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A SYSTEM FOR RECORDING AND ANALYZING MACROINVERTEBRATE BEHAVIOUR IN LABORATORY STREAM CHANNELS

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

McNicol, R.E., and E. Scherer. 1985. A system for recording and analyzing macroinvertebrate behaviour in laboratory stream channels. Can. Tech. Rep. Fish. Aquat. Sci. 1391: iv + 15 p.

Described is a laboratory system for the recording and analysis of the microdistribution, locomotor activity, and drift behaviour of stream-dwelling macroinvertebrates under visible and infrared lighting. A photographic apparatus automatically takes black and white, infrared photographs of test animals, housed in two identical, recirculating stream channels, at regular intervals. The X-Y position coordinates of the head and abdomen of each animal are determined from the photographic negatives using a digitizer interfaced with a microcomputer. From these coordinates a computer program calculates the behavioural parameters of interest.

Key words: locomotor activity; biological drift; microdistribution; infrared photography; computer programmes; statistics; pollutants; sublethal effects.

RESUME

McNicol, R.E., and E. Scherer. 1985. A system for recording and analyzing macroinvertebrate behaviour in laboratory stream channels. Can. Tech. Rep. Fish. Aquat. Sci. 1391: iv + 15 p.

Le document décrit un système en laboratoire d'enregistrement et d'analyse, sous une lumière visible et infra-rouge, de la microdistribution, de l'activité locomotrice et du comportement de dérive des invertébrés macroscopiques fréquentant les ruisseaux. Un dispositif photographique prend automatiquement, à intervalles réguliers, des clichés en noir et blanc et à la lumière infra-rouge des animaux faisant l'objet de l'expérience. Ceux-ci sont alors placés dans deux canaux identiques d'eau courante à débit continu. Les coordonnés X-Y de la position de la tête et de l'abdomen de chaque animal sont établies à partir de négatifs photographiques traités à l'aide d'un convertisseur numérique couplé à un micro-ordinateur. D'après ces coordonnées, un programme informatique calcule les paramètres du comportement intéressants.

Mots-clés: activité locomotrice; dérive biologique; microdistribution; photographie à infra-rouge; programmes informatiques; statistiques; polluants; effets sublétaux.

INTRODUCTION

So far, research on the behavioural ecology of lotic invertebrates has been largely descriptive, due in part to a lack of suitable techniques for detailed study of underlying behavioural mechanisms. However, a significant number of recent studies on invertebrate drift, predator/prey interactions, and microhabitat selection have taken experimental approaches, aimed at identifying and studying these mechanisms (e.g. Gersabeck and Merritt 1979; Peckarsky 1980a, b; Peckarsky and Dodson 1980; Sheldon and Haick 1981; Ciborowski 1983; Kohler 1983; Maude and Williams 1983; Williams and Moore 1982). Indications that drift patterns, locomotor activity, and habitat selection can be altered when such organisms are exposed to pollutants (e.g. Flannagan 1973; Wallace and Hynes 1975; Hall et al. 1980; Flickinger et al. 1982; Williams and Moore 1982) underscore the need for closer study of their behaviour in order to understand its importance to these animals' survival both under normal as well as stress conditions. However, the tendency of many species to reside in turbulent water, under rocks or in crevices, obscured from easy viewing, has hampered field studies of their behaviour. Even when specimens are observed in laboratory streams, their photophobic and thigmotactic tendencies often make direct viewing and recording of their behaviour difficult.

One behavioural parameter which has received much attention in the past (see references above) is locomotor activity. Though several systems have been devised for automatic recording of locomotor activity of aquatic invertebrates (Lehmann 1972; Wildish and Polar 1972; Wallace et al. 1975; Idoniboye-Obu 1977; Rebach 1977; Fingerman et al. 1979; Batac-Catalan and White 1983; others reviewed in Atkinson et al. 1974), only two (Wallace et al. 1975; Lehmann 1972) have been applied to the study of lotic species. In this report we describe a system with which we can automatically record locomotor activity, microdistribution and drift behaviour of macroinvertebrates in laboratory stream channels under close to natural conditions.

APPARATUS DESCRIPTION

STREAM CHANNELS

The laboratory stream system (Fig. 1a,b) consists of two side-by-side, rectangular, open glass channels (120 cm L x 7.5 cm W x 12 cm H), similar to those described by Scherer (1965). Water flow to each channel is provided by two 1-HP submersible pumps (up to ≈ 1 L$\cdot$s^{-1} each) placed in separate, temperature-controlled reservoirs (315 L capacity each). Water enters one end of each channel and exits from the opposite, open end, returning to the reservoirs which are equipped with overflow and replacement water feeder lines ("partially recirculating channel", following the classification by Hammons (1981)). Flow velocity within each channel is controlled by valves on the main inflow-line and on a bypass line. Maximum velocity at a depth of 4.3 cm above the substrate plate is 30 cm$\cdot$s^{-1}. The minimum possible depth is contingent on the mesh size of the screen which dams the water to some extent. The insertion of solid barriers, such as glass plates, in front of the screen provides further depth control.

A plexiglass plate (1 cm thickness), upon which a thin layer of clear, silica sand is glued, is inserted into each channel. This provides a removable substrate which not only affords adequate footing for crawling invertebrates under high water velocity conditions, but is translucent enough to allow viewing of the animals from beneath the channels. These plates are supported 1 cm above the channel bottom by 1-cm diameter plexiglass posts, and extend to within 2-3 cm from the upstream end of the channel. This allows some water to flow under the plate, thus preventing the build-up of trapped air bubbles or debris between the plate and the channel bottom. Test animals are confined to a 75-cm long section of each channel by screens made from a rectangular piece of plastic "egg-crating", consisting of 1.3 cm^2 open cells one cm deep, over which is stretched a piece of nylon wedding veil (3-mm mesh size). The thread diameter of this material provides low resistance to flow, while providing a relatively strong and rot-resistent barrier. An additional screen (1 mm Nitex®) is placed between the upstream screen and the water inflow to collect debris and to reduce turbulence. Sponge-rubber weather stripping (0.6 x 1.3 cm) around the edge of each screen provides an escape-proof seal between it and the channel walls, while allowing the screen to be easily removed for cleaning.

