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INFLUENCE OF STRIP-MINING ON THE MORTALITY OF A WETLAND CADDISFLY, *LIMNEPHILUS INDIVISUS* (TRICHOPTERA: LIMNEPHILIDAE).

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ABSTRACT

A coal mine about 2.2 km upstream from Stillfork Swamp Nature Preserve, Carroll Co., Ohio was suspected of causing a reduction in *Limnephilus indivisus* caddisflies in the south half of the preserve. Second instar *L. indivisus* larvae collected from the south half of the preserve and from two control areas were reared in cages at the site of collection and at the other two sites in a replicated experiment. Elevated total dissolved solids in water samples from within rearing enclosures displayed strong correlation ($r^2 = 0.864$) with increased mortality when compared to larvae reared in unaffected areas. This investigation suggests that larvae of *L. indivisus* are useful in biomonitoring of wetlands impacted by acid-mine drainage, and potentially other perturbations.

The Blum Coal Company began mining activities on 26 November 1985, ca. 2.2 km upstream from Stillfork Swamp Nature Preserve, Carroll Co., Ohio (Fig. 1). Because pre-perturbation data on Trichoptera existed (Usis and MacLean 1986), an intensive survey of the caddisflies inhabiting Stillfork Swamp was conducted from the spring of 1986 through the fall of 1988 to evaluate changes (Usis 1990).

Based upon 56 light-trap collections made in 1984, Usis and MacLean (1986) reported that *Limnephilus indivisus* Walker represented the most abundant caddisfly at Stillfork Swamp. However, equal numbers of light-trap collections made during 1986, 1987, and 1988 indicated that their population had dramatically declined (853 in 1984, 94 in 1986, 33 in 1987, and 98 in 1988). Nimmo (1966) suggested that increases or declines in light-trap catch size might be attributed to variations in factors such as temperature, wind, moonlight, and trap placement. Night-time temperature has even been shown to influence the percentage of females in light trap catches (Andersen 1978). Analysis of air temperature records at the time of nightly collection revealed that they did not vary by more than a few degrees in subsequent years. Field observations also did not reveal other physical factors that would account for the substantial declines in numbers of *L. indivisus*. Unfortunately, only a few studies have monitored populations of Trichoptera for several seasons (McElravy et al. 1982, Resh 1976, 1982; Haag et al. 1984, McElhone et al. 1987, McElravy and Resh 1987), and the natural variability in size of trichopteran populations is relatively unknown. As a consequence of its population reduction, *L. indivisus* was selected for a field rearing experiment to determine if numerical reduc-

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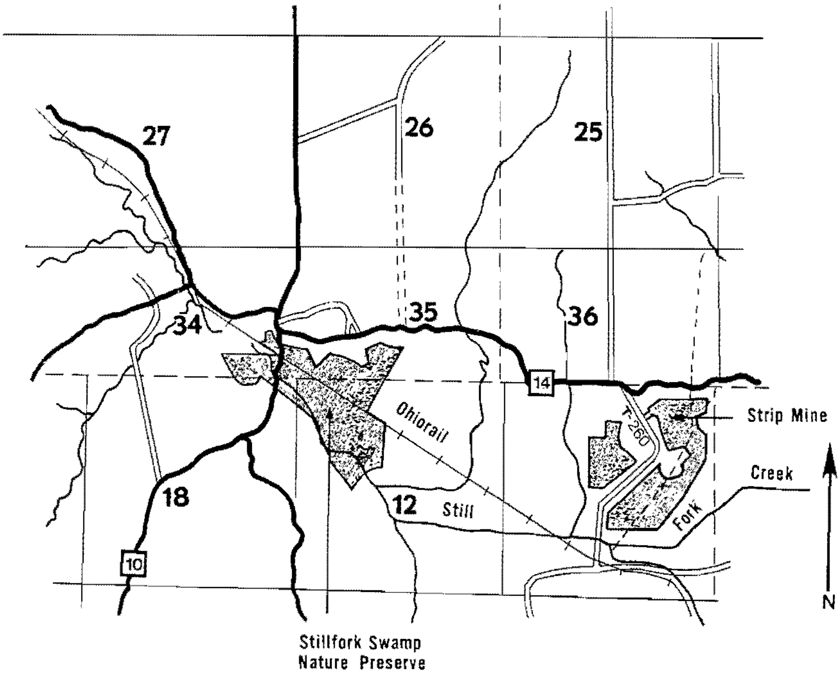

