The decline of *Mysis relicta* Lovén in response to experimental acidification of a whole lake.

by

Redwood William Nero

A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
Master of Science
in
Zoology

Winnipeg, Manitoba, 1981

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FOREWORD

This thesis has been organized in a non-traditional format, better suited for conversion into manuscripts suitable for publication in a scientific journal. The 'General Introduction' briefly summarizes literature on acid precipitation and mysid biology. Three papers follow the 'Introduction', the first on sampling *Mysis*, the second on population characteristics of *Mysis* at the Experimental Lakes Area, and the third is on the acidification effects on *Mysis*. These three chapters are written as separate units for publication. The final chapter is the 'General Discussion'. Printed copies of this thesis have been produced using a Waterloo SCRIPT text formatting program (SYSPUB 1979, SCRIPT 1979).

ABSTRACT

Mysis relicta in Lake 223 (L223) of the Experimental Lakes Area (ELA), northwestern Ontario, are benthic during the day, with their shoreward distribution limited by the boundary between the 18C isotherm and the lake bottom. A requirement for oxygen concentrations of more than $2mg \cdot l^{-1}$ excluded Mysis from hypoxic waters of the hypolimnion. Overnight monitoring of densities along a single transect in ELA, L239, using vertical hauls of a net with a $1m^2$ mouth opening, indicates that the optimal time for sampling is one hour after sunset, with numbers of Mysis decreasing slowly to about 60% of the maximum densities prior to sunrise. The July to August period is optimal for population estimates and year to year comparisons because seasonal migration of Mysis is restricted by the thermocline during summer stratification.

In late summer of 1978, there were 5,300,000 ± 1,060,000 (±95% confidence limits) Mysis in L223, as determined by stratified random sampling. During August 1979, the number of Mysis decreased by 96% to 220,000 ± 59,000. Prior to 1978, the lowest the pH within the region inhabited by Mysis had declined to was 5.9. Between 1978 and 1979 the lowest pH was 5.6 . A rapid decrease in pH from a high summer val-

ue of 6.2, to a value of 5.6, during fall turnover in 1979, resulted in the disappearance of the remaining 4% of the population. Therefore, the critical pH lies between 5.6 and 5.9.

The mechanism by which the low pH affected the *Mysis* population remains unknown. The decline of *Mysis* was not a result of the toxic effects of trace metals. Concentrations of Zn, Al, Mn, Fe, Cd, Cu, Ni, and Hg in *Mysis* samples from L223 were less than or equal to concentrations in animals from 5 control lakes. Reduced growth and reproductive failure were eliminated as mechanisms. No changes were evident in available food supplies. The decline was most likely due to direct action on a physiological mechanism.

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Chapter I

GENERAL INTRODUCTION

Acid precipitation has been a growing problem in North America since before 1950 (Cogbill and Likens 1974, Likens and Bormann 1974). Soft water lakes underlain by non calcareous bedrock are highly vulnerable to acidification (Likens et al. 1979, and Scheider et al. 1979) with the most affected areas lying downwind from major emission sources in the eastern portions of the United States and Canada (Likens et al. 1972, Likens 1976, and Dillon et al. 1978).

A number of studies has been done on the effects of acidification of lakes on aquatic fauna, reviewed by Giddings and Galloway (1976), Leivestad et al. (1976), Wright et al. (1976), and Almer et al. (1978). Daye (1980) and Spry et al. (1981) reviewed effects of acid on fishes. Studies have been carried out on benthic organisms (Bell 1970, Bell 1971, Wiederholm and Eriksson 1977, Fryer 1980, Okland, J. 1980, Okland K. 1980, Okland and Okland 1980, and Raddum 1980), crustacean zooplankton (Sprules 1975, Hendrey et al. 1976, Roff and Kwiatkowski 1978, Raddum et al. 1980, and Yan and Strus 1980), and phytoplankton (Hendrey et al. 1976, Yan 1978, Yan and Stokes 1978, Raddum et al. 1980 and Findlay and Saesura 1980).

The effects of acidification on the relict crustaceans *Mysis relicta* Lovén and *Pontoporeia hoyi* (Smith 1874), have never been thoroughly studied. Both species may be highly susceptible to acidic water because distribution studies of *Gammarus lacustris* (G. O. Sars), a similar amphipod in Scandinavia, and *Mysis relicta* in North America and Europe show they are among the most sensitive organisms (Dadswell 1974, Okland, K. 1980, and W. Dickson pers. comm.).

Mysis has long been recognized as a valuable food organism in many lakes (Van Oosten and Deason 1937, and Rawson 1961). Their size, intermediate between crustacean zooplankton and small fish, and their ubiquitous feeding habits (Bowers and Grossnickle 1978, Grossnickle 1979, Cooper and Goldman 1980, and Parker 1980) make them an important food for fish in many lakes. Thus, they are frequently transplanted into new systems (reviewed by Gosho 1975). Hendrey et al. (1976) have suggested that in many lakes the loss of fish food organisms around pH 6.0 could reduce fish growth well before pH values become low enough to result in reproductive failure of the fishes. *Mysis relicta* was therefore a key organism to study during the experimental acidification of Lake 223 (L223) of the Experimental Lakes Area (ELA), northwestern Ontario (Schindler et al. 1980a, and Schindler 1980).

The purpose of this study was first, to develop reliable sampling methods for *Mysis* ; second, to examine several natural populations of *Mysis* in order to determine variability of life cycles, growth, and abundance of *Mysis* ; and third, to use this information in the interpretation of the effects of experimental acidification of a small oligotrophic lake on its *Mysis* population.

In order to quantify any changes in the *Mysis relicta* population of L223, sampling methods more reliable than benthic tows (Reynolds and DeGraeve 1972), SCUBA quadrat counts (Lasenby 1971), and horizontal tows (Brownell 1970) had to be developed. On the basis of preliminary tests, a depth-stratified sampling scheme like that described by Hakala (1978), using vertical hauls described by Grossnickle and Morgan (1979), was believed to provide the most representative population estimates.

Chapter II

FACTORS AFFECTING THE RELIABILITY OF POPULATION ESTIMATES OF *MYSIS RELICTA* IN SMALL PRECAMBRIAN SHIELD LAKES.

2.1 INTRODUCTION

Mysis relicta Lovén migrates diurnally, moving off the bottom at night to feed in the water column (Beeton 1960, Teraguchi 1969). In a number of studies, this migration pattern has led to the use of vertically-towed nets at night to obtain quantitative estimates of mysid abundance (Grossnickle and Morgan 1979, and Malley and Reynolds 1979). In addition, seasonal changes in abundance have been attributed to seasonal onshore or offshore movements of *Mysis* (Tattersall and Tattersall 1951, Holmquist 1959, and Carpenter et al. 1974).

A night time estimate of the total number of *Mysis relicta* in a small Precambrian Shield lake required three critical assumptions (Nero and Davies 1981; Appendix B). First, the ascent phase of the vertical migration of *Mysis* is completed by 1h after sunset on moonless nights. The entire *Mysis* population must be pelagic for quantitative sampling using night-time vertical net tows. Knowledge of the timing and extent of the vertical migration will allow efficient sampling to be done. Second, the seasonal migration pattern of *Mysis* results in a population which remains in the same areas of the lake during August. Seasonal changes in the distribution of the *Mysis* population could bias a population extimate derived from samples collected during a number of nights. An examination was made of seasonal changes in the L223 and L239 populations to elucidate the seasonal migration of *Mysis* in these lakes. Third, their spatial distribution justifies the use of depth stratified sampling. Nero and Davies (1981) used stratified sampling with strata defined by bottom contours because the *Mysis* distribution was most closely related to the depth at the bottom. However, differences in *Mysis* densities due to different substrates are more difficult to test and should be accounted for. Tests were made of data collected for Lake 223 (L223) and Lake 239 (L239) to determine if substrate type affects *Mysis* density. The purpose of this study was to determine if the above assumptions are appropriate for populations of *Mysis* in L223 and L239 at the Experimental Lakes Area (ELA), northwestern Ontario.

2.2 METHODS

2.2.1 SCUBA Observations of *Mysis relicta*.

Observations of the substrate preferences and depth distribution during the daytime were made, using SCUBA (self contained underwater breathing apparatus), for *Mysis* in Lakes 161, 223, 228, 239, 305 and 468 during 1978, 1979, and 1980. The lower depth limits of *Mysis* usually were not obtained, as diving was restricted to a 20m limit for safety reasons. Temperatures were measured with a hand held field thermometer or estimated from temperature profiles.

2.2.2 Heterogeneity Within Depth Contours

To test for equal densities within similar depth zones in L223, a total of 79 samples were collected using vertical tows of a $1m^2$ net at night (Appendix A). During August, 1978 six transects between 5.0m and 14.4m were sampled (Fig. 5, Chapter III). Due to the low numbers of samples collected from the shallow and deep sections of most transects, the 5.0-6.5m, 11.0-12.5m and 12.5-14.5m strata were excluded in the analysis. Few samples were collected from transects C and D. Because both transects had similar slopes and bottom types, they were combined. This left five transects covering three depth zones (6.5-8.0m, 8.0-9.5m, and 9.5-11.0m). For the analysis of variance, 15 experimental cells were created by treating transects as treatment effects and similar depths as block effects. The median of the *Mysis* densities within each cell was analyzed using the Friedman test (Hollander and Wolfe 1973). A similar test was carried out in L239 on July 7, 1980, using data from samples collected at 6 depths (6.8m, 9.0m, 10.0m, 14.0m, 23.0m, and 31.0m) along transects A, B, and C (Fig. 8, Chapter III). Transects were chosen to represent differing bottom types. A - represented a smooth sand bottom with a gradual slope, B - a sand bottom with an intermediate slope, and C - a rock strewn bottom with a steep slope.

2.2.3 Diurnal Migration

Diurnal changes in the distribution of mysids in L223 were observed during late summer 1978 by the use of diurnal SCUBA observations as well as night-time vertical net tows of a $1m^2$ net. Counts of *Mysis* from SCUBA quadrat counts and vertical net tows were used to derive night and day distribution maps of *Mysis*. A distinct horizontal migration was found in L223 (Appendix B), so there was some concern that night sampling would be biased by population movements. To determine the best time for quantitative sampling, a comparison was made of samples collected from 1 1/2 to 5 1/2 hours after sunset during August 1978.

An additional test of abundance changes during the night was carried out on August 24, 1979 in L239. One vertical net haul was collected from each of six stations at 6.0, 8.8, 10.2, 13.7, 23.0, and 30.0m at 21:30h, 24:30h, and 3:30h. The Friedman test was used to determine significant differences in numbers between the sampling times.

The ice melt of spring 1980 was very rapid so a number of ELA lakes; including L239 did not mix completely. Oxygen profiles using the modified Winkler technique showed that the lower 7m of the lake had oxygen concentrations $<2mg.l^{-1}$ (P. F. Olesiuk unpublished data). Under these conditions, a horizontal diurnal migration like that observed in L223 was expected because of anoxic conditions in the bottom waters of L239, similar to those in L223 during summer, 1978.

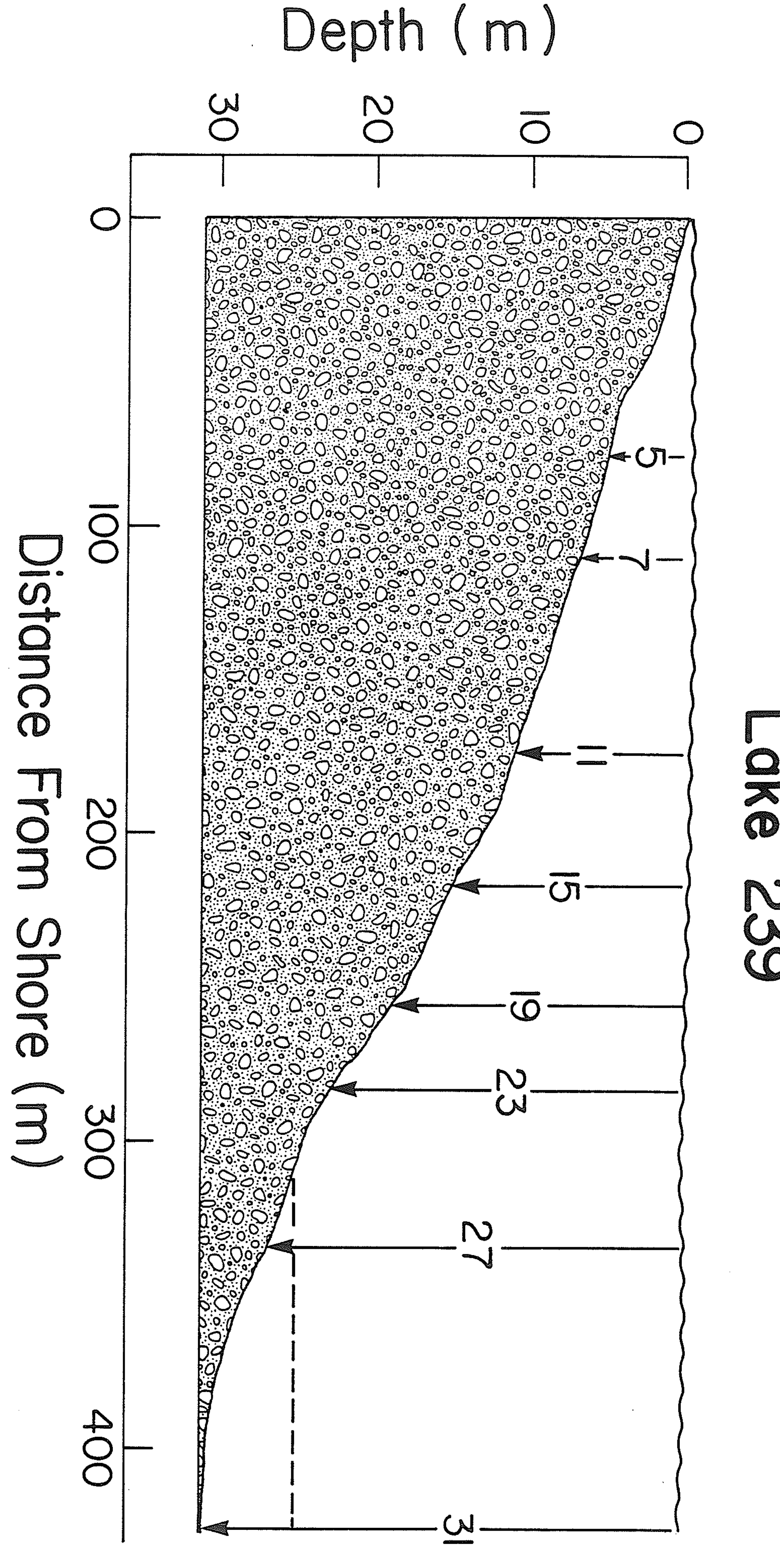
Therefore, a test was carried out to see if L239 Mysis exhibited any horizontal distribution changes during their diurnal migration. On September 6, 1980, eight stations along a transect at 5.0m, 7.0m, 11.0m, 15.0m, 19.0m, 23.0m, 27.0m, and 31.5m were sampled with vertical tows of the $1m^2$ net (Fig. 1). Two boats serviced four of the stations each. One boat began at the 15m station and sampled 11.0m, 7.0m, and 5.0m; the other began at the 31.5m station and sampled 27.0m, 23.0m, and 19.0m. Each station was sampled at 1/2 hour intervals from 1900h to 2100h, then at 2h intervals to 0500h, and at 1/2h intervals untill 0800h. Five additional 27.0m and 31.5m samples were collected up to 1100h. All samples were preserved in 95% ethanol. Counts were made of juvenile and adult Mysis.

Information obtained from daytime SCUBA observations combined with observations of the success of net tows for life history samples (Chapter III) allows generalizations to be made about diurnal movements of Mysis in lakes 161, 224, 228, 305, and 468.

2.2.4 Changes in Seasonal Distribution

Generalizations about seasonal changes in the distribution pattern of Mysis relicta within the study lakes are based on summer data collected using SCUBA and night time net hauls (Appendix B) and winter data collected by net through the ice (Chapter III).

Figure 1: The position of 8 sampling stations along the northwest transect in L239 are depicted in this vertical cross-section of the lake. The vertical scale is exaggerated 10x. The horizontal line at 25m represents the beggining of water with insufficient oxygen for *Mysis* (i.e. < 2mg.l^{-1} O_2).


Lake 239
Depth (m)
0
10
20
30
Distance From Shore (m)
0
100
200
300
400
5
7
11
15
19
23
27
31

2.3 RESULTS

2.3.1 SCUBA Observations of the Distribution of *Mysis relicta*

Mysis were found equally over sand or flocculent organic sediments in all lakes studied. Some shoaling of *Mysis* was evident around the bases of boulders and rock outcrops. Few *Mysis* were observed on bare rock outcrops. *Mysis* were usually found below 15C. However, in L223 during August 1978, *Mysis* were encountered at 17.6C in 6.5m of water, during a period when oxygen concentrations dropped from $10.2 mg.l^{-1}$ at 8m to $0.3 mg.l^{-1}$ at 10m. In the very cold, clear lakes such as 161, 228, and 305, *Mysis* did not occur above the 8C isotherm. In less oligotrophic and more humic lakes such as 239 and 468, *Mysis* were found up to the 15C isotherm.

2.3.2 Heterogeneity Within Depth Contours

Differences in the density of *Mysis* along depth contours were not significant ($P=.17$, Friedman test), for data obtained from net hauls in L223 during 1978. Major differences between transects were not evident because of the high variability at each depth. Similarly, for L239 the Friedman test gave non-significant results ($P>.25$), indicating that the variability at each station exceeded differences in density due to the different substrates and slopes in the lake.

2.3.3 Diurnal Migration

Horizontal migration of *Mysis* does not appear to have affected abundance estimates in L223. No changes in densities were obvious between 1 1/2 hours after sunset and 5 1/2 hours after sunset. At a 9.8m station sampled at 1 1/2 and 5 1/2 hours after sunset, only a 2.6% change in numbers was observed. Vertical distribution in the water column also remained the same during the 4 hour period. Therefore, the assumption can be made that most of the migration occurred within the first 1 1/2 hours after sunset. A preliminary test to see if the abundance of *Mysis* at six stations in L239 varied during the night was carried out on data collected at 2130h, 2430h, and 0330h, and showed no significant differences among the three sampling times (Friedman test, $P>0.25$).

More rigorous sampling of transect A in L239 on the night of September 6, 1980 showed that the maximum abundance in the water column, for both juveniles and adults occurred 1h after sunset (Fig. 2 and 3). Overall densities decreased slightly until about 0500h, at which time they decreased rapidly as the sun rose.

A diurnal horizontal migration of *Mysis* was also observed in L239. In the early evening, no *Mysis* were present in the water column above the region of the lake with oxygen concentrations $<2mg.l^{-1}$ (27m - 31m). During the 4h period from 0100h to 0500h, mysids invaded this region, presumably

Figure 2: Isopleths joining equal densities of juvenile *Mysis relicta* along transect A (Y axis) of L239 during the night of September 6, 1980 (X axis). Sunset and sunrise are shown by the downward pointing and upward pointing arrows on the X - axis respectively.

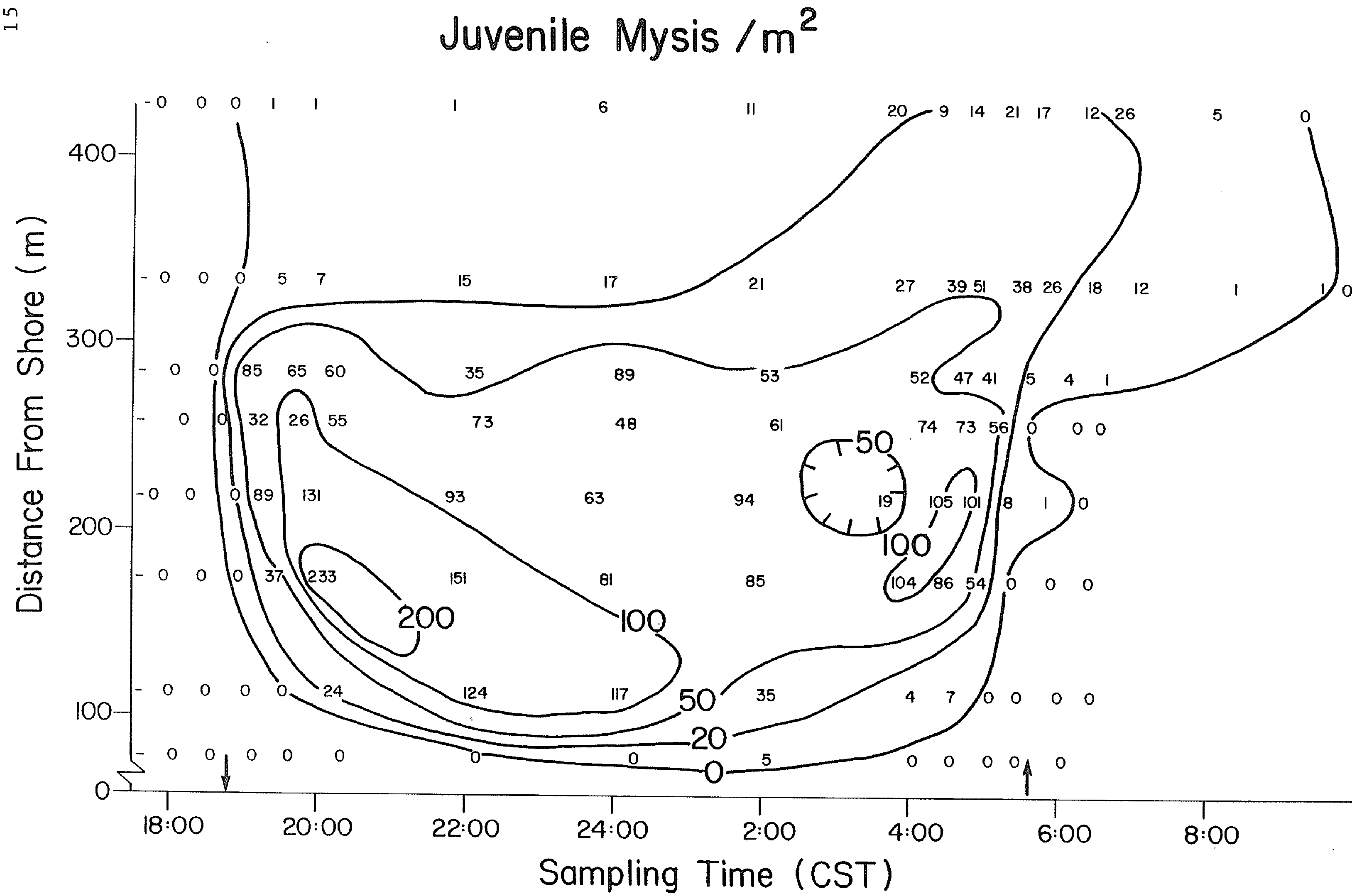
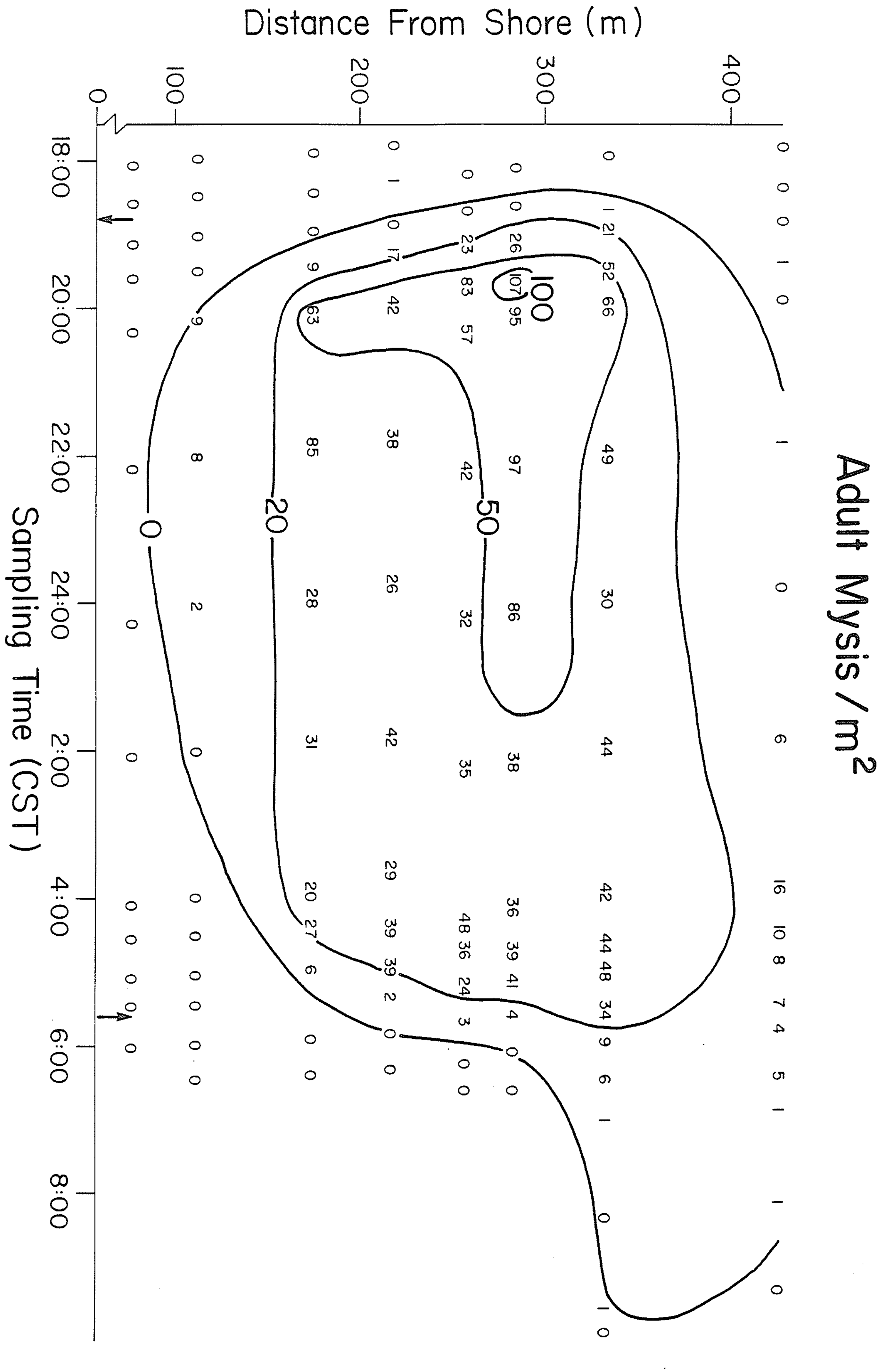

Juvenile Mysis / m2
Distance From Shore (m)
0
100
200
300
400
Sampling Time (CST)
18:00
20:00
22:00
24:00
2:00
4:00
6:00
8:00
200
100
50
20
0

Figure 3: Isopleths joining equal densities of adult *Mysis relicta* along transect A (Y axis) of L239 during the night of September 6, 1980 (X axis). Sunset and sunrise are shown by the downward pointing and upward pointing arrows on the x - axis respectively.


