of Number 2 fuel oil, which is very similar to diesel, on the ecology of a US stream has also been reported (Hoehn *et al*. 1974). There have been studies of the impacts on freshwaters of crude oil in the United States of America (e.g. McCauley 1966; Masnik *et al*. 1976; Harrel 1985; Crunkilton & Duchrow 1990; Poulton *et al*. 1998) and Brazil (Couceiro *et al*. 2006) and impacts of a petrol (gasoline) spill in the United States of America (Pontasch & Brusven 1988). Comparing impacts between different studies is complicated by the fact that the size of spill and size of receiving water varies. Furthermore, the toxicity of different hydrocarbon mixtures (e.g. various crude oils and distillate fractions) is very variable. Most toxicity studies on oils are carried out using the water soluble fraction (WSF), but in a significant spill event there will also be acute toxic impacts on invertebrates due to coating of their respiratory surfaces and contamination of their food (detritus, bacteria, algae, higher plants and invertebrates) (Bhattacharyya *et al*. 2003). The WSF of diesel is generally considered to be more toxic than crude oil, due to its composition (Bhattacharyya *et al*. 2003). However, the WSF of diesel is less toxic than coal-derived oils (Ullrich & Millemann 1983).

Diesel is very similar to Number 2 fuel oil used for domestic heating. It is a complex mixture of alkanes (normal, branched and cyclic; forming 60–90% of the volume), aromatic compounds, especially alkylbenzenes (5–40% of volume) and small quantities of alkenes (0–10% of volume) (World Health Organisation 1996). The water solubility is low, only 0.2–5.0 mg/L (World Health Organisation 1996). Diesel has a density usually in the range 0.82–0.85, so the bulk of a spill to a stream or river will float. Volatilisation rates are dependent upon temperature, which also affects the toxicity of the diesel. The WSF of diesel is more toxic at higher temperatures (Ullrich & Millemann 1983).

Background

On 23 January 2005 approximately 9800 L of diesel was lost from several underground tanks at commercial premises in Swindon, Wiltshire. The diesel entered a long culvert which led to the Westlea Brook at National Grid Reference (NGR) SU 1259 8438 (Fig. 1). On 24 January, specialist contractors (Adler and Allan Ltd.) constructed a sandbag weir at the outfall to reduce the amount of diesel entering Westlea Brook. They also put in oil booms on Westlea Brook and the River Ray. Diesel was removed from behind these booms and natural obstacles, such as debris and tree branches, using a mobile vacuum tank. Diesel was also recovered from the site interceptor. Adler and Allan Ltd. estimate that they recovered ~7000 L of diesel in liquid form and up to 2000 L in absorbents and contaminated materials (H. Simpson, pers. comm., Adler and Allan Ltd.). However, much of this would have entered the watercourses and had some toxic impacts (e.g. forming a WSF that could not be removed) before being recovered.

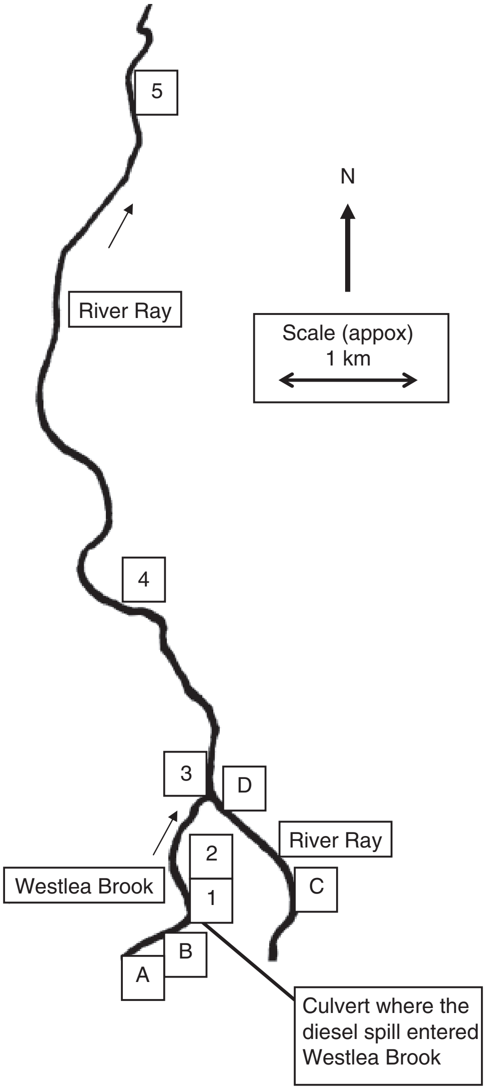

Fig. 1. Sampling sites.

The Westlea Brook is a small urban stream which enters the River Ray ~1.3 km below the spill. The source of the Westlea Brook is ~1.2 km above the spill. Widths at our sampling sites were 0.95–2.2 m. Depths were typically 0.1–0.2 m on the first survey and 0.16–0.25 m on the second survey.

The River Ray is approximately 20.9 km long, of which 8.6 km is above the confluence with the Westlea Brook (Fig. 1). From the Westlea Brook to the River Thames is ~12.3 km. It has a shallow gradient of 1.23 m/km in the stretch below Westlea Brook. Its mean flow at the confluence with the Thames is 1.34 m^3/s.

The River Ray had widths ranging from 3 m at our most upstream sampling site to 7 m at the most downstream. Water depths in the River Ray on the first survey ranged from ~0.18 m at the most upstream site to an estimated 1 m at the most downstream site. Owing to heavy rain before the March 2006 survey, the water depths on the River Ray ranged from ~0.50 m at the most upstream site to well over a metre at the other sites.