Figure 1. Map showing Stillfork Swamp Nature Preserve, located in Augusta and Washingtown townships, and strip-mine permit area, located in Washingtown township, Carroll County, Ohio.

tions could be attributed to surface mining activities. The life history and behavior of *L. indivisus* are relatively well known (Mickel and Milliron 1939, Noval and Sehnal 1963, 1965; Wiggins 1973, Richardson and Mackay 1984).

MATERIALS AND METHODS

Submerged screen enclosures allow water, fine silt, and detritus to exchange between enclosure and pool, providing largely natural conditions for trichopteran larvae (Colburn 1984). Nine wood-framed enclosures (31cm × 31cm × 31cm) with bottom and sides covered by 2 mm mesh PVC-coated fiberglass screen were placed in wetland pools at south Stillfork Swamp and each of two control areas, which were vegetatively similar, one week prior to beginning the experiment. These control areas were the north Stillfork Swamp which is isolated from mine drainage by a railroad (Fig. 1), and a wetland near Leetonia, which is in a different watershed. Enclosure tops were covered with 6 mm (1/4") plate glass permitting easy access. At the start of the experiment decaying water smartweed (*Polygonum natans*) and giant bur-reed (*Sparganium eurycapum*) were placed into the enclosures to provide a substrate for larval feeding and case construction. These substrates represent the preferred feeding and casemaking materials for *Limnephilus indivisus* at Stillfork and Leetonia

Source Location of *Limnephilus indivisus*

Rearing Enclsoure Location	North RR 2nd instar Larvae	South RR 2nd instar Larvae	Leetonia Swp. 2nd instar Larvae
North RR Stillfork Swp.	25 individuals per enclosure a,b,c (CN)	25 individuals per enclosure a,b,c (SN)	25 individuals per enclosure a,b,c (LN)
South RR Stillfork Swp.	25 individuals per enclosure a,b,c (NS)	25 individuals per enclosure a,b,c (CS)	25 individuals per enclosure a,b,c (LS)
Leetonia Swamp	25 individuals per enclosure a,b,c (NL)	25 individuals per enclosure a,b,c (SL)	25 individuals per enclosure a,b,c (CL)

Figure 2. Experimental design utilized for rearing *Limnephilus indivisus* (Walker), a 3 x 3 contingency table (ANOVA Model 1) with nine treatments each with three replicates (a, b, c). Symbols for treatments 1st letter indicates the larval source (N = north, S = south, L = Leetonia, C = control when larval source and rearing habitat are the same); 2nd letter indicates larval rearing location.

swamps. On 30 March 1988 second instar larvae were collected at each of the three study sites. Figure 2 illustrates the experimental design. The 25 larvae reared in each enclosure were checked biweekly at each site from 30 March – 2 June. Measurements on temperature, dissolved oxygen, pH, conductivity, and total dissolved solids (TDS) were gathered from rearing enclosures during each visit. On 2 June, enclosures were removed from rearing locations and larval cases (occupied or empty) were sorted by instar and placed in 80% ethanol.

By transferring larvae to different rearing locations and establishing controls, a 3 x 3 contingency table containing 9 separate treatment groups generated a Model 1 analysis of variance (ANOVA) (Zar 1984). The results were analyzed with ANOVA after the data were transformed to arcsin $\sqrt{(\%\ \text{mortality})}$ to obtain a normal distribution (Steel and Torrie 1960). Significantly different means ($P \leq 0.05$) were separated by Tukey's multiple range test (Zar 1984).

RESULTS AND DISCUSSION

Larvae construct cases of vegetation and as they grow attach more vegetation to increase the size of their cases. When they reach their 5th and final instar, cases typically measure 22-25mm. If the larva dies, the case remains. Its size can be used to determine at what instar mortality occurred. Survivorship calculations depended on accounting for all cases. Table 1 lists mortality of reared *Limnephilus indivisus*. Of 675 larvae, 173 completed their 5th instar and were sealed in their cases as pupae or pre-pupae and had attached themselves to decaying giant bur-reed or water

Table 1.—Larval survivorship of *Limnephilus indivisus* Walker reared in encosures in wetland areas impacted or not impacted by strip-mining between March 30 and June 2, 1988 (refer to Fig. 2 for listing of treatment symbols).