Adult Mysis / m²
Distance From Shore (m)
0
100
200
300
400
Sampling Time (CST)
18:00
20:00
22:00
24:00
2:00
4:00
6:00
8:00
0
20
50
100

coming from shallower parts of the lake. *Mysis* were still captured over the bottom at the 27m and 31m stations at dawn because they could not approach the waters with insufficient oxygen to support *Mysis*. The *Mysis* required about 3 1/2h to move laterally out of this region of the lake during the early morning. The presence of *Mysis* in occasional zooplankton hauls from the deeper parts of the lake in the morning (D. F. Malley, unpublished data), gives further evidence of their slow return to shallower bottom areas at dawn.

Mysis were entirely benthic in lakes 161, 224, 228, 305, and 468 on cloudless days but were frequently up to 1/2m off the bottom during cloudy weather. Because *Mysis* are easily caught just below the thermocline in these lakes at night during summer, *Mysis* in these lakes probably have diurnal migration patterns similar to those observed in L223 and L239, with the degree of horizontal movements dependent on the extent of summer anoxia.

2.3.4 Seasonal Distribution Changes

Adult *Mysis* occupied the deeper portion of L239 during the summer of 1979, while the young were most abundant just below the thermocline. *Mysis* remained below the thermocline and moved downward as it deepened. Some of the *Mysis* were observed on the lake bottom, during days in autumn when epilimnion temperatures had decreased to about 9C, at depths as shallow as 3m. After ice formation in November, almost all *Mysis* were in the deepest part of the lake, most of them below 20m. Not until late winter (March) did the gravid females move into the shallows, where broods were released just before ice melt in the spring. At that time few *Mysis* were in the deep portions of the lake. After ice melt the adults were back at the center of the lake, while the juveniles stayed in the shallows. During summer, 1980, adult *Mysis* were present in lower numbers and at times absent from the deepest parts of L239 because of low oxygen concentrations (Fig. 3).

In the other ELA lakes (223, 224, 228, 305, and 468) similar movements were suggested by life history data from summer and winter net hauls. One exception was L468, where *Mysis* were absent from the greatest depth of 23m during midwinter and were also rare at all other depths. These *Mysis* possibly moved into a specific region of the lake as noted by Lasenby (1971) for Stony Lake, Ontario.

2.4 DISCUSSION

The distribution of *M. relicta* in L223 was similar to that found by Dadswell (1974) in Murray and Danford Lakes, southwestern Quebec, where *Mysis* were restricted above by temperatures over 18C and below by oxygen concentrations less than $2mg.l^{-1}$. The upper temperature limit for *Mysis* of 17.6C observed in L223 is similar to that observed by Thienemann (1925) and Dadswell (1974) and probably represents the upper tolerance limit for the species (Smith 1970).

Because SCUBA observations in L223 and L239 during the daytime suggested that mysids are most abundant over flocculent sand bottoms, differences in Mysis densities between different slopes and bottom types in the lakes would be expected. Low densities are usually observed over bare rock outcrops. Such habitat differences have also been observed during the daytime by Holmquist (1959), Aleksandrov (1963), and Lasenby (1971). However, at night the low variability of mysid densities about a depth contour in L223 and L239 suggest that these differences are insignificant in comparison to overall variability at a single station. The diurnal migration of animals off the bottom probably has a mixing effect on the animals reducing the local density differences. At night, the greater dependence of Mysis densities on bottom depth rather than bottom type justifies the use of depth-stratified sampling without consideration for bottom types. True stratified random sampling as used by Hakala (1978) or stratified systematic sampling (Mendenhall et al. 1971) may be best. The use of randomly or systematically placed stations depends on the ease of locating stations at night.

The timing of the diurnal migration is similar to that observed by Teraguchi (1969) in Green Lake, Wisconsin and by Grossnickle and Morgan (1979) in Lake Michigan. The rapid movement of *Mysis* as the light decreases, achievement of maximum numbers just after sunset, and the slow decrease in numbers during the night make the period from 1-2h after sunset ideal for sampling *Mysis* in the water column. Sampling later in the evening would result in a slight underestimate of numbers because of reduced abundance in the water column. Whether these animals are moving away from the transect or are moving back to the bottom is unknown.

The seasonal migration pattern found in the ELA lakes is not unique. Similar patterns have been observed in the Great Lakes (Reynolds and DeGraeve 1972). Seasonal migrations do not cause sampling problems unless the sampling program requires a series of nights to obtain a sufficient number of samples to obtain the desired statistical precision. During the fall or spring when the populations are moving lakeward or shoreward, respectively, some bias may be introduced. Movements of the populations are restricted during late summer by stratification, making this period an ideal time to census populations.

Chapter III

VARIATIONS IN GROWTH AND FECUNDITY OF *MYSIS RELICTA* IN FIVE LAKES AT THE EXPERIMENTAL LAKES AREA, NORTHWESTERN ONTARIO.

3.1 INTRODUCTION

Factors determining the life history characteristics of Mysis relicta Lovén have recently been studied (Morgan 1980). A variety of aquatic habitats has been examined (Brownell 1970, Lasenby and Langford 1972, Reynolds and DeGraeve 1972, Carpenter et al. 1974, Hakala 1978, and Morgan and Beeton 1978), but none of the studies has examined the life history characteristics of populations in small ultra-oligotrophic lakes on the Precambrian Shield. The purpose of this study was to obtain information on the variability of growth, life history patterns, fecundity, and abundance of natural populations of Mysis relicta at the Experimental Lakes Area (ELA). This information would serve as a data base for interpreting the effects of experimental manipulations on Mysis populations, and to facilitate studies examining the factors governing life history patterns in Mysis relicta.

Measurements of animal size obtained from qualitative samples would allow an analysis of life history patterns. Growth can be determined from the life history data. Tied in with life history and growth is fecundity, and the size at onset of sexual maturity, a measure used by Wenner et al. (1974) as an indicator of environmental perturbations of natural populations. The interacting effects of the primary production of each lake on the population's mean size at onset of sexual maturity is considered. The above measures ultimately allow generalizations to be made about the relationships between relative fish production, *Mysis* production, and primary production in 6 ELA lakes.

3.2 DESCRIPTION OF THE STUDY LAKES

The lakes studied are part of ELA, northwestern Ontario, and lie in the area bounded by 93:35′ - 94:00′ west and 49:35′ - 49:45′ north (Fig. 4). A more detailed description of the local geography is given in Brunskill and Schindler (1971).

3.2.1 Morphometric Characteristics

The physical characteristics of the study lakes are summarized in Table 1. Altitudes were determined from 1:50,000 scale national topographic series maps. Bathymetric charts for Lakes 223, 224, 228, 239, and 305 are given in Figures 5 - 9. Lakes 161 and 228 are large and very oligotrophic with maximum depths over 100m. Lake 239 is a small headwater lake. L224 drains into L223. L305 drains into L468 which flows through a small creek into L228. Lake 310 drains a number of smaller lakes and bogs. Lakes 223, 224, 228, 305, 310, and 468 drain north into the Eagle Lake watershed. Lakes 161 and 239 drain south into the Dryberry Lake watershed. Lake 468 has a more irregular shoreline than the other lakes studied and is intermediate in size.

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Figure 4: Locations of the lakes sampled during the study of the life history of *Mysis relicta*. Lakes surveyed in the Experimental Lakes Area, northwestern Ontario are shaded. Solid line denotes road, triangle the ELA camp (After Johnson and Vallentyne 1971).

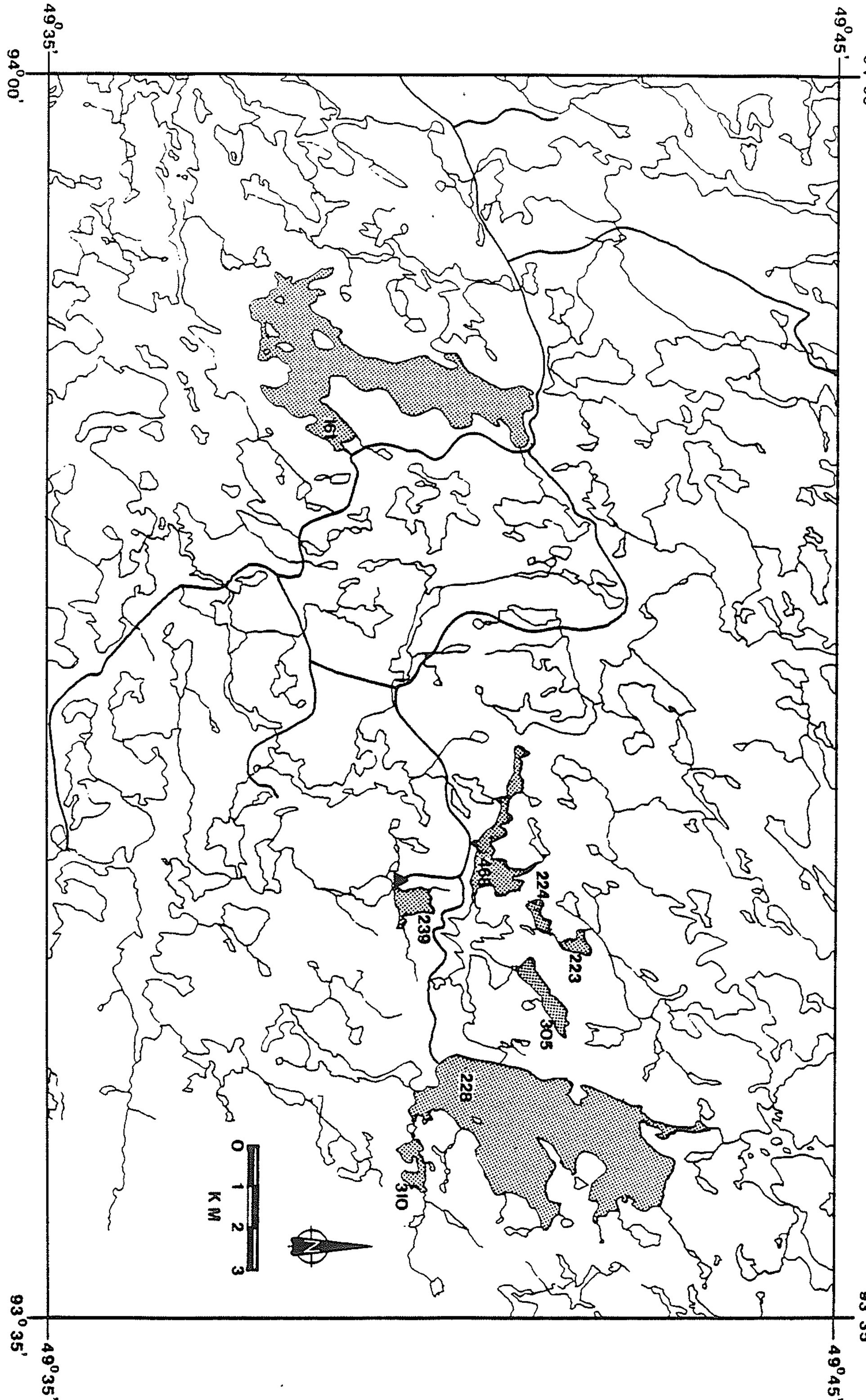
49°35'
94°00'
49°45'
94°00'
93°35'
49°35'
93°35'
49°45'
161
468
224
239
223
305
228
310
0 1 2 3
KM

TABLE 1

Physical characteristics of the 8 study lakes.

Abreviations: Zm maximum depth (m); Alt., altitude (m); R.T., renewal time (yrs); SDV, Secchi disc visibility (m); C, conductivity (micromhos/cm at 25C); Ex, summer range of extinction coefficients; and Color, visual color. Data have been compiled from Cleugh and Hauser (1971), Brunskill and Schindler (1971), Reid et al. (1975a), Beamish et al. (1976), Newbury and Beaty (1980), and Hesslein et al. (1980).

Lake	Area (ha)	Zm	Alt.	R.T.	SDV	C	Ex	Color
161	1008	117	378		10.1	28	.21b	Blue
223	27.3	14.4	408	3.3	5.0	23	.45-.67	Orange
224	25.9	27.4	411	12.5	7.0	18	.24-.34	Blue
228	1677	167	367		9.0	23	.20-.29	Blue
239	54.3	32.0	392	6.0	4.8	25	.72-1.10	Orange
305	52.0	32.7	403		7.5	24	.30-.42	Green
310	49.7	19.7	396		2.5	23	1.39c	Orange
468a		23.0	402			22		Green

a - West of narrows.
b - 20/8/69 only.
c - 16/9/69 only.

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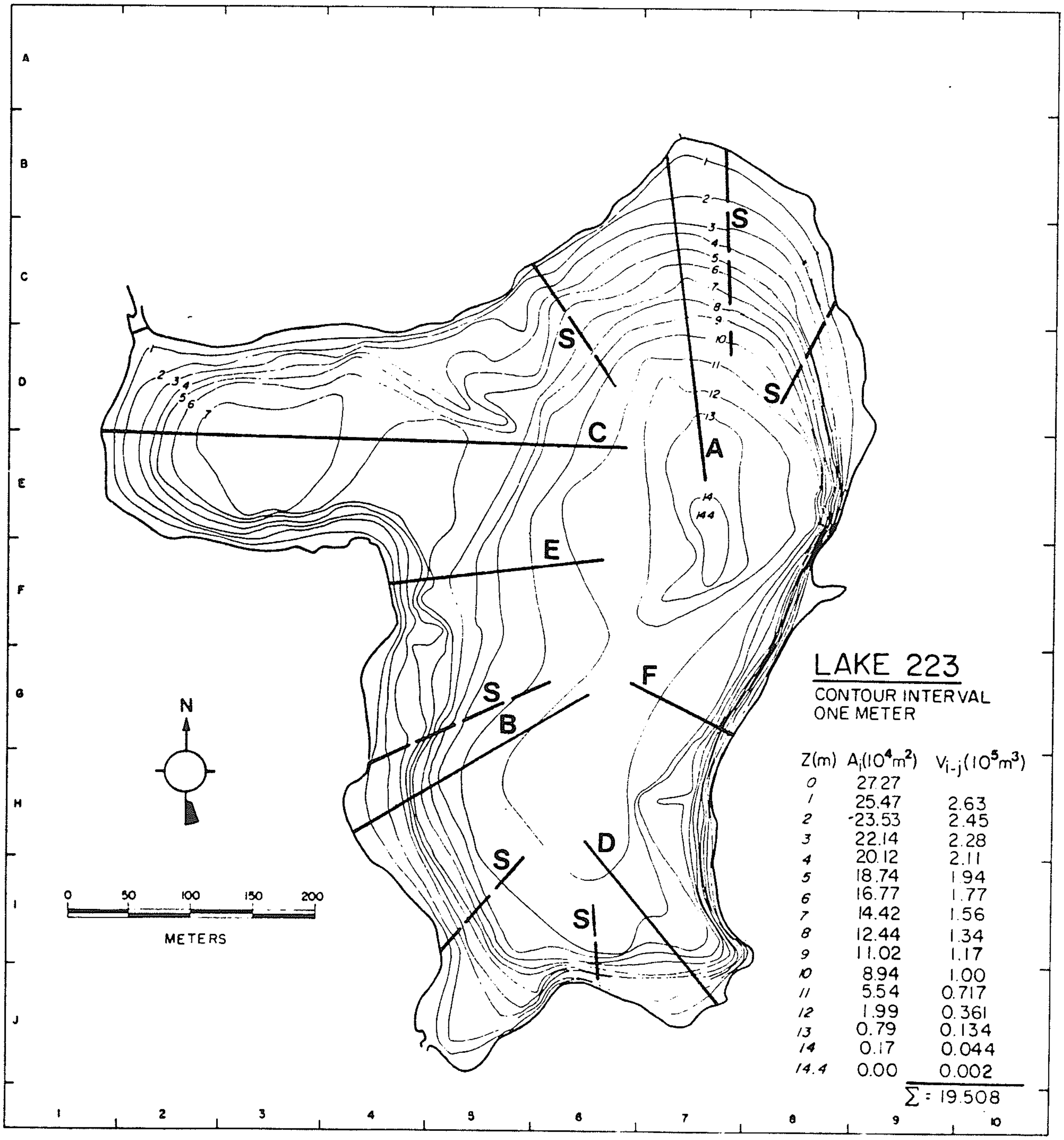
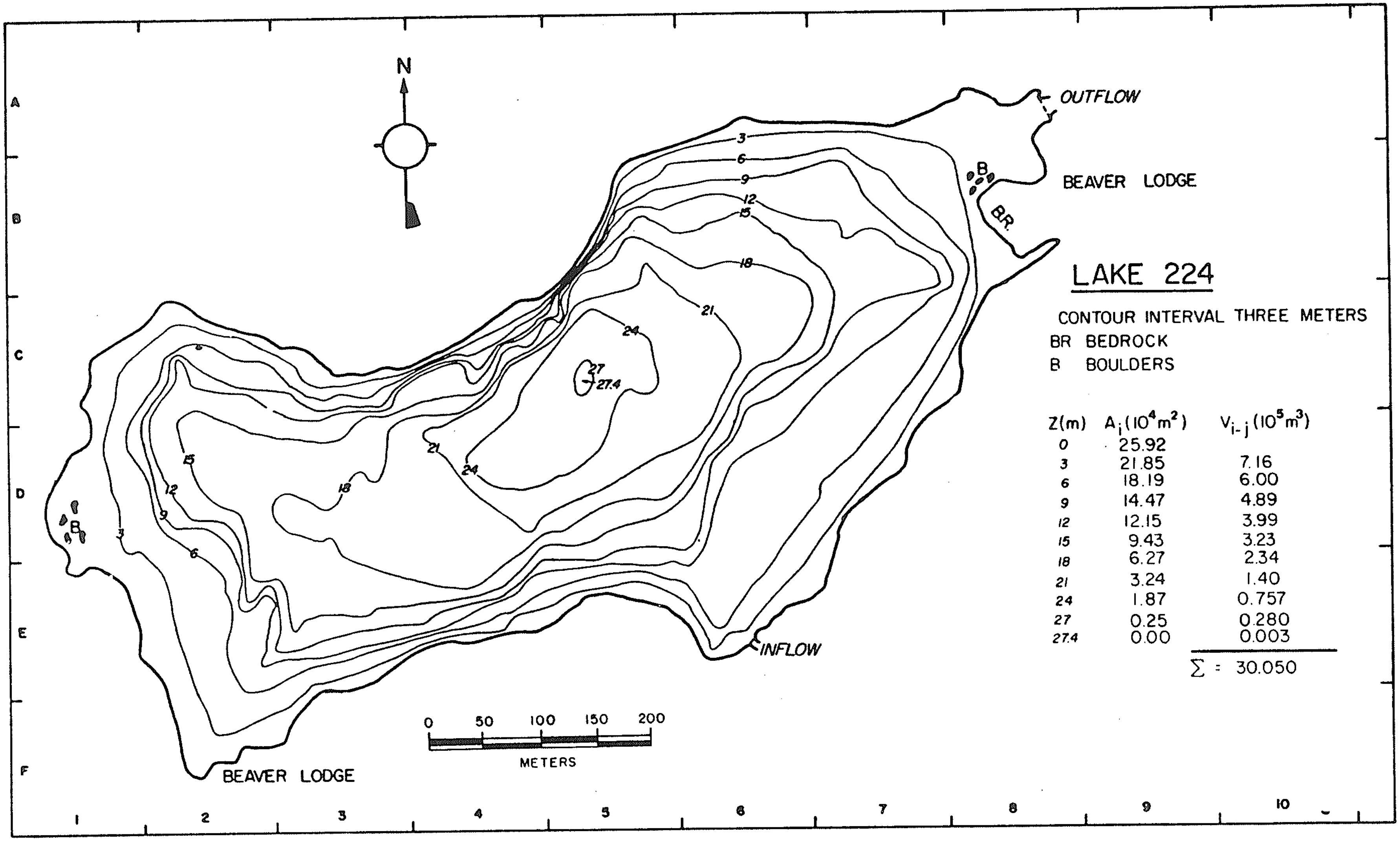
Figure 5: Bathymetric map of L223 showing the transects used for SCUBA quadrat counting of *Mysis* as Broken lines "S" and the night net tows as Solid lines "A-F", (After Nero and Davies 1981).


LAKE 223
CONTOUR INTERVAL
ONE METER
Z(m) A_i(10^4m^2) V_i-j(10^5m^3)
0 27.27
1 25.47 2.63
2 23.53 2.45
3 22.14 2.28
4 20.12 2.11
5 18.74 1.94
6 16.77 1.77
7 14.42 1.56
8 12.44 1.34
9 11.02 1.17
10 8.94 1.00
11 5.54 0.717
12 1.99 0.361
13 0.79 0.134
14 0.17 0.044
14.4 0.00 0.002
Σ = 19.508
A
B
C
D
E
F
S
N
0 50 100 150 200
METERS

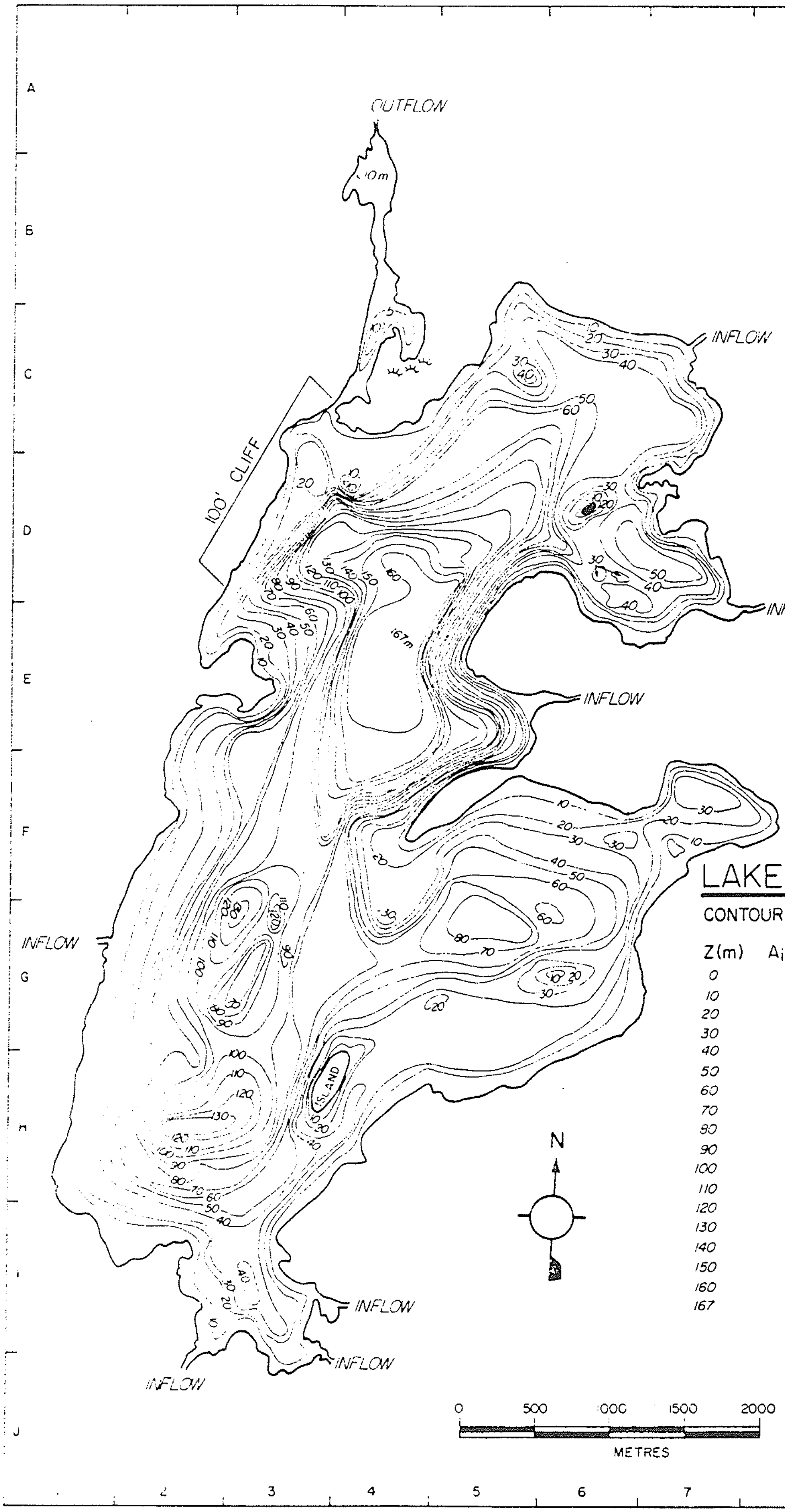
30

Figure 6: Bathymetric map of L224 (After Hesslein et al. 1980).



Z(m)	$A_i (10^4 m^2)$	$V_{i-j} (10^5 m^3)$
0	25.92	
3	21.85	7.16
6	18.19	6.00
9	14.47	4.89
12	12.15	3.99
15	9.43	3.23
18	6.27	2.34
21	3.24	1.40
24	1.87	0.757
27	0.25	0.280
27.4	0.00	0.003
		Σ = 30.050

Figure 7: Bathymetric map of L228 (D. W. Schindler unpublished data).