The surveyed reaches of the Westlea Brook were variably shaded. More open stretches exhibited marginal and emergent vegetation dominated by *Apium nodiflorum* (fool's watercress) and overhanging or trailing amphibious bankside species such as *Urtica dioica* (nettles) and *Rubus fruticosus* (brambles). Limited submerged flora included *Elodea canadensis/nuttallii* (Canadian/Nuttall's waterweed), *Cladophora* (blanket weed) and *Vaucheria* (mole's-pelt alga).

The River Ray exhibited little significant channel shading among sampled stretches. Flora within the upper reaches was largely similar to that found in the Westlea Brook; marginal species included *A. nodiflorum* and *Phalaris arundinacea* (reed canary grass), with little or no submerged species. Sites downstream of the confluence with the Westlea Brook typically exhibited greater diversity of marginal species, including *A. nodiflorum*, *Carex* sp. (sedges), *Glyceria* sp. (sweet-grass), *Juncus* sp. (rushes), *Typha latifolia* (bulrush) and *Veronica beccabunga* (brooklime). As in the Westlea Brook submerged vegetation was typically limited; species included *Fontinalis antipyretica* (willow-moss), *Callitriche* sp. (water starwort), *Cladophora* and *Vaucheria*.

In addition to the impacts on invertebrates described in this paper, there was a fish kill of approximately 2000 fish, mainly three-spined sticklebacks, but also bullheads and minnows. Other known vertebrate mortalities were three frogs, three kingfishers, one swan and one moorhen.

Given the urban setting of the area of the spill, the river receives frequent low level inputs of hydrocarbons. Water sampling on the impacted section of Westlea Brook over the first 4 weeks following the spill showed total petroleum hydrocarbon concentrations of 70–150 μg/L (mean 110 μg/L). Concentrations in unaffected upstream reaches ranged from 80 to 210 μg/L (mean 128 μg/L) on Westlea Brook and 60–260 μg/L (mean 120 μg/L) on the River Ray.

Survey and analytical methods

Survey design

A walk-over survey on 1 February 2005 was used to assess visual aspects of the impacts of the spill (e.g. sheen, dead invertebrates and vertebrates) and choose suitable sampling sites. Sampling sites were selected according to their location relative to the spill and whether the Environment Agency had invertebrate data for them. Inclusion of these Environment Agency sites aided interpretation of the data. No Environment Agency data were available for the Westlea Brook. The Environment Agency had invertebrate data for four sites on the River Ray, and our survey included three of these sites. The fourth site was too far upstream to be a useful reference site for this study.

Two surveys were carried out after the diesel spill. The first, on 1–2 February 2005, aimed to assess short-term impacts. Sites impacted by the spill were sampled on 2 February 2005, 10 days after the spill. The second survey, on 9 March 2006 (13.5 months after the spill) aimed to assess medium-term impacts and recovery.

Both surveys examined the same nine sites (Fig. 1). Four sites were on Westlea Brook (two upstream reference sites and two downstream of the culvert and source of the diesel spill). Five sites were on the River Ray (two upstream reference sites and three downstream of the confluence with Westlea Brook). NGRs of sampling sites are shown in Table 1. Upstream sites that could not have been affected by the diesel spill are denoted by letters (A and B on Westlea Brook; C and D on the River Ray). Potentially impacted sites are numbered 1–5 progressing downstream (1 and 2 on Westlea Brook; 3, 4 and 5 on the River Ray). Sites C, 4 and 5 were at the same locations as Environment Agency routine monitoring sites. Environment Agency invertebrate data for these sites was obtained for the period 1995–2005. The number of sampling occasions in this period ranged from 11 to 16. The Environment Agency also provided data on their postspill monitoring of Westlea Brook and the River Ray.

Sampling and laboratory analysis

A standardised 3-min kick sampling technique (Environment Agency 1997) was used at all sites where the water depth was not too deep. In the second survey water depths at Sites D, 3 and 5 on the River Ray were too deep for kick sampling, so a sweep sampling method was used instead. At Sites D and 3 the sampling effort was split, with 1-min sampling bed sediments and the remaining 2-min sampling marginal vegetation. At Site 5 a 3 min sweep sample was also used in 2005 and the results are therefore directly comparable.

We normally fix and preserve freshwater samples in the field by the addition of buffered formalin. They are then sorted in the laboratory, by which time all the invertebrates present are dead. However, in the first survey we noted that some of the dead invertebrates in Westlea Brook were well-preserved by the diesel, and would be extremely difficult to distinguish from those killed by the formalin. In order to distinguish invertebrates killed by the diesel from other individuals, live sorting in the laboratory was carried out for samples from potentially impacted sites (Sites 1–3). All invertebrates were removed from these samples and separated into those found live or dead; both were then preserved in ethanol for later identification. This live sorting was completed within 2 days of the completion of the survey. The remaining six samples were fixed and preserved with buffered formalin on site and sorted in the usual way at the laboratory.

On the second survey there was no need for live sorting and all the samples were preserved in the field with buffered formalin.

Identification of invertebrates was to family level (or class level for oligochaetes). The number of individuals in each group was recorded.

Data analysis

Two indices of organic pollution, the Biological Monitoring Working Party (BMWP) score and Average Score Per Taxon (ASPT) were calculated. The BMWP scoring system was established in the United Kingdom in 1976 to provide a method of assessing the biological quality of streams and rivers. Not all families of freshwater invertebrates are included in the BMWP scoring system (Armitage *et al.* 1983). Those that are have been assigned a score from 10 (least tolerant of organic pollution, e.g. sewage) to 1 (most tolerant). Oligochaetes are taxonomically a class, rather than a family, but it is normal in the BMWP system to count them as a family. The BMWP score is the total of individual scores for each family found at a site. The ASPT is the BMWP score divided by the number of scoring families.

