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Drift Response of Stream Invertebrates to Aerial Applications of Glyphosate

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Carnation Creek flows into the southwest corner of Barkley Sound, near Bamfield, on the west coast of Vancouver Island, British Columbia, and drains a 10 km^2 area in the coastal hemlock and cedar zone (Figure 1). The watershed has been intensively studied for 14 years under the jurisdiction of the Fisheries Research Branch of the Pacific Biological Station, Nanaimo, B.C., with emphasis on multidisciplinary research into the effects of forest harvesting on the watershed (Hartman 1982). In September 1984, the herbicide glyphosate (Roundup®) was applied aerially for conifer release to part of the lower valley, and research activities were conducted to investigate the fate and effects of the herbicide on various components of the watershed. Although the toxicity of glyphosate to aquatic invertebrates (Folmar 1978; Folmar et al. 1979) and the effects of a glyphosate application on lentic daphnids (Hildebrand et al. 1980) have been studied, the response of stream invertebrates to an aerial application of glyphosate has not been previously reported. This paper presents the drift response of aquatic invertebrates to glyphosate contamination from an aerial application to the Carnation Creek watershed.

MATERIALS AND METHODS

Three sites were selected for sampling invertebrate drift: 1) in the main channel of Carnation Creek approximately 30 m below B-weir and downstream of most treated portions of the valley, 2) in C Creek, a 1000-m ephemeral tributary buffered from the applications, and 3) in 1600 Tributary, a 800-m stream directly oversprayed (Figure 1). The drift site below B-weir was typical of most of lower Carnation Creek, with a section of riffle approximately 4 m wide comprising scoured gravel with small amounts of sand and silt. Water velocity ranged from 0.55 to 1.15 m/sec during sample collection. Water levels in C Creek fluctuated drastically throughout the sampling period such that the drift site varied from 0.5 m wide with almost no flow to 2 m wide with a water velocity of 0.97 m/sec. The stream bottom at the C Creek sampling site and through most of the 100 m section bordering the spray blocks consisted of small rubble and gravel

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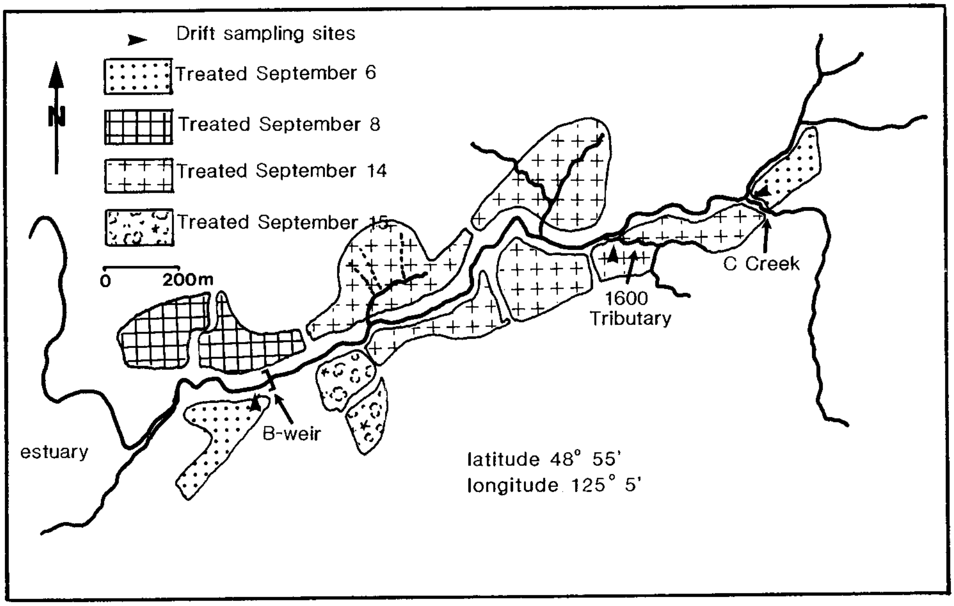

Figure 1. Glyphosate treatment blocks and drift sampling sites in the Carnation Creek watershed, September 1984.

with patches of sand and silt in quieter areas. Although riparian vegetation was dense in certain sections, C Creek contained little organic debris. The 1600 Tributary drained a swampy portion of the valley south of the main channel with a number of small pools and sections of moderate flow in constricted areas. In contrast to the other two sampling sites, this tributary contained a large amount of organic debris with a bottom type mostly consisting of silt, detritus, sand and small sections of fine gravel. The drift sampling site in 1600 Tributary was approximately 0.5 m wide with a flow rate that varied much less (0.33 to 0.58 m/sec) than the main channel or C Creek.