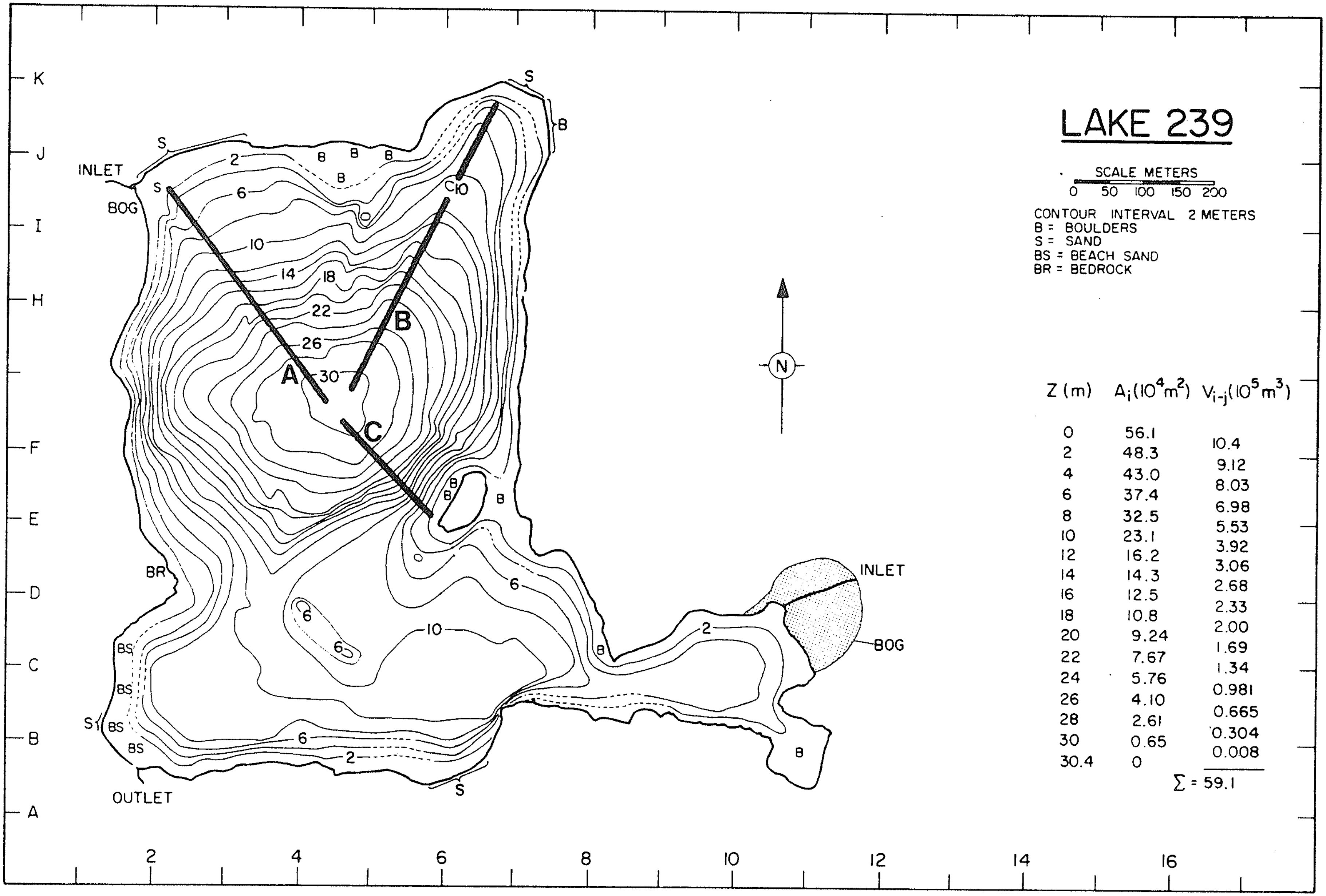
LAKE 228 (TEGGAU)

CONTOUR INTERVAL TEN METRES

Z(m)	A_i ($10^4 m^2$)	V_{i-j} ($10^5 m^3$)
0	1676.5	
10	1472.5	1573.3
20	1332.2	1401.7
30	1096.2	1212.3
40	884.1	988.2
50	728.3	804.9
60	655.2	691.4
70	552.5	603.1
80	447.1	498.9
90	376.6	411.4
100	232.3	301.6
110	201.4	216.7
120	152.0	176.1
130	119.5	135.4
140	97.7	108.4
150	78.5	87.9
160	46.9	62.0
167	0.0	10.9
		Σ = 9284.2

34

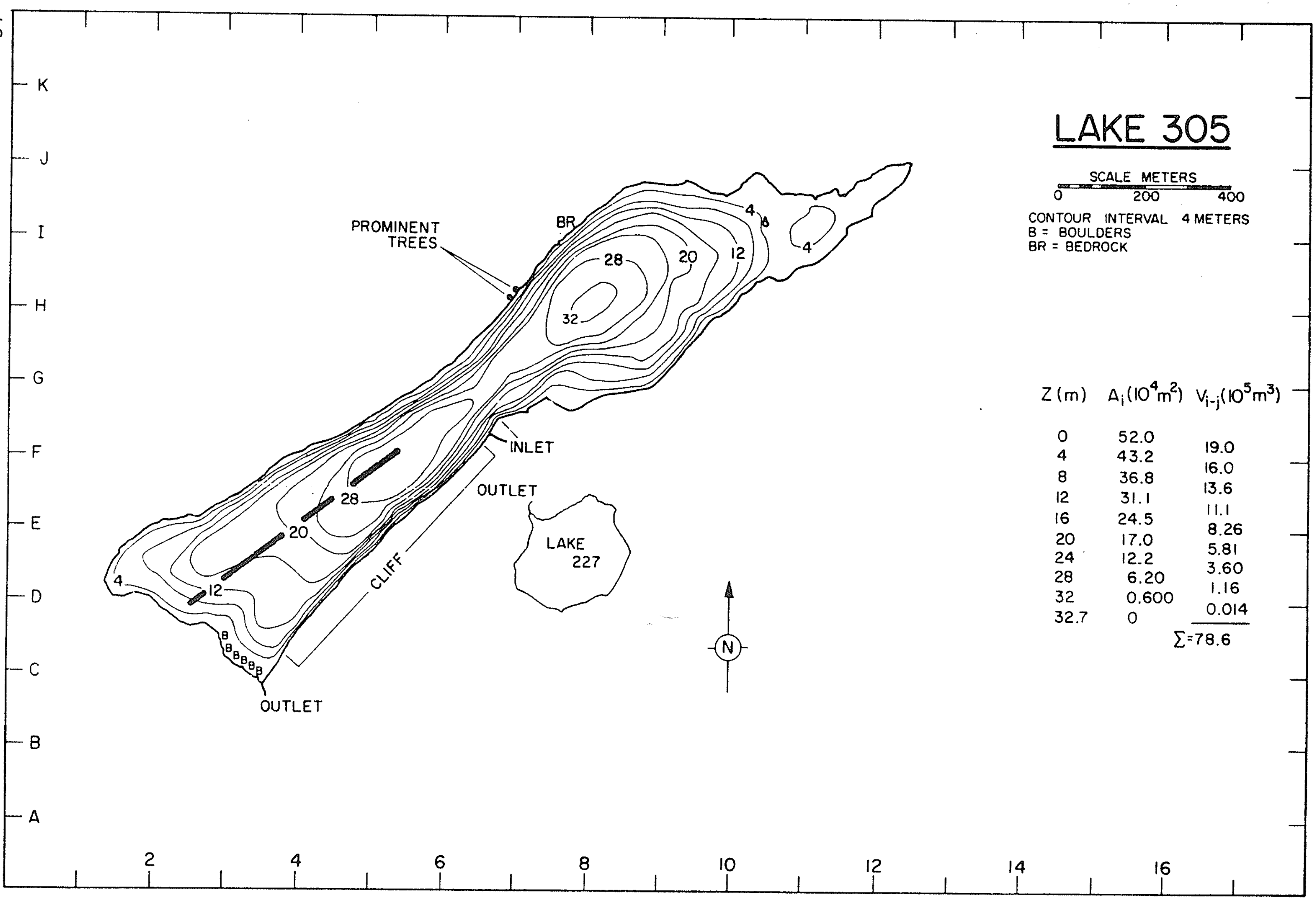
Figure 8: Bathymetric map of L239 with transects A, B, and C, represented by the solid lines (After Brunskill and Schindler 1971).



Z (m)	$A_i (10^4 m^2)$	$V_{i-j} (10^5 m^3)$
0	56.1	10.4
2	48.3	9.12
4	43.0	8.03
6	37.4	6.98
8	32.5	5.53
10	23.1	3.92
12	16.2	3.06
14	14.3	2.68
16	12.5	2.33
18	10.8	2.00
20	9.24	1.69
22	7.67	1.34
24	5.76	0.981
26	4.10	0.665
28	2.61	0.304
30	0.65	0.008
30.4	0	
		$\Sigma = 59.1$

36

Figure 9: Bathymetric map of L305. Solid line denotes sampling transect (After Brunskill and Schindler 1971).



Z (m)	A_i ($10^4 m^2$)	V_{i-j} ($10^5 m^3$)
0	52.0	
		19.0
4	43.2	
		16.0
8	36.8	
		13.6
12	31.1	
		11.1
16	24.5	
		8.26
20	17.0	
		5.81
24	12.2	
		3.60
28	6.20	
		1.16
32	0.600	
		0.014
32.7	0	
		Σ=78.6

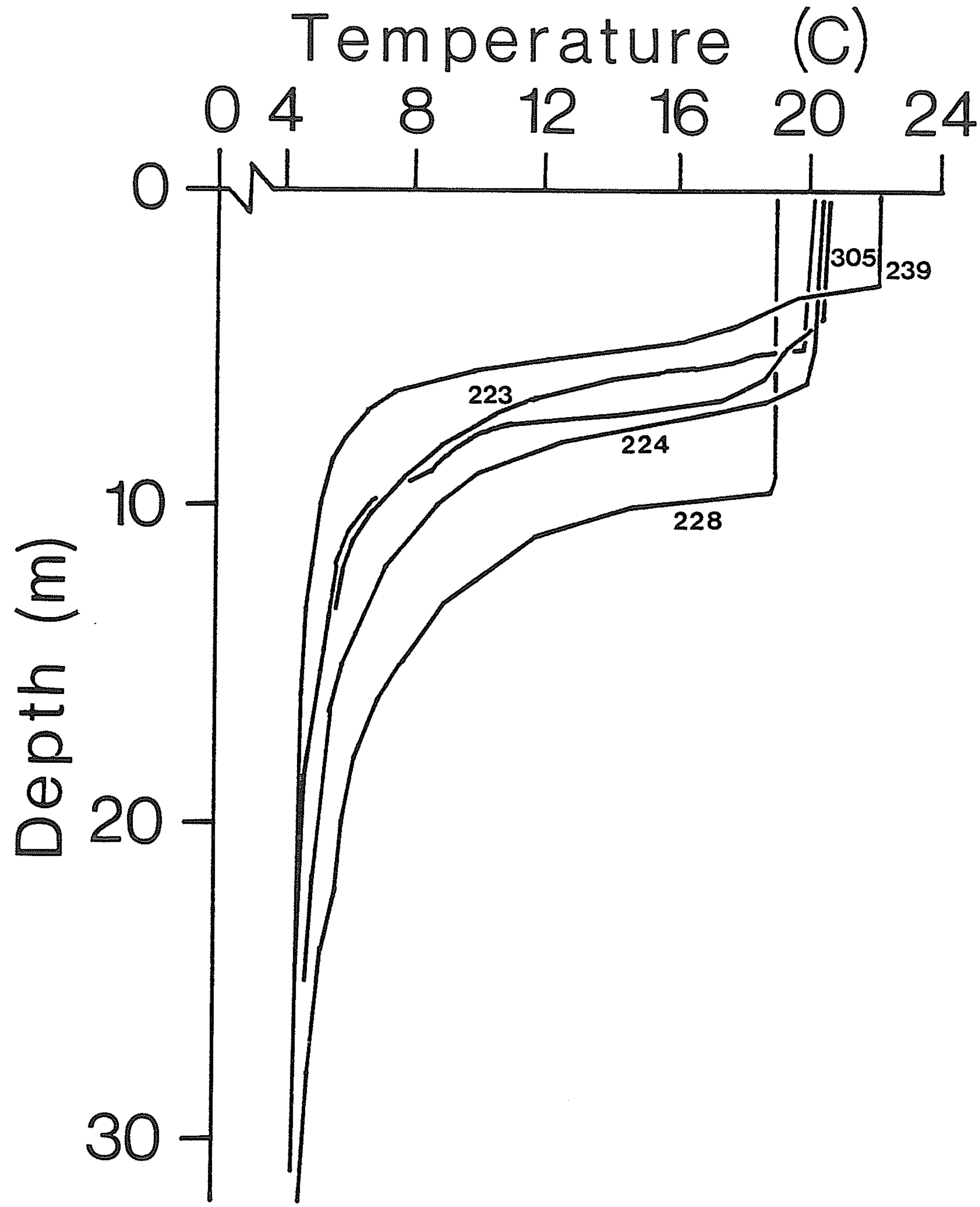
3.2.2 Light, Temperature, and Oxygen Regimes

The water transparencies of Lakes 161 and 228 are similar to those of distilled water except for a second transmission maximum at green wavelengths (Schindler 1971). Their Secchi disk values were among the highest recorded in the area (9 to 10 meters), Table 1. Most of the other ELA lakes are less tranparent than lakes 161 and 228, transmitting in the yellow or orange wavelengths (Schindler 1971).

Light extinction coefficients for lakes 223, 224, 228, 239, and 305 were measured from May to November 1974 (Shearer 1976). The ranges for each lake during this period are given in Table 1. Single measurements were made on lakes 161 and 310 during summer 1969 (Reid et al. 1975a). Lakes 223, 239, and 310 are humic lakes with an orange color while lakes 161, 224, 228, 305, and 468 are clear lakes (Fee 1980).

All of the study lakes are dimictic with ice cover normally lasting from November through April, except for Lakes 161 and 228 where the onset and end of ice cover are delayed for about two weeks because of their larger volumes. Maximum epilimnion temperatures range from 20C to 23C (Fig. 10). Temperatures at the lake bottom during winter are around 3.9C. Total heat budgets (summer + winter) for Lakes 239 and 305 are 18,300 $cal \cdot cm^{-2}$ and 24,200 $cal \cdot cm^{-2}$ respectively (Schindler 1971).

Figure 10: August 1974 midsummer temperature profiles for 5 ELA lakes (Schindler unpublished data, 1974 was the only year that temperature data were gathered from the 5 lakes during a comparable period).


Temperature (C)
0 4 8 12 16 20 24
0
10
20
30
Depth (m)
305
239
223
224
228

Overall, L223 has the highest rate of oxygen depletion in the lower 3m of the water column. Anoxic conditions prevail during summer and winter stratification (1974-77, Schindler et al. 1980a; 1978-80, Schindler unpublished data).

Anoxic conditions have not been observed in Lakes 161 and 228. In L228 on August 16, 1971 10.82 mg oxygen.l^{-1} were present at 160m (Schindler unpublished data).

Concentrations of dissolved oxygen in L239 decrease to 1 to 2 mg $O_2 \cdot l^{-1}$ at 30m during winter and summer stratification (1969-80, Schindler unpublished data). Rates of oxygen depletion under the ice range from 15.0 mg $O_2 \cdot m^{-3} \cdot day^{-1}$ to 17.4 mg $O_2 \cdot m^{-3} \cdot day^{-1}$ (1968-69 and and 1969-70 respectively, Schindler 1971).

Concentrations of dissolved oxygen in L305 usually drop to 2 to 3 mg $O_2 \cdot l^{-1}$ at 30m during late summer and late winter. Rates of oxygen depletion under ice are 13.4 to 13.7 mg $O_2 \cdot m^{-3} \cdot day^{-1}$ (1968-69, 1969-70 respectively, Schindler 1971).

Very few data have been collected for Lakes 310 and 468. L310 probably has an oxygen regime similar to L239. L468 has been observed to become anoxic below 27m during late summer (P. Olesiuk, unpublished data).

3.2.3 Lake Disturbances

All of the lakes being studied are considered as controls except for 223 which is undergoing experimental acidification (Schindler et al. 1980a). Representative publications dealing with effects on L223 and its biota are: Findlay and Saesura (1980), Schindler (1980), Schindler et al. (1980), Kennedy et al. (1980), Malley (1980), and Malley et al. (1981). Results relevant to this study are considered later in Chapter IV.

In 1974 a forest fire burned parts of the watersheds of Lakes 228, 239, 305, 310, and 468. However, effects on the water chemistry of L239 were minimal (Schindler et al. 1980c). On June 19, 1980 a second fire reburned a portion of the area burned by the 1974 fire and remaining unburned portions of the L239 watershed. Some parts of the L468 watershed were also burned. The last life history samples were collected 2 weeks after this fire, so any effects on the data from the lakes and their biota would have been minimal.

On July 29, 1976, a whole-lake radioisotope spike of 200 to 500 mCi of each of six gamma emitting isotopes was added to the epilimnion of L224 (Hesslein et al. 1980). The resulting radioactivity was too low to have adversely affected the biota of L224.

3.3 METHODS

Mysis populations were estimated using stratified random sampling (Elliott 1973) of night-time vertical hauls of a net with a $1 m^2$ mouth opening (Appendix A). Stratification was based on depth. Four population estimates in Lake 239 (L239) were made over a two year period to show year to year fluctuations (Table 2). The L239 estimate and one population estimate from L305 (Table 2), shows lake to lake variability. For all estimates in both lakes, samples were collected along a single transect.

Specimens were enumerated as juvenile or adult mysids based on measurements of total animal length (<10mm and ≥10mm respectively). For each estimate the juveniles, adults, and total mysids were estimated separately.

Densities (as number/m^3) have been calculated by dividing the total number of *Mysis* in the lake by the volume of water which they inhabit during summer stratification. Water volumes are considered more realistic than surface area because during the summer months, *Mysis* are primarily filter feeders relying on the density of food within a given volume. The calculations are very simplistic and are not intended to indicate that water volumes determine densities of *Mysis* in lakes. More complex calculations at this stage would be unrealistic as the type of food organisms available will affect the efficiency with which *Mysis* can feed and utilize the available production (Lasenby and Langford 1973, Cooper and Goldman 1980).

TABLE 2

Dates and depths for which samples of *Mysis relicta* were collected in L239 and L305.

Depths (m) from which samples were collected for population estimates of *Mysis* in L239 and L305. 'n' denotes the number of samples collected at each station.

L239								L305	
7/79	n	8/79	n	7/80	n	9/80	n	7/80	n
7.0	3	6.0	3	6.8	3	7.0	3	7.6	2
9.2	3	8.8	3	9.0	3	11.0	3	13.8	2
10.7	3	10.2	3	10.0	3	15.0	3	18.7	2
14.4	3	13.7	3	14.0	3	19.0	3	22.9	2
23.0	7	23.0	3	23.0	3	23.0	3	28.1	2
30.4	4	30.0	3	31.0	3	27.0	3	33.4	4
						31.5	3		
total =	23		18		18		21		14

In Lakes 224, 228, 239, 305 and 468, collections for life history began in May 1979 and continued every two weeks until October 1979, after which monthly samples were collected when ever ice and weather conditions permitted until July 1980. Most lakes were inaccessible during November 1979. One sample was collected from L161 on September 1, 1979 and one sample was obtained from L310 on July 13, 1979.

During open water periods, a horizontal tow of a $1m^2$ net at a depth of about 7m for one-half minute was usually sufficient to obtain a sample of 50 to 200 mysids. More samples were collected elsewhere in the lake if an expected cohort was poorly represented. During the winter, a collapsing version of the $1m^2$ net was used to sample through the ice (Appendix A).

Samples were placed in 500ml Nalgene bottles with enough ethanol to prevent decomposition or freezing of the samples while in the field. Samples were transferred within 72h to 12 dram vials with a permanent preservative of 70% ethanol denatured with formalin. As most samples were biased towards one cohort of *Mysis*, with other cohorts often poorly represented, more emphasis was placed on ensuring that a sufficient number of each cohort was measured than on careful "statistically correct" subsampling. For most samples, 2-3 cohorts could easily be identified prior to measuring. Twelve animals of each cohort were measured to give a total of 36 animals. When cohorts could not be identified, a subsample of about 36 animals was randomly selected from the sample to be measured.

Measuring of specimens took place between 3 days and 1 year after collection. An analysis of variance of three measures of size indicated that antennal scale length as suggested by Grossnickle and Beeton (1979) should be used (Appendix D). Measurements were made at 50X magnification giving a resolution of .025mm.

Preliminary observations of *Mysis* placed in 70% ethanol during summer 1979 suggested that within the first few days some shrinkage occurred, perhaps due to the dis-solution of ethanol-soluble fats (Sladeckova and Pieczynska 1971). Further tests to find out how this might affect measurements on preserved samples were carried out on a sample collected on August 4, 1980. From the freshly collected sample, 48 randomly selected mysids were measured for antennal scale length. Animals were each placed in a 15ml glass vial with 5ml of 70% ethanol. Mysids were remeasured after 1, 6, and 13 days. Antennal scale length decreased only 2.2% after the first 24h (P=.0001, analysis of variance). Because this change was small and occurred immediately, it was ignored for the remainder of the study.

In addition to measures of size, animals were examined for sex, and stage of sexual maturity. The sex and sexual maturity stages have been adapted from Reynolds and DeGraeve (1972) (See Appendix C). The brood pouch of each gravid female was opened and the number of eggs and embryos counted. Embryos were classed into stages I - VI (Berrill 1969).

A MANTES (Manitoba text editing system) file was set up which contained 3,804 cases and all identifying information. For the life history analysis a WATFIV program was used to derive length-frequency histograms for each sample. From these, the demarcations between cohorts were estimated by eye and the age in years for each animal was added to the MANTES file. SAS MEANS and PLOT procedures (Statistical Analysis System, SAS 1979) were used to derive plots for each lake of the mean size (±95% confidence limits) for the three size classes of each cohort through time.

Female mysids appear to continue growing until their third molt into the fourth instar (Morgan and Beeton 1978), after which fertilization and brooding commences. Growth is greatly reduced until the release of young in the spring (Holmquist 1959, Lasenby and Langford 1972, Hakala 1978, Hakala 1979). Because growth is greatly reduced during maturity, the mean size at onset of sexual maturity for one cohort can be easily calculated. Samples collected during a period that the cohort is reaching maturity are required. From these samples the size of all mature females produces a normal distribution of the initial size at onset of sexual maturity. Differences in the mean size of the sexually mature females for each lake should indicate if environmental limiting factors or disturbances are affecting the populations (Wenner et al. 1974). A larger size will indicate that a population has been exposed to better growing conditions. A smaller size will indicate less favorable growing conditions.

For each lake, the mean size of female mysids at onset of sexual maturity was determined by selecting all cases between days 125 and 274 where females were sexually mature and less than 2 years old. The day limit removed bias introduced by the absence of winter samples for some lakes. Lakes were the treatment effect in an unbalanced analysis of variance design with size of sexually mature 1+ yr old females as observations (SAS GLM procedure). A multiple t test was used to further analyze differences between the means (Snedecor and Cochrane 1967).

A SAS plot procedure was used to produce plots of the number of offspring versus antennal scale length for each lake. The embryological stage of each brood was imposed on each plot to determine if correlations existed between brood size, female size, and brood age.

Statistical analyses were carried out on an Amdahl V7 computer.

3.4 RESULTS

The August 1979 estimate for L239 is lower than the other three estimates for the lake, possibly due to an unexpected change in the distribution of *Mysis* along the transect sampled (Table 3). The estimate has been excluded from further analysis. Confidence intervals for the 1979 L239 estimate, the 1980 L239 estimates, and the L305 1980 estimate overlap, indicating that year to year as well as lake to lake densities are comparable. The mean number of *Mysis* per cubic meter of inhabitable volume is 17.5 ± 1.8 ($\pm$ 2x standard deviation boundary on the error of estimation).

Data from population estimates of juvenile and adult *Mysis* was used to calculate the percent of the population represented by adult animals for the three L239 estimates (Table 4). The percentage of adult animals was approximated for data from three lakes obtained from the literature (Table 4). Overall, the percentage of the population as adults for L239 is similar to values for Great Slave Lake, Stony Lake, and Lake Superior.

Plots of antennal scale length for each cohort and sex for all sampling dates are presented in Figures 11, 12a, 12b, 13a, and 13b. The presence of brooding females during their second year indicates that generation times for *Mysis* from all lakes except L468 are two years. *Mysis* in Lakes 161 and 310 also have a two year life cycle based on measurements obtained from single samples. L468 has a one year

TABLE 3

Average densities of *Mysis relicta*.

Estimated average densities of *Mysis relicta* determined from population estimates in two ELA lakes and density estimates in six lakes obtained from previous studies. Abbreviation 'n' denotes sample size. The mean number of *Mysis* was calculated by dividing the total number of *Mysis* in the lake by the volume of water which they inhabit during summer stratification.

Lake	Date	Source	Inhabitable Volume ($10^5 m^3$)	n	Total±95%CI ($X10^6$)		Mean Number ±95%CI (m^{-3})	
239	7/79	a	31.49	23	60.2	1.06	19.1	.337
239	8/79	a	31.49	18	30.8	7.04	9.78	2.24
239	7/80	a	31.19	18	55.0	24.8	17.6	7.95
239	9/80	a	31.19	18	53.8	32.6	17.2	7.95
305	7/80	b	47.33	14	77.2	16.3	16.3	3.44
Paajarvi	8/75	c	198.3	26	180.	60	9.08	3.03
Char	7/70	d	53.65	20	5.22		.973	
Ontario	71	e					1.79	
Huron	71	e					1.91	
Superior	71	e					2.33	
Michigan	7/76	f					3.09	

a - This study.
b - Olesiuk unpublished data.
c - Hakala (1978).
d - Lasenby (1971).
e - Carpenter et al. (1974).
f - Morgan (1976).

TABLE 4

Percentage of adults in the population.

Estimated percentage of the population represented by adults for L239 as well as three lakes examined by previous authors (±95% confidence intervals).

Lake	Date	Total	Juveniles	Adults	%Adults
239	7/79	60.25 ±4.62	49.14 ±4.28	11.11 ±.740	18.4
239	7/80	54.98 ±24.8	25.11 ±13.5	29.87 ±14.7	54.3
239	8/80	53.82 ±32.6	38.10 ±20.5	15.71 ±7.01	29.2
Great Slave		Larkin 1948			47.5
Stony Lake		Lasenby and Langford 1972			37.9
Superior		Carpenter et al. 1974			36

life cycle, based on the presence of brooding juvenile females during their first winter. Molting of spent females into a second mature stage was found in the May 9, 1980 sample in L239 wich indicates that production and release of a second brood is possible during the females 3rd year. The production of a second brood may be widespread as third year females have been observed in the other lakes during early spring and summer.