	CN			SN			LN			CS			NS			LS			CL			NL			SL			
Description. of Specimen	CNa	CNb	CNc	SNa	SNb	SNc	LNa	LNb	LNc	CSa	CSb	CSc	NSa	NSb	NSc	LSa	LSb	LSc	CLa	CLb	CLc	NLa	NLb	NLc	SLa	SLb	SLc	Totals
2nd Instar Case Empty (open)	0	2	1	3	4	4	0	6	3	3	11	7	8	9	7	3	9	10	1	0	1	3	2	0	4	5	2	108
3rd Instar Case Empty (open)	3	1	4	4	5	1	2	1	4	5	6	4	5	0	11	1	3	0	2	3	1	4	2	3	3	3	4	85
4th Instar Case Empty (open)	4	2	1	2	3	1	1	1	1	0	3	1	3	0	0	0	1	0	4	1	3	2	2	4	1	0	2	43
5th Instar Case Empty (open)	3	4	3	6	7	5	3	2	3	1	2	4	1	4	0	2	0	4	3	6	5	5	3	3	5	7	5	96
5th Instar Case (closed)																												
Larva inside – Live	2	0	1	1	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	1	10
Larva inside – Dead	2	3	0	1	1	2	3	1	4	0	0	0	0	0	0	0	0	0	1	0	2	1	1	2	1	0	1	26
Pupa inside – Live	8	7	9	4	2	4	7	5	6	2	2	2	2	4	3	3	2	4	10	9	8	9	6	9	4	5	4	140
Pupa inside – Dead	3	2	4	1	0	1	2	0	0	0	1	0	1	1	0	0	0	0	1	0	1	1	3	0	0	1	0	23
Adult – Male	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2
Adult – Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Larva Location Unknown	0	4	2	2	3	6	7	9	4	14	0	7	5	6	3	16	10	7	2	5	3	0	5	4	7	4	6	141
Totals	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**675**
No. of Survivors	10	7	10	6	2	5	7	5	6	2	2	2	2	5	4	3	2	4	11	10	9	9	7	9	4	5	5	153
Mean No. of Survivors/Treatmt.		9			4.33			6			2			3.67			3			10			8.33			4.67		
Mean Survivorship Rate/Treatmt.		0.36			0.17			0.24			0.08			0.15			0.12			0.4			0.33			0.19		
Survivorship Rate	**0.40**	**0.28**	**0.40**	**0.24**	**0.08**	**0.20**	**0.28**	**0.20**	**0.24**	**0.08**	**0.08**	**0.08**	**0.08**	**0.20**	**0.16**	**0.12**	**0.08**	**0.16**	**0.44**	**0.40**	**0.36**	**0.36**	**0.28**	**0.36**	**0.16**	**0.20**	**0.20**	**0.23**

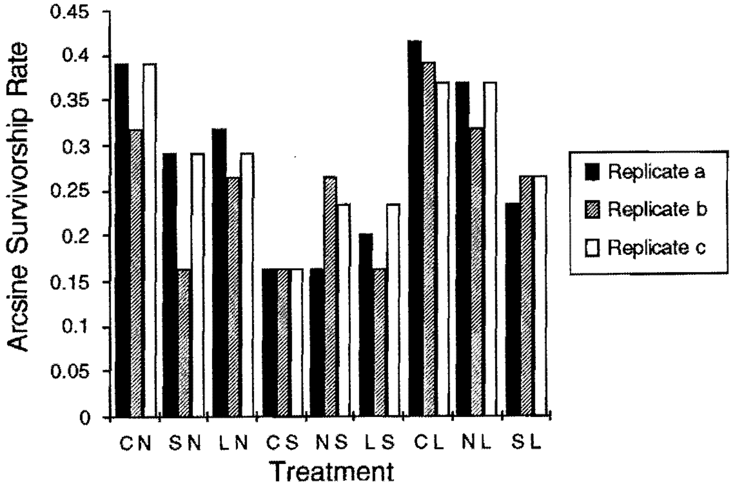

Figure 3. Histogram showing *Limnephilus indivisus* survivorship for all treatments and replicates (refer to Fig. 2 for listing of treatment symbols).