At Sites 1–3, where live and dead specimens were separated in the first survey, BMWP, number of scoring families and ASPT were calculated separately for live and combined live and dead invertebrates. The calculated scores for the combined live and dead invertebrates was used as an estimate of the BMWP and ASPT values before the spill.

Table 1 Sampling locations and observations

Location	Site and date	Observations
Upstream (reference) sites		
Westlea Brook, 125 m upstream of culvert and diesel spill SU 1249 8431	A Feb 2005	No oil sheen
	A March 2006	Very small amount of oil sheen seen during disturbance of sediment
Westlea Brook, 40 m upstream of culvert and diesel spill SU 1255 8437	B Feb 2005	No oil sheen
	B March 2006	No smell on entry into stream. No smell during sampling, but minute sheen seen as streaks on water towards end of sampling
River Ray at EA Morris Street site, 910 m above confluence with Westlea Brook SU 1336 8494	C Feb 2005	Larger substrate man-made rubble, etc.
	C March 2006	Heavy rain during sampling
River Ray, 25 m above confluence with Westlea Brook SU 1273 8552	D Feb 2005	A very small amount of oil disturbed from sediment (not from spill)
	D March 2006	High flows required use of sweep sampling. Very turbid. Recent silt deposits especially on left hand bank. Site probably affected by environmental enhancement and nearby development site
Downstream sites		
Westlea Brook 50 m downstream of source of diesel spill in culvert SU 1257 8443	1 Feb 2005	One live gammarid in sample, others dead. Live worm and chironomid seen
	1 March 2006	Slight oil sheen noted when sediment disturbed. Smell of oil in air before sampling
Westlea Brook, 475 m downstream of source of diesel spill in culvert SU 1241 8481	2 Feb 2005	Large numbers of live leeches nearby on surface of streambed, but behaving abnormally. Lots of dead invertebrates including gammarid amphipods
	2 March 2006	Oil mobilised by sediment disturbance during sampling
River Ray, 1385 m downstream of culvert, 35 m downstream of confluence with Westlea Brook SU 1270 8559	3 Feb 2005	Diesel on both banks, strong smell and strong sheen. Mixture of dead and live invertebrates in sample. Caddis often dead. Live species included a baetid mayfly.
	3 March 2006	High flows required use of sweep sampling. Very turbid. May be affected by environmental enhancement and development site upstream
River Ray at EA Moredon Bridge site, 3750 m downstream of culvert, 2400 m from confluence with Westlea Brook SU 1208 8722	4 Feb 2005	Sheen very obvious at all times. Strong smell of diesel. Over 95% of invertebrates in sample alive when collected. 1 *Hydropsyche* caddis dead
	4 March 2006	Faint smell in air, oil sheen seen in sample
River Ray at EA Seven Bridges Cricklade site, 11 500 m downstream of culvert, 10 150 m from confluence with Westlea Brook SU 1189 9253	5 Feb 2005	Sweep sample from bank due to water depth and safety requirements. Oil sheen very obvious
	5 March 2006	No oil sheen. Slight smell noticed during sampling. Sweep sample from bank, as in 2005

The invertebrate fauna of a stream (and hence the BMWP score) is affected by a variety of natural factors such as distance from source, altitude, water hardness, flow, channel width and depth. It is therefore difficult to compare BMWP scores from different locations. To overcome this problem a predictive method called RIVPACS has been developed (Moss *et al*. 1987). This uses information on natural variables to predict the expected BMWP and ASPT values in the absence of human influences. These predicted values can then be compared with values obtained in surveys. We have predicted the RIVPACS scores for our survey sites using RIVPACS III+ software (available from Centre for Ecology and Hydrology). RIVPACS predictions of BMWP and number of scoring families were rounded to the nearest integer. The RIVPACS software was used to predict values for the spring season.

Results and discussion

BMWP and ASPT values before the diesel spill

The BMWP, number of scoring families and ASPT values for both survey dates are summarised in Table 2. The prespill values were estimated by three methods. This variety of methods was necessary in order to produce the best possible estimation of values before the spill. Explanation of the rationale is provided below:

(i) For Sites 1–3, where live sorting was used, the BMWP and ASPT scores were calculated from combined live and dead families. Estimated BMWP scores prespill at Sites 1 and 2 (18 and 27, respectively), were similar to those at reference Sites A and B (19 and 20, respectively). The method of estimating the scores from combining live and dead invertebrates collected at each site 10 days after the spill may have caused the slightly higher value for Site 2, as live or dead specimens could have moved downstream from locations nearer to the spill. However, the predicted number of scoring families at Site 1 prespill was 6, identical to the number found at the nearest upstream reference site (Site B). This suggests that it is unlikely that