Estimates of Mysis growth are based on the life cycle plots presented in Fig. 11 - 13b. Growth was determined as the increase in antennal scale length on a monthly basis (Fig. 14). Mysis from L239 and L468 exhibit higher growth rates than Mysis in Lakes 224, 228, and 305 when comparisons of growth are based on the maximum rate of growth during the summer. Data on Mysis summer growth, primary production (Fee 1980), and growth of lake trout (Salvelinus namaycush) and white sucker (Catostomus commersoni) (K. Mills, unpublished data) have been used to rank growth and production in the study lakes (Fig. 5). In general, faster summer growth rates for Mysis and fish in Lakes 468 and 239 are associated with higher production rates.

The mean size of sexually mature female Mysis is significantly different among the lakes (P=0.0001). A multiple comparison t - test (Snedecor and Cochrane 1967) was used for these comparisons as the SAS Duncan's multiple t test was not robust for an analysis of the means due to low F

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Figure 11: Life cycle of *Mysis* in L224, open circles denote females, triangles males, and closed circles undifferentiated females and males. Bars indicate approximate 95% confidence intervals based on Student's t distribution.

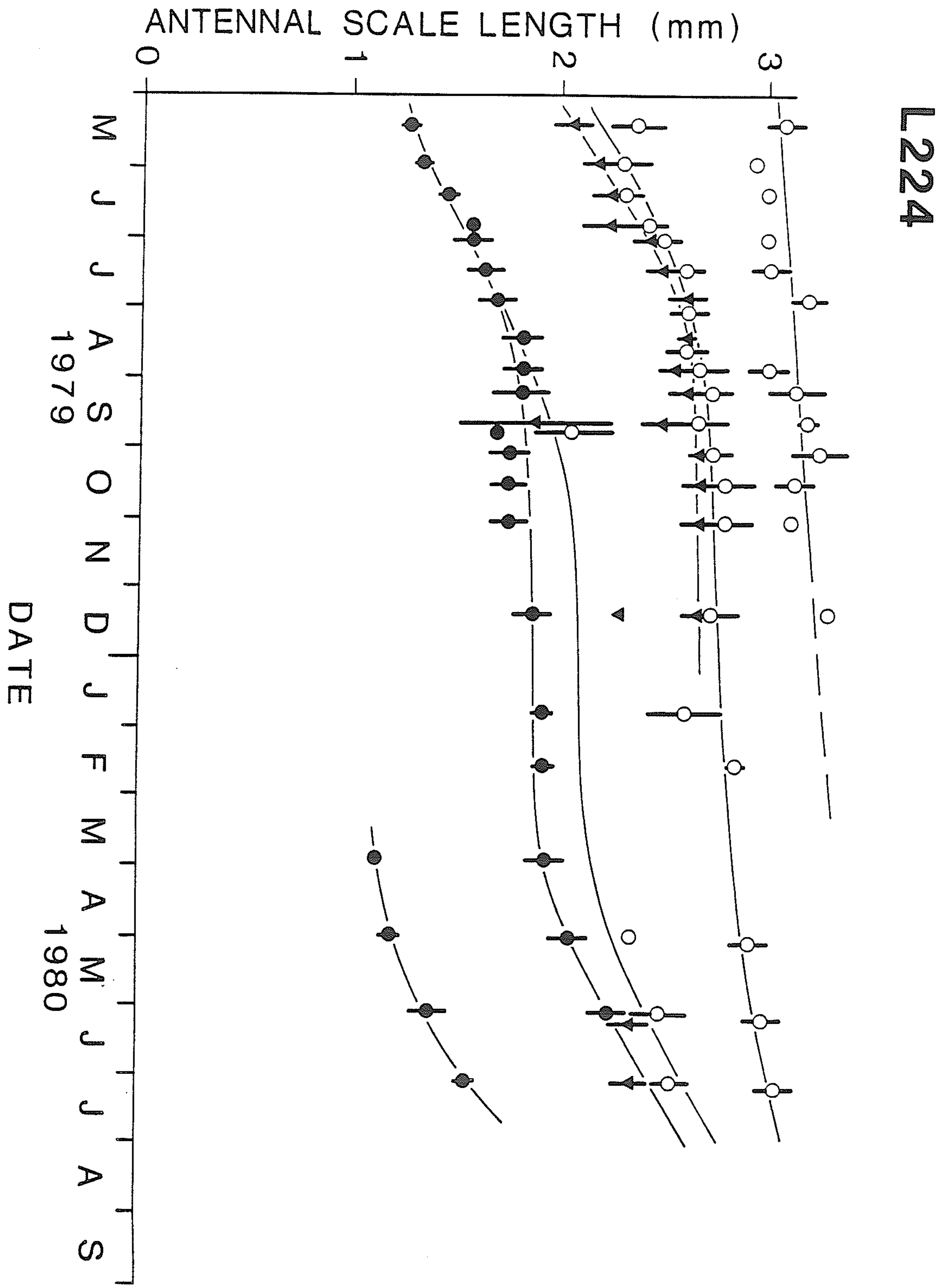
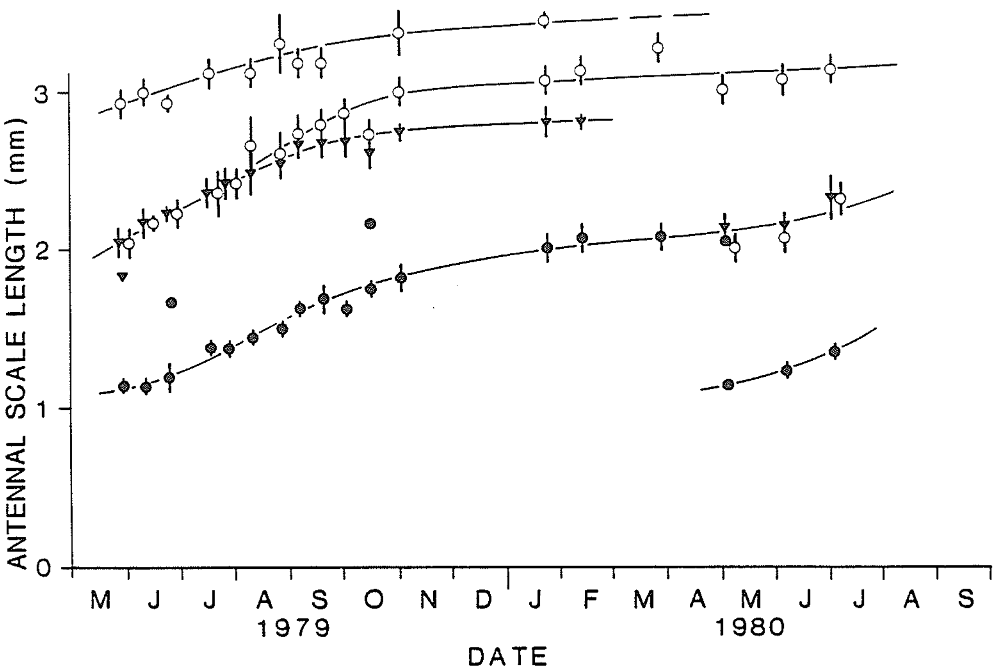
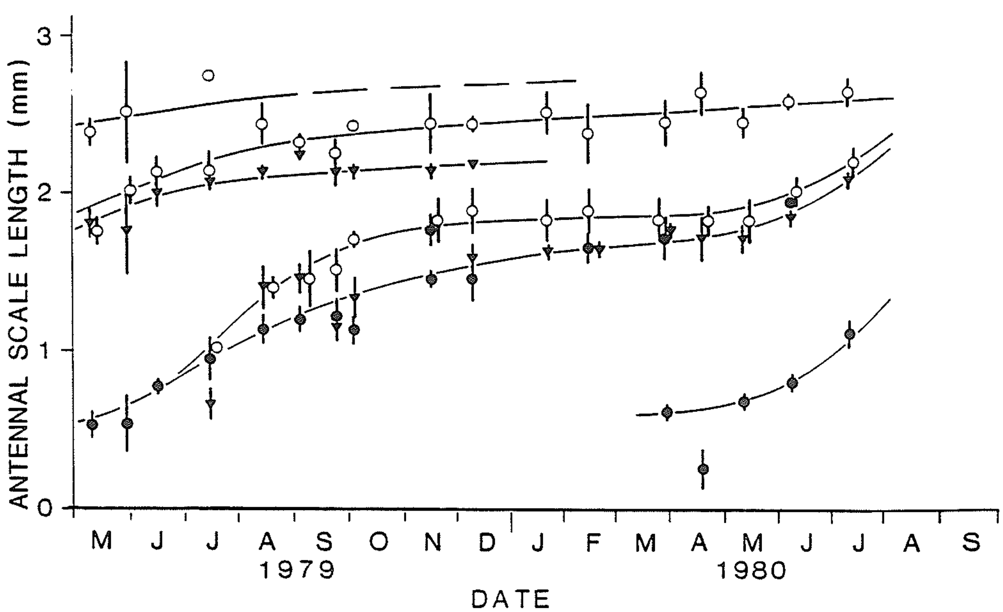

L224
ANTENNAL SCALE LENGTH (mm)
0
1
2
3
M J J A S O N D J F M A M J J A S
1979
1980
DATE

Figure 12: Life cycle of *Mysis* in A - L228 and B - L239. Symbols as in previous figure.

A L228

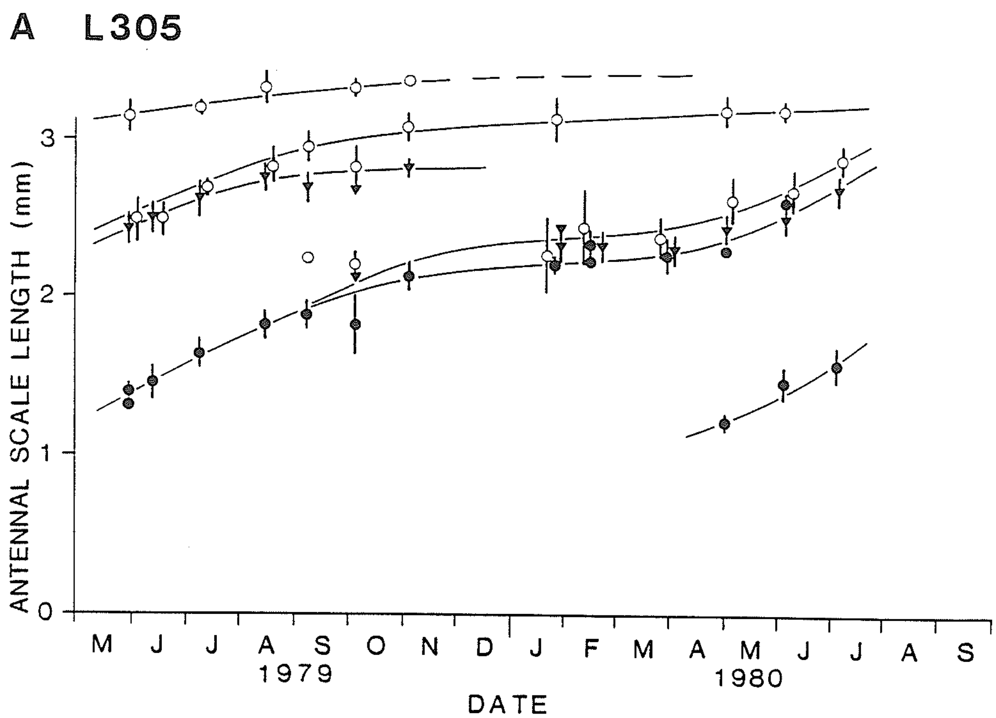

ANTENNAL SCALE LENGTH (mm)
0
1
2
3
M J J A S O N D J F M A M J J A S
1979
1980
DATE

B L239


ANTENNAL SCALE LENGTH (mm)
0
1
2
3
M J J A S O N D J F M A M J J A S
1979
1980
DATE

57

Figure 13: Life cycle of *Mysis* in A - L305 and B - L468. Symbols as in previous figure.


A L305
ANTENNAL SCALE LENGTH (mm)
0
1
2
3
M J J A S O N D J F M A M J J A S
1979
1980
DATE

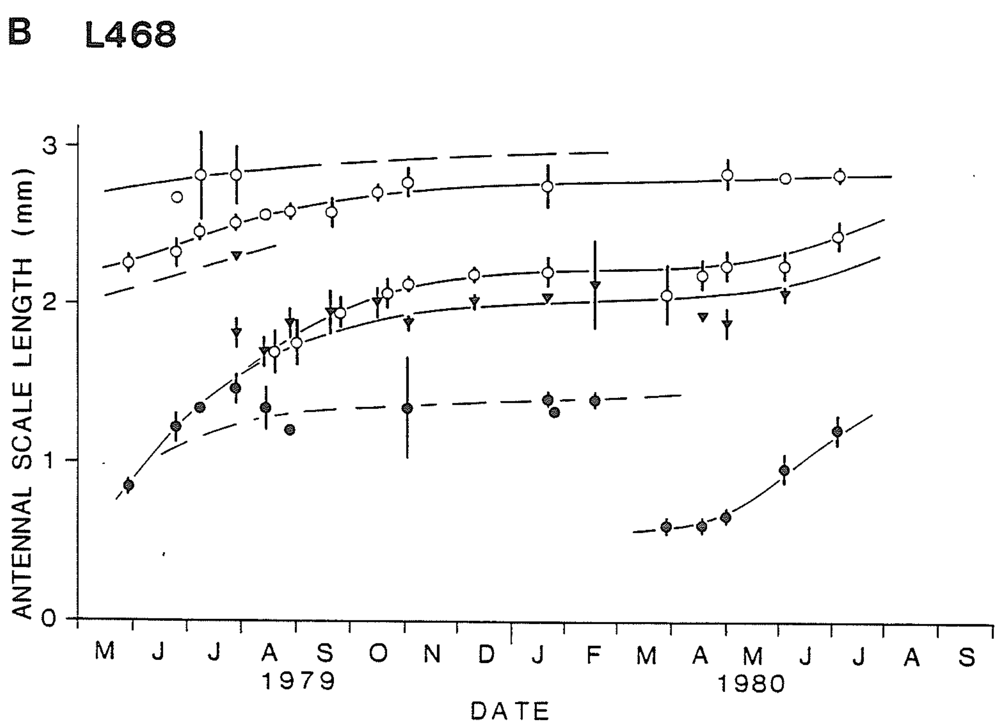

B L468
ANTENNAL SCALE LENGTH (mm)
0
1
2
3
M J J A S O N D J F M A M J J A S
1979
1980
DATE

TABLE 5

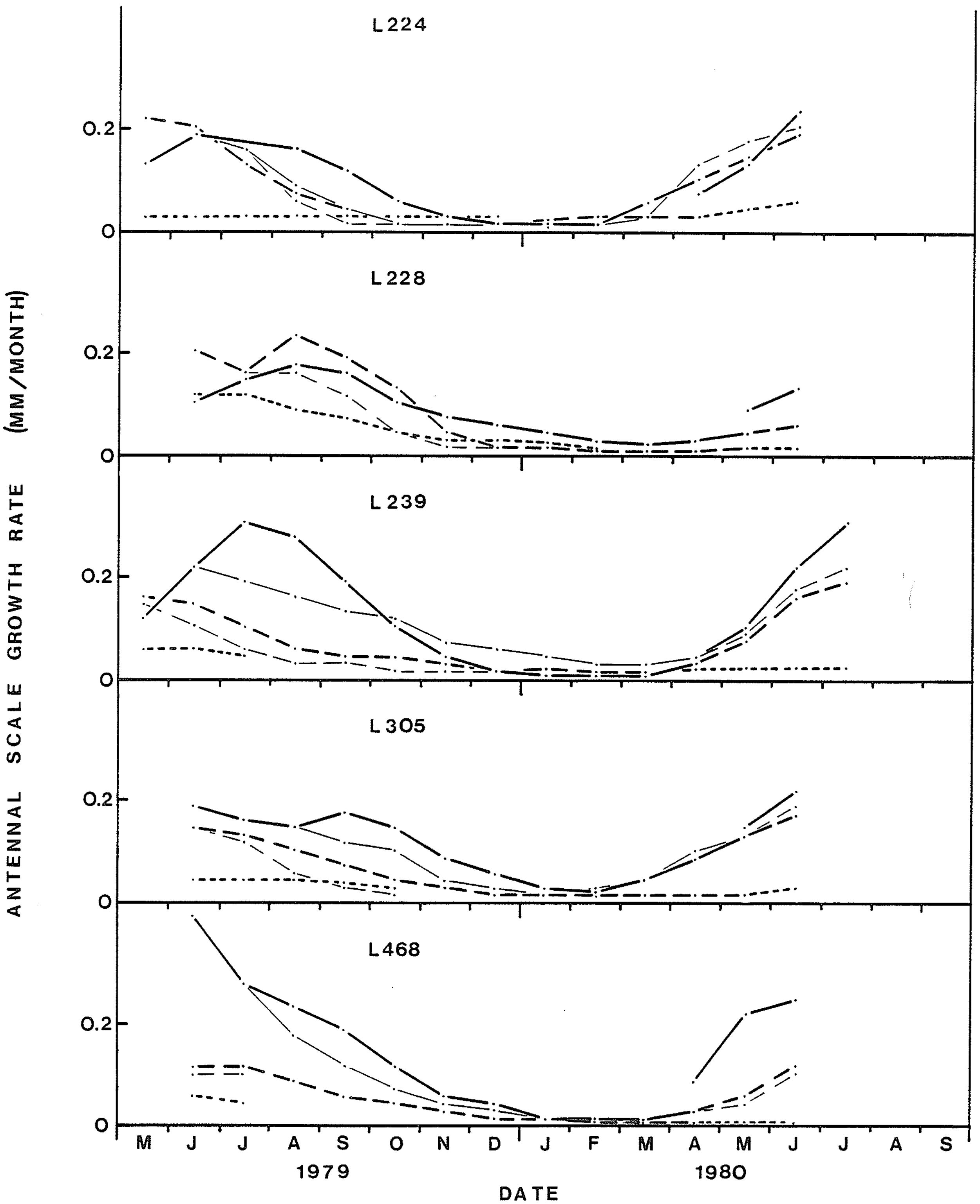
Growth and production in six of the study lakes.

Ordering of the study lakes for primary production (Fee 1980), mysid growth, and growth of two fish species (K. Mills unpublished data). Orders are based on general trends observed in the growth and primary production data.

Measure	Lake Ordering (High → Low)
Primary Production	468>239>224=305>228=161
Mysid Growth	468>239>224>228>305
Lake Trout Growth	468>239=228=161>305>224
White Sucker Growth	468>239>305=228=310>224

60

Figure 14: Antennal scale growth rate of _Mysis relicta_ in five ELA lakes. Solid lines denote age 0+ yr, partly broken 1+ yr, and fully broken 2+ yr. Thick lines are females, thin lines males.


L224
L228
L239
L305
L468
0.2
0
ANTENNAL SCALE GROWTH RATE (MM/MONTH)
M J J A S O N D J F M A M J J A S
1979
1980
DATE

values. Four comparisons were found to be significantly different ($P \geq .002$, significance level adjusted for 21 comparisons, Dunn and Clark 1974). In order to help explain differences in the mean size at onset of sexual maturity between lakes, a measure of production dependent on lake volume was sought. As Mysis are primarily planktonic filter and encounter feeders (Lasenby and Langford 1973, Bowers and Grossnickle 1978, Grossnickle 1979, and Cooper and Goldman 1980), values of primary production calculated as $g.carbon.m^{-3}.yr^{-1}$ for 1974 were used (Fee 1980 column 12, his values are for cloudless weather production corrected for morphometry). For the four control lakes in Fig. 15, a regression of moderate fit, with a negative slope, explains 70% of the variance.

For fecundity data from all lakes there is a significant positive correlation ($P=.0001$, $n=172$, Spearman correlation, Hollander and Wolfe 1973) between the size of the brood and the antennal scale length of the mother (Appendix E). Because few data have been collected for most of the lakes, and high variability is associated with the data, no statistical tests were made for fecundity relationships in individual lakes. In all lakes, broods became more mature as winter progressed. The mean size of the broods in all lakes peaked at 23 in mid February and reached a low of 7 by the end of May.

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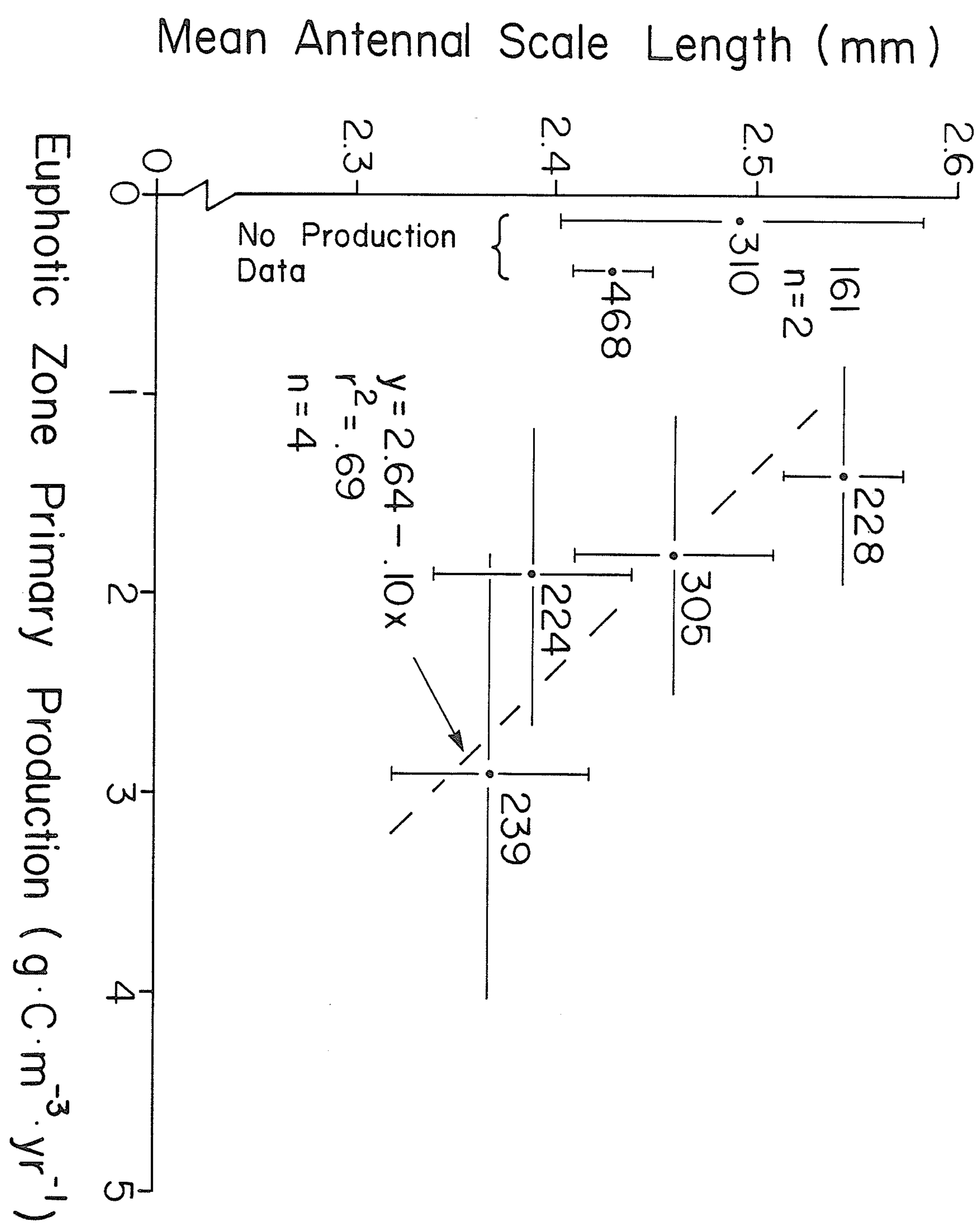
Figure 15: Mean antennal scale length for female *Mysis* at onset of sexual maturity for the 4 study lakes and euphotic zone primary production (Production data from Fee 1980).



Mean Antennal Scale Length (mm)
2.6
2.5
2.4
2.3
0
Euphotic Zone Primary Production (g·C·m-3·yr-1)
0
1
2
3
4
5
No Production Data
161
n=2
310
468
228
305
224
239
y=2.64 - .10x
r2=.69
n=4

3.5 DISCUSSION

The densities of Mysis in Lakes 239 and 305 are similar yet, L305 has a greater surface area and is more oligotrophic than L239. In the larger Lake Paajarvi, Finland, a lake larger than either L239 or L305, lower densities have been obtained (Hakala 1978). Even lower densities occur in the Great Lakes, probably due to the pelagic habits of animals in these lakes (Morgan 1976, Carpenter et al. 1974). In Char Lake, an oligotrophic arctic lake, very low densities were found, probably due to the lakes much lower primary production (Table 3).

The percent of the population represented by adults is a measure of reproductive success which can be used for comparisons between populations with similar life history patterns. Under normal conditions the ratio should range from 18% to 54% (Table 4).

The life history pattern of Mysis relicta in all the lakes studied, except L468, is similar to that found in other oligotrophic lakes such as Great Slave Lake (Larkin 1948), Char Lake (Lasenby and Langford 1972), and Lake Superior (Carpenter et al. 1974). Juveniles are released from March to April. Rapid growth occurs until mid summer at which time the sexes can be differentiated. Continued growth occurs the following spring and summer, at which time the animals reach sexual maturity. Breeding occurs over winter with the males dying by late February. Young are released in March to April, at which time the parents are two years old. Another molt after the release of young indicates that females may live into their third year, breeding with 1^+ yr age males and releasing a second brood at 3 yrs of age. A second brood is often released by Mysis relicta (Larkin 1948, Holmquist 1959, McWilliam 1970, Morgan and Beeton 1978). This is a 2 yr life history pattern (described by Furst (1972). The life history pattern in L468 is similar to the two year life history except that there is only one year between birth and release of young. This is Furst's one year pattern, found in warmer, less oligotrophic lakes such as Stony Lake (Lasenby and Langford 1972), Cayuga Lake (Brownell 1970), and Lake Mälaren (Furst 1965).