smartweed foliage or the screen mesh of the enclosure. Upon examination of these sealed cases, 23 pupae were recorded as dead with only the exoskeleton remaining; several had a white fungal growth covering much of the inside of the case. Bert(1982) reported that a species of *Entomophthora* can infect *Limnephilus externus* Hagen. Three adults had emerged from their sealed cases, not by removing the thick filter plugs at the ends of the case which the larva constructs before pupation, but by cutting through the silken linings of the case approximately 2-3mm from the anterior end. This behavior has not previously been reported for *L. indivisus* by other observers. The rearing experiment was terminated before adult emergence to facilitate collection and quantification of mortality while live individuals were still inside their cases.

Survivorship rates in all enclosures are shown in Fig. 3. Larval survival was not the same for all treatment groups or replicates. Most survivorship rates [proportion (X/*n*)] observed for this experiment were below the 30% value and required arcsine transformation. The arcsine survivorship values resulted in an F statistic equal to 12.53***, highly significant, since F0.001(1)8,18 = 6.48 (Table 2), indicating that treatments do not share means in common. Because a significant F value resulted from the analysis of variance, the Tukey test was applied to the means ranked in order of magnitude. This multiple comparison test revealed that the mean survival ratios described three distinct groups - (Low survival: CS, LS); (Medium survival: NS, SN, SL, LN); (High survival: NL, CN, CL) (refer to Fig. 2 for description of treatments).

It was the control groups (CN, CS, CL) that were of primary interest, because these groups could reveal whether wetland areas of Stillfork Swamp Nature Preserve were being impacted by surface-mining activities. Figure 4a compares mortality in control treatments. In both Stillfork Swamp north of the RR tracks (CN) and Leetonia swamp (CL) similar mortalities of approximately 60% were observed during development from 2nd instar to adult; but larvae occupying southern Stillfork Swamp locations (CS) suffered 92% mortality rates and especially high mortality during the 2nd instar.

Table 2 — Single factor analysis of varinace (ANOVA) of arcsine transformed survival ratios for reared *Limnephilus indivisus* Walker (refer to fig. 2 for treatment headings.).

HO = There is no difference in survival of larvae among treatments.
(i.e., Is variability among treatments greater than variability within treatment?)

Treatment	CN	SN	LN	CS	NS	LS	CL	NL	SL
Replicate a	0.3923	0.2933	0.3195	0.1643	0.1643	0.2027	0.4155	0.3687	0.2358
Replicate b	0.3195	0.1643	0.2657	0.1643	0.2657	0.1643	0.3923	0.3195	0.2657
Replicate c	0.3923	0.2933	0.2933	0.1643	0.2358	0.2358	0.3687	0.3687	0.2657

Source of Variation	SS	DF	MS
Total	0.1778	26	-
Groups	0.1505	8	0.0188
Error	0.0274	18	0.0015

F = Group MS/Error MS = 12.53 ***

F 0.05(1) 8,18 = 3.01
F 0.01(1) 8,18 = 4.28
F 0.001(1) 8,18 = 6.48

Mortality of all larvae (CS, NS, LS) reared in the southern area of Stillfork Swamp was similar with 50% mortality or greater observed between the 2nd and 3rd instars (Fig. 4b). Larvae taken north of the RR tracks and transferred to other locations (NS, NL) only showed substantial reductions when reared in southern Stillfork Swamp locations (NS) (Fig.4c). Survivorship in the Leetonia swamp was similar to those observed north of the RR tracks at Stillfork Swamp (compare Fig. 4d & 4e). Southern source larvae transferred to rearing locations north of the RR tracks (SN) and Leetonia swamp (SL) showed improved levels of survivorship by almost double when compared to the southern control treatment (CS) (Fig. 4f). However, levels of survivorship were still relatively low.