Table 2 Biotic scores for sampling sites

		Westlea Brook				River Ray				
		Upstream		Downstream		Upstream		Downstream		
	Site Date	A	B	1	2	C	D	3	4	5
BMWP										
Observed	2005 pre-spill	19	20	18	27	80	76	78	68	89
	Feb 2005	19	20	9	9	80	76	21	63	89
	Mar 2006	36	34	28	34	101	78	61	83	49
Predicted	RIVPACS	104	113	109	108	117	117	120	123	109
EQI	2005 pre-spill	0.18	0.18	0.17	0.25	0.68	0.65	0.65	0.55	0.82
	Feb 2005	0.18	0.18	0.08	0.08	0.68	0.65	0.18	0.51	0.82
	Mar 2006	0.35	0.30	0.26	0.31	0.86	0.67	0.51	0.67	0.45
% change	Pre-spill to 2005	0.0	0.0	− 50.0	− 66.7	0.0	0.0	− 73.1	− 7.4	0.0
	Feb 2005–2006	89.5	70.0	211.1	277.8	26.3	2.6	190.5	31.7	− 44.9
No. of scoring families										
Observed	2005 pre-spill	5	6	6	8	18	16	17	17	18
	Feb 2005	5	6	4	4	18	16	8	15	18
	Mar 2006	10	9	9	10	21	17	15	18	11
Predicted	RIVPACS	21	23	22	22	23	23	24	24	22
EQI	2005 pre-spill	0.24	0.26	0.27	0.36	0.78	0.70	0.71	0.71	0.82
	Feb 2005	0.24	0.26	0.18	0.18	0.78	0.70	0.33	0.63	0.82
	Mar 2006	0.48	0.39	0.41	0.45	0.91	0.74	0.63	0.75	0.50
% change	Pre-spill to 2005	0.0	0.0	− 33.3	− 50.0	0.0	0.0	− 52.9	− 11.8	0.0
	Feb 2005–2006	100	50.0	125.0	150.0	16.7	6.3	87.5	20.0	− 38.9
ASPT										
Observed	2005 pre-spill	3.8	3.33	3	3.38	4.44	4.75	4.59	4.2	4.94
	Feb 2005	3.8	3.33	2.25	2.25	4.44	4.75	2.63	4.2	4.94
	Mar 2006	3.6	3.78	3.11	3.4	4.81	4.59	4.07	4.61	4.45
Predicted	RIVPACS	4.87	4.86	4.9	4.91	5.17	5	5.05	5.09	4.83
EQI	2005 pre-spill	0.78	0.69	0.61	0.69	0.86	0.95	0.91	0.83	1.02
	Feb 2005	0.78	0.69	0.46	0.46	0.86	0.95	0.52	0.83	1.02
	Mar 2006	0.74	0.78	0.63	0.69	0.93	0.92	0.81	0.91	0.92
% change	Pre-spill to 2005	0.0	0.0	− 25.0	− 33.4	0.0	0.0	− 42.7	0.0	0.0
	Feb 2005–2006	− 5.3	13.5	38.2	51.1	8.3	− 3.4	54.8	9.8	− 9.9

At Sites 1–3 the calculated scores for the combined live and dead invertebrates were used as an estimate of the BMWP and ASPT values before the spill.

For Site 4 we used the mean of Environment Agency data for this location on 16 dates before the spill (March 1995 to November 2004).

For reference sites (A–D) and the most downstream site (Site 5) the BMWP and ASPT scores pre-spill were estimated to be the same as those 10 days after the spill.

EQI refers to the Environmental Quality Index, calculated by dividing observed by the predicted values. An EQI approximately equal or greater than one infers good biological water quality; low values indicate poor biological water quality.

% change refers to the percentage change between observed values; between pre-spill and post-spill 2005 values and between post-spill 2005 and 2006 values.

BMWP, Biological Monitoring Working Party; ASPT, Average Score Per Taxon.

many families were absent from Site 1 due to the spill. The only invertebrate families in Westlea Brook for which we have evidence of movement downstream were leeches of the families Erpobdellidae and Glossiphonidae (see 'Short-term impacts').

(ii) For Site 4 the families present in the postspill survey were compared with those recorded at this site by the Environment Agency on 16 occasions between 1995 and 2004. These data suggested that some families may have been missing from our postspill sample (e.g. due to downstream drift of live or dead invertebrates). We therefore used the mean of Environment Agency results for this site for the period 1995–2004.

(iii) For reference sites (A–D) and the most downstream site (Site 5) the BMWP and ASPT scores prespill were assumed to be the same as those ~10 days after the spill. In the case of Site 5, the BMWP ~10 days after the spill was 89, compared with a long-term mean from Environment Agency surveys of 94.5. However, the ASPT at Site 5, ~10 days after the spill was 4.94, slightly higher than the long-term Environment Agency mean of 4.81. Therefore we did not adjust the estimated value of 89 for Site 5.

The RIVPACS predictions in Table 2 for Westlea Brook may be too high, as the RIVPACS method tends to overestimate scores at sites that are close to the source of the watercourse (Furse 2000).

RIVPACS is a method for predicting scores in pristine streams and rivers. It is quite common for the actual scores at sites in urban watercourses to be well below the RIVPACS predicted scores. This can be due to urban watercourses being affected by diffuse and point source discharges. Extensive channel shading or minimal vegetative habitat, for example, can limit potential faunal richness.

Short-term impacts

Dead and dying individuals could have been swept downstream before the first survey. This could have caused a decrease in the estimated pre-spill diversity at downstream sites. However, Table 2 shows that the predicted pre-spill BMWP value at Site 1, immediately downstream of the spill, was very similar to those at Sites A and B, above the spill.

During the first survey many leeches were observed in depressions in the stream bed of Westlea Brook. These depressions had been caused by people attending the spill and clean-up operations. The accumulations of leeches were most obvious near Site 2 (approximately 500 m downstream of the culvert) and none were seen at Site 1. Many of the leeches were alive, but their behaviour was abnormal as they were writhing in an unusual manner. They may have been affected by some components of the diesel. Densities of the leeches in the depressions were not accurately assessed, but there were about 20–100 in each footprint. We are not aware of any other reports of this behaviour in leeches following a diesel or oil spill.

Tables 3 and 4 show the survival at family level and the total number of live and dead individuals (excluding oligochaete worms) at each of the sites downstream of the spill. Oligochaetes were excluded from the calculation as their high densities and survival rates skewed the overall results.

The survival rate of all families combined was relatively high (Table 3 and Fig. 2). Both the impacted sites on Westlea Brook (Sites 1 and 2) had a combined family survival rate of 56%. At the next site downstream (Site 3, River Ray) the family survival rate was lower (45%). This was probably due to the presence before the spill of a greater proportion of sensitive families in the River Ray (e.g. Leptoceridae, Limnephilidae, Phryganeidae and Sialidae).