Observations of Mysis growth in ELA lakes agree well with differences expected due to differing productivities of the lakes. Based on water color, transparency, and production data, lakes 468 and 239 are more productive than Lakes 224, 228, and 305. The highest summer growth rates have been observed in lakes 468 and 239. Similar results were obtained for the growth of white sucker and lake trout indicating that faster mysid and fish growth is associated with the more productive lakes.

In all lakes, reproduction was normal throughout the study period. The relationship between the size of the brood and the female's size lies within the range of similar relationships found for Mysis in Lake Michigan (McWilliam

1976). Higher fecundity values of 10 - 40 eggs/female have been noted by Reynolds and DeGraeve (1972) for Lake Michigan, Hakala (1978) for Lake Pääjärvi, Finland, and Morgan (1980) for Emerald Bay, Lake Tahoe. Fecundity as low as that observed in ELA lakes (10 - 20 eggs/female) has been found in Green Lake, Wisconsin (Juday and Birge 1927), Cayuga Lake, New York (Brownell 1970), and Char Lake, N.W.T. (Lasenby and Langford 1972). McWilliam (1970) found that, in Lake Michigan, at a deeper, more oligotrophic, station brooding females had smaller broods. This explains the low fecundity found for ELA *Mysis* were the primary production values of these Precambrian Shield lakes is very low (Schindler and Holmgren 1971).

Reynolds and DeGraeve (1972) have shown that the number of young per female decreases as the breeding season progresses, probably due to abortion or absorption of embryos. This is evident in data from the ELA lakes where the brood sizes decreased during the breeding season.

Optimal growing conditions, as indicated by the largest mature females, occur in the more oligotrophic lakes (L224, L228, and L305). However, the fastest summer growth rates have been found in Lakes 239, and 468; Lakes 224, 228, and 305 show slower summer growth rates. These discrepancies are explainable in terms of overall growth rates during the year. The *Mysis* in more oligotrophic lakes (224, 228 and 305) show low summer growth rates, but growth rates during the fall, winter, and spring seasons is higher than in the more eutrophic lakes (239 and 468). Therefore, factors such as length of the growing season, or the mean water temperatures may be influencing the molt increment or frequency and therefore size at maturity (Annala et al. 1980). The interacting effects in these systems may be complex and may involve the role of fish in cropping Mysis and reducing intraspecific competition (Magnus Furst pers. comm.). A larger data-base must be obtained to explain the factors causing differences in Mysis growth, life history, and abundance.

Chapter IV

THE DECLINE OF *MYSIS RELICTA* LOVÉN IN RESPONSE TO THE EXPERIMENTAL ACIDIFICATION OF A WHOLE LAKE.

4.1 INTRODUCTION

Many Precambrian Shield lakes in southern Canada are acidic (Conroy 1974, Dillon et al. 1979, Pitblado et al. 1980) and thousands more may be acidified during the next few decades (Beamish and Harvey 1972, Harvey 1975, Beamish 1976, Dillon et al. 1978). Hendrey et al. (1976) have suggested that the loss of fish-food organisms could reduce fish growth well before pH levels become low enough to result in reproductive failure of the fishes. In deep oligotrophic lakes, the loss of an important fish-food organism such as *Mysis relicta* could affect the growth of a number of fish species (Van Oosten and Deason 1937, Rawson 1961, Dryer et al. 1965, Wells and Beeton 1963, Bailey 1972). *Mysis relicta* was therefore a key organism to study during the experimental acidification of Lake 223 (L223) of the Experimental Lakes Area (ELA), northwestern Ontario (Schindler et al. 1980a, and Schindler 1980).

The only available pH ranges for natural populations of *Mysis relicta* are from studies of their distribution with respect to Pleistocene glaciation. Holmquist (1959) found *Mysis relicta* in lakes ranging from pH 6.2 to pH 8.6 . In a later study Dadswell (1974) found *Mysis* present in lakes with pH as low as 5.7 . However, as he used indicator solutions to measure pH and collected only surface and bottom samples, his pH values may not be accurate or representative of the entire water column. In some mountain lakes in Wyoming, introduced Mysis populations appear to be healthy at pH 5.8 (J. Grabowski pers. comm. 1981). In Sweden W. Dickson (pers. comm. 1980) found that introduced populations of Mysis relicta began declining at values around pH 5.9.

The lower pH tolerance of Mysis relicta would be obtained by following the decline of Mysis in L223 during experimental acidification. Effects on the population were expected to be due to decreased pH and not toxic metals because only the lake waters are being acidified. Leaching of metals from terrestrial watersheds by acid precipitation has been observed in lakes with acidified watersheds (Malmer 1976, Herman and Baron 1980, and Troutman and Peters 1980). This is not expected in L223. However, some increases in metals were observed due to mobilization from lake sediments (Schindler et al. 1980a, b). Although this study began after two years of acidification, in 1978, major changes were expected in the L223 biota with the mean summer 1979 epilimnion pH set at 5.75 (Schindler unpublished data).

To determine the mechanism by which the Mysis relicta population of L223 would decline, animal size, sex, sexual maturity, and fecundity were measured. Concentrations of Al, Zn, Cd, Cu, Hg, Ni, Mn, and Fe were determined in preserved samples of L223 Mysis and animals collected from five control lakes. Differences in these parameters between the L223 population and five control lake populations (Chapter III) would help elucidate the mechanism by which the decline was brought about.

4.2 METHODS

Values of pH in L223 had declined to below 6.0 before summer 1978. In order to get an indication of pre-acidification *Mysis* abundance in L223, an extrapolation was made from data on the 1979 and 1980 summer abundance of *Mysis* in L239 as well as 1980 abundances for L305 (Chapter III). The volume of the lake inhabited by *Mysis* during late summer was assumed to be the dominant factor in determining mean *Mysis* densities expressed as number of *Mysis* per m^{-3} of inhabited volume. The expected abundance of *Mysis* for L223 was obtained by multiplying the mean density for lakes 239 and 305 by the inhabitable volume in L223. Lake productivity could not be used in the calculations as the relationship between *Mysis* abundance and lake productivity is not clearly known.

The limits of pH encountered by *Mysis* in L223 were determined using a combination of monthly pH profiles and temperature profiles (Schindler et al. 1980a, and unpublished data). The portion of the water column available to *Mysis* was determined on the basis of their upper temperature tolerance (18C) and their lower limit of oxygen concentration (2 $mg.l^{-1}$) (Brownell 1970). Within the water column bounded by these limits the highest and lowest pH values were chosen as the limits that *Mysis* would encounter.

Samples for life history analysis were collected from L223 during June - September 1978 and during May - September 1979. For each animal, the antennal scale length was measured and the sex and stage of development was recorded. Growth was measured as the increase in antennal scale length per month. The mean size at onset of sexual maturity (Wenner et al. 1974) was determined for 1+ age female mysids.

As a result of experimental acidification of L223 concentrations of Zn, Al, and Mn in the oxygenated water column of L223 had increased. Approximate mean concentrations of Zn rose from $2 \mu g \cdot l^{-1}$ in 1976 to $4 \mu g \cdot l^{-1}$ in 1979. Al concentrations increased from $8 \mu g \cdot l^{-1}$ in 1976 to $20 \mu g \cdot l^{-1}$ in 1979. Mn increased from about $15 \mu g \cdot l^{-1}$ in 1976 to about $90 \mu g \cdot l^{-1}$ in 1979. Concentrations of Cd, Cu, Cr, Pb, and Co were usually $<1 \mu g \cdot l^{-1}$ (Schindler et al. 1980; and Schindler unpub. data). In the unacidified L224 concentrations of Cd, Co, Cr, Cu, and Pb were low, ranging on average from 0.5 to 2.0 $\mu g \cdot l^{-1}$. Iron concentrations were about $20 \mu g \cdot l^{-1}$, Mn $6 \mu g \cdot l^{-1}$, Zn $3 \mu g \cdot l^{-1}$, and Al $15 \mu g \cdot l^{-1}$ (Schindler unpub. data).

Metal determinations were carried out on specimens collected from lakes 223, 224, 228, 239, 305, and 468. All samples were taken in 1979-1980 except for the L223 samples which were taken in summer 1978. No L223 samples from 1979 were analyzed because the low number of <u>Mysis</u> captured. Metals were analyzed by the Analytical Chemistry Unit at the Freshwater Institute, Winnipeg, Manitoba by flame atomic absorption spectrophotometry using a Varian AA-5 atomic absorption spectrophotometer equipped with a BC-6 simultaneous background corrector (Stainton et al. 1977).

Samples from each lake were ranked according to storage time (scale 1-7) to determine if storage time had an effect on mean metal concentration. An analysis of variance design was used for each metal with lake and storage time rankings as two treatment effects having one observation per cell. Duncan's multiple range test was used to examine differences between the means (Snedecor and Cochrane 1967, and Duncan 1975).

Population estimates of <u>Mysis relicta</u> were made for L223 during August 1978 and August 1979. The 1978 estimate was based on 83 samples collected along 6 transects between August 2 and August 27 (Appendix B). Similarities in <u>Mysis</u> densities between the different transects in 1978 (Chapter II) led to the use of a single transect in 1979. Forty five samples were collected between August 14, and September 9, 1979, from 9 permanent stations (6.0m, 7.0m, 8.0m, 8.7m, 9.8m, 10.4m, 11.4m, and 13.7m) located along a transect running from the north shore of L223 to the center of the lake.

Samples were enumerated as juveniles or adult mysids based on measurements of total animal length ($<$10mm and $\geq$10mm respectively). Population estimates were calculated using depth-stratified random sampling (Elliot 1973) with areas of each stratum calculated from hypsographic curves. Juveniles, adults, and total mysids were estimated separately.

Statistical analyses were made using SAS (Statistical Analysis System; SAS 1979) procedures on an Amdahl V7 computer.

4.3 RESULTS

The population of *Mysis* in L223 decreased from 5,300,000 ± 1,060,000 (±95% confidence limits) in 1978 to 220,000 ± 59,000 in 1979 (Fig. 16). Observations using SCUBA during 1978 and 1979 corroborated the changes in population size and showed that on October 6, 1979 *Mysis* were no longer present in the lake. During this period, the pH within the water column inhabited by *Mysis* had declined from a range of 5.51 to 5.89 (spring 1978) to a range of 5.23 to 5.64 (March 1979). A decrease in the depth of the thermocline during October, 1979, resulted in a decrease of the pH in the region inhabited by *Mysis* from pH 6.0 to pH 5.6 (Fig. 16).

An attempt has been made to determine the carrying capacity of L223 for *Mysis* because no data are available on the *Mysis* population of L223 prior to acidification. The mean density (which includes a 2x standard deviation boundary on the error of estimation) in L239 and L305 was 17.5 1.8 *Mysis* m^{-3} (Chapter III). This mean was multiplied by the volume of water inhabited by *Mysis* in L223 during August to give a total of 7.12×10^6 ($\pm 0.74 \times 10^6$) *Mysis*. The lower limit of this estimate (6.4×10^6) and the upper limits

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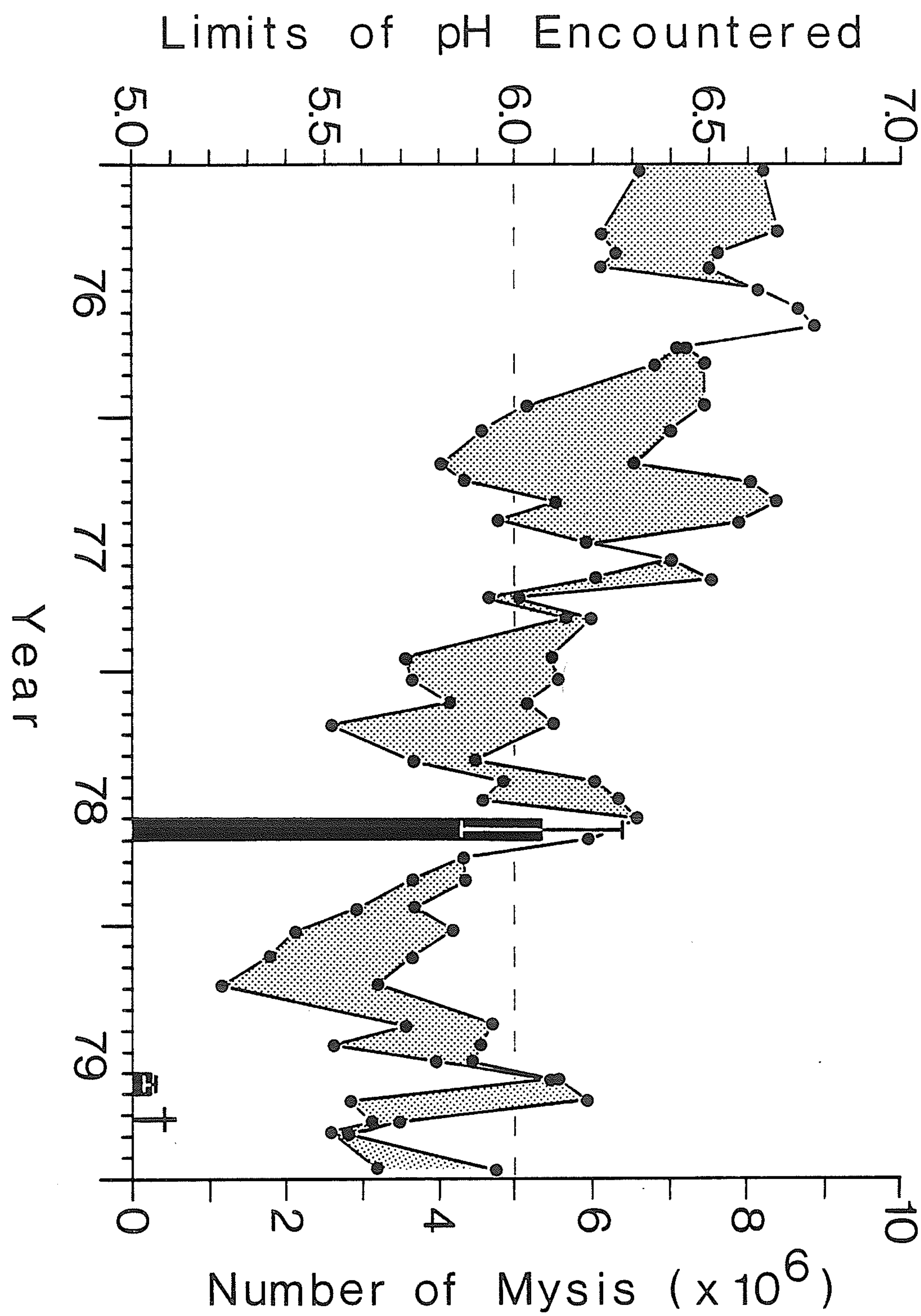
Figure 16: The maximum and minimum pH found within the water column of L223 within the region which could be inhabited by Mysis relicta (Shading). Dark vertical bars represent population estimates and their 95% confidence intervals. The cross indicates date at which Mysis were no longer observable in L223.


Limits of pH Encountered
5.0
5.5
6.0
6.5
7.0
76
77
78
79
Year
0
2
4
6
8
10
Number of Mysis (x 10^6)

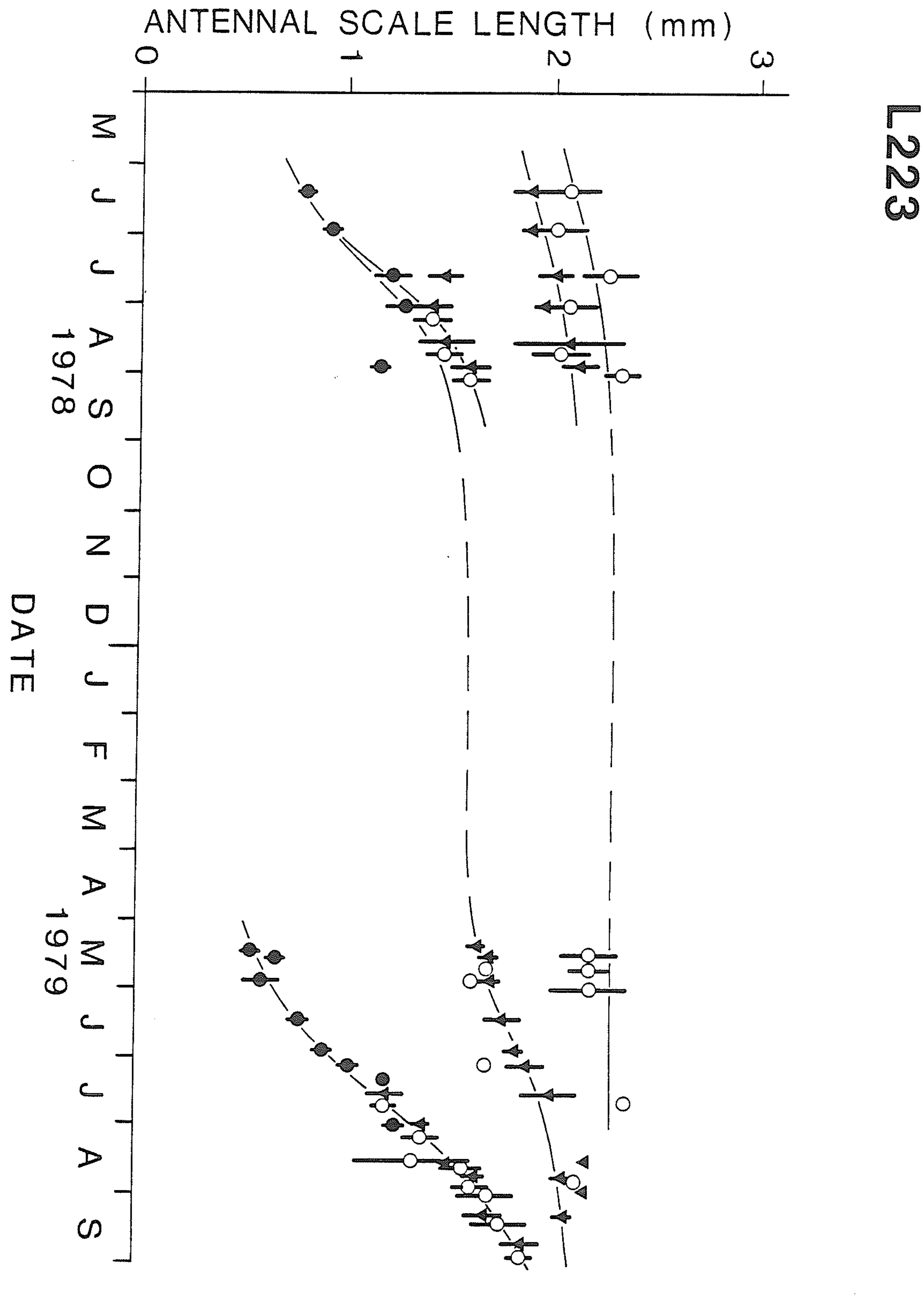
of the 1978 population estimate (6.3×10^6) almost overlap, indicating that the number of *Mysis* present in L223 in 1978 may have not been severely affected by decreasing pH values up to that time. However, the results are inconclusive and a slight decline from pre- acidification years to 1978 may have occurred.

The two L223 estimates for juvenile and adult *Mysis* permit a calculation of the percentage of the population represented by adults (number of adults/(adults + juveniles)X 100%). The 1978 value of 7.0% and the 1979 value of 12.6% in L223 are low in comparison to values of 18% to 54% for natural populations with a similar two year life cycle (Chapter III).

Acidification of L223 did not affect the life history pattern of *Mysis* (Fig. 17) or growth rates prior to their disappearance (Fig. 18). The life history pattern and growth rates of the cohorts in L223 during summer 1978 and 1979 were normal compared with a number of ELA control lakes (Chapter III).

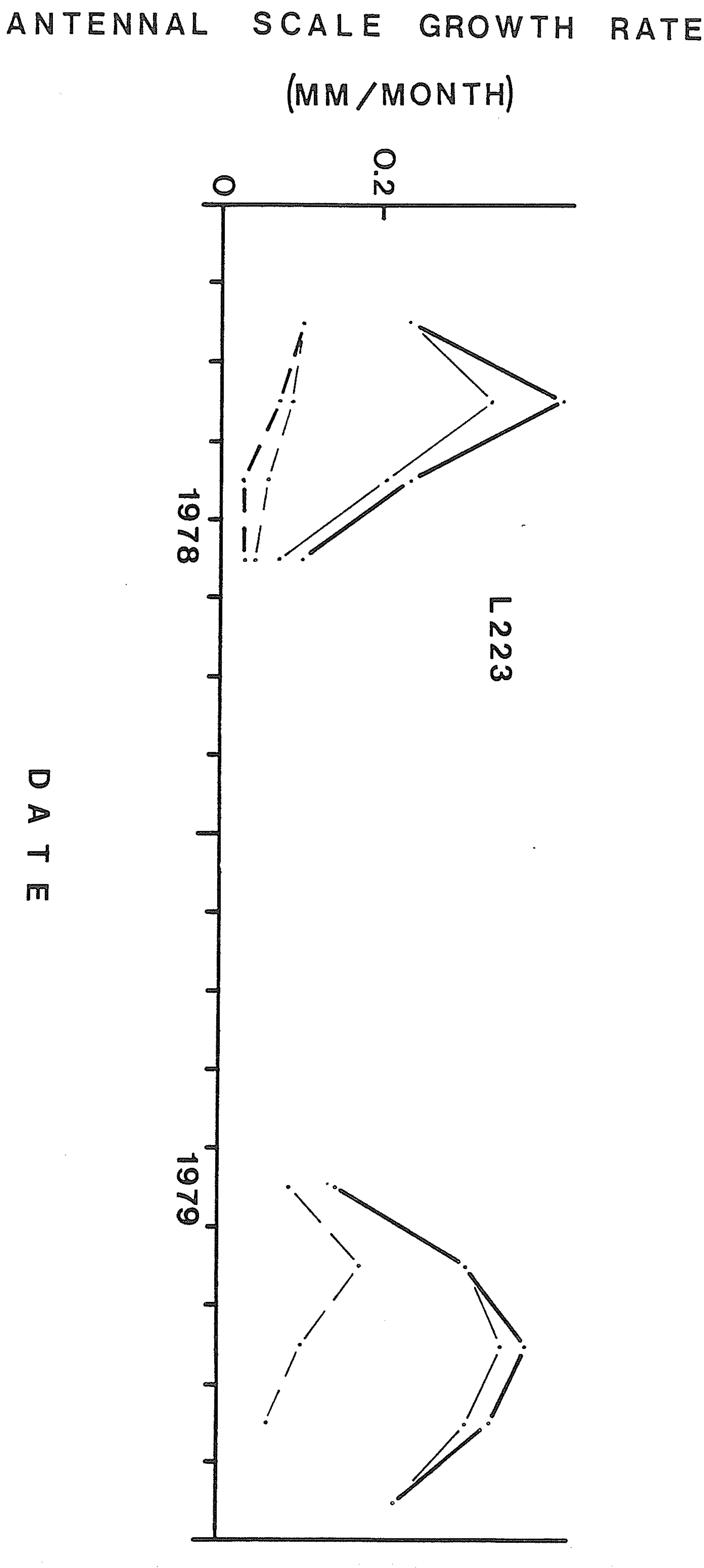
The mean antennal scale length at onset of sexual maturity for L223 mature females of 1+ yr age was 2.24mm. This value is significantly lower than the means for females of the same age during similar seasons for four ELA control lakes (2.37 - 2.54) (Duncan's multiple range test, P=0.05, control data from Chapter III).

Figure 17: Life cycle plot for L223 *Mysis relicta*. Open circles denote females, triangles males, and closed circles undifferentiated females and males. Bars indicate approximate 95% confidence intervals based on Student's t distribution.



L223
ANTENNAL SCALE LENGTH (mm)
0
1
2
3
M
J
J
A
S
O
N
D
J
F
M
A
M
J
J
A
S
1978
1979
DATE

Figure 18: Antennal scale growth rate of *Mysis relicta* in L223 at ELA. Solid lines denote age 0+ yr, and partly broken 1+ yr. Thick lines are females, thin lines males.



ANTENNAL SCALE GROWTH RATE
(MM/MONTH)
0
0.2
1978
L223
1979
DATE

As L223 was not sampled during the winter months, only one gravid female was obtained in spring 1979. This animal's brood appeared as a whitish mass, possibly a resorptive condition (Reynolds and DeGraeve 1972).

Significant differences ($P \geq .002$) were found for the mean concentrations of Zn, Al, Cd, and Hg in the *Mysis* samples from the six lakes (Table 6). For those metals that showed significant differences Duncan's multiple range test was used to determine the order of mean concentrations for the lakes. L223 *Mysis* had lower mean concentrations of Zn, Al, Cd, and Hg than the other five lakes (Table 6). For Hg, L223 animals had lower concentrations than L305, L224, and L468 animals. The storage time of the sample had no significant effect on the mean concentrations of the metals ($P=0.11$ to $P=0.98$).

4.4 DISCUSSION

The 96% decrease in the *Mysis relicta* population of L223 between August 1978 and August 1979 coincided with a .3 unit decrease in the limits of the pH encountered within the water column inhabited by *Mysis* (5.51 - 5.89 to 5.23 - 5.64). Tests of the sampling efficiency of the net and sampling program (Chapter II, and Appendix B) indicate that the change in the L223 population is real and cannot be attributed to natural fluctuations which, at most, would result in a 50% decrease (Hakala 1978).

TABLE 6

Trace metal concentrations in *Mysis relicta* samples which are significantly different among the control lakes.

Results of the statistical analysis showing whole body concentrations of metals which are significantly different among *Mysis* populations in the different lakes (P values are the observed significance level of the analysis of variance F statistic). Lakes have been arranged on the basis of the mean concentrations of each metal in *Mysis* samples (Duncan's multiple range t test). Significant differences between lakes are represented by '>', and nonsignificant differences by '='.