A specific drift net location was established at each site and used throughout the sampling period. Drifting invertebrates were collected in 0.47 x 0.32 m nets with a 363μ mesh positioned mid stream to sample a water column from the surface to the stream bottom. Current velocity and depth of water at the net opening were measured with each sample, and the collected invertebrates were subsequently quantified as number per 10 m^3 of water flowing through the drift net. Drift samples of 5 to 15 min duration were taken hourly during sunset and sunrise periods on collection dates, corresponding to the times when stream invertebrate drift normally peaks and declines (Waters 1972). Additional samples were collected at hourly intervals immediately following the applications of herbicide.

Differences in drift levels between sample periods were tested with ANOVA ($p<0.05$). Because of the small size of the creeks, especially C Creek and 1600 Tributary, spatial replication of drift nets was impossible. The values tested were mean drift numbers from collections over equal periods of time, i.e., means from morning or evening drift periods. Mean values were tested for statistical significance, but peak drift numbers provided a clearer indication of drift magnitude and are presented.

Stream water was collected from the B-weir and 1600 Tributary sites for analysis of residual glyphosate. No water samples were collected from C Creek. Only residue data relevant to the assessment of drift response are presented here. The concentration and persistence of glyphosate in stream water following the applications are reported by Feng et al. (1988). Collection, handling, and analytical procedures are described by Feng and Klassen (1986).

Glyphosate was applied as Roundup® at a concentration of 2.0 kg a.i./ha (maximum registered rate for conifer release) in a total volume of 252 L/ha using a Microfoil® boom with 1.5 mm hayrack nozzles, mounted on a Bell G-47 helicopter. Glyphosate was applied to 11 spray blocks (Figure 1) at various times over four separate days. Only those with potential for contaminating the portion of the watershed above drift sampling sites are discussed. On the evening of September 6, 1984 between 1940 and 2005 h, a spray block immediately adjacent to the east edge of C Creek was treated with herbicide (Figure 1). Most of the remaining creek valley, including the portion on the west side of C Creek, was sprayed between 1425 and 1945 h on September 14. The main channel of Carnation Creek and C Creek were buffered by a 10-m no-spray zone, while 1600 Tributary was intentionally oversprayed.

RESULTS AND DISCUSSION

The application of glyphosate on or adjacent to small tributaries of Carnation Creek did not result in undue disturbance of stream invertebrates. Drift densities of most aquatic invertebrates did not increase in response to the herbicide applications. None of the post-spray mean drift values for total invertebrate catches were significantly higher than pre-spray mean densities (ANOVA $p > 0.05$). High water conditions at both the beginning (0.41 m^3/sec) and end (0.51 m^3/sec) of the sampling period, compared to mid-period discharges (0.14 m^3/sec), probably accounted for most of the variations in drift density (discharge values were obtained from the permanent flow monitoring station at B-weir). Since many of the organisms in the drift samples occurred in low numbers, only the more frequently collected taxa are listed for each site with their peak drift levels (Table 1). Some groups, such as *Baetis* sp. at B-weir and *Capnia* sp. in C Creek, demonstrated an increase in drift after a glyphosate application, but the levels were comparable to previous pre-spray evening drifts. Several other taxa, including *Lepidostoma* sp. at B-weir and early instar limnephilid caddisflies in 1600 Tributary, showed an increase in drift density at the end of the sampling period that corresponded to heavy rainfall and an approximately five-fold increase in stream discharge. Since elevated drift levels of these same groups had occurred in high water conditions at the beginning of the sampling period (September 5,6) (Table 1), the post-spray drift increases were more likely a result of the sharp increase in discharge than contamination by the herbicide.

Table 1. Drift of selected aquatic invertebrates (no. per 10 m^3) collected in Carnation Creek. Values for each taxon represent peak numbers collected in morning or evening drift samples. Totals represent the total numbers of all taxa in the largest of hourly samples from morning or evening sample periods.