On top of the plexiglass/silica sand plate is placed a glass plate (40 cm L x 7 cm W x 0.2 cm H) supported 5 mm above the substrate by four 1-mm diameter glass posts. The top surface of each plate is painted with a thin, translucent layer of black, epoxy paint. These plates provide a refuge attractive to invertebrates with photophobic or thigmotactic tendencies, yet do not obscure their images from beneath the channel. The size and number of plates, as well as their height above the substrate may be varied depending on the size of the test animals and type of experiment.

To facilitate observing and recording the positions of animals in these channels, a mirror is placed underneath at a 45° angle. With sufficient lighting above the channel, sharp images of the animals, both in the open and under the glass plates, are clearly visible. Daytime lighting is provided by two 40-W fluorescent plus two 15-W incandescent lamps, while nighttime "lighting" is provided by six 40-W incandescent lamps fitted with infrared (IR) filters (>690 nm) (Fig. 1). The fluorescent and IR lighting is controlled by a double-throw interval timer, while an automatic light-dimming apparatus (Graham and Hutchinson 1977) controls the incandescent lamps which provide 40-min simulated dawn and dusk periods.

RECORDING APPARATUS

To record the positions of animals within the channels, we developed an automatic set-up

which allows us to photograph mirror images of the organisms at regular intervals of from <1 s to 24 h, day and night, over long periods. It consists of a 35-mm camera plus motor drive controlled by an intervalometer which is in turn controlled by a programmable, interval timer (Fig. 2). Such a timer is usually necessary for intervals greater than 15 min; it also allows recording at irregular intervals. More than one camera can be operated from the same intervalometer by splicing several leads into the connecting cable. This will allow simultaneous photographing of the channels from different angles. It is advantageous to provide an external power source for the camera (Fig. 2), thereby eliminating the possibility of premature battery failure of the camera's internal batteries during long periods of recording.

The use of Kodak high-speed black and white IR film allows us to record day and night. Intensity of the fluorescent lighting must be adjusted to match that of the IR lamps to ensure the same film exposure at the same camera settings regardless of light source. With our camera shutter set at 0.5 s, the aperture at f8, and the daytime lighting adjusted to 0.01 watts$\cdot$cm^{-2} (700 lux), we get similar film exposures under both light sources.

DATA PROCESSING

Once the IR film has been developed (similar procedure as for regular black and white film), the negatives are placed in a photographic enlarger and projected onto the active surface of a Hipad® digitizer interfaced with a Heathkit® H-90 microcomputer. A BASIC program (Appendix 1) requests the user to input the file name under which the data will be stored, followed by a reference name, treatment name and replicate number for each channel, as well as the date and time of the initial photograph to be digitized. The program then requests at what time increment subsequent photos were taken, followed by a request to digitize two reference points on the photograph. These allow the computer to calculate the correct x-y position coordinates of the animals regardless of the position of the negative image on the digitizer surface. The program then prompts the user to digitize the positions of the head and abdomen of each animal in the first channel. When all are digitized, the user can convey any one of six commands to the computer by digitizing a point within one of six contiguous 52 x 46 mm program-designated areas along the upper edge of the active surface of the digitizer. These six commands are (from left to right): 1) increment the time by the specified amount (when the user is ready to digitize the next negative; the program will request that the reference points be redigitized from this next photo), 2) reset the reference points, 3) switch channels (when the user has finished digitizing one channel and wishes to digitize the other from the same negative), 4) change the treatment name, the date, time or increment interval, 5) change only the time and increment interval, 6) close the file (data are stored on diskette). These "built-in" commands minimize keyboard interactions during digitizing. A partial listing of a data file is given in Appendix 2.

These data can now be directly transferred from diskette to a mainframe computer, using appropriate communications software, for data analysis. We then run our data through an SAS (SAS Institute Inc. 1982) program to calculate values of the behavioural parameters of interest. For example, the program listed in Appendix 3 calculates the number of animals changing position between frames (as a measure of locomotor activity), and numbers facing into the current (rheotactic response). However, this program can be easily modified to compute any information which can be calculated from x-y position coordinates (e.g. microdistribution or habitat preference). Even drift rates can be derived from such data, when calculated as the number of animals leaving the field of view (i.e. leaving the channel or becoming entrapped on the downstream screen). However, under most circumstances such behaviour is better quantified by using an IR-sensitive video camera.

At this stage, the data are ready for statistical analysis. For studies investigating the natural periodicity of certain behaviour parameters, several methods of analysis are possible (see Broom 1979 for a recent review). Our studies have been directed at investigating the time course of behavioural responses of invertebrates exposed to sublethal concentrations of contaminants. Typically, this involves exposing a group of animals to the contaminant and taking successive, behavioural measurements on the same group at regular time intervals over some period of time. Such experiments are of a "repeated-measures" design requiring special univariate (Rowell and Walters 1976; Walters and Rowell 1982), or multivariate methods of analysis (Danford et al. 1960; Cole and Grizzle 1966; Morrison 1972).

DISCUSSION

Previously described methods for recording locomotor activity and drift behaviour of aquatic invertebrates have relied on the use of light-beam (UV, red, or IR) interruption systems (Wildish and Polar 1972; Atkinson et al. 1974; Wallace et al. 1975; Rebach 1977; Williams and Moore 1982; Batac-Catalan and White 1983), electrical action potential (Idoniboye-Obu 1977), electrical impedance (Hoggarth and Trueman 1967) or other techniques (Guyselman 1957; Naylor 1958; Heusner and Enright 1966; Fielder and French 1970; Fingerman et al. 1979). However, most of these techniques require that test animals be confined to "open" habitats with no refuge. For crevice-dwelling stream invertebrates this would probably be a stressful environment, certainly not one approximating natural conditions. In addition, systems employing electrical impedance, action potentials, or mechanical devices to monitor animals are either unuseable in flowing water, or unable to monitor more than one animal at a time. Light-beam interruption systems also present problems because they measure locomotor activity through the beam paths only. Therefore, unless

a large number of beams are used, only a small proportion of an experimental chamber area can be monitored. This could lead to inaccurate measurements, depending on the size, number, motility, and microhabitat selectivity of test animals.