By comparing results from Sites 4 and 5 with Environment Agency data before the spill, we were able to estimate the likely impacts of the diesel spill on the number of families present. At Site 4 on the River Ray (Moredon Bridge) we estimated that between 0 and 3 families had been lost due to the spill. The most downstream site on the River Ray that we sampled (Site 5, Seven Bridges, Cricklade) did not appear to have any lost any invertebrate families.

The survival rate at our most impacted sites (Sites 1–3, 50 m to 1.4 km downstream of the spill) was similar to a 55% survival of taxa (mainly identified to generic level) recorded 0.7 km downstream of a larger diesel spill (26 500 L) in New York State, USA (Lytle & Peckarsky 2001). However, these authors reported no improvement at 5 km downstream, whereas at Site 4 (3.75 km

Table 3 Survival at the family level, 10 days after spill

	Invertebrate families alive	Invertebrate families dead	Survival at family level (%)
Site 1 (Westlea Brook)	5	4	56
Site 2 (Westlea Brook)	9	7	56
Site 3 (River Ray)	10	12	45
Site 4 (River Ray)	20	0–3 (absent)	87–100
Site 5 (River Ray)	21	0	100

Table 4 Survival rates of all invertebrates, 10 days after spill (oligochaete worms excluded)

Sites in downstream order	Specimens alive	Specimens dead	Overall survival of individuals (%)
Site 1 (Westlea Brook)	9	86	9
Site 2 (Westlea Brook)	58	300	16
Site 3 (River Ray)	76	188	29
Site 4 (River Ray)	2714	≥1	95–99
Site 5 (River Ray)	153	0	100

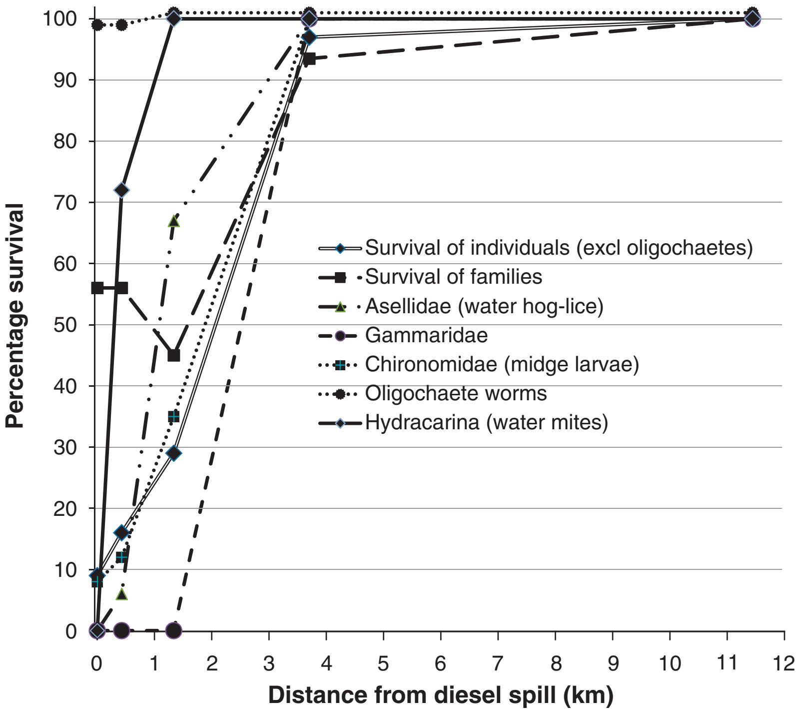

Fig. 2. Downstream impacts, 10 days after diesel spill.

downstream of the spill) we estimated a survival rate of 87–100% (Table 3). It seems likely that in our study all impacts on invertebrate distributions and densities occurred within approximately 4 km of the spill. No impacts were discernible at Site 5, 11.5 km downstream of the spill.

The lack of significant impacts on aquatic ecology at Site 4 is confirmed by information provided by the Environment Agency (R. Preston, unpublished report, Environment Agency). They reported that a fisherman caught roach, dace and chub on 25 January 2005, 2 days after the spill, at a point about 100 m upstream of Site 4 and retained them alive in a keep-net.

The percentage survival of all individuals (excluding oligochaete worms) showed a clear increase progressing downstream from the spill (Fig. 2 and Table 4). At Site 1 on Westlea Brook, closest to the spill, the estimated survival rate was 9%. Site 2 had an estimated survival rate of 16%, and the nearest downstream site on the River Ray (Site 3) had an estimated survival rate of 29%. At Site 4 (Moredon Bridge) examination of the sample in the field indicated a survival rate of at least 95% and at Site 5 (Seven Bridges Cricklade) it was probably 100%.

Figure 2 shows the percentage survival of ubiquitous groups (i.e. those found at every site downstream of the spill). These data allow estimation of their relative sensitivities to the diesel spill. Leeches appeared to be less sensitive than chironomids, but more sensitive than water mites (Appendix A). The overall order (in decreasing sensitivity) is: Gammarids > Water hog-lice > Chironomids > Leeches > Water mites > Oligochaetes.

These results are in broad agreement with those from toxicity testing of South Louisiana crude oil and diesel using three freshwater species (Bhattacharyya *et al.* 2003). This study showed that the tubificid oligochaete *Tubifex tubifex* was the least sensitive, with the gammarid *Hyallela azteca* the most sensitive and the chironomid *Chironomus tentans* intermediate in sensitivity. Studies of the impact of oil spills on freshwater invertebrates have shown that leeches and oligochaete worms are relatively tolerant of hydrocarbon pollution, while groups such as gammarid amphipods are very sensitive (McCauley 1966; Crunkilton & Duchrow 1990; Lytle & Peckarsky 2001). In freshwater toxicity studies, amphipod crustaceans (including the family Gammaridae) are one of the most sensitive groups to pollution (Environmental Protection Agency 2000), and this was the case in the current study.