Date (September)	5	6	6	7	11	12	13	14	15	15
Collection time	PM	AM	PM	AM	PM	PM	AM	PM	AM	PM
C Creek[1]										
Baetis sp.	15.5	11.4	12.2	2.5	2.9	0.4	0.0	2.2	0.0	0.0
Paraleptophlebia sp.	2.0	1.4	21.9	1.2	1.1	0.0	0.0	2.2	0.0	0.9
Amphinemeura sp.	4.0	4.3	2.4	1.2	0.7	0.4	0.0	0.0	0.0	0.4
Capnia sp.	1.3	4.3	4.9	0.0	0.7	0.8	0.0	4.3	0.0	0.2
Limnephilidae	2.7	1.4	0.0	2.5	0.0	1.3	0.0	0.0	0.0	1.9
Total invertebrates	33.8	22.8	43.9	7.4	7.3	4.0	0.0	6.5	1.4	6.1
1600 Tributary[2]										
Paraleptophlebia sp.	0.9	0.0	0.9	0.0	0.7	0.7	0.0	0.8	0.0	7.4
Amphinemeura sp.	0.9	0.9	2.0	0.0	0.7	0.0	0.0	0.8	0.0	0.9
Limnephilidae	8.2	10.4	6.1	7.5	3.0	3.0	2.6	1.5	0.9	4.8
Chironomidae	7.1	1.7	1.0	2.8	3.5	3.0	1.3	1.5	0.9	3.3
Gammarus sp.	7.1	6.0	2.7	4.7	3.8	7.5	4.0	12.4	3.0	18.2
Total invertebrates	18.8	13.9	7.1	9.3	7.1	13.5	7.9	15.5	3.0	33.5
B-weir[3]										
Baetis sp.	19.4	24.2	21.4	16.9	35.4	38.5	4.3	42.6	3.6	29.4
Paraleptophlebia sp.	3.6	4.4	1.0	1.6	1.4	1.9	0.0	0.5	0.3	15.5
Alloperla sp.	1.5	1.9	0.5	0.5	1.9	2.7	0.1	3.4	0.0	1.9
Amphinemeura sp.	3.8	1.1	1.6	1.3	0.2	1.4	0.0	0.5	0.0	1.9
Capnia sp.	5.2	2.0	3.6	2.9	7.4	8.1	1.4	11.8	0.3	6.4
Lepidostoma sp.	43.7	12.7	8.6	1.9	0.5	8.4	1.6	1.0	0.3	19.6
Limnephilidae	2.6	2.3	1.4	0.8	0.7	1.4	2.2	0.5	0.3	5.4
Chironomidae	6.5	2.5	4.2	2.4	1.4	6.1	1.4	2.4	0.5	7.8
Dixidae	1.4	1.3	0.4	0.8	0.0	0.5	0.3	0.5	0.0	6.4
Simuliidae	1.2	1.1	3.6	1.1	0.9	0.4	0.5	1.0	0.3	0.3
Total invertebrates	88.4	55.1	47.2	32.8	51.2	65.0	12.7	63.7	4.4	82.6

1 East periphery of C Creek sprayed at 1940 h to 2000 h on September 6, and west periphery sprayed at 1515 h to 1535 h on September 14, 1984
2 Oversprayed at 1425 h to 1510 h on September 14, 1984
3 Located in untreated main channel downstream of C Creek and 1600 Tributary

Although the drift patterns of most invertebrates were not measurably affected by the glyphosate applications, the drift response of two particular organisms, *Gammarus* sp. and *Paraleptophlebia* sp., may suggest a slight and ephemeral herbicide induced disturbance in and downstream of the treatment areas. Post-spray drift of these taxa was not significantly different from prespray levels (ANOVA $p>0.05$), but a measurable alteration in the drift patterns of these two genera, especially of *Paraleptophlebia*, was demonstrated. This disruption of drift patterns may have resulted from natural causes, but was coincident with glyphosate contamination and therefore cannot be dismissed as being unrelated to the herbicide applications.

The numbers of drifting *Gammarus* sp. collected in 1600 Tributary were approximately twice as high on the evening of the overspray as they were in prespray evening drifts (Table 1) but this increase is not directly attributable to the application. This amphipod is a common stream drift component but is not particularly susceptible to glyphosate. In a toxicity test of aquatic organisms including four invertebrates, *Gammarus* sp. was found to be the least sensitive to Roundup® with a 48-h EC50 of 43,000 μg/L (Folmar et al. 1979). In 1600 Tributary, residual glyphosate in integrated water samples did not exceed 162 μg/L and was less than 50 μg/L within 10 h after the overspray (Table 2). The magnitude of the drift increase was not statistically significant (ANOVA $p>0.05$) and the duration was less than 2 h but the peak drift value from the evening of the application represents a disruption of the previous evening drift patterns and may indicate a disturbance of *Gammarus* sp. by glyphosate contamination in 1600 Tributary. Definite indications of a toxic or behavioral response of *Gammarus* sp. to the herbicide application are precluded by the minimal drift increase.