Our system overcomes these problems. Test animals are unimpeded and can be studied in a fully monitored, heterogeneous experimental habitat. Water surface turbulence does not seriously affect IR photo quality, so that animals can be studied in still or flowing water. We used IR instead of UV light for "invisible" night illumination because literature data indicate that insects are insensitive to infrared wavelengths, while being capable of detecting UV light (Mazokin-Porshniakov 1959; Wigglesworth 1972). Furthermore, we found no evidence that our test animals reacted to the IR lighting which we subjected them to. We have successfully used our system to study the effects of sublethal exposure to chemical stressors on the locomotor activity, microdistribution and drift behaviour of Acroneuria lycorias, a large, riffle-dwelling, perlid stonefly (Fig. 3). However, in addition to these parameters, others, such as those related to habitat preference or predator/prey studies, can be just as easily derived from the same photographs through small changes to the SAS program. In short, this relatively simple photographic method provides a versatile recording device for the simultaneous study of several aspects of the behaviour of groups of one or more invertebrate species under any water-flow conditions. The required equipment and materials are relatively inexpensive (Appendix 4), with many of the items commonly found in research laboratories.

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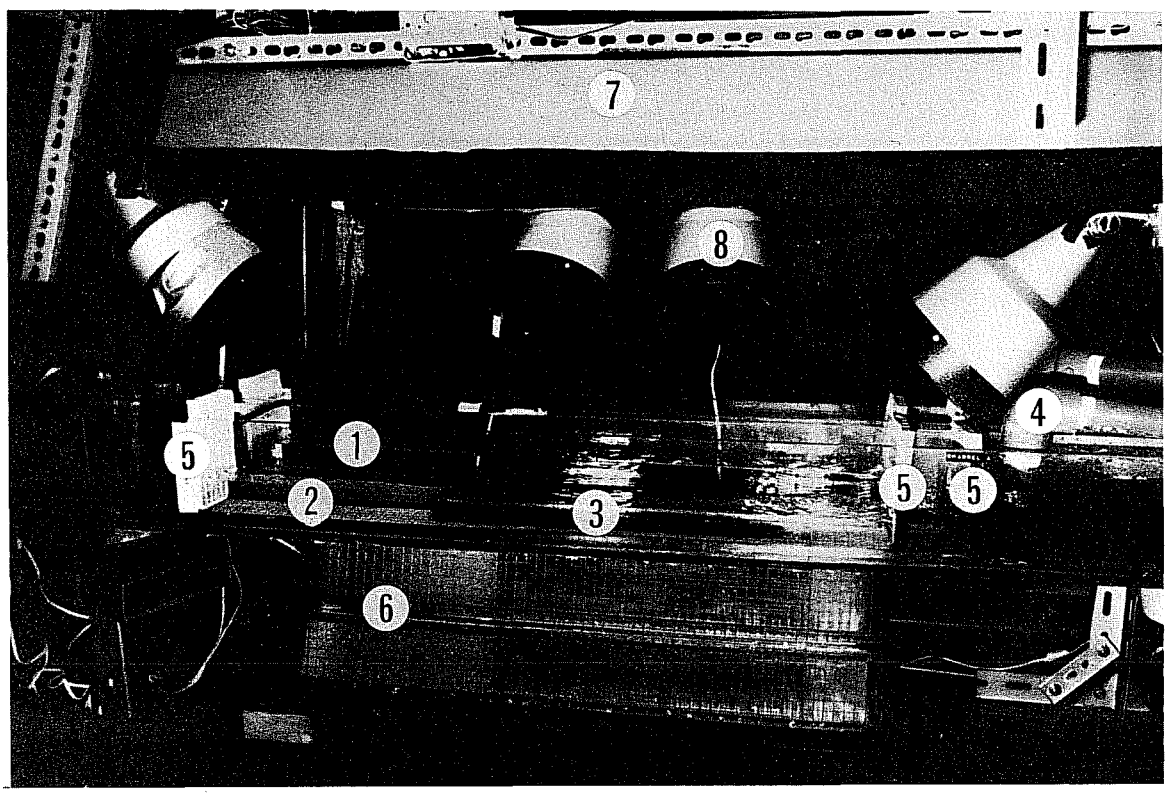

Fig. 1. Recirculating laboratory stream system.
a: Stream channels (two infrared lamps removed for clarity). 1) rectangular, open channel, 2) substrate plate, 3) darkened glass plate, 4) channel inflow pipe, 5) screens, 6) mirror showing view from underside of channels, 7) fluorescent light fixture, 8) infrared lamp.