Metal	P	Order of Means High - - - Low
Zn	.0002	239>228=224=305>468>223
Al	.0024	239>228>224>305>468=223
Cd	.0001	224>305>228=239=468=223
Hg	.0001	305>224=468>223=239>228
Fe	.0085	224>239=223>468=305=228

The exact pH at which the population became stressed is difficult to assess as the water column inhabited by Mysis often extended over a range of pH values (Fig. 16). The greatest range was during midwinter. Mysis may be able to avoid pH values between 6.0 and 5.0 if high levels of DIC (dissolved inorganic carbon) are associated with low acidity (Hoglund 1961). Measurements of DIC, especially during the critical overwinter periods 1977-78, and 1978-79 indicate that in L223 pH values between 5.0 and 6.0 are usually associated with higher DIC levels (Schindler et al. 1980). Assuming that Mysis can avoid the lower pH values in the water column, the upper line in Fig. 16 would represent the pH that Mysis were exposed to. The critical pH would lie between 5.89 and 5.64. However, the length of time spent at low pH and the severity of sudden changes is more realistic. Most of the 1977-78 values were above pH 6.0 while most of the 1978-79 values were below pH 5.8. An indicator of the vulnerability of the population to sudden drops in pH, like that produced by spring snow melt (Johannessen and Henriksen 1978, Scheider et al. 1979, and Jeffries et al. 1979), is shown by the final disappearance of Mysis in October 1979, at which time the pH dropped from 6.2 to 5.7. The effects of spring depression of pH would be more severe in lakes being acidified by precipitation because it would occur when ovigerous females are releasing their broods.

Evidence of mysid mortalities in Swedish lakes supplied by W. Dickson (pers. comm. 1980) indicates that pH values above 5.6 - 5.9 are critical for the survival of *Mysis*. Introductions of *Mysis* into Ostra Lake, pH 5.5-6.1, were not successful. Other lakes with pH 5.8-6.0 gave similar results. In Olen Lake at pH 5.1-6.4 *Mysis* were lost and in Unden Lake, at pH 5.9-6.2 the population of *Mysis* has been reduced. *Mysis* introductions have been successful in lakes with a higher pH.

Okland and Okland (1980) state that owing to their high sensitivity to low pH values, crustaceans like *Gammarus lacustris* may be used as indicators of acidification at an early stage. *Mysis relicta* is also sensitive to low pH values and may be suitable as an early warning organism.

The number of *Mysis* present in L223 during August 1978 was just below the expected number of *Mysis* calculated using abundance data from two ELA lakes. There is the possibility that a decrease in the population by as much as 15% occurred prior to 1978 due to pH values around 5.9.

Estimates of the percent of the population represented by adults for L223, are lower than values for other populations with a 2 yr life history. A possible change in the life history pattern of L223 *Mysis* from a one year cycle to a two year cycle as a result of acidification would not account for the low percentage of adults. In addition there is no evidence to suggest a shift in the life cycle. The low percentage of adults may have occurred through size-selective predation by fish or physiological effects selective for the larger adults.

No changes in the 2 yr life history pattern of L223 *Mysis* were evident during the short study period, although, some changes in the mean size of the larger cohorts occurred. During 1979, the older cohorts appeared smaller than they should have been in comparison with control lakes and L223 for 1978 (Chapter III). In addition, the older cohorts appeared to have been lost before the younger ones during fall 1979. This may have been an artifact due to the small number of *Mysis* in samples collected at the time (i.e. older individuals were less abundant and may not have shown up in samples).

The pH regime of L223 during the summers of 1978 and 1979 appears to have been favorable for *Mysis* growth. Growth rates for L223 *Mysis* during this period were within the normal range for ELA lakes.

No measures are available of fecundity changes in L223, because samples were not collected during March and April in 1978 and 1979. The release of immature *Mysis* in spring 1979 suggests that for at least some percentage of the population, breeding was successful. The rest of the population either died before breeding or during the brooding period.

Female *Mysis* in L223 were found to have a significantly smaller mean size at onset of sexual maturity than in the other lakes. This indicates that environmental conditions were stressing the L223 population (Wenner et al. 1974). However, the inverse relationship between primary production per m^3 of the euphotic zone and the mean size of sexually mature females ($r^2 = .69$, n=4) in the control lakes accounts for the low mean size of the L223 females on the basis of high primary production in L223 (Chapter III).

Concentrations of most metals in the L223 *Mysis* were significantly lower than the control lakes indicating that trace metals were not accumulated in sufficient amounts to be toxic in 1978 and probably did not reach toxic levels during the major population decline. These results are contrary to those expected on the basis of higher concentrations of metals in L223 water and the greater toxicity of metals under acidic conditions (Hutchinson and Collins 1978, Baker and Schofield 1980, Grahn 1980, and Muniz and Leivestad 1980).

Franzin and McFarlane (1980) and McFarlane and Franzin (1980) have hypothesized that some factor(s) such as Ca^{++} concentration of lake waters may have a modifying role in metal uptake by biota. This hypothesis is supported by the data on the metal concentrations in *Mysis* in L223 and the 5 control lakes. The study lakes can be placed in order from low to high Ca^{++} concentrations based on data obtained from Beamish et al. (1976), Prokopowich (1979), and D. W. Schindler (unpublished data) as follows:

$$224<228=468<305<223<239.$$

These observed trends for water Ca^{++} concentrations help explain the observed trends for Cd. Lakes 223, 468, and 239 had higher Ca^{++} concentrations and had lower concentrations of Cd in their Mysis than did Lakes 224 and 228, lakes lower in Ca^{++}. L305 Cd concentrations are an exception. For Zn and Al in L239, and Hg in L228 the relationship does not hold true. Excluding the Zn and Al values for L239 Mysis and the Hg values for L228 Mysis the Zn, Al, and Hg concentrations in the Mysis are inversely related with Ca^{++} concentrations in the water column.

The mechanisms by which water hardness inhibits Cd uptake (Kinkade and Erdman 1975) and, more specifically, the "accidental" uptake of Cd, Mg, Zn, Ni, and Be in place of Ca^{++} ions in low Ca^{++} waters have been examined in laboratory studies by Zitko and Carson (1976), Ellis and Oliver (1979), and Wright (1980).

A number of mechanisms which may have caused the decline of *Mysis relicta* in L223 have been considered. Decreased growth, expected if inhibition of Ca^{++} uptake occurs (Borgstrom and Hendrey 1976; Malley 1980) was not evident during 1978 or 1979. Reproductive failure due to exposure to acid waters, often seen among fishes (Schofield 1976) and crustaceans (Borgstrom and Hendrey 1976, and Furst 1977), was not a factor in reducing the Mysis population of L223. Tissue concentrations of Zn, Mn, and Al lower than tissue concentrations in Mysis from the control lakes ruled out the possibility that these metals were toxic to the Mysis. In addition to these mechanisms there are a number which have not been studied. Increased light intensities, decreased bacterial decomposition, and direct physiological effects of increased H^+ ion concentrations may have resulted in the decline.

Light transmission in the water column of L223 increased as a result of acidification (J. Shearer unpublished data). Greater light intensities may have adversely affected the Mysis population of the lake. Smith (1970) found that light intensities of 60fc (foot candles) produced mortalities of Mysis in aquaria. A maximum surface illumination of 2,787fc (30,000 lux) was used to calculate the light present at 9m, the lower limit of Mysis occurrence in L223. Minimum attenuation coefficients obtained from Shearer (1974), Shearer and DeClercq (1975, 1976, 1977, 1978, 1979, and 1980) for L223 during the June - July period were used for the calculations. Maximum light intensities in L223 at 9m steadily rose from about 42fc in 1974 to 59fc in 1979 (excluding 1977). All these values are below Smith's 60fc value and probably were not harmful. The 1977 value was 130fc, well above that believed detrimental. However, the major die-off occurred over the winter of 1978 - 1979, when light intensities were much lower than during the summer. In addition, Mysis have been observed between 6 and 8m in Lakes 224 and 305 at light intensities above 200fc, indicating that these animals in their natural environment can tolerate light intensities much higher than 60fc.

Increased light intensities in L223 may have allowed the lake trout population to prey on the *Mysis* to a greater extent than in previous years, but observations by K. Mills (pers. comm.) indicate this is not the case. No increase in lake trout growth was evident during fall 1978, which would have occurred if 96% of the *Mysis* population had been consumed by the fish. Lake trout growth rtes were very low over the winter of 1978 - 1979 indicating that the food intake was very low (K. Mills pers. comm.).

Mysis are suspected of relying on detrital feeding during the winter months (Lasenby and Langford 1973, and Tattersall and Tattersall 1951) and could thereby be affected by changes in microbial decomposition in acidic lakes (Hendrey and Wright 1975). Decreases in the ciliate fauna of the detritus could decrease the overwinter food supply for *Mysis*. Up until 1979 in L223 no changes are believed to have occurred in the overall microbial decomposition of detritus in L223 because total CO_2 production has remained constant (Schindler 1980). A decrease in food supply would be expected to cause reduced growth in the *Mysis*, which has not been the case in L223.

Most likely, the decline of *Mysis* in L223 was a direct result of increased H^+ ions in the water column. Shaw (1960) found that the pH values below 6.0 inhibited the absorption of Na^+ by the crayfish Astacus pallipes Lereboullet. Borgstrom and Hendrey (1976) speculate a blocking of the Ca^{++} uptake mechanism by low pH during molt in the amphipod Gammarus lacustris G. O. Sars and the branchipod Lepidurus arcticus (Pallas). Malley (1980) has examined the inhibition of Ca^{++} uptake in post molt crayfish (Orconectes virilis [Hagen]) in greater detail and suggests three possible mechanisms as: '1) the scarcity of HCO_3^- to accompany Ca^{++} for electrical neutrality, 2) the high concentration of H^+ in the external medium which may interfere with the exchange of internal H^+ for external Ca^{++}, or 3) direct effects of H^+ on the active transport system such as by altering molecular configuration.' Any one or combination of these mechanisms could have resulted in the decline of Mysis over the winter of 1978 to 1979. The few animals present in summer of 1979 were probably the genetically more tolerant 4% of the population.

The chronological sequence of events in L223 leading to the demise of the Mysis population suggests that 5.9 is the critical pH. Changes in the percentage of adults in the population of L223 probably occurred when pH values fell below 5.9 during spring 1978, resulting in the loss of 1+ year adults. Heavy mortality occurred again in spring of 1979, with 96% of the population being lost due to pH values below 5.6. Rapid growth and partial recovery occurred during summer of 1979 at pH 6.0. Mysis were probably exterminated when the pH rapidly dropped from 6.2 to 5.6 during October, 1979.

Chapter V

GENERAL SUMMARY

1. Vertical net hauls taken on moonless nights represent a reliable method for obtaining estimates of *Mysis* abundance in the water column as suggested by Malley and Reynolds (1979) and Grossnickle and Morgan (1979). Such hauls appear to be free of avoidance behavior caused by bow waves and interference from the towing bridle or local reduction of the population in the water column from replicate sampling.
2. Tests carried out in L239 indicated that the highest *Mysis* densities were obtained between 1h and 2h after sunset.
3. During summer stratification seasonal movements of *Mysis* are minimal, allowing the pooling of samples from a number of nights where the statistical precision is required.
4. In Lakes 224 and 239 the mean number of *Mysis* per m^3 of inhabitable volume is 17.5 ± 1.8 ($\pm$ 2x standard deviation boundary on the error of estimation).
5. For *Mysis* populations with a 2 yr life cycle the percentage of the population represented by adults should range from 18.4% to 54.3%.

6. Summer growth rates are faster in the more productive lakes. However, yearly growth and size of mature females is greater in the more oligotrophic lakes.
7. Fecundity in ELA lakes is low (10 to 20 eggs per female) probably because of the low productivities of the lakes.
8. Experimental acidification in 1978 and 1979 decreased the maximum pH that Mysis could encounter from 5.9 to 5.6. The resulting population decrease was 96%. By fall 1979 a further decline to just below pH 5.6 resulted in the disappearance of all Mysis from the lake. These pH values are similar to those reported in Sweden where problems with Mysis transplants have occurred (W. Dickson pers. comm.).
9. Estimates of the population size of M. relicta in L223 prior to experimental acidification, based on population estimates for Lakes 239 and 305, indicate pre-acidification numbers of $7.12 \times 10^6 \pm 7.4 \times 10^5$ Mysis ($\pm$ 2x standard deviation boundary on the error of estimation). Thus, part of the decline occurred prior to summer 1978 at pH values around 6.0.
10. An examination of sex ratios in L223 and life cycle plots suggest that larger mysids were more susceptible to low pH values than smaller mysids.
11. The decline in Mysis could not be attributed to the toxic effects of heavy metals, reproductive failure,

the loss of food supplies, physiological impairment of growth, or increased water transparency.

REFERENCES

Aleksandrov, B. M. 1963. O nektonobentosykh reliktovykh rakoobraznykh Onezhskogo ozera. [Relict crustaceans from the nektonic benthos of Lake Onega.] In: Problemy isopol′zovanii promyslovykh resursov Belogo morya i vnutrennykh vodoemov Karelii. [Problems in the utilization of fisheries resources in the White Sea and inland waters in Karelia.] Akad. Nauk SSSR: Moscow-Leningrad. 1. 232-243. From: REF ZH BIOL, 1965, No. 3D61. (Translation) [In Biological Abstracts. 1967. Vol. 48, no. 566.]

Almer, B., W. Dickson, C. Ekstrom, E. Hornstrom. 1978. Sulfur pollution and the aquatic ecosystem, p. 271-311. In J. O. Nriagu [ed.] Sulfur in the environment: Part II Ecological impacts. John Wiley and Sons, New York, N.Y. 482p.

Annala, J. H., J. L. McKoy, J. D. Booth, and R. B. Pike. 1980. Size at onset of sexual maturity in female Jasus edwardsii (Decapoda: Palinuridae) in New Zealand. N. Z. Jour. Mar. Freshw. Res. 14:217-227.

Armstrong, F. A. J. and D. W. Schindler. 1971. Preliminary chemical characterization of waters in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Canada 28:171-187.

Bailey, M. M. 1972. Age growth and food of the burbot Lota lota (Linaeus), in Southwestern Lake Superior. Trans. Amer. Fish. Soc. 100:667-674.

Baker, J. P., and C. L. Schofield. 1980. Aluminum toxicity to fish as related to acid precipitation and Adirondack surface water quality. P. 292-293. In Drablos,D., and A. Tollan [ed.]. Ecological impact of acid precipitation. SNSF Project, Oslo. 383p.

Beamish, R. J. 1976. Acidification of lakes in Canada by acid precipitation and the resulting effects on fishes. Pp. 479-498 In Proc. of the First Intl. Symp. on Acid Prec. and the Forest Ecosystem.

Beamish, R. J., and H. H. Harvey. 1972. Acidification of the LaCloche Mountain lakes, Ontario, and resulting fish mortalities. 1972. J. Fish. Res. Board Can. 29:1131-1143.

Beamish, R. J., and J. C. Van Loon. 1977. Precipitation loading of acid and heavy metals to a small acid lake near Sudbury, Ontario. J. Fish. Res. Board Can. 34:649-658.

Beamish, R. J., L. M. Blouw and G. A. McFarlane. 1976. A fish and chemical study of 109 lakes in the Experimental Lakes Area (ELA), northwestern Ontario, with appended reports on lake whitefish ageing errors and the northwestern Ontario baitfish industry. Fish. Mar. Serv. Res. Dev. Tech. Rep. 607: 116 p.

Bell, H. L. 1970. Effects of pH on the life cycle of the midge Tanytarsus dissimilis. The Can. Entomologist. 102:636-639.

Bell, H. L. 1971. Effect of low pH on the survival and evergence of aquatic insects. Water Res. 5:313-319.

Berrill, M. 1969. The embryonic behavior of the mysid shrimp, Mysis relicta. Can. J. Zool. 47:1217-1221.

Borgstrom, R., and G. R. Hendrey. 1976. pH tolerance of the first larval stages of Lepidurus arcticus (Pallas) and adult Gammarus lacustris G. O. Sars. Internal Rep. Norw. Inst. Water Res. Oslo, Norway. 37 p.

Bowers, J. A., and N. E. Grossnickle. 1978. The herbivorous habits of Mysis relicta in Lake Michigan. Limnol. Oceanogr. 23:767-776.

Brownell, W. 1970. Studies on the ecology of Mysis relicta in Cayuga lake. M. S. Thesis. Cornell Univ., Ithaca, N. Y. 67 p.

Brunskill, G. J. and D. W. Schindler. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Canada. 28:139-155.

Buikema, A. L., J. Cairns and G. W. Sullivan. 1974. Rotifers as monitors of heavy metal pollution in water. Virginia Water Res. Res. Center. Bull. 74:74p.

Carpenter, G. F., E. L. Mansey, and N. H. F. Watson. 1974. Abundance and life history of Mysis relicta in the St. Lawrence Great Lakes. J. Fish. Res. Board Can. 31:319-325.

Cogbill, C. V., and G. E. Likens. 1974. Acid precipitation in the northeastern United States. Water Res. Res. 10:1133-1137.

Conroy, N. I, D. S. Jeffries, and J. R. Kramer. 1974. Acid shield lakes in the Sudbury Ontario region. Proc. Symp. Wat. Poll. Res. Can. 9:45-61.

Cooper, S. D., and C. R. Goldman. 1980. Opossum shrimp (Mysis relicta) predation on zooplankton. Can. J. Fish. Aquat. Sci. 37:909-919.

Cleugh, T. R. and B. W. Hauser. 1971. Results of the initial survey of the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Canada. 28:129-137.

Dadswell, M. J. 1974. Distribution ecology and postglacial dispersal of certain crustaceans and fishes in eastern North America. Publications in Zoology, National Museuom of Natural Sciences (Ottawa). (11):110p.

Daye, P. G. 1980. Effects of ambient pH on fish: an annotated bibliography. Can. Tech. Rep. Fish. Aquat. Sci. 950, iii + 28p.

Dickson, W. 1978. Some effects of the acidification of Swedish lakes. Verh. Internat. Verein. Limnol. 20:851-856.

Dillon, P. J., D. S. Jeffries, W. Snyder, R. Reid, N. D. Yan, D. Evans, J. Moss, and W. A. Scheider. 1978. Acidic precipitation in south-central Ontario: recent observations. J. Fish. Res. Board Can. 35:809-815.

Dillon, P. J., N. D. Yan, W. A. Scheider, N. Conroy. 1979. Acidic lakes in Ontario, Canada: characterization, extent and responses to base and nutrient additions. Ergebn. Limnol. 13:317-336.

Dryer, W. R., L. F. Erkkila, and C. L. Tetzloff. 1965. Food of lake trout in Lake Superior. Trans. Amer. Fish. Soc. 94:169-176.

Duncan, D. B. 1975. t Tests and intervals for comparisons suggested by the data. Biometrics 31:339-359.

Dunn, O. J., and V. A. Clark. 1974. Applied statistics: analysis of variance and regression. John Wiley & Sons, Toronto. 387p.

Elliott, J. M. 1973. Some methods for the statistical analysis of benthic invertebrates. Freshw. Biol. Assoc. Sci. Publ. 25:144p.

Fee, E. J. 1980. Important factors for estimating annual phytoplankton production in the Experimental Lakes Area. Can. J. Fish. Aquat. Sci. 37:513-522.

Findlay, D. L. and H. J. Kling. 1979. A species list and pictorial reference to the phytoplankton of central and northern Canada (in two parts). Can. Fish. Mar. Serv. MS Rep. 1503: iv + 619 p.

Findlay, D. L. and G. Saesura. 1980. Effects on phytoplankton biomass, succession and composition in Lake 223 as a result of lowering pH levels from 7.0 to 5.6. Data from 1974 to 1979. Can. MS Rep. Fish. Aquat. Sci. 1585: iv + 16 p.

Franzin, W. G., and G. A. Mcfarlane. 1980. An analysis of the aquatic macrophyte, Myriophyllum exalbescens, as an indicator of metal contamination of aquatic ecosystems near a base metal smelter. Bull. Environ. Contam. Toxicol. 24:597-605.

Fryer, G. 1980. Acidity and species diversity in freshwater crustacean faunas. Freshw. Biol. 10:41-45.

Furst, M. 1965. Experiments on the transplantation of Mysis relicta Loven into Swedish Lakes. Rept. Inst. Freshwater Res., Drottningholm, 46:69-87.

Furst, M. 1972. On the biology of the opossum shrimp Mysis relicta Loven and its introduction in impounded lakes in Scandinavia. Abstracts of Uppsala dissertations from the Faculty of Science, No. 207. Univ. of Uppsala, Uppsala, Sweden. 7 pp.

Furst, M. 1977. Information fraan Sotvattens laboratoriet. Nordic Crayfish Symposium 14:90-93.

Galloway, J. N., and G. E. Likens. 1979. Atmospheric enhancement of metal deposition in Adirondack lake sediments. Limnol. Oceanogr. 24:427-433.

Giddings, J., and J. N. Galloway. 1976. The effects of acid precipitation on aquatic and terrestrial ecosystems. In Literature reviews on acid precipitation. Center for Environ. Quality Manag., and Water Resoc. and Mar. Sciences, Cornell Univ. Ithca, New York.

Gosho, M. E. 1975. The introduction of Mysis relicta into freshwater lakes. Fisheries research institute, college of Fisheries. Univ. of Washington. Circular No. 75:1-65.

Grahn, O. 1980. Fish kills in two moderately acid lakes due to high aluminum concentration. P. 310-311. In Drablos,D., and A. Tollan [ed.]. Ecological impact of acid precipitation. SNSF Project, Oslo. 383p.

Grossnickle, N. E. 1979. Nocturnal feeding patterns of *Mysis relicta* in Lake Michigan, based on gut content fluorescence. Limnol. Oceanogr. 24:777-780.

Grossnickle, N. E., and A. M. Beeton. 1979. Antennal scale length as a measure of relative size in the opposum shrimp, *Mysis relicta* (Loven). Crustaceana. 36:141-146.

Grossnickle, N. E. and M. B. Morgan. 1979. Density estimates of *Mysis relicta* in Lake Michigan. J. Fish. Res. Board Can. 36:694-698.

Hakala, I. 1978. Distribution, population dynamics and production of *Mysis relicta* (Loven) in southern Finland. Ann. Zool. Fennici. 15:243-258.

Hakala, I. 1979. Seasonal variation in the carbon and energy contents and assimilation of a *Mysis relicta* population in Paajarvi, southern Finland. Ann. Zool. Fennici. 16:129-137

Hamilton, A. L. 1971. Zoobenthos of fifteen lakes in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Canada. 28:257-263.

Hartnoll, R. G. 1978. The determination of relative growth in crustacea. Crustaceana. 37:281-293.

Harvey, H. H. 1975. Fish populations in a large group of acidstressed lakes. Verh. Int. Ver. Limnol. 19:2406-2417.

Hendrey, G. R., and R. F. Wright. 1975. Acid precipitation in Norway: effects on aquatic fauna. Proc. First Specialty Symposium on Atmospheric Contribution to the Chemistry of Lake Waters. Internat. Assoc. Great Lakes Res. Sept. 28-Oct. 1, 192-207.

Hendry, G. R., K. Baalsrud, T. S. Traaen, M. Laake, and G. Raddum. 1976. Acid precipitation: some hydrobiological changes. Ambio 5:224-227.

Henriksen, A., and R. F. Wright. 1978. Concentrations of heavy metals in small Norwegian lakes. Water Research. 12:101-112.

Herrmann, R., and J. Baron. 1980. Aluminum mobilization in acid stream environments, Great Smoky Mountains National Park, U.S.A. P. 218-219. *In* Drablos,D., and A. Tollan [ed.]. Ecological impact of acid precipitation. SNSF Project, Oslo. 383p.

Hesslein, R. H., W. S. Broecker, and D. W. Schindler. 1980. Fates of metal radiotracers added to a whole lake: sediment-water interactions. Can. J. Fish. Aquat. Sci. 37:378-386.

Hoglund, L. B. 1961. The reactions of fish in concentration gradients. Rep. Inst. Freshw. Res. Drottningholm. 43:1-147.

Hollander, M., and D. A. Wolfe. 1973. Nonparametric statistical methods. John Wiley and Sons, Toronto. 503p.

Holmquist, C. 1959. Problems on marine-glacial relicts on account of investigations on the genus Mysis. Berlinska Boktryckeriet, Lund. 270p.

Hutchinson, T. C., and F. W. Collins. 1978. Effects of H^+ ion activity and Ca^{2+} on the toxicity of metals in the environment. Environ. Health Perspectives. 25:47-52.

Jeffries, D. S., C. M. Cox, and P. J. Dillon. 1979. Depression of pH in lakes and streams in central Ontario during snowmelt. J. Fish. Res. Board Can. 36:640-646.

Johannesssen, M., and A. Henriksen. 1978. Chemistry of snow meltwater: changes in concentration during melting. Water Res. Res. 14:615-619.

Johnson, L. 1975. Distribution of fish species in Great Bear Lake, Northwest Territories, with reference to zooplankton, benthic invertebrates, and environmental conditions. J. Fish. Res. Bd. Canada. 32:1989-2004.

Juday, C., and E. A. Birge. 1927. Pontoporeia and Mysis in Wisconsin lakes. Ecology. 8:445-452.

Kennedy, L. A. 1980. Teratogenesis in Lake Trout (Salvelinus namaycush) in an experimentally acidified lake. Can. J. Fish. Aquat. Sci. 37:2355-2358.

Kinkade, M. L., and H. E. Erdman. 1975. The influence of hardness components (Ca^{2+} and Mg^{2+}) in water on the uptake and concentration of cadmium in a simulated freshwater ecosystem. Environm. Res. 10:308-313.

Larkin, P. A. 1948. Pontoporeia and Mysis in Athabasca, Great Bear and Great Slave Lakes. Bull. Fish. Res. Board Can. 78:3.

Lasenby, D. 1971. The ecology of Mysis relicta in an arctic and a temperate lake. Ph.D. Thesis, University of Toronto, Ontario. 119p.

Lasenby, D. C., and R. R. Langford. 1972. Growth, life history, and respiration of Mysis relicta in an arctic and temperate lake. J. Fish. Res. Bd. Canada. 29:1701-1708.

Lasenby, D. C., and R. R. Langford. 1973. Feeding and assimilation of Mysis relicta. Limnol. Oceanogr. 18:280-285.

Leivestad, H., G. Hendrey, I. P. Muniz, and E. Snekvik. 1976. Effects of acid precipitation on freshwater organisms, p. 87-111. In F. H. Braekke [ed.] Impact of acid precipitation on forest and freshwater ecosystems in Norway. SNSF Res. Report 6/76:111p. Norway FR 6/76.

Likens, G. E. 1976. Acid precipitation. Chem. Eng. News 54: 29-44.

Likens, G. E., and F. H. Bormann. 1974. Acid rain: a serious regional environmental problem. Science 184:1176-1179.

Likens, G. E., F. H. Bormann, and N. M. Johnson. 1972. Acid rain. Environment. 14:33-40.