Table 3 lists water quality data which were collected at the three habitat locations from within enclosures (Stillfork Swamp—north and south of RR tracks and Leetonia Swamp) during the rearing of *Limnephilus indivisus*. Water temperatures during larval development at the three different locations reflected similar warming rates. Leetonia swamp remained only slightly warmer during the study. Richardson and Mackay (1984) have observed that temperature primarily controls the rate of development for *L. indivisus* which was probably similar for these three locations. Dissolved oxygen, inversely related to water temperature, reflected similar saturation levels (all near or at 100%) for all enclosures and rearing locations. On 23 May 1988 the dissolved oxygen level within southern Stillfork Swamp rearing locations was 9.4 mg/ml (93% saturation), the lowest oxygen concentration recorded. Hydrogen ion concentrations (pH) although not similar, were not statistically different (F = 2.07, where F0.05, 2, 15 = 3.68). The mean pH at southern locations was 5.17; at northern locations, 5.59; and at Leetonia Swamp, 6.27. In all locations, pH slowly increased from March to June. The lowest pH, 4.7, was recorded on 30 March 1988 for locations south of the RR tracks, Stillfork Swamp. North RR track locations registered 5.1 at that same time.

Conductivity readings between locations, although not statistically different (F = 3.26, F0.05, 2, 15 = 3.68), did show some variability (Fig. 5). There was a general trend toward decreasing conductivity over the course of the experiment and the lowest levels recorded on 2 June. Total dissolved solids (TDS) proved to be the most

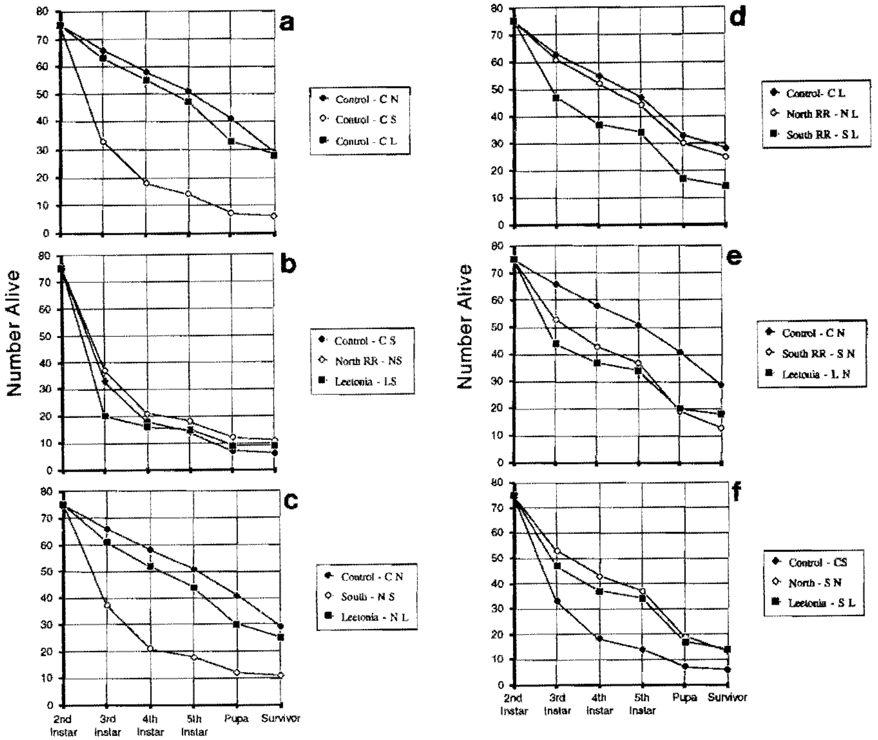

Figure 4. Larval mortality of *Limnephilus indivisus* Walker: (a) control enclosures, (b) reared in south Stillfork Swamp, (c) transferred from northern portions of Stillfork Swamp to locations (NS, NL), (d) reared in Leetonia Swamp, (e) reared north Stillfork Swamp, (f) transferred from southern portions of Stillfork Swamp to locations (SN, SL) (refer to Fig. 2 for treatment symbols).

variable (Fig.6) and were statistically different between locations with an F = 5.65* (F0.025, 2, 15 = 4.77). More significant is the difference in dissolved solids on 30 March 1988 when larvae were placed in their enclosures. Southern locations had two to three times higher TDS and conductivity readings on this date than northern or Leetonia habitats. We are uncertain whether intensity in mining activity remained constant during this rearing experiment.