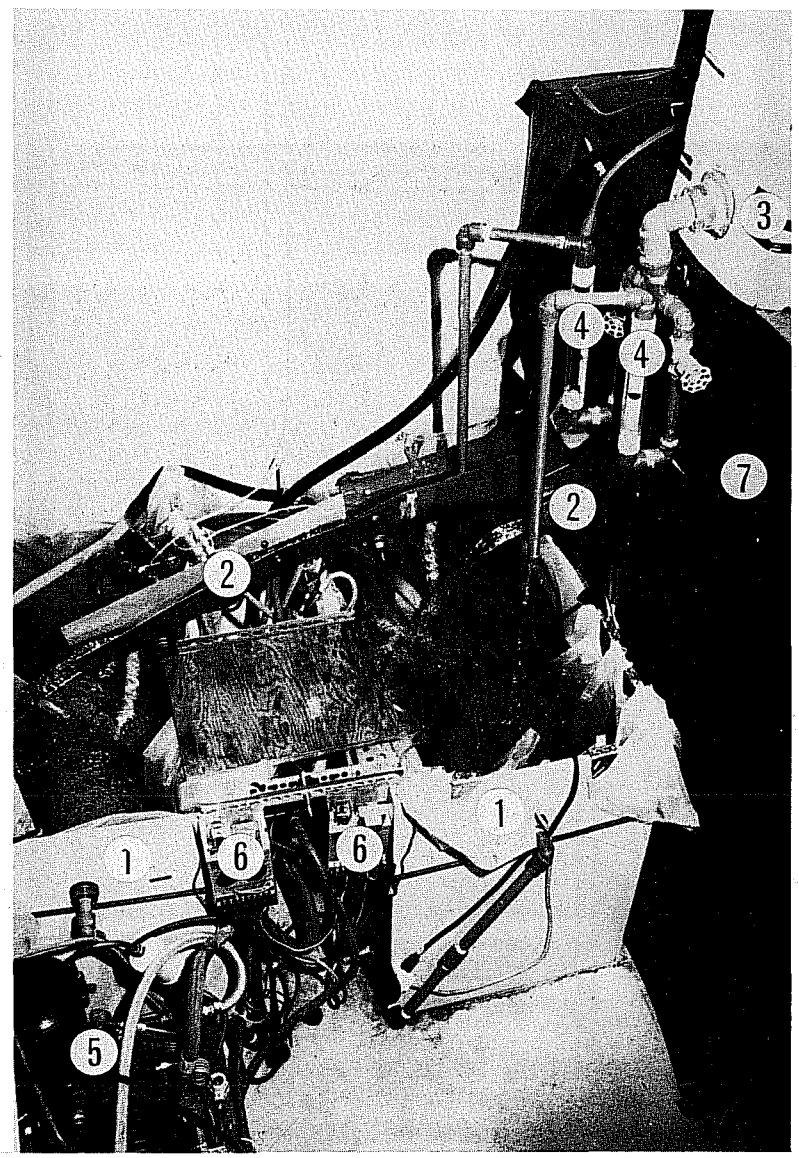

Fig. 1. Recirculating laboratory stream system.
b: Temperature-controlled reservoirs. 1) reservoirs, 2) channel outflows, 3) replacement water head tank, 4) flowmeters, 5) water-cooling apparatus, 6) temperature controllers, 7) enclosure surrounding stream channels.

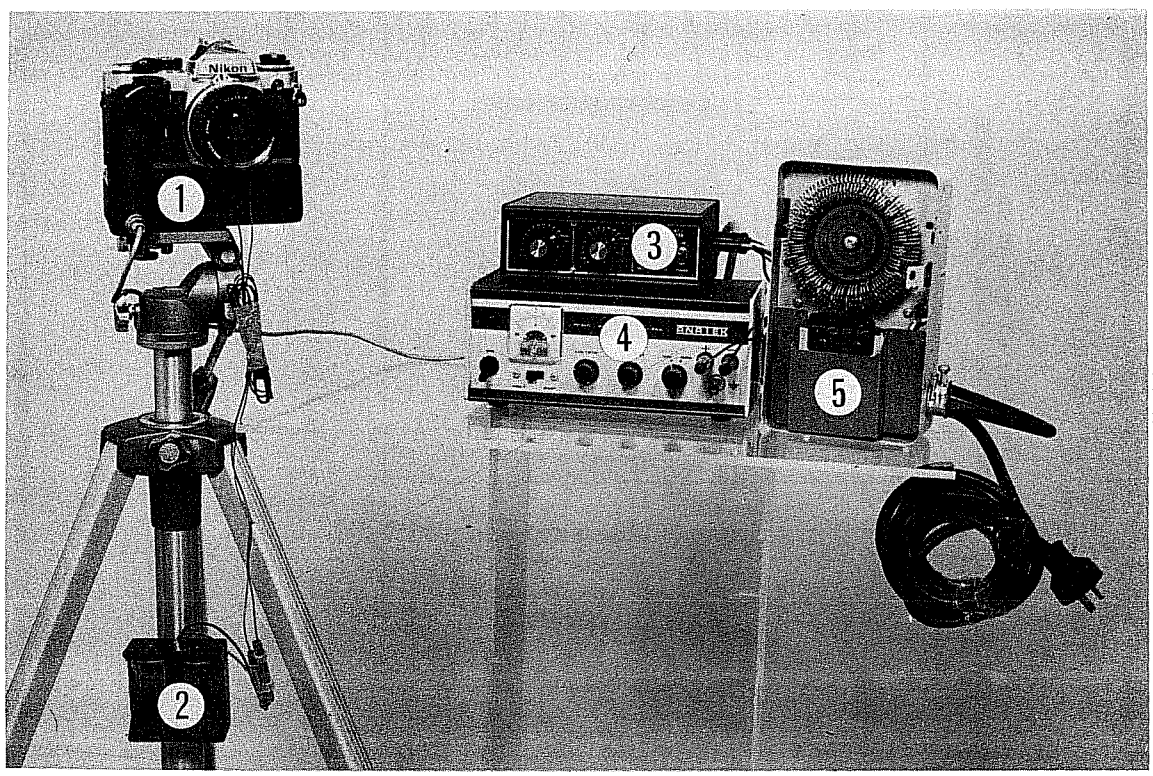

Fig. 2. Apparatus for automatic, regular-interval photographing of macroinvertebrates in laboratory stream channels. 1) 35-mm SLR camera with motor drive, 2) external camera power supply (two 1.5 V batteries), 3) intervalometer, 4) intervalometer DC power supply, 5) tab-programmable interval timer.