In laboratory toxicity tests using *Asellus aquaticus* exposed to Dubai crude oil, severe toxicity was noted after only a few hours at concentrations ≥9.8 mg/L (Ramusino & Zanzottera 1986). The sensitivity of water hog-lice (*Asellus* spp.) in the present study is an important finding, as this group is widely recorded in streams and lakes, even at sites impacted by organic enrichment, e.g. from sewage discharges. If a diesel spill affected a watercourse that was already impacted by organic enrichment and therefore lacking many pollution-sensitive species, the densities of

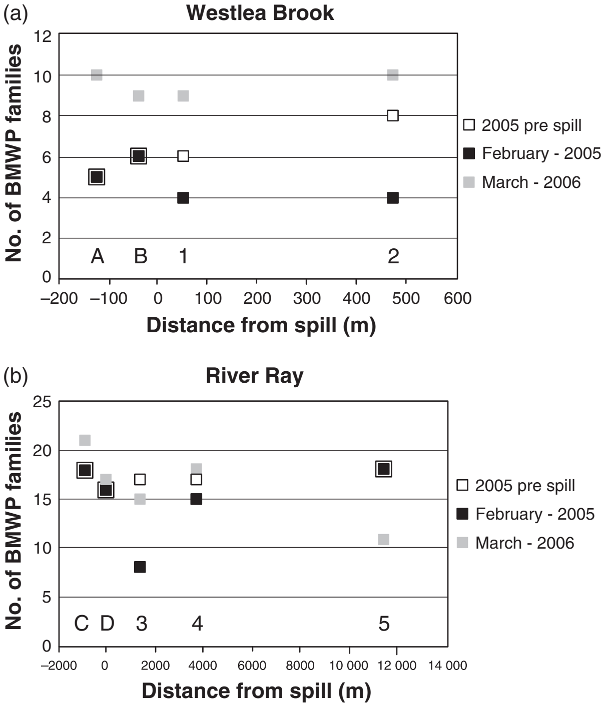

Fig. 3. Number of BMWP families upstream and downstream of the spill on (a) Westlea Brook and (b) River Ray.

Asellus spp. could be a good method of monitoring impacts and recovery.

Medium-term impacts and recovery

BMWP, number of scoring families and ASPT values for the second survey are shown in Table 2. With the exception of Site 5, sites showed an increase in the BMWP and number of scoring families (Fig. 3) compared with the values 10 days after the spill. As expected, the increases in BMWP and number of scoring families were greatest at sites that were most affected by the spill (Sites 1–3). The variations in sampling method at Sites D and 3 in the second survey may have resulted in a small decrease in the calculated BMWP scores at these sites.

Increases in BMWP scores and the number of families at reference sites (A–D) may be due to the slightly later sampling date in 2006. However, Environment Agency data for routine monitoring sites show even larger fluctuations, which do not appear to be related to sampling date. This suggests that sites on the River Ray go through periods of pollution impacts and recovery, and it is possible that both the Westlea Brook and River Ray were less impacted by other pollution sources in March 2006 than in February 2005.

In contrast to the general pattern of increased BMWP and number of families, the ASPT scores were not consistently higher at reference sites in the second survey. However, there were large increases in the ASPT scores at Sites 1–3.

The survey in March 2006 showed that Westlea Brook had recovered well (Fig. 3 and Table 2), and it was only at Site 1, closest to the culvert, where there were still some clear but small reductions in BMWP and ASPT values relative to other sites on Westlea Brook (Table 2). Site 2 on the Westlea Brook had apparently recovered fully (in terms of BMWP, scoring families and ASPT), but lack of prespill data makes it difficult to be certain.

Site 3, on the River Ray (1.4 km downstream of the spill), showed considerable improvement after 13.5 months, but was probably still impacted, as its BMWP and ASPT values were lower than at Site D, immediately upstream. It also had a lower BMWP and ASPT value than we predicted that it had in 2005 before the diesel spill. Owing to the high flows in the River Ray during the second survey the sampling method was slightly different, and this complicates interpretation of the results. Interpretation was further complicated by impacts from channel engineering works (environmental enhancements) carried out after the spill, which would have significantly disturbed the river. There was also a construction site immediately upstream that appeared to be adding additional sediment to the river and this would also affect the invertebrate communities.

Further downstream on the River Ray, at Site 4 (Moredon Bridge), it was possible to use the same sampling method in 2006 as in 2005. The results show that the site improved in quality since the spill and in March 2006 there was no evidence of any impact due to the diesel spill.

The low BMWP score (49) at Site 5 on the River Ray (Seven Bridges, Cricklade) in March 2006 was well below the normal range for this site (Environment Agency minimum for the previous 10 years was 62). However, the ASPT score was within the normal range. Although the results could be interpreted as indicating an impact due to the diesel spill it is more likely that they reflect some other pollution incident during the intervening period.

The recovery from the diesel spill occurred within the 13.5-month sampling period at all but the most impacted site. This is consistent with the results from a spill of 26 500 L of diesel to a small US stream, where the number of individuals had recovered after 3 months and the number of taxa had recovered after 15 months (Lytle & Peckarsky 2001). A large crude oil spill (1.5 million litres) to a Missouri river had significant impacts for 9 months (Crunkilton & Duchrow 1990). Harrel (1985) stated that complete recovery of a small Texan stream from a spill of 25 440 L of oil had not occurred 26 months after the spill. However in that case the recovery process was interrupted by cessation of stream flow during the period 6–11 months after the spill. Significant but incomplete recovery of invertebrates was observed 1 year after an oil spill in Missouri (Poulton *et al.* 1998).