Table 2. Glyphosate levels (μg/L) in stream water. Values are the hourly mean concentration of integrated samples to the end of September 14 and actual concentrations of point samples at given times starting September 15. ns = not sampled.

Date	Time interval (PDT)	Hours after application	Glyphosate conc. ug/L B-weir	1600 Tributary
September 6	1950-2050	1.2	0.0	ns
	2100-2150	2.2	0.0	ns
September 14	1440-1530	1.0	0.0	138.00
	1540-1630	2.0	0.0	162.00
	1640-1730	3.0	0.0	ns
	1740-1830	4.0	0.0	150.00
	1840-1930	5.0	0.94	121.50
	1940-2030	6.0	1.40	79.40
	2030-2130	7.0	1.33	54.40
	2140-2230	8.0	0.66	ns
	2400	9.5	ns	37.10
September 15	0130	11.0	0.57	ns
	0430	14.0	0.0	ns
	0600	15.5	ns	36.50
	1030	20.0	0.17	ns
(rain began 1430)	1800	27.5	ns	109.40
	1900	28.5	0.42	ns
	2000	29.5	2.00	78.60
	2100	30.5	2.80	ns
	2200	31.5	3.20	31.90
(rain ended 2230)	2300	32.5	3.10	ns
	2335	33.0	1.80	20.60

Immediately following the herbicide treatment to the eastern periphery of C Creek on September 6, the numbers of drifting

Paraleptophlebia sp. distinctly increased (Table 1). The mean number of these mayflies collected on the evening of the application adjacent to C Creek was not significantly different from the previous evening (ANOVA $p>0.05$), but the drift of *Paraleptophlebia* sp. attained a higher level (approximately 10X the prespray peak) than at any other time in C Creek (Figure 2). The lack of significant difference in *Paraleptophlebia* sp. drift levels occurred despite the nearly tenfold increase because one sample on the evening of the increase contained no mayflies, consequently producing high variability and no significant difference from other evening drifts.

Paraleptophlebia sp. drift rates in C Creek did not increase with treatment of the other adjacent spray block on September 14. A 10-m buffer on both sides of C Creek was observed for both applications adjacent to the creek, but recorded field observations suggested that the helicopter flew closer to the creek on the evening of September 6 than during the 1600 Tributary overspray on September 14. During the first application adjacent to C Creek, the aircraft flew a spray pattern roughly parallel to the stream and given the circuitous nature of the creek, the likelihood of inadvertently overspraying parts of C Creek was greater than when flight patterns approached the creek at right angles during the application on September 14. The greater opportunity for contamination of the stream during the first peripheral application may account for the differential response of *Paraleptophlebia* sp. to the two applications adjacent to C Creek.

This particular mayfly also demonstrated increased drift in 1600 Tributary and B-weir in evening drift samples 30 h after the overspray of 1600 Tributary (Figure 2). This increase was less than 9X the prespray peak and may be a response to increases in stream discharge (from 0.13 to 0.51 m^3/sec at B-weir) resulting from a 39-mm rainfall prior to and during the evening sampling period of September 15. Elevated drift levels associated with increases in stream discharge have been frequently observed (Waters 1972; Anderson and Lehmkuhl 1968). In this instance, however, elevated drift of *Paraleptophlebia* sp. at these two sites was not previously associated with the high water conditions on September 5 (Figure 2), and was coincident with a recurrence of glyphosate from runoff. Shortly after rain began on September 15 (approximately 1430 h), concentrations of glyphosate in stream water increased at both B-weir and 1600 Tributary sites (Table 2). The occurrence of glyphosate residues may have combined with the effects of increasing discharge to produce these slightly elevated drift levels of *Paraleptophlebia* sp.