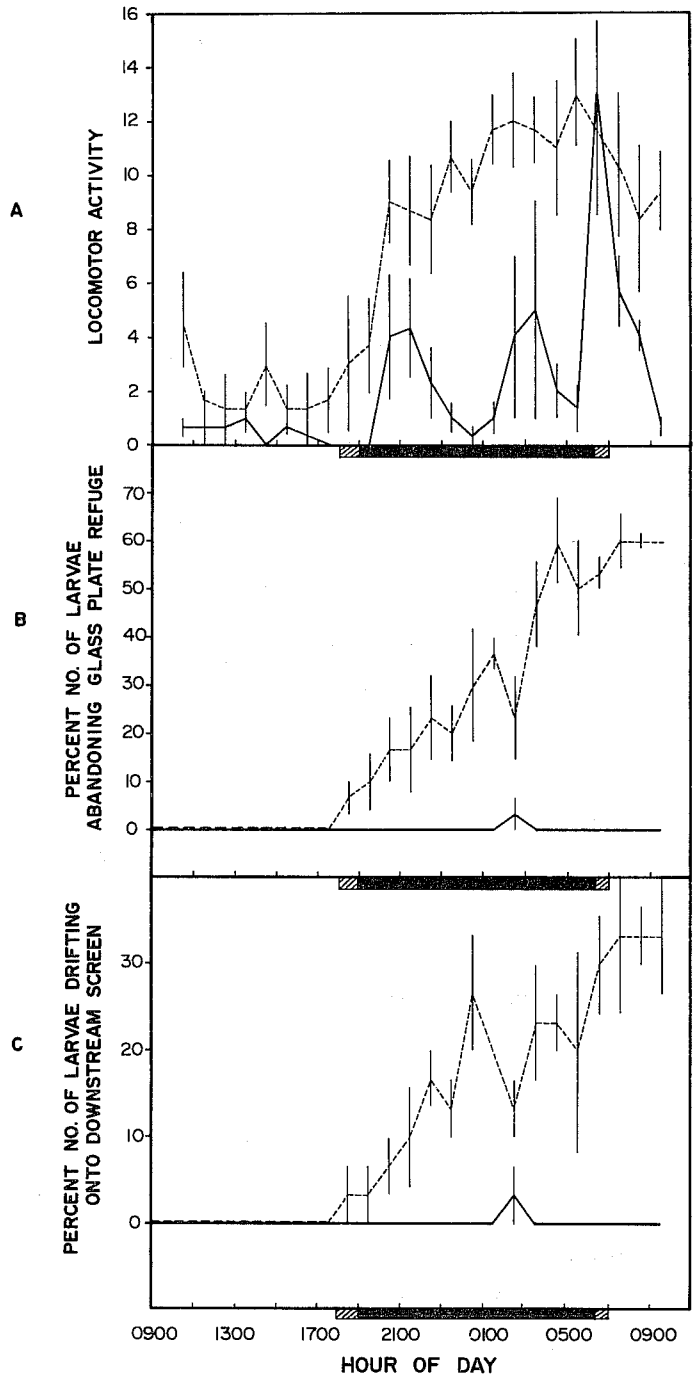

Fig. 3. Application example: effect of sublethal exposure to the insecticide fenitrothion on the A) locomotor activity, B) microdistribution, and C) drift of Acroneuria lycorias in laboratory stream channels. Locomotor activity of control (——) and insecticide-exposed (---) animals recorded as mean (of 3 replicates) number of larvae (out of groups of 10) moving a distance >0.5 body lengths per hour; ±1 SE indicated by solid, vertical bars; temperature 14.0°C; photoperiod 13 L:11 D with the 40 min "dawn" and 60 min "dusk" periods indicated by hatched, horizontal bars, and darkness by solid, horizontal bars; daytime light intensity 0.01 watts·cm^{-2} (700 lux); insecticide applied as a single 12 ppb spike at 0930 h, with a fresh water replacement rate of 90% in 24 h.

Appendix 1. Program (Microsoft® BASIC) for storing digitizer-generating x-y coordinates of the head and tail positions of test animals as recorded on photographic negatives.

```
1200 WIDTH 255
1250 DEFINT A-Z : DEFSNG X
1300 DIM BUTTON(5), MONTH(12)
1350 DIM DC$(15)
1400 DIM RECS$(50)
1450 DIM TIME(3), CTIME(3), ITIME(3)
1500 DIM DATE(3), CDATE(3)
1550 DIM TTYPE$(1), TNAME$(1), TNUM(1), TREP(1)
1600 '
1650 NO = 0 : YES = NOT NO
1700 TTYPE$(0) = "TOP" : TTYPE$(1) = "BOTTOM"
1750 HOME$ = CHR$(27) + "E"
1800 EEOL$ = CHR$(27) + "K"
1850 NR = 0
1900 XPI = 3.14159
1950 '
2000 DEF FNLC$(L, C) = CHR$(27) + "Y" + CHR$(L+32) + CHR$(C+32)
2050 DEF FNZF$(N) = MID$("0123456789",N\10+1,1)+MID$("0123456789",N MOD 10+1,1)
2100 DEF FNSZ$(N) = RIGHT$("       " + STR$(N), 6)
2110 DEF FNTF$(N) = RIGHT$("    " + STR$(N), 4)
2150 '
2200 ' Read in digitizer button addresses
2250 DATA 2400, 340, 845, 1351, 1866, 2392
2300 FOR I=0 TO 5 : READ BUTTON(I) : NEXT
2350 '
2400 ' Read in days in months
2450 DATA 31, 28, 31, 30, 31, 30, 31, 31, 30, 31, 30, 31
2500 FOR I=1 TO 12 : READ MONTH(I) : NEXT
2550 '
2600 ' Initialize output
2650 GOSUB 10400 : ' Get file name in NAM$
2700 OPEN "O", #1, NAM$
2750 GOSUB 3500 : 'get treatment, date and time
2800 '
2850 ' Get DX, DY from digitizer, branch to correct routine
2900 GOSUB 8150 : 'get x, y from digitizer
2950 FOR I=1 TO 5 : IF DX < BUTTON(I) THEN GOTO 3000 ELSE NEXT
3000 BRANCH = I
3050 IF DY < BUTTON(0) THEN BRANCH = 7
3100 ON BRANCH GOSUB 7100, 6600, 11850, 3500, 5000, 3250, 7450
3150 GOTO 2900
3200 '
3250 GOSUB 5800
3300 PRINT HOME$;
3350 CLOSE
3400 END
3450 '
3500 T = 0 : GOSUB 3700
3550 T = 1 : GOSUB 3700
3600 GOTO 4500
3650 '
3700 PRINT HOME$; FNLC$(10,0);
3750 PRINT "Enter "; TTYPE$(T); " treatment name : ";
3800 INPUT "", TNAME$(T)
3850 TNAME$(T) = LEFT$(TNAME$(T) + "          ", 10)
3900 PRINT "Treatment name is '"; TNAME$(T); "'"
3950 PRINT "Enter "; TTYPE$(T); " treatment number : ";
4000 INPUT "", TNUM(T)
4050 PRINT "Treatment number is "; TNUM(T)
4100 PRINT "Enter "; TTYPE$(T); " replication number : ";
```