Despite the recovery from the diesel spill impacts, sites on the River Ray (both upstream and downstream of the confluence with Westlea Brook) had lower BMWP scores and families than expected. Unfortunately many watercourses in urban areas have chronic water quality problems, from a variety of sources, e.g. storm water discharges (Robson *et al.* 2006). The impacts of the diesel spill on the River Ray and Westlea Brook would probably have been more severe if the sites had supported the full range of families expected at sites unaffected by discharges.

Conclusions

(1) A diesel spill of ~9800 L had severe short-term impacts on the macroinvertebrate fauna of Westlea Brook and the River Ray, UK. Impacts were discernible for about 4 km. After 13.5 months the impacts were minor, even at sites very close to the spill.

(2) The diesel spill affected urbanised watercourses. The short-term impacts would probably be greater in watercourses less impacted by minor (diffuse and point source) discharges.

(3) The use of live sorting of samples from impacted sites in the first survey allowed us to distinguish between specimens killed by the diesel and those still alive at the time of sampling. We recommend live sorting for any pollution impact studies where recently dead specimens may be present.

(4) Gammarid amphipods were the most sensitive group, followed by water hog-lice (*Asellus* spp.), chironomids, leeches, water mites and oligochaete worms. The diesel spill had little impact on the densities of oligochaete worms.

(5) Apart from leeches, there was no evidence that live or dead specimens were transported downstream after the spill. It would be interesting to monitor downstream movements of invertebrates immediately after a hydrocarbon spill to determine if this is a correct interpretation.

(6) The percentage loss of families was greatest at Site 3 on the River Ray (1.4 km downstream of the spill), probably due to the presence of a greater proportion of sensitive families before the spill.

(7) The sensitivity of *Asellus* spp. is an important finding, as this genus is widely recorded in streams and lakes, even at sites impacted by organic enrichment from sewage discharges. The survival and densities of *Asellus* spp. could be a good method of monitoring impacts and recovery from a diesel spill into a watercourse already significantly impacted by organic enrichment.

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Appendix A

Table A1

Table A1 Data for Westlea Brook

		February 2005						March 2006			
Order	Family	A	B	1D	1L	2D	2L	A	B	1	2
Trichoptera	Psychomyiidae	0	0	0	0	0	0	0	0	0	0
	Hydropsychidae	0	0	0	0	0	0	0	0	0	0
	Polycentropidae	0	0	0	0	0	0	0	0	0	0
	Rhyacophilidae	0	0	0	0	0	0	0	0	0	0
	Glossosomatidae	0	0	0	0	0	0	0	0	0	0
	Hydroptilidae	0	0	0	0	0	0	0	0	0	0
	Leptoceridae	0	0	0	0	0	0	0	0	0	0
	Limnephilidae	3	0	0	0	0	0	1	0	0	0
	Phryganeidae	0	0	0	0	0	0	0	0	0	0
Plecoptera	Nemouridae	0	0	0	0	0	0	0	0	0	0
Ephemeroptera	Baetidae	0	0	0	0	1	0	0	0	2	0
	Caenidae	0	0	0	0	0	0	0	0	0	0
Odonata	Calopterygidae	0	0	0	0	0	0	0	0	0	0
	Coenagriidae	0	0	0	0	0	0	0	1	0	0
Megaloptera	Sialidae	0	0	0	0	0	0	0	0	0	0
Coleoptera	Dytiscidae	0	0	0	0	0	0	0	0	0	1
	Elmidae	0	0	0	0	0	0	0	0	0	0
	Haliplidae	0	0	0	0	0	0	0	0	0	0
	Hydrophilidae	0	0	0	0	0	0	0	0	0	0
Hemiptera	Corixidae	0	0	0	0	0	0	0	0	0	0
Crustacea	Asellidae	214	145	17	0	49	3	398	537	22	59
	Gammaridae	365	406	4	0	158	0	186	315	80	117
	Crangonyctidae	0	0	0	0	0	0	4	2	0	1
	Ostracoda	0	0	0	0	0	0	0	2	0	0
Mollusca	Ancylidae	0	0	0	0	0	0	0	0	0	0
	Acroloxidae	0	0	0	0	0	0	0	0	0	0
	Hydrobiidae	0	0	0	0	0	0	0	0	27	0
	Lymnaeidae	0	0	0	1	0	0	0	0	20	4
	Physidae	0	0	2	2	0	0	1	0	3	0
	Planorbidae	0	0	0	0	0	0	0	0	0	0
	Sphaeriidae	4	4	0	0	2	0	7	40	16	2
Diptera	Ceratopogonidae	1	0	0	1	2	1	4	‘1	2	0

Table A1 Continued.

		February 2005						March 2006			
Order	Family	A	B	1D	1L	2D	2L	A	B	1	2
	Chironomidae	178	212	59	5	35	5	173	176	198	33
	Ephydridae	0	0	0	0	0	0	0	0	0	2
	Dolichopodidae	0	0	0	0	2	1	0	0	0	0
	Empididae	0	0	0	0	0	0	0	0	0	0
	Muscidae	0	0	0	0	0	0	0	0	0	0
	Psychodidae	2	0	0	0	1	0	8	2	1	0
	Sciomyzidae	0	0	0	0	0	0	0	0	0	0
	Simuliidae	0	1	0	0	0	0	0	0	0	0
	Stratiomyidae	1	0	0	0	0	0	0	0	0	0
	Tipulidae	0	0	0	0	1	0	1	2	0	0
	Unidentified Diptera	1	0	3	0	19	0	0	0	0	0
Acari	Hydracarina	0	1	1	0	11	28	0	0	0	0
Collembola	Collembola	0	0	0	0	0	0	0	0	0	0
Hirudinea	Erpobdellidae	0	0	0	0	18	14	0	0	0	45
	Glossiphoniidae	0	0	0	0	1	5	3	0	0	15
	Hirudidae	0	0	0	0	0	0	4	2	0	0
Tricladida	Dendrocelidae	0	0	0	0	0	0	0	0	0	0
	Dugesiidae	0	0	0	0	0	0	0	0	0	0
	Planariidae	0	0	0	0	0	0	0	1	0	3
Oligochaeta	Oligochaeta	53	242	10	1336	6	603	529	323	697	421
Nematoda	Nematoda	0	0	0	0	0	0	0	0	0	1
Nematomorpha	Nematomorpha	0	0	0	0	0	1	0	0	0	0
Total invertebrates		822	1011	96	1345	306	661	1319	1404	1068	704
Total invertebrates excluding oligochaete worms		769	769	86	9	300	58	790	1081	371	283

D, specimens dead when sample was sorted; L, specimens alive when sample was sorted.