Likens, G. E., R. F. Wright, J. N. Galloway, and T. J. Butler. 1979. Acid rain. Sci. Am. 241:43-51.

Malley, D. F., and J. B. Reynolds. 1979. Sampling strategies of non-insectan freshwater invertebrates. J. Fish. Res. Board Can. 36:311-318.

Malley, D. F. 1980. Decreased survival and calcium uptake by the crayfish Orconectes virilis in low pH. Can. J. Fish. Aquat. Sci. 37:364-372.

Malley, D. F., D. L. Findlay, and P. S. S. Chang. 1981. Ecological effects of acid precipitation on zooplankton. Proc. Conf. Effects of acid prec. on ecological systems: Great Lakes Region. April 1-3, 1981. McFarlane, G. A., and W. G. Franzin. 1980. An examination of Cd, Cu, and Hg concentrations in livers of northern pike Esox lucius, and white sucker, Catostomus commersoni, from five lakes near a base metal smelter at Flin Flon, Manitoba. Can. J. Fish. Aquat. Sci. 37:1573-1578.

McFarlane, G. A., and W. G. Franzin. 1980. An examination of Cd, Cu, and Hg concentrations in livers of northern pike, Esox lucius, and white sucker, Catostomus commersoni, from five lakes near a base metal smelter at Flin Flon, Manitoba. Can. J. Fish. Aquat. Sci. 37:1573-1578.

McWilliams, P. S. 1970. Seasonal changes in abundance and reproduction in the opossum shrimp, Mysis relicta Loven, in Lake Michigan. M.S. thesis, Univ. Sydney, Sydney, Australia. 94 p.

Mendenhall, W., L. Ott, and R. L. Scheaffer. 1971. Elementary survey sampling. Wadsworth Pub. Co., Belmont California. 243p.

Morgan, M. D. 1976. Life history and annual net secondary production of Mysis relicta (Loven) in Westcentral Lake Michigan. MSc thesis. 57 p.

Morgan, M. D. 1980. Life history characteristics of two introduced populations of Mysis relicta. Ecology 6:551-561.

Morgan, M. D., and A. M. Beeton. 1978. Life history and abundance of Mysis relicta in Lake Michigan. J. Fish. Res. Board. Can. 35:1165-1170.

Muniz, I. P., and H. Leivestad. 1980. Toxic effects of aluminium on the brown trout, Salmo trutta L. P. 320-321. In Drablos,D., and A. Tollan [ed.]. Ecological impact of acid precipitation. SNSF Project, Oslo. 383p.

Nero, R. W., and I. J. Davies. 1981. A comparison of two sampling methods for estimating the abundance and distribution of Mysis relicta. Can. J. Fish. Aquat. Sci. 38:0000-0000 In Press.

Newbury, R. W., and K. G. Beaty. 1980. Water renewal efficiency of watershed and lake combinations in the ELA region of the Precambrian Shield. Can. J. Fish. Aquat. Sci. 37:335-341.

Okland, J. 1980. Environment and snails (Gastropoda): studies of 1,000 lakes in Norway. P. 322-323. In Drablos,D., and A. Tollan [ed.]. Ecological impact of acid precipitation. SNSF Project, Oslo. 383p.

Okland, K. A. 1980. Mussels and crustaceans: studies of 1,000 lakes in Norway. P. 324-325. In Drablos,D., and A. Tollan [ed.]. Ecological impact of acid precipitation. SNSF Project, Oslo. 383p.

Okland, J., and K. A. Okland. 1980. pH level and food organisms for fish: Studies of 1,000 lakes in Norway. P. 326-327. In Drablos,D., and A. Tollan [ed.]. Ecological impact of acid precipitation. SNSF Project, Oslo. 383p.

Parker, J. I. 1980. Predation by Mysis relicta on Pontoporeia hoyi a food chain link of potential importance in the great lakes. J. Great Lakes Res. (Internat. Assoc. Great Lakes Res.) 6:164-166.

Patalas, K. 1971. Crustacean plankton communities in forty-five lakes in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Canada. 28:231-244.

Pitblado, J. R., W. Keller, and N. I. Conroy. 1980. A classification and description of some northeastern Ontario lakes influenced by acid precipitation. J. Great Lakes Res. 6:247-257.

Prokopowich, J. 1979. Chemical characterization of epilimnion waters of unenriched lakes in the Experimental Lakes Area, northwestern Ontario. Can. Fish. Mar. Serv. Tech. Rep. 873: iv + 41 p.

Raddum, G. G. 1980. Comparison of benthic invertebrates in lakes with different aidity. P. 330-331. In Drablos,D., and A. Tollan [ed.]. Ecological impact of acid precipitation. SNSF Project, Oslo. 383p.

Raddum, G. G., A. Hobaek, E. R. Lomsland, and T. Johnsen. 1980. Phytoplankton and zooplankton in acidified lakes in South Norway. P. 332-333. In Drablos,D., and A. Tollan [ed.]. Ecological impact of acid precipitation. SNSF Project, Oslo. 383p.

Rawson, D. S. 1961. The lake trout of Lac la Ronge, Saskatchewan. J. Fish. Res. Bd. Canada. 18:423-462.

Reid, R. A., D. W. Schindler and R. W. Schmidt. 1975a. Light measurements in the Experimental Lakes Area, 1969-73. Fish mar. Serv. Res. Dev. Tech. Rep. 559: 167 p.

Reid, R. A., D. W. Schindler and R. W. Schmidt. 1975b. Phytoplankton production in the Experimental Lakes Area, 1969-1972. Fish Mar. Serv. Res. Dev. Tech. Rep. 560: 164 p.

Reynolds, J. B., and G. M. DeGraeve. 1972. Seasonal population characteristics of the opossum shrimp Mysis relicta in southeastern Lake Michigan, 1970 - 71. Proc. 15th Conf. Great Lakes Res, 1972: 117-131. Internat. Assoc. Great Lakes Res.

Roff, J. C., and R. E. Kwiatkowski. 1978. Zooplankton and zoobenthos communities of selected northern Ontario lakes of different acidities. Can. J. Zool. 55:899-911.

SAS. 1979. SAS user's guide, 1979 edition. J. T. Helwig and K. A. Council ed. SAS (Statistical Analysis System) Institute Inc., North Carolina. 494 p.

Scheider, W. A., D. S. Jeffries, and P. J. Dillon. 1979. Effects of acidic precipitation on precambrian freshwaters in southern Ontario. J. Great Lakes Res., Internat. Assoc. Great Lakes Res. 5:45-51.

Schindler, D. W. 1971. Light, temperature, and oxygen regimes of selected lakes in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Canada. 28:157-169.

Schindler, D. W. 1980. Experimental acidification of a whole lake: a test of the oligotrophication hypothesis. P. 370-374. In Drablos,D., and A. Tollan [ed.]. Ecological impact of acid precipitation. SNSF Project, Oslo. 383p.

Schindler, D. W., and S. K. Holmgren. 1971. Primary production and phytoplankton in the Experimental Lakes Area, northwestern Ontario, and other low-carbonate waters, and a liquid scintillation method for determining ^{14}C activity in photosynthesis. J. Fish. Res. Bd. Canada. 28:189-201.

Schindler, D. W., R. Wagemann, R. B. Cook, T, Ruszczynski and J. Prokopowich. 1980a. Experimental acidification of Lake 223, Experimental Lakes Area; background data and the first three years of acidification. Can. J. Fish. Aquat. Sci. 37:342-354.

Schindler, D. W., R. H. Hesslein, R. Wagemann, and W. S. Broecker. 1980b. Effects of acidification on mobilization of heavy metals and radionuclides from the sediments of a freshwater lake. Can. J. Fish. Aquat. Sci. 37:373-377.

Schindler, D. W., R. W. Newbury, K. G. Beaty, J. Prokopowich, T. Ruszynski, and J. A. Dulton. 1980c. Effects of windstorm and forest fire on chemical losses from forested watersheds and on the quality of receiving streams. Can. J. Fish. Aquat. Sci. 37:328-334.

Schofield, 1976. Acid Precipitation: Effects on fish. Ambio 5:228-230.

SCRIPT. 1979. SCRIPT user's guide. Dept. of Computing services, University of Waterloo, Ontario. 69p.

Sladeckova, A., and E. Pieczynska. 1971. Methods of collection: Periphyton, p. 109-122. In W. T. Edmondson and G. G. Winberg [ed.]. A manual on methods for the assessment of secondary productivity in fresh waters. IBP No 17. Blackwell, England. 358p.

Smith, W. E. 1970. Tolerance of Mysis relicta to thermal shock and light. Trans. Amer. Fish. Soc. 99:418-422.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods. Iowa State Univ. Press., Ames Iowa. 593 p.

Sprules, W. G. 1975. Midsummer crustacean zooplankton communities in acid-stressed lakes. J. Fish. Res. Board Can. 32:389-395.

Spry, D. J., C. M. Wood, and P. V. Hodson. 1981. A literature review: The effects of environmental acid on fishes with reference to heavymetals. Can. J. Fish. Aquat. Sci. Tech. Rep. In Press.

Stainton, M. P., M. J. Capel, and F. A. J. Armstrong. 1977. The chemical analysis of fresh water, 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. 25:180p.

SYSPUB. 1979. SYSPUB user's guide. Dept of Computing services, University of Waterloo, Ontario. 57p.

Tattersall, W. M., and O. S. Tattersall. 1951. The British Mysidacea. Roy Society, London. 460p.

Teraguchi, M. 1969. Diel vertical migration of Mysis relicta (Loven) in Green Lake, Wisconsin. Phd. thesis. Univ. Wis. p.

Thienmann, A. 1925. Mysis relicta. Zeit. Morphol. und Oekol. der Tiere. 3:389-440.

Tomlinson, G. H., R. J. P. Brouzes, R. A. N. McLean, and J. Kadlecek. 1980. The role of clouds in atmospheric transport of mercury and other pollutants: the link between acid precipitation, poorly buffered waters. mercury and fish. P. 134-137. In Drablos,D., and A. Tollan [ed.]. Ecological impact of acid precipitation. SNSF Project, Oslo. 383p.

Troutman, D., and N. Peters. 1980. Comparison of lead, manganese, and zinc transport in three Adirondack lake watersheds, New York. P. 262-263. In Drablos,D., and A. Tollan [ed.]. Ecological impact of acid precipitation. SNSF Project, Oslo. 383p.

Van Oosten, J., and H. J. Deason. 1937. The food of the lake trout Cristivomer namaycush and the lawyer, Lota maculosa of Lake Michigan. Trans. Am. Fish. Soc. 67: 155-177.

Wells, L., and A. M. Beeton. 1963. Food of the bloater, Coregonus hoyi in Lake Michigan. Trans. Amer. Fish. Soc. 92:245-255.

Wenner, A. M., . Fusaro, and A. Oaten. 1974. Size at onset of sexual maturity and growth rate in crustacean populations. Can. J. Zool. 52:1095-1106.

Wiederholm, T., and L. Eriksson. 1977. Benthos of an acid lake. Oikos. 29:261-267.

Wright, R. F. 1975. Acid precipitation and its effects on freshwater ecosystems: an annotated bibliography. In Acid precipitation - Effects on forest and fish. SNSF - project, 1432 Aas-NLH, Norway. FA 3/75.

Wright, R. F., T. Dale, E. T. Gjessing, G. R. Hendrey, A. Henriksen, M. Hohannessen, and I. P. Muniz. 1976. Impact of acid precipitation on freshwater ecosystems in Norway. Water Air Soil Pollut. 6:483-499.

Yan, N. D. 1979. Phytoplankton community of an acidified, heavy metal-contaminated lake near Sudbury, Ontario: 1973-1977. Water, Air and Soil Pollution 11:43-55.

Yan, N. D., and P. Stokes. 1978. Phytoplankton of an acidified lake and its responses to experimental alterations of pH. Environ. Conserv. 5:93-100.

Yan, N. D., and R. Strus. 1980. Crustacean zooplankton communities of acidic, metal contaminated lakes near Sudbury, Ontario. Can. J. Fish. Aquat. Sci. 37:2282-2293.

Zitko, V., and W. G. Carson. 1976. A mechanism of the effects of water hardness on the lethality of heavy metals to fish. Chemosphere. 5:299-303.

Appendix A

A DESCRIPTION OF THREE NETS SUITABLE FOR QUANTITATIVE ESTIMATES OF OPOSSUM SHRIMP (*MYSIS RELICTA*) ABUNDANCE.

A.1 ABSTRACT

Nero, R. W. 1981. A description of three nets suitable for quantitative estimates of opossum shrimp (Mysis relicta) abundance.

The basic net design used for vertical tows takes the form of an inverted pyramid which tapers to the apex in two stages, first as a 1mm mesh and second as a fine mesh (250μ) cod end. Overall net length is 1.5m; the cross sectional area of the net mouth is $1m^2$. Two modifications are 1) a collapsing version used for sampling through winter ice and 2) a downward sampling type which can be closed when contact is made with the lake bottom. The latter design is particularly suited to tests of vertical tow net efficiency.

A.2 INTRODUCTION

In order to study the effects of experimental, whole lake acidification on a population of *Mysis relicta* (Nero 1981), the need arose to develop a sampling net which would be simpler to use and lighter than the benthic sleds equipped with nets which are usually used to catch *Mysis* on or near the lake bottom during the daytime (Beeton 1960; Reynolds and DeGraeve 1972; Grossnickle and Morgan 1979; Malley and Reynolds 1979), and would be suitable for sampling through the ice in winter. *Mysis* may be collected at night when populations are planktonic by towing a net vertically through the water column (Hakala 1978, Morgan et al. 1978, Grossnickle and Morgan 1979, Malley and Reynolds 1979). The nets described here are larger than those used in previous studies, as well are less expensive and simpler to construct from easily available materials. Apart from the downward sampling version, the nets are lightweight and can be used from craft as small as a canoe.

In addition to sampling *Mysis*, the nets offer some potential as samplers for planktonic populations of other large crustaceans and larval fishes.

Evaluation of the relative catch performance of the nets was given in Nero and Davies (1981).

For testing if *Mysis* avoid the towing bridle and whether or not *Mysis* close to the substrate are sampled when using the vertical tow net a downward sampling net was designed. The downward net collects an undisturbed sample as it descends through the water column and closes a few centimeters above the substrate.

A.3 VERTICAL TOW NET

The vertical tow net is used for obtaining quantitative estimates of *Mysis* density in a m^2 column of water. The large mesh size and mouth opening reduces the bow wave to a minimum yet allows a reasonable towing speed of $1/3 m \cdot s^{-1}$.

A.3.1 Materials List

Rip Stop Nylon	2.1m
6 x 26mm Bar Stock Aluminum	4.2m
4mm Nylon Cord	3m
25mm Brass Snap Swivel	1ea
1mm mesh "Charcoal Grey Bridal Veil"	4m
4mm Plastic Coated Steel Cable	As required
250µ Mesh Nitex	.5m
500ml Nalgene Bottle	1ea
20mm Tygon Tubing	10cm
Pinch Clamp	1ea
Lead Fishnet Weights	.5kg

The estimated cost of the net is $100.

A.3.2 Construction

The net frame is made from a single piece of 6x26mm aluminum bar 4.2m in length with a 20cm overlapping joint on one side secured with two 6mm steel bolts (Fig. 1-D). The net is constructed from 8 pieces of material: four sides of 1mm mesh netting for the upper net (Fig. 1-F), two pieces of 250μ mesh net for the cod end (Fig. 1-G), and two strips of rip-stop Nylon for the frame sleeve (Fig. 1-E). The four bridle lines are attached to holes drilled through the frame at the corners (Fig. 1-C). These lines are tied with sufficient tension to keep the bridle flat, lying within the plane formed by the net frame and to force the net to lie flush with the substrate before vertical tows. The collecting funnel with attached drain tube and pinch clamp along with four 20g lead weights are attached to the cod end using water-proof tape (Fig. 1-H,I,J). The lead weights help keep the net from becoming tangled during descent.

A.3.3 Operation

The net is lowered, cod end first, through the water column. A speed of about 1/4 $m.s^{-1}$ prevents the net from tangling in the suspension bridle or from becoming fouled in the loose bottom sediments. Once the frame is in contact with the bottom, a 30-60s pause is usually sufficient to allow mysids to redistribute over the net. An upward towing speed of about $1/3 m.s^{-1}$ is recommended (Nero and Davies

1981). Once the net reaches the surface it is lifted clear of the water then dropped and raised, without submerging the net mouth, three times to ensure that all animals caught in the net are washed into the collecting funnel. Samples are drained into sample bottles from the collecting funnel by opening the pinch clamp.

A.4 COLLAPSING NET

A folding version of the vertical tow net can be passed through a 30cm hole in the ice, folds open and samples in the usual manner, and then folds shut for retrieval through the ice. This net has been used through ice up to 70cm thick.

A.4.1 **Materials List**

Materials in addition to those required for the vertical tow net are:

5cm diameter Steel Ring	1ea
3cm diameter Steel Ring	1ea
4mm Nylon Cord	6m
6mm Eye Bolts	4ea
6mm Nuts	16ea
6mm Washers	8ea

A.4.2 **Construction**

Apart from changes in the bridle, frame, and frame sleeve the net design is identical to the vertical tow net previously described. A larger frame sleeve with reinforced corners and reinforced corner holes accommodates the frame and eye bolts. The frame is made from 4 pieces of 6x26mm aluminum bar. Eyebolts securing the corners of the frame function as hinges (Fig. 2-H). The corners of the frame have been rounded and polished to prevent the frame sleeve from being torn.

The frame is opened by two lines from the far corners of the frame which pass through the 5cm steel ring (Fig. 2-c), join together, and attach to the 3cm steel ring. To limit opening of the net to a one meter square, two fixed lines (Fig. 2-F) attach to the remaining eye bolts and the 5cm diameter ring (Fig. 2-D). The closing line runs from an eyebolt on the far corner (Fig. 2-H), by-passes the 5cm ring and attaches to the 3cm ring.

A.4.3 Operation

During cold weather sampling, the net is kept frozen in the closed position and is wrapped in a nylon tarp to form a package 2m long and 20cm in diameter. At the sampling site, the net is unwrapped, a sampling line attached, and the net is placed in the hole in the ice and into the water to thaw. Once the net is thawed it can be untangled and lowered until the frame clears the bottom of the ice. By pulling on the main sampling line (Fig. 2-A) the net is forced open against the bottom of the ice. A vertical haul is then taken in an identical manner to that used for the standard net. As the net approaches the ice the retrieval line (Fig. 2-D) is used to draw one corner of the net into the sampling hole, forcing the net to collapse. This must be done quickly to ensure that *Mysis* are not lost from the net. The net is drawn though the ice and re-opened to allow rinsing of the cod end. After the sample has been removed, the net is allowed to refreeze in the collapsed position and is re-wrapped in the tarp.

A.5 BOTTOM CLOSING NET

For testing the effects of towing bridles and whether or not *Mysis* close to the substrate are sampled when using the vertical tow net, a downward-sampling net was designed. This net samples downwards through the water column without prior disturbance and closes a few centimeters above the substrate.

A.5.1 Materials List

Materials in addition to those required for the vertical tow net are:

Lead Weights	3.0kg
5cm diameter Steel Ring	1ea.
6 x 26mm Bar Stock Aluminum	1.5m
1cm Grommets	24ea.
4mm Nylon Cord	20m
Second Sampling Line	As Required
1mm Steel Cable	5m

A.5.2 Construction

The bottom-closing net itself is a frameless, inverted version of the vertical tow net with an enlarged nylon sleeve to accommadate a drawstring closure (Fig. 3-G). An additional tension line attached to the cod end holds the net upright while at the surface (Fig. 3-C).

During descent, the net is held open by tension on the opening lines (Fig. 3-E) produced by the weighted aluminum frame (Fig. 3-I). The opening lines pass through holes drilled through the frame at the corners and attach by nylon tabs to the net sleeve. One half meter above the net the opening lines are joined together by a 5cm steel ring (Fig. 3-B) attached to the main descent line (Fig. 3-A).

The ascent line (Fig. 3-D) attaches to the ends of a drawstring closure (Fig. 3-F) which passes through two holes on one side of the frame through grommets along the nylon sleeve and encircles the net.

A.5.3 Operation

The downward sampling net is somewhat more cumbersome to use than the simpler vertical nets and as such requires calm weather for optimum performance. To begin a tow, at the surface, the drawstring is opened and the ascent line is left slack. The descent line is held taught, keeping the net open due to the weight of the frame on the opening lines. The net is lowered through the water column at about

$1/4 m.s^{-1}$. After contact with the bottom, an additional 2m of the descent line are paid out and the ascent line is drawn tight, closing the net and raising it to the surface. At the side of the boat the net is rinsed and the sample removed as in the operation of the standard net.

A.6 ACKNOWLEDGMENTS

Bill Dentry assisted in designing a prototype of the vertical tow net and Jim Beaty provided the initial design for the collapsing net.

A.7 REFERENCES

Beeton, A. M. 1960. The vertical migration of Mysis relicta in lakes Huron and Michigan. J. Fish. Res. Bd. Canada. 17:517-539.

Grossnickle, N. E. and M. B. Morgan. 1979. Density estimates of Mysis relicta in Lake Michigan. J. Fish. Res. Board Can. 36:694-698.

Hakala, I. 1978. Distribution, population dynamics and production of Mysis relicta (Loven) in southern Finland. Ann. Zool. Fennici. 15:243-258.

Malley, D. F., and J. B. Reynolds. 1979. Sampling strategies of non-insectan freshwater invertebrates. J. Fish. Res. Board Can. 36:311-318.

Morgan, M. D., S. T. Threlkeld, and C. R. Goldman. 1978. Impact of the introduction of kakanee (Oncorhynchus nerka) and opossum shrimp (Mysis relicta) on a subalpine lake. J. Fish. Res. Board Can. 35:1572-1579.

Nero, R. W. 1981. The decline of Mysis relicta in response to acidification of a whole lake. MSc. Thesis University of Manitoba.

Nero, R. W., and I. J. Davies. 1981. A comparison of two sampling methods for estimating the abundance and distribution of Mysis relicta. Can. J. Fish. Aquat. Sci. 38:0000-0000 In Press.

Reynolds, J. B., and G. M. DeGraeve. 1972. Seasonal population characteristics of the opossum shrimp Mysis relicta in southeastern Lake Michigan, 1970 - 71. Proc. 15th Conf. Great Lakes Res, 1972: 117-131. Internat. Assoc. Great Lakes Res.

A.8 FIGURES

Figures 1(19), 2(20), and 3(21) on the following pages show the net designs and name various parts. All dimensions are given in centimeters.

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Figure 19: The vertical tow net.

A. Sampling line (4mm plastic coated steel cable).

B. Snap swivel (25mm diameter).

C. Bridle (4mm nylon cord).

D. Net frame (6x26mm bar stock aluminum).

E. Frame sleeve (rip stop nylon).

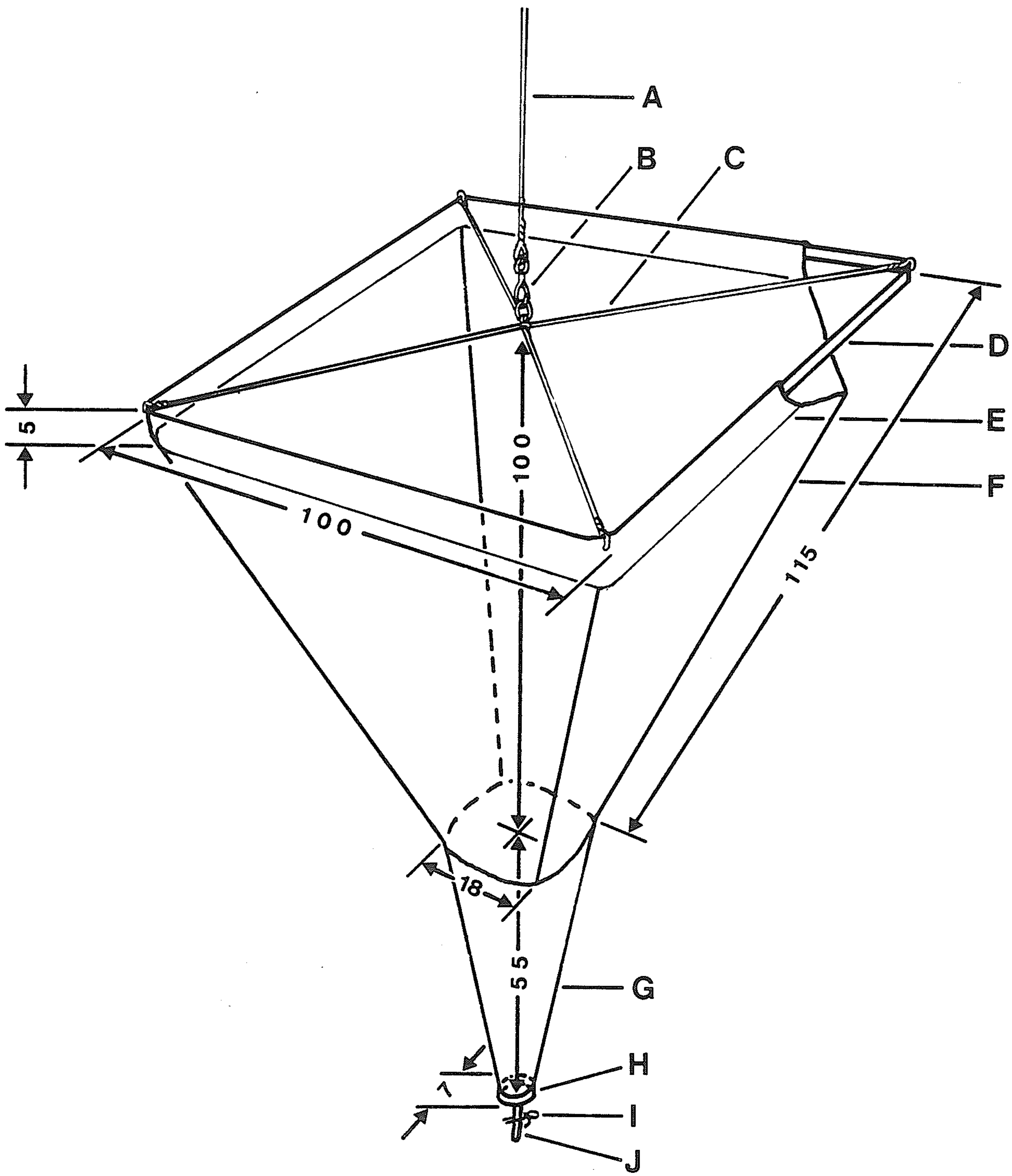
F. Netting (1mm mesh).