Appendix 1. continued

```
4150 INPUT "", TREP(T)
4200 PRINT "Replication number is "; TREP(T)
4250 PRINT : INPUT "Is this data OK"; A$
4300 A$ = LEFT$(A$, 1)
4350 IF A$ <> "Y" AND A$ <> "y" THEN GOTO 3700
4400 RETURN
4450 '
4500 PRINT HOME$; FNLC$(10,0);
4550 INPUT "Enter date in form YYMMDD : ", DATE$
4600 DATE$ = LEFT$(DATE$ + "      ", 6)
4650 GOSUB 8700 : IF NOT OK THEN PRINT "Invalid date" : GOTO 4550
4700 PRINT "Date is "; DATE$; "."
4750 INPUT "Is that OK"; A$
4800 A$ = LEFT$(A$, 1)
4850 IF A$ <> "Y" AND A$ <> "y" THEN GOTO 4500
4900 FOR I=1 TO 3 : CDATE(I) = DATE(I) : NEXT
4950 '
5000 MSG$ = "Enter time in form HHMMSS : " : GOSUB 5350
5050 FOR I=1 TO 3 : CTIME(I) = TIME(I) : NEXT
5100 MSG$ = "Enter time increment in the form HHMMSS : " : GOSUB 5350
5150 FOR I=1 TO 3 : ITIME(I) = TIME(I) : NEXT
5155 CH = 0
5200 GOSUB 6600
5250 RETURN
5300 '
5350 PRINT HOME$; FNLC$(10,0); MSG$;
5400 INPUT "", TIME$
5450 TIME$ = LEFT$(TIME$ + "      ", 6)
5500 GOSUB 9450 : IF NOT OK THEN PRINT "Invalid time" : GOTO 5400
5550 PRINT "Time is "; TIME$; "."
5600 INPUT "Is that OK"; A$ : A$ = LEFT$(A$, 1)
5650 IF A$ <> "Y" AND A$ <> "y" THEN GOTO 5350
5700 RETURN
5750 '
5800 FOR I=1 TO NR : PRINT #1,RECS$(I) : NEXT
5850 DUMMY = FRE("")
5900 NR = 0
5950 '
6000 PRINT HOME$;
6010 PRINT FNLC$(1,5); "Channel is "; TTYPE$(CH);
6050 PRINT FNLC$(3,5); "Treatment name : "; TNAME$(CH);
6055 PRINT FNLC$(4,5); "Treatment number : "; TNUM(CH);
6060 PRINT FNLC$(5,5); "Replication number : "; TREP(CH);
6100 PRINT FNLC$(3,40); "Origin : "; XO; ","; YO; EEOL$;
6110 PRINT FNLC$(5,40); "Rotation (degrees) : "; 180 * XDTHETA / XPI
6150 PRINT FNLC$(7,5); "Date : ";
6200 PRINT FNZF$(CDATE(1)); FNZF$(CDATE(2)); FNZF$(CDATE(3));
6250 PRINT FNLC$(9,5); "Time : ";
6300 PRINT FNZF$(CTIME(1)); FNZF$(CTIME(2)); FNZF$(CTIME(3));
6350 PRINT FNLC$(11,5); "Time increment : ";
6400 PRINT FNZF$(ITIME(1)); FNZF$(ITIME(2)); FNZF$(ITIME(3));
6450 PRINT FNLC$(13,5); "Head : "; FNLC$(15,5); "Abdn : "; FNLC$(13,12);
6500 RETURN
6550 '
6600 PRINT HOME$; FNLC$(10,0);
6650 PRINT "Position cursor at left bottom edge and press digitize button ";
6700 GOSUB 8150
6750 XO = DX : YO = DY
6800 PRINT : PRINT
6850 PRINT "Position cursor at right bottom edge and press digitize button ";
```

Appendix 1. continued

```
6900 GOSUB 8150
6950 IF DX-XO=0 THEN XDTHETA=SGN(DY-YO)*XPI/2 ELSE XDTHETA=ATN((DY-YO)/(DX-XO))
7000 GOTO 5800
7050 '
7100 FOR I=1 TO 3 : CTIME(I) = CTIME(I) + ITIME(I) : NEXT
7150 IF CTIME(3) > 59 THEN CTIME(3) = CTIME(3) - 60 : CTIME(2) = CTIME(2) + 1
7200 IF CTIME(2) > 59 THEN CTIME(2) = CTIME(2) - 60 : CTIME(1) = CTIME(1) + 1
7250 IF CTIME(1) > 23 THEN CTIME(1) = CTIME(1) - 24 : GOSUB 10150
7255 CH = 0
7300 GOSUB 6600
7350 RETURN
7400 '
7450 GOSUB 11500 : HX = DX : HY = DY
7500 PRINT  FNLC$(13,12); HX; ","; HY; EEOL$; FNLC$(15,12); EEOL$;
7550 GOSUB 8150
7600 GOSUB 11500
7650 PRINT  FNLC$(15,12); DX; ","; DY; EEOL$;
7700 '
7750 NR = NR + 1
7800 RECS$(NR) = TNAME$(CH)+" "+FNTF$(TNUM(CH))+" "+FNTF$(TREP(CH))
7810 RECS$(NR) = RECS$(NR)+" "+FNZF$(CDATE(1))+FNZF$(CDATE(2))+FNZF$(CDATE(3))
7850 RECS$(NR) = RECS$(NR)+" "+FNZF$(CTIME(1))+FNZF$(CTIME(2))+FNZF$(CTIME(3))
7900 RECS$(NR) = RECS$(NR)+" "+FNSZ$(HX)+" "+FNSZ$(HY)
7950 RECS$(NR) = RECS$(NR)+" "+FNSZ$(DX)+" "+FNSZ$(DY)
8000 PRINT FNLC$(19,5); EEOL$; RECS$(NR);
8050 RETURN
8100 '
8150 GOSUB 8550 : IF DC = 10 OR DC = 13 THEN GOTO 8150
8200 DC$(1) = CHR$(DC) : FOR I=2 TO 15 : GOSUB 8550 : DC$(I) = CHR$(DC) : NEXT
8250 DP = VAL(DC$(1))
8300 DX$ = "" : FOR I=2 TO 7 : DX$ = DX$ + DC$(I) : NEXT : DX = VAL(DX$)
8350 DY$ = "" : FOR I=8 TO 13 : DY$ = DY$ + DC$(I) : NEXT : DY = VAL(DY$)
8400 IF DC$(15) = CHR$(10) THEN PRINT CHR$(7); : RETURN
8450 GOSUB 8550 : IF DC <> 10 THEN GOTO 8450 ELSE GOTO 8150
8500 '
8550 IF (INP(&0325) AND &01) = 0 THEN GOTO 8550
8600 DC = INP(&0320) AND &0177: RETURN
8650 '
8700 FOR I = 1 TO 6
8750    IF MID$(DATE$, I, 1) < "0" OR MID$(DATE$, I, 1) > "9" THEN GOTO 9350
8800 NEXT
8850 '
8900 DATE(1) = VAL(MID$(DATE$, 1, 2))
8950 DATE(2) = VAL(MID$(DATE$, 3, 2))
9000 DATE(3) = VAL(MID$(DATE$, 5, 2))
9050 '
9100 IF DATE(1) < 70 OR DATE(1) > 90 THEN GOTO 9350
9150 IF DATE(2) < 1 OR DATE(2) > 12 THEN GOTO 9350
9200 IF (DATE(1) MOD 4) = 0 THEN MONTH(2) = 29 ELSE MONTH(2) = 28
9250 IF DATE(3) < 1 OR DATE(3) > MONTH(DATE(2)) THEN GOTO 9350
9300 OK = YES : RETURN
9350 OK = NO : RETURN
9400 '
9450 FOR I=1 TO 6
9500    IF MID$(TIME$, I, 1) < "0" OR MID$(TIME$, I, 1) > "9" THEN GOTO 10050
9550 NEXT
9600 '
9650 TIME(1) = VAL(MID$(TIME$, 1, 2))
9700 TIME(2) = VAL(MID$(TIME$, 3, 2))
9750 TIME(3) = VAL(MID$(TIME$, 5, 2))
```