Appendix B

Table B1

Table B1 Data for River Ray

		February 2005						March 2006				
Order	Family	C	D	3D	3L	4	5	C	D	3	4	5
Trichoptera	Psychomyiidae	26	1	0	0	0	0	8	1	1	0	0
	Hydropsychidae	0	0	0	0	43	0	0	0	0	0	0
	Polycentropidae	0	0	0	0	0	0	0	0	0	0	0
	Rhyacophilidae	1	0	0	0	0	0	0	0	0	0	0
	Glossosomatidae	0	0	0	0	0	0	2	0	0	0	0
	Hydroptilidae	54	2	0	0	10	1	1	0	0	8	0
	Leptoceridae	0	2	3	0	0	1	3	0	0	0	0
	Limnephilidae	21	1	6	0	0	0	27	3	2	2	0
	Phryganeidae	0	0	1	0	0	0	0	0	0	0	0
Plecoptera	Nemouridae	0	0	0	0	0	0	0	1	0	1	0
Ephemeroptera	Baetidae	569	4	6	0	81	2	88	8	1	48	1
	Caenidae	0	0	0	0	6	37	0	0	0	1	1
Odonata	Calopterygidae	0	0	0	0	0	20	1	0	0	2	2
	Coenagriidae	0	0	0	0	0	0	0	0	0	0	0
Megaloptera	Sialidae	0	0	2	0	0	0	0	0	0	0	0
Coleoptera	Dytiscidae	0	4	0	0	0	0	0	1	0	0	0
	Elmidae	25	1	1	0	49	18	14	4	3	6	1
	Haliplidae	0	0	0	0	0	1	0	0	0	0	0

Table B1. Continued.

		February 2005						March 2006				
Order	Family	C	D	3D	3L	4	5	C	D	3	4	5
	Hydrophilidae	0	0	0	0	0	0	0	0	1	0	0
Hemiptera	Corixidae	0	0	0	0	0	0	0	1	0	0	0
Crustacea	Asellidae	167	23	10	20	112	3	59	8	7	715	0
	Gammaridae	406	12	3	0	6	3	419	9	3	94	0
	Crangonyctidae	0	0	0	0	0	0	0	0	0	20	1
	Ostracoda	0	0	0	0	0	0	0	0	0	0	0
Mollusca	Ancylidae	103	0	2	0	0	0	10	1	0	0	0
	Acroloxidae	0	0	0	0	0	2	0	0	0	0	0
	Hydrobiidae	175	47	18	3	1995	23	22	3	9	310	0
	Lymnaeidae	23	0	1	1	1	0	5	0	5	0	1
	Physidae	0	0	0	0	0	13	0	0	0	0	1
	Planorbidae	12	2	6	3	0	0	14	1	2	0	0
	Sphaeriidae	55	25	63	6	52	3	72	13	2	98	0
Diptera	Ceratopogonidae	0	0	0	0	3	1	4	3	3	2	0
	Chironomidae	121	39	56	30	279	17	86	43	10	242	13
	Ephydridae	0	0	0	0	0	0	0	0	0	0	0
	Dolichopodidae	0	0	0	0	0	0	0	0	0	0	0
	Empididae	7	2	2	0	0	0	0	0	0	0	0
	Muscidae	4	0	1	0	0	0	1	0	0	7	0
	Psychodidae	5	2	1	0	0	1	1	13	9	3	0
	Sciomyzidae	0	0	0	0	0	1	0	0	0	0	0
	Simuliidae	285	3	6	0	14	2	45	14	1	71	25
	Stratiomyidae	0	0	0	0	1	0	0	0	0	0	0
	Tipulidae	0	1	0	0	0	0	0	2	0	0	2
	Unidentified Diptera	0	0	0	0	0	0	0	3	2	0	0
Acari	Hydracarina	3	0	0	5	0	0	1	5	0	1	0
Collembola	Collembola	0	0	0	0	0	0	0	1	3	0	0
Hirudinea	Erpobdellidae	0	0	0	1	19	0	1	0	0	35	0
	Glossiphoniidae	2	1	0	6	1	0	12	0	1	7	0
	Hirudidae	0	0	0	0	0	0	0	0	0	0	0
Tricladida	Dendrocelidae	0	0	0	0	2	0	0	0	0	3	0
	Dugesiidae	2	0	0	0	34	1	1	0	0	92	0
	Planariidae	0	0	0	0	6	0	0	0	0	22	0
Oligochaeta	Oligochaeta	349	480	0	666	273	28	348	60	29	515	58
Nematoda	Nematoda	0	8	0	1	0	0	2	4	0	0	0
Nematomorpha	Nematomorpha	0	0	0	0	0	0	0	0	0	0	0
Total invertebrates		2415	660	188	742	2987	181	1247	202	94	2305	106
Total invertebrates excluding oligochaete worms		2066	180	188	76	2714	153	899	142	65	1790	48

D, specimens dead when sample was sorted; L, specimens alive when sample was sorted.