The design of the field experiment allowed several statements about the impact of surface mining on *Limnephilus indivisus* to be made. *Limnephilus indivisus* is a shredder that inhabits the temporary pools within many northeastern Ohio wetlands and swamps. Wetland areas within Stillfork Swamp which were regularly flooded by Still Fork Creek in the spring of 1988 received effluents commonly associated with acid-mine drainage. Increased conductivity and elevated total dissolved solid concentrations were strongly correlated ($r^2 = 0.864$) with decreased survivorship of larvae reared at southern swamp locations. Controls established in northern areas and in a similar wetland habitat of another watershed known not to be receiving acid-mine drainage did not suffer these high mortality rates (92%). But mortality in

Table 3 — Water quality data in enclosures during rearing of *Limnephilus indivisus* larvae.

Date/Location	Temperature °C	pH	Conductivity µmhos/cm	Dissolv.Oxy. mg/ml	TDS mg/L
South RR					
3/30/88	3	4.7	766	13.5	798.1
4/13/88	4.5	4.8	513	15.2	489.3
4/24/88	6	5.6	746	12.5	451.7
5/14/88	11.2	6.4	453	10.8	423.6
5/23/88	13	6.4	445	9.4	296.8
6/2/88	14	6.2	261	12.2	472.1
Mean Value	8.62	5.17	630.67	12.27	488.60
Std. Dev	4.69	0.78	193.99	2.03	166.40
North RR					
3/30/88	2	5.1	380	13.1	232.5
4/13/88	4	5.3	472	14.9	280.3
4/24/88	5	6.2	551	11.2	379.5
5/14/88	10.1	6.5	287	11	200.4
5/23/88	11	5.9	327	12.2	286.9
6/2/88	13	6.5	275	9.7	432
Mean Value	7.52	5.59	382.00	12.02	301.93
Std. Dev	4.43	0.60	109.69	1.82	88.04
Leetonia					
3/30/88	3	5.9	425	12.2	295.8
4/13/88	5	6.4	451	13.3	370.7
4/24/88	7	6	254	10.9	189.4
5/14/88	11	6.2	381	11.2	291.5
5/23/88	11.5	7.2	233	11.7	351.7
6/2/88	15	6.8	240	10.8	154.1
Mean Value	8.75	6.36	330.67	11.68	275.53
Std. Dev	4.51	0.50	99.55	0.95	86.82

controls was high (60%); however, type 2 or 3 survivorship curves are common in many aquatic insect species (Resh and Rosenberg 1984). It is felt that this mortality rate reflects a true mortality estimate experienced by this species under natural conditions.

Hydrogen ion concentration (pH) has been shown to be critical for most Trichoptera (Havas and Hutchinson 1982), but some limnephilids, e.g., *Limnephilus pallens* (Banks), apparently can tolerate extended periods at pH's much lower than 4.5. Chris Stefanov, the ODNR field inspector for the mining site, stated that sediment ponds located on the permit site routinely had pH's readings in the 5.0—4.5 range. At no time during this study did we obtain water samples from Still Fork Creek or from rearing enclosures with pH values below 4.7. Wiederholm (1984) has indicated that the effects of low pH in acid-mine waste water are often difficult to separate from the effects of suspended solids and heavy metals. Consequently, it is highly unlikely that pH was the single critical variable affecting larval mortality. It is more likely that an interaction involving a number of factors produced poor survival for this species.

Colburn (1983) reported that larval survival and adult development of *Limnephilus assimilis* (Banks) was reduced as temperature increased in desert ponds with high