Appendix 1. continued

```
9800 '
9850 IF TIME(1) < 0 OR TIME(1) > 23 THEN GOTO 10050
9900 IF TIME(2) < 0 OR TIME(2) > 59 THEN GOTO 10050
9950 IF TIME(3) < 0 OR TIME(3) > 59 THEN GOTO 10050
10000 OK = YES : RETURN
10050 OK = NO : RETURN
10100 '
10150 CDATE(3) = CDATE(3) + 1
10200 IF CDATE(3) > MONTH(CDATE(2)) THEN CDATE(2) = CDATE(2) + 1 : CDATE(3) = 1
10250 IF CDATE(2) > 12 THEN CDATE(2) = 1 : CDATE(1) = CDATE(1) + 1
10300 RETURN
10350 '
10400 PRINT HOME$; FNLC$(10,0);
10450 INPUT "Enter output file name : ", NAM$
10500 GOSUB 10950
10550 IF OK = -2 THEN PRINT "Invalid file name" : GOTO 10450
10600 PRINT "File name is '"; NAM$; "'"
10650 IF OK = -1 THEN PRINT "This file already exists and will be erased"
10700 INPUT "Is that OK"; A$
10750 A$ = LEFT$(A$, 1)
10800 IF A$ <> "y" AND A$ <> "Y" THEN GOTO 10400
10850 RETURN
10900 '
10950 ON ERROR GOTO 11300
11000 OPEN "I", #1, NAM$
11050 CLOSE #1
11100 OK = -1
11150 ON ERROR GOTO 0
11200 RETURN
11250 '
11300 IF ERL = 11000 AND ERR = 64 THEN OK = -2 : RESUME 11150
11350 IF ERL = 11000 AND ERR = 53 THEN OK = 0 : RESUME 11150
11400 ON ERROR GOTO 0 : END
11450 '
11500 DX = DX - XO : DY = DY - YO
11550 XR = SQR(DX*DX + DY*DY)
11600 IF DX = 0 THEN XTHETA = SGN(DY) * XPI / 2 ELSE XTHETA = ATN(DY / DX)
11610 IF DX < 0 THEN XTHETA = XTHETA - SGN(DY) * XPI
11650 XTHETA = XTHETA - XDTHETA
11700 DX = XR * COS(XTHETA)
11750 DY = XR * SIN(XTHETA)
11800 RETURN
11849 '
11850 CH = (CH+1) MOD 2
11855 GOSUB 5800
11900 RETURN
```

Appendix 2. Partial listing of a data file created by the program in Appendix 1. Each line consists of a reference name, treatment no., replicate no., data, time, and the x and y position coordinates of the head and abdomen, respectively, of one animal. The first 20 animals (10 per channel) were digitized from one negative followed by 20 animals from the next channel (30 min increment), etc.

FEN	12	1	831004	093000	993	637	1061	617
FEN	12	1	831004	093000	1300	511	1222	546
FEN	12	1	831004	093000	1376	623	1285	629
FEN	12	1	831004	093000	1496	600	1450	535
FEN	12	1	831004	093000	1732	594	1716	505
FEN	12	1	831004	093000	1793	612	1868	570
FEN	12	1	831004	093000	2018	484	1943	524
FEN	12	1	831004	093000	2127	575	2043	573
FEN	12	1	831004	093000	2306	599	2229	576
FEN	12	1	831004	093000	2347	419	2273	414
FEN	0	1	831004	093000	1124	26	1064	56
FEN	0	1	831004	093000	1324	153	1221	134
FEN	0	1	831004	093000	1419	117	1421	203
FEN	0	1	831004	093000	1544	60	1564	136
FEN	0	1	831004	093000	1713	71	1637	85
FEN	0	1	831004	093000	1743	148	1787	201
FEN	0	1	831004	093000	1816	47	1875	72
FEN	0	1	831004	093000	1982	186	1911	188
FEN	0	1	831004	093000	2110	127	2040	74
FEN	0	1	831004	093000	2276	38	2362	44
FEN	12	1	831004	100000	996	632	1062	609
FEN	12	1	831004	100000	1153	599	1217	554
FEN	12	1	831004	100000	1258	633	1344	607
FEN	12	1	831004	100000	1562	530	1484	580
FEN	12	1	831004	100000	1748	520	1705	588
FEN	12	1	831004	100000	1796	607	1860	569
FEN	12	1	831004	100000	2022	496	1943	525
FEN	12	1	831004	100000	2147	575	2060	567
FEN	12	1	831004	100000	2208	526	2281	548
FEN	12	1	831004	100000	2347	439	2276	416
FEN	0	1	831004	100000	1115	22	1062	55
FEN	0	1	831004	100000	1335	140	1236	140
FEN	0	1	831004	100000	1443	97	1424	188
FEN	0	1	831004	100000	1542	65	1563	136
FEN	0	1	831004	100000	1724	82	1648	82
FEN	0	1	831004	100000	1706	159	1779	183
FEN	0	1	831004	100000	1811	78	1877	68
FEN	0	1	831004	100000	1897	129	1950	172
FEN	0	1	831004	100000	2132	143	2062	87
FEN	0	1	831004	100000	2278	37	2359	45
FEN	12	1	831004	103000	990	635	1060	610
FEN	12	1	831004	103000	1152	602	1221	550
FEN	12	1	831004	103000	1252	627	1338	599
FEN	12	1	831004	103000	1446	502	1414	575
FEN	12	1	831004	103000	1730	497	1704	569
FEN	12	1	831004	103000	1853	548	1775	570
FEN	12	1	831004	103000	2020	495	1939	522
FEN	12	1	831004	103000	2135	575	2054	568
FEN	12	1	831004	103000	2205	528	2280	547
FEN	12	1	831004	103000	2314	427	2232	426
FEN	0	1	831004	103000	1119	24	1061	53
FEN	0	1	831004	103000	1333	127	1234	131
FEN	0	1	831004	103000	1435	103	1423	192
FEN	0	1	831004	103000	1523	63	1562	124
FEN	0	1	831004	103000	1713	81	1641	87
FEN	0	1	831004	103000	1709	145	1781	183
FEN	0	1	831004	103000	1858	63	1782	64
FEN	0	1	831004	103000	1892	124	1946	171
FEN	0	1	831004	103000	2133	145	2085	88
FEN	0	1	831004	103000	2224	73	2292	47