The peak measured concentrations of 109.40 μg/L at 1600 Tributary and 3.20 μg/L at B-weir are 1/90 to 1/3,000 the reported concentration of 10,000 μg/L required to induce movement of *Ephemerella* sp. mayflies in an avoidance chamber (Folmar 1978). In the same experiment, Folmar found no avoidance of Roundup® by *Ephemerella* sp. at concentrations of 1,000 μg/L. Our peak residue

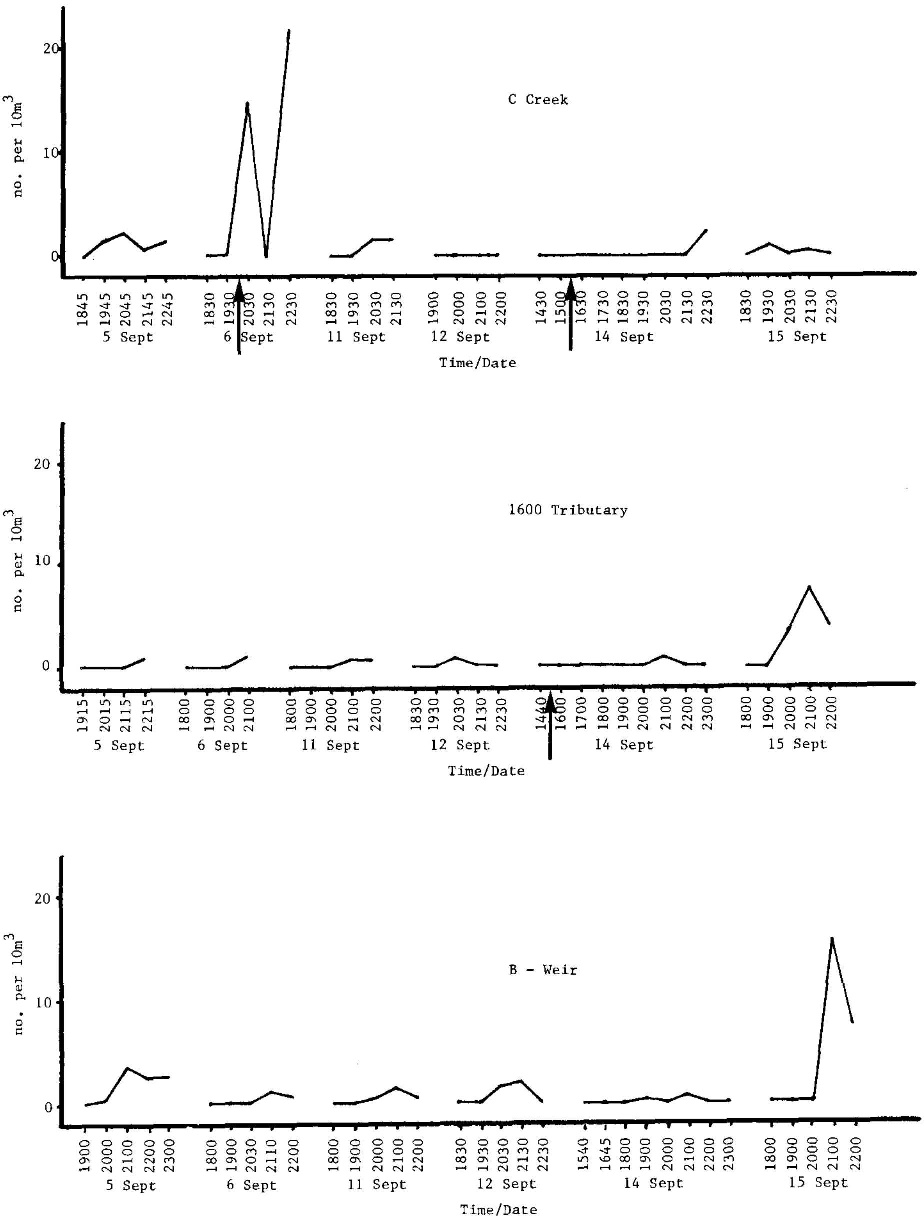

Figure 2. *Paraleptophlebia* sp. drift densities in evening samples. Arrows indicate glyphosate applications.

values represent what would appear to be a substantial margin of safety from the reported effective concentrations of glyphosate.

If the drift increases of *Paraleptophlebia* sp. were due to glyphosate contamination, they occurred at much lower concentrations than would be expected, suggesting a lower level of toxicant perception or a particular susceptibility of this genus to glyphosate, especially when exposed to the herbicide in actual field applications. If the drift responses were attributable to the herbicide treatment, the impact was limited to *Paraleptophlebia* sp. since none of the other groups demonstrated a similar increase. Small postspray increases in drifting *Gammarus* sp. were inconclusive. This short term impact study has shown that when glyphosate was aerially applied to or near salmon nursery streams, it produced, at most, slight and ephemeral drift increases of one mayfly species.

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