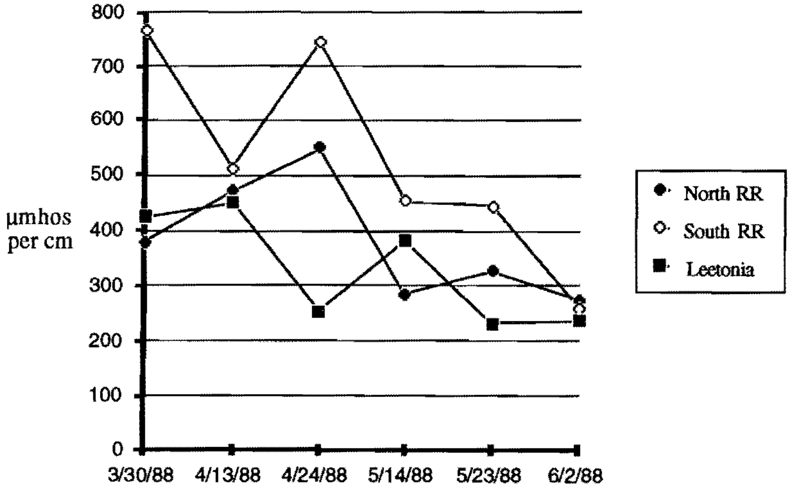

Figure 5. Water conductivities within rearing enclosures during *Limnephilus indivisus* larval development.

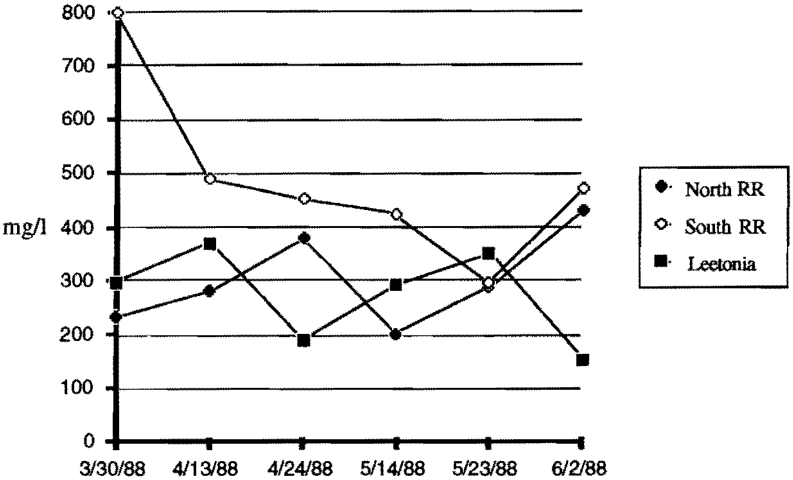

Figure 6. Total dissolved solids (TDS) within rearing enclosures during *Limnephilus indivisus* larval development.

salinities. Apparently, high temperatures coupled with the high conductivities due to elevated ion concentrations interrupted the larva's osmo-regulatory system and caused increased mortality. We suspect that some similar condition might have affected *L. indivisus*. Conductivity ($\approx$780 μmhos/cm) and dissolved solid concentrations ($\approx$ 800 mg L^{-1}) were extremely high in southern swamp areas in early spring of 1988. In general, dissolved metals are more toxic than metals in other forms (e.g., as precipitates) and their solubility is strongly affected by pH and temperature. Apparently such synergistic effects increase the negative influence of acid-mine water on many freshwater invertebrates (Wiederholm, 1984). After loss of ice cover in early spring, water temperature increases and larval growth and development rapidly occurs. Death for many larvae occurred during their 2nd instar, and was especially noticeable in southern rearing locations (Table 1). Solem (1983) reported that a critical time for survival of 1st or 2nd instars larvae of *Limnephilus stigma* Curt., an inhabitant of temporary vernal pools in Norway, was when ice was present and the substrate frozen. Perhaps, spring is a critical time for larval development of *L. indivisus* before the pools dry-out in mid-June. Stresses caused by elevated total dissolved solids and conductivity may have affected this insect's ability to regulate ion exchange within its chloride epithelium. Wichard and Komnick (1973) indicated that larvae of Limnephilidae possess circumscribed areas, known as chloride epithelia, on abdominal segments II—VII which function as osmoregulatory organs able to remove chloride ions and other electrolytes from the water passing through their cases. These ions are subsequently transferred to the hemolymph in compensation for ions lost from excretion.

Regardless of the exact mechanism which caused *L. indivisus* mortality, the evidence suggests that acid-mine drainage was the contributing factor in the decline of this species. The high degree of sensitivity shown by this species to acid-mine drainage also suggests that this caddisfly or congeneric species are potential candidates for wetland managers to utilize when assessing the influence of upstream strip-mining, and possibly other perturbations.

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