Appendix 3. Listing (A) and documentation (B) of an SAS program for calculating numbers of test animals changing position between photographs (based on head and abdomen x-y position coordinates), and their orientation to water flow.

A

```
3.  DATA;
4.  INPUT NAME $ TREAT_NO REP_NO DATE YYMMDD6. +1 (HOUR MINUTE) (2.) +2
5.        X1:4.1 Y1:4.1 X2:4.1 Y2:4.1;
6.  DATETIME=DHMS(DATE,HOUR,MINUTE,0);
7.  X0=(X1+X2)/2; Y0=(Y1+Y2)/2;
8.  COS=(X1-X2)/SQRT((X1-X2)**2+(Y1-Y2)**2);
9.  DROP  DATE HOUR MINUTE X1 Y1 X2 Y2;
10. CARDS;
11. ++EMBED FENITRO
12. ;
13. PROC SORT;  BY NAME REP_NO TREAT_NO DATETIME;
14. DATA;
15. ARRAY X(I) X1-X10; ARRAY Y(I) Y1-Y10;
16. I=0; NUP=0;
17. DO UNTIL(LAST.DATETIME);
18.   SET; BY NAME  REP_NO TREAT_NO DATETIME;
19.   I+1;
20.   X=X0; Y=Y0;
21.   IF COS>0 THEN NUP+1;
22. END;
23. FRACT=NUP/I;
24. DROP X0 Y0 COS;
25. DATA;
26. ARRAY T1X(I)   X1-  X10; ARRAY T1Y(I)   Y1-  Y10;
27. ARRAY T2X(J) T2X1-T2X10; ARRAY T2Y(J) T2Y1-T2Y10;
28. RETAIN START HOUR NO;
29. OLD_NO=NO;
30. NO=.;
31. SET _LAST_(FIRSTOBS=2);
32. TREAT2=TREAT_NO;
33. REP2=REP_NO;
34. TIME2=DATETIME;
35. N2=I;
36. NUP2=NUP; FRACT2=FRACT;
37. DO J=1 TO N2;
38.   I=J; T2X=T1X; T2Y=T1Y;
39. END;
40. SET;
41. IF _N_=1 THEN DO;
42.   START=DATETIME;
43. END;
44. IF NAME2 NE NAME OR  TREAT2 NE TREAT_NO OR REP2 NE REP_NO OR
45.    (TIME2-DATETIME)>'0:30'T THEN GOTO EXIT;
46. NO=0;
47. N1=I;
48. DO J=1 TO N2;
49.   MIN=5; N=0;
50.   DO I=1 TO N1;
51.     IF T1X=. OR T1Y=. THEN GOTO NEXTI;
52.     D=SQRT((T2X-T1X)**2+(T2Y-T1Y)**2);
53.     IF D<MIN THEN DO;
54.       MIN=D; N=I;
55.     END;
56.   NEXTI:END;
57.   IF N THEN DO;
58.     I=N; T1X=.; T1Y=.;
59.   END;
60.   ELSE NO+1;
61. END;
62. NO+MAX(N1-N2,0);
63. EXIT:IF TREAT2 NE TREAT_NO OR REP2 NE REP_NO THEN START=TIME2;
64. OLD_HOUR=HOUR;
65. HOUR=HOUR(TIME2);
66. IF NO=. THEN HOUR=.;
67. IF HOUR=OLD_HOUR;
68. NT=(TIME2-START)/'1:00'T;
69. UPSTR=(FRACT+FRACT2)/2;
70. NO_SUM=NO+OLD_NO;
71. KEEP NAME2 TREAT2 REP2 HOUR  UPSTR NO_SUM PNO;
72. PROC DELETE DATA=DATA1 DATA2;
73. /*
```

B

Lines 3-13: This section of the program follows the job control language specific to the mainframe computer used. The data file "Fenitro" is read in and the mid-body position coordinates of each animal are calculated.

Lines 14-24: The percent number of animals facing upstream is calculated, based on whether they are oriented at an angle <90° to the stream flow direction (program assumes the water flows from right to left).

Lines 25-73: The number of animals changing position between half-hour intervals is calculated. The position of each animal at time x is compared to those at time x + 1. Where coordinates match, within a specified distance (here 5 mm, as measured on the projected image of the negative), an animal is considered not to have changed positions. Otherwise a position change is recorded. This method, therefore, does not make it necessary to keep track of the identity of each animal between photographs. Animals moving up onto and down from end screens are also counted as having changed positions. Half-hourly counts are then summed to give hourly values.

Appendix 4. Approximate cost (Can. $) of components of the laboratory stream channels plus recording apparatus

Stream Channels (2)

Glass (10 mm thickness)	$ 60
PVC pipe and fittings	70
4 submerisble pumps (Cole-Parmer)	640
1 fluorescent fixture (2 bulb)	30
6 infrared illuminators (H., B. and W. Company, San Diego, CA)	1000
2 plywood reservoirs (epoxy painted)	60
1 water-cooling unit (2 stainless steel coils)	800
2 temperature controllers	600
1 mirror	14
2 flowmeters	300
2 interval timers	200
1 incandescent dimming device [see Graham and Hutchinson (1977) for details]	100
2 LED display digital clocks	30

Photographic apparatus

SLR camera	$ 300
Motor drive	270
Intervalometer	1000
Interval timer	125
Camera tripod	75
Digitizer	1500
TOTAL	$7174

Other equipment

Microcomputer (minimum 64K memory)	1000 & up
Photographic enlarger	200