G. Cod end (250μ mesh).

H. Collecting funnel.

I. Pinch clamp.

J. Drain tube.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7

128

Figure 20: The collapsing net.

A. Sampling line (4mm plastic coated steel cable).

B. Snap swivel (25mm size).

C. Opening line (4mm nylon cord).

D. Close line (4mm nylon cord).

E. Steel ring (5cm diameter).

F. Fixed line (4mm nylon cord).

G. Opening line (4mm nylon cord).

H. Hinge (6mm eyebolt).

I. Scissor frame (6x26mm bar stock aluminum).

J. Standard $1m^2$ net (from vertical tow net).

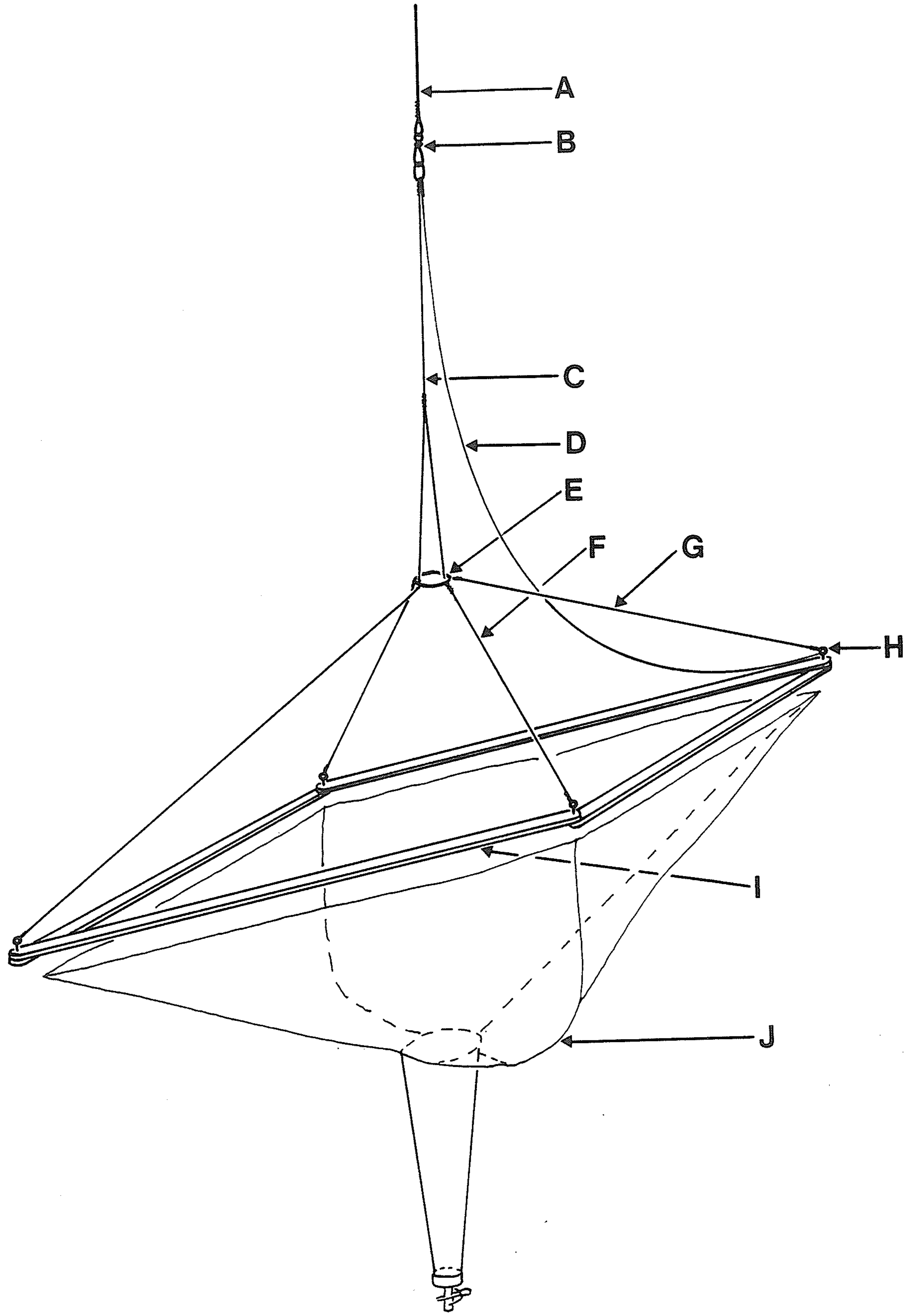
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Figure 21: The bottom closing net.

A. Descent line (4mm nylon cord).

B. Steel ring (5cm diameter).

C. Tension line (4mm nylon cord).

D. Ascent line (4mm nylon cord).

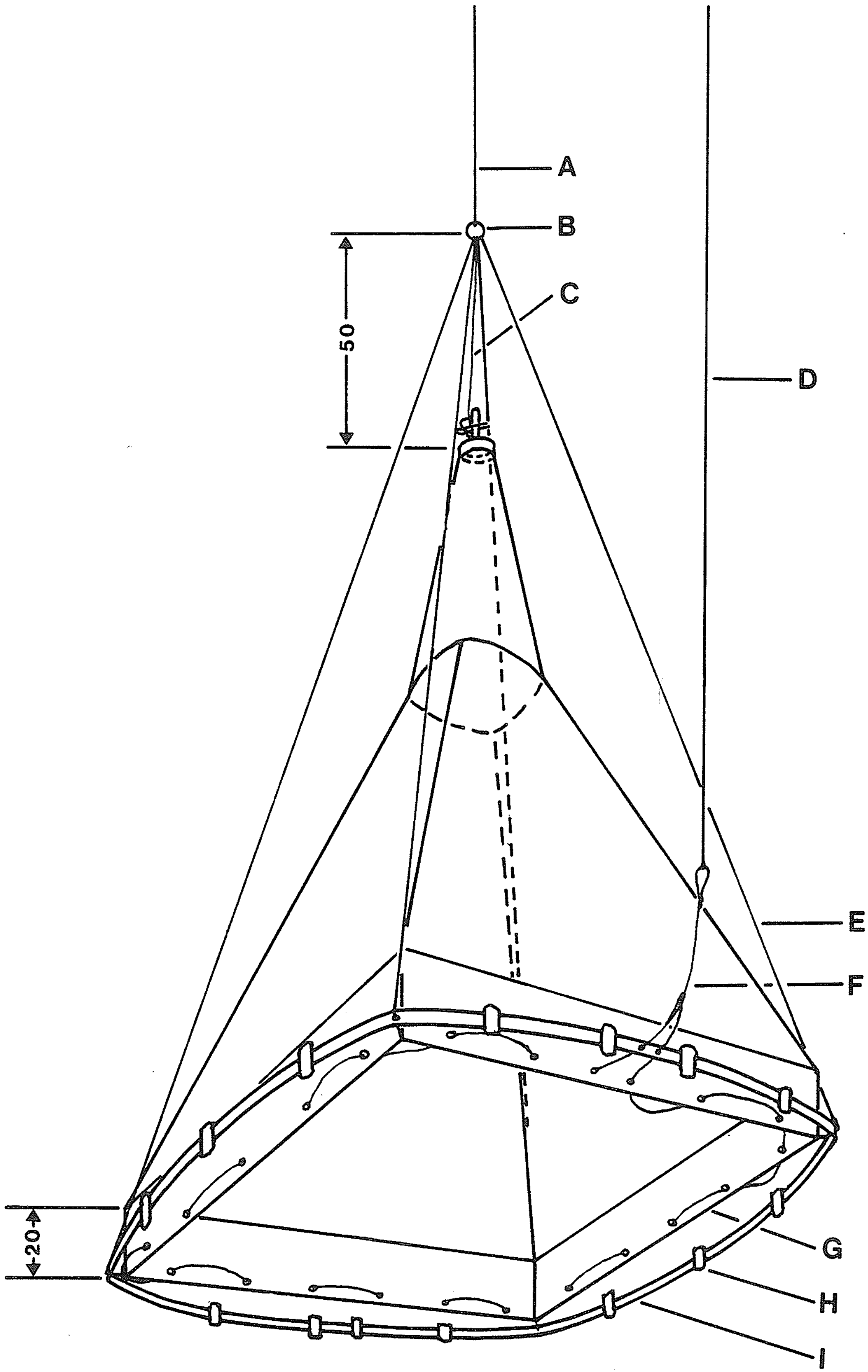
E. Opening line (4mm nylon cord).

F. Drawstring (1mm steel cable).

G. Sleeve with grommets (rip stop nylon 20cm width).

H. Weight (20g lead).

I. Frame (6x26mm bar stock aluminum).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Appendix B

A COMPARISON OF TWO SAMPLING METHODS FOR ESTIMATING THE ABUNDANCE AND DISTRIBUTION OF *MYSIS RELICTA*.

by

R. W. Nero and I. J. Davies

ABSTRACT

Nero, R.W., and I.J. Davies. 1981. A comparison of two sampling methods for estimating the abundance and distribution of Mysis relicta. Can. J. Fish. Aquat. Sci. 38: 0000-0000.

Two independent methods for determining the abundance of Mysis relicta gave widely differing results. Vertical net hauls taken at night yielded a population estimate of 5,300,000 ± 1,060,000 ($p \leq .05$) while quadrat counts done by SCUBA divers in the daytime, fixed the population size at 1,100,000 ± 170,000. Observations by divers suggested that the behaviour and distribution of Mysis during the day resulted in an underestimate of their abundance by the quadrat method. Net hauls showed that at night mysids migrated both vertically to the base of the epilimnion and horizontally within the metalimnion. Performance tests were conducted to evaluate the net technique. A vertical tow rate of 1/3 $m.s^{-1}$ used for the nighttime population estimates, lay within the optimum sampling speed of the net. Repeated sampling at a single station had little or no effect on catch totals for up to 9 consecutive hauls. Beyond this number some local depletion was noted. Comparisons between the standard vertical tow net and a modified version, which sampled on descent then closed once contact was made with the substrate, showed that this change in design did not improve catch performance except when the shallowest stations were sampled repeatedly. Temporal variation in the catch of young-of-the-year Mysis at shallow stations, however, contributed at most ±8% error to the whole-lake population estimate.

Key Words: Mysidacea; Diurnal migration; SCUBA; vertical net tows.

INTRODUCTION

Estimates of population size from sample data rely on two critical assumptions: first, that the sampling scheme takes into account the spatial distribution of the population and second, that the sampling gear provides unbiased estimates of density. If the animals are randomly distributed the first assumption is easily satisfied. Spatial heterogeneity can be overcome by a stratified sampling scheme, however, gear bias is often a more difficult problem to evaluate. The purpose of this study was to estimate the total number of *Mysis relicta* and the spatial distribution of this population in Lake 223 (L.223) at the Experimental Lakes Area, northwestern Ontario, by two independent techniques, then to examine each method for evidence of sampling bias.

The occurrence of *Mysis relicta* on or near the lake bottom during the daytime has led a number of workers to sample them by towing benthic sleds equipped with nets across the lake bottom (Beeton 1960; Reynolds and DeGraeve 1972; Grossnickle and Morgan 1979; Malley and Reynolds 1979). The small size of L.223 (27.27 ha), combined with extremes of bottom type found in the zone of *Mysis* occurrence (loose flocculent sediment or gravel interspersed with large boulders and granite outcrops) made sampling with a benthic sled impractical. Alternatively, daytime abundance estimates have been based on a series of quadrat counts done by SCUBA divers (Lasenby 1971). Quantitative samples of *Mysis* have also been taken at night when the *Mysis* population is planktonic by towing a net vertically through the water column (Hakala 1978; Morgan et al. 1978; Grossnickle and Morgan 1979; Malley and Reynolds 1979). Both daytime SCUBA counts and nighttime vertical net hauls were employed and compared in this study.

METHODS

Mysis abundance was determined as part of an overall study aimed at evaluating the response of L.223 to artificial acidification (Schindler 1980; Schindler et al. 1980).

Initial SCUBA surveys showed that during late August 1978 (the time of the population census) mysids were restricted to a region of the central lake basin between the top of the thermocline (5 m) and the upper boundary of the anoxic water in the hypolimnion (9 m). A total of 197 direct counts, by SCUBA divers, were made on cloudless days between August 29 and September 4, 1978, at approximately solar noon when the mysids were completely benthic. Between August 2 and August 27, 1978, 83 net hauls were made at least 1 hr after sunset on moonless nights, the optimum light conditions for *Mysis* vertical migration (Beeton 1960; Nero, unpublished data).

Prior to making diver estimates of mysid abundance, several sizes and types of quadrats were tested by the authors. Mysids avoided visible objects, escaped from open frame quadrats, and were displaced from the counting area by a bow wave when quadrat boxes with closed tops were placed on the substrate. The quadrat chosen for use was a clear, acrylic-plastic, square box (25 cm x 25 cm x 10 cm high) with an open bottom and a 5 cm wide lip around the top inside perimeter. This partial top design proved to be the best compromise between bow wave effect and counting accuracy. An acrylic handle attached to one side of the quadrat allowed the diver to place it on the lake bottom ahead of him without shading the substrate or blocking water flow through the box with his hand. An underwater light was used to illuminate the counting area once the quadrat was in position. Mysids caught within the box swam near the substrate until they encountered one of the quadrat sides, whereupon they typically attempted an escape by darting upward. With their escape route blocked by the top inside lip of the box, mysids returned to the substrate allowing the diver sufficient time to make an accurate count. Divers used a two-way voice communications system to relay counts and detailed observations to a surface attendant for recording.

A depth stratified counting scheme (Elliott 1973) with 0.3 m of depth per stratum was used by the divers over the zone of Mysis occurrence. Several replicate counts were made within each stratum along 6 transects perpendicular to the shore spaced at regular intervals around the central lake basin. Within a given stratum samples from all transects gave similar densities (Freedman test, $P<0.05$, Hollander and Wolfe 1973).

Vertical net hauls were used to determine the nighttime abundance of Mysis in the water column. Samples were collected along 6 transects, arranged in a similar manner to those used for the SCUBA estimates. Catch in each sample was expressed as total mysids per m^2. A poststratification technique was used to segregate samples, on the basis of total column depth, into five 1.5 m strata over the depth range 5 m to 12.5 m and one 1.9 m stratum (12.5 - 14.4 m).

The vertical tow net was shaped like an inverted pyramid (basal opening 1 m x 1 m) with sides tapering towards the end in two stages. Coarse mesh (1 mm x 1 mm opening) converged to an apical diameter of 22 cm one meter below the mouth of the net. To the apex was attached a 1/2 m long cod end of 210 μm mesh Nitex cloth which terminated in a plastic fitting equipped with a rubber drain tube, held closed by a pinch clamp. The combination of two mesh sizes permitted the user to haul the net vertically at a reasonable rate with a minimum bow wave while assuring that Mysis swept into the cod end suffered little damage from the net and could easily be removed. Lines from each corner of an aluminum support frame at the mouth of the net formed a bridle with a central attachment to a single tow rope. Based on the results of preliminary tests, we used a bottom time of 30 s to 1 min prior to retrieving the net and a towing speed of approximately 1/3 m s^{-1} for opitmum results.

Differences between population estimates derived from SCUBA counts and net hauls subsequently led to a more detailed investigation of the sampling characteristics of the net. Due to a drastic reduction of the *Mysis* population size in L.223 over the winter of 1978-79, probably as a result of experimental acidification (Nero, unpublished data), net performance tests were conducted in nearby lake 239 (L.239) in 1979 and 1980. A physical description of L.239 is available in Brunskill and Schindler (1971).

To evaluate the effects of repetitive sampling several replicate total column samples were taken over a half hour period at each of 5 sample stations (depths: 6 m, 9 m, 14 m, 23 m, and 30 m).

To determine whether or not vertical haul rate affected the catch efficiency of the net, 3 replicates of four hauling speeds (1/8 m s^{-1}, 1/4 m s^{-1}, 1/2 m s^{-1}, and 3/4 m s^{-1}) were tested in random sequence at the 14 m station.

Additional tests were conducted as paired comparisons between the vertical tow net and a downward-sampling closing net to determine whether or not catches varied due to mysids being: (i) trapped beneath the vertical tow net as it was lowered into position, (ii) attracted or repelled by sediment stirred up when the net contacted the lake bottom, or (iii) influenced by vibrations or bow wave effects from the towing bridle as the sample was taken.

The closing net was similar in design to the vertical tow net except that it sampled from the lake surface to the bottom, then closed. The descent line was attached to four cords which passed through a weighted aluminum frame and were attached to the net. Tension on the cords from the weight of the frame held the net open. Rate of fall through the water column could be regulated from the surface by the descent line. Once bottom contact was made the descent line was slackened. A second rope from the surface, used for retrieval, functioned as a drawstring to close the mouth of the net. Performance tests were conducted at eight depths (5.9 m, 7.0 m, 9.4 m, 9.6 m, 13.6 m, 13.7 m, 13.8 m, and 29.9 m) in L.239 on eight nights between June 24 and September 3, 1979, by sampling alternately with each net design.

Using the standard net design the vertical distribution of *Mysis* in the water column was determined on July 1, July 8, and September 3, 1979 in L. 239 at the 5.9 m, 13.6 m, and 14.8 m stations respectively. The number of *Mysis* in progressively deeper 1 m^3 volumes of water were determined by subtracting the number caught from z m depth from the number caught at z + 1 m depth.

Sample catches from each of the net-test experiments were enumerated as young-of-the-year or adult mysids based on measurements of total animal length (<10 mm and $\geq$10 mm respectively). Information on oxygen and temperature profiles for the lakes was furnished by D. W. Schindler (Freshwater Institute, Winnipeg).

RESULTS AND DISCUSSION

During the daytime the entire *Mysis* population was benthic (Fig. 1a). The shoreward limit of *Mysis* occurrence was marked by the top of the thermocline (17.6°C) while the lower limit of their distribution coincided with a region of the lower metalimnion where oxygen concentrations dropped from 10.2 mg^{-1} at 8 m to 0.3 mg L^{-1} at 10 m. No mysids were found outside these limits.

At night, the *Mysis* in the water column moved horizontally to cover a larger portion of the central lake basin (Fig. 1b). The limit of their shoreward occurrence remained the same as in the daytime. Observations made using SCUBA at night showed that mysids remained above the anoxic waters of the hypolimnion. Differences between daytime and nighttime distribution patterns suggest that each night mysids migrate vertically and horizontally (2 m vertically and on average 50 m horizontally) away from the areas of maximum daytime density while staying within physiologically tolerable conditions in the water column.

Although the two census methods gave reasonable information on the relative distribution of *Mysis* in the lake during the day and at night, they did not compare favourably when used to construct population estimates. Data from the diver-taken quadrat counts fixed the total population at 1,100,000 ± 170,000 (95% confidence limits) while the net haul method gave a significantly different estimate ($p < 0.01$) of 5,300,000 ± 1,060,000.

This discrepancy between abundance estimates could not be attributed to anything other than methodological differences. Water samples taken from depths corresponding to the zone of *Mysis* occurance on 31 July, 28 August and 25 September showed a pH change of only 0.2 units from 6.3 to 6.1, well above a value of 5.9 recorded in May of the same year. During the experiment the lake was thermally stratified with the thermocline slowly declining 1 m. between July 31 and September 25. Oxygen concentrations during the same period remained constant at 10 mg·1-1 and 0.4 mg·1-1 for the 8 m and 10 m depths respectively. No evidence of a temporal decline of the Mysis population, beyond the 10% expected from considerations of life history (Hakala, 1978), could be found in the data from either method. Less than 30 hr. elapsed between the final net tow sampling and the onset of the quadrat counts. Because lake conditions were stable during this brief interval between sampling periods, a catastrophic population failure could not account for the observed discrepancy in abundance estimates.

Several sources of error may have acted to give an artifically low value to the quadrat estimates of abundance. Even though divers made extensive observations which showed that during the day mysids were completely benthic and present only between 5 m and 9 m, within that zone Mysis tended to aggregate around boulders, rock outcrops and in areas of cobbles where often a quadrat could not be effectively placed. The rocky nature of the bottom together with the fact that portions of it are overlain by plate-like ferromanganese deposits, frequently undercut by tunnels and cavities, raises the possibility that a substantial portion of the mysid population remained hidden from view as suggested by Lasenby (1971). Mysids also tended to swim away from divers and the quadrat. Despite careful quadrat placement, some Mysis were occasionally displaced by its bow wave. Because few of the above errors could be easily quantified, we turned instead to a detailed examination of the net haul method.

Efficiency factors aside, vertical net tows should give reasonable estimates of abundance provided that the population sampled is entirely planktonic. At night the catch of a series of net hauls taken from progressively deeper starting points at a deep station showed that mysid abundance was maximum within the metalimnion (Fig. 2a). The same station sampled 2 months earlier during a full moon gave similar results except that a second peak of *Mysis* occurred at the bottom (Fig. 2b). This lower peak is probably due to an inhibition of their vertical migration by moonlight (Beeton 1960; Bowers and Grossnickle 1978; Morgan et al. 1978). At a shallow station sampled on a moonless night, most of the *Mysis* were confined to the lower 2 m of the water column by the presence of the thermocline 1.7 m above the bottom (Fig. 2c). Observations using SCUBA on moonless nights confirmed that mysids were only seen near the bottom in the zone where the thermocline contacted the substrate.

Tests of relative catch size vs. towing speed of the net (Fig. 3) showed that a vertical tow rate of 1/3 m s-1, used for the population estimates lay within the range of optimum sampling speeds for the net. For a haul rate of 3/4 m·s-1 the catch was significantly less ($p \leq 0.05$, Freedman Test, Hollander and Wolfe 1973) than at lower speeds. At the highest towing speeds resistance to water flow through the net created a substantial bow wave which displaced mysids away from the net mouth resulting in a reduced catch. We found no evidence of net avoidance behaviour even at the slowest hauling rates. Similarly, Fleminger and Clutter (1965) showed that nighttime avoidance of nets by marine mysids was marginal. The lowest catches (Fig. 3) were obtained for the last 3 hauls in a series of 12 suggesting that local depletion of *Mysis* may have occurred due to repeated sampling over a short period of time.

A single pair of samples from each of 8 stations was used to compare the catch performance of the vertical-tow net and a downward-sampling closing net. Overall, there was no significant catch difference between the two net types (paired t test, $p \leq 0.05$), indicating that even if mysids became trapped beneath the vertical tow net as it was lowered into position prior to taking a sample, or if their behaviour was influenced by the rope towing bridle, these factors did not bias the relative catch characteristics of the tow nets (Table 1).

For shallow stations, where mysid vertical migration was restricted to a narrow range of depth by the upper boundary of the thermocline (Fig. 2c), the number of young Mysis caught per haul increased as sampling continued (Fig. 4a). The increases may have been due to a temporal variation in the abundance of Mysis at the stations. Alternatively, changes may have been brought about by repeated sampling. Both nets may initially under sample the young but as sampling continued young Mysis, previously located near the substrate, moved into the water column and became available for capture. Another possibility is that the initial samples were representative of the station but as sampling continued young-of-the-year Mysis were attracted to the sediment cloud stirred up by each successive sampling resulting in an overestimate for the later hauls. For samples taken toward the end of the series catches were higher for the closing net than for the vertical tow net. In addition, young-of-the-year Mysis made up a higher proportion of the catch in samples from the closing net. These differences suggest that vertical tows may under sample Mysis located near the bottom, particularly young-of-the-year, by trapping them beneath the net prior to each sampling haul. At deeper stations on moonless nights (Fig. 4b) where both adults and young are located higher in the water column, the discrepency between the net catches was negligible. The importance of variation in the catch of shallow stations to a whole lake population estimate is minor. For example, the maximum range of values for young-of-the-year Mysis in Fig. 4b were only introducing an 8% error for L.

239 and a 2% error for L.223. The magnitude of this error varies among lakes depending on the population distribution and basin morphometry.

Determining *Mysis* abundance by diver-taken quadrat counts may be a useful method for lakes where the population is entirely benthic such as those at high arctic latitudes exposed to 24 hr daylight (Lasenby 1971). In most cases, though, mysids avoid divers, remain hidden from view or aggregate around rocks where quadrat placement is difficult, making net hauls a more attractive census tecnique. Except in the case where shallow stations are sampled, vertical net hauls taken on moonless nights appear to be free of avoidance and depletion effects caused by bow waves, interference from the towing bridle or local reduction of the population in the water column from replicate sampling and as suggested by Malley and Reynolds (1979) and Grossnickle and Morgan (1979) represent a reliable method for obtaining estimates of *Mysis* abundance in the water column.

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REFERENCES

Beeton, A. M. 1960. The vertical migration of *Mysis relicta* in Lakes Huron and Michigan. J. Fish. Res. Board Can. 17: 517-539.

Bowers, J. A. and N. E. Grossnickle. 1978. The herbivorous habits of *Mysis relicta* in Lake Michigan. Limnol. Oceanogr. 23: 767-776.

Brunskill, G. J. and D. W. Schindler. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 139-155.

Elliott, J. M. 1973. Some methods for the statistical analysis of benthic invertebrates. Freshw. Biol. Assoc. Sci. Publ. 25: 144 p.

Fleminger, A. and R. I. Clutter. 1965. Avoidance of towed nets by zooplankton. Limnol. Oceanogr. 10: 96-104.

Grossnickle, N. E. and M. B. Morgan. 1979. Density estimates of *Mysis relicta* in Lake Michigan. J. Fish. Res. Board Can. 36: 694-698.

Hakala, I. 1978. Distribution, population dynamics and production of *Mysis relicta* (Lovén) in southern Finland. Ann. Zool. Fennici 15: 243-258.

Hollander, M. and D. A. Wolfe. 1973. Nonparametric statistical methods. John Wiley and Sons, Toronto. 503 p.

Lasenby, D. 1971. The ecology of *Mysis relicta* in an arctic and a temperate lake. Ph.D. Thesis, University of Toronto, Ontario. 119 p.

Malley, D. F. and J. B. Reynolds. 1979. Sampling strategies and life history of non-insectan freshwater invertebrates. J. Fish. Res. Board Can. 36: 311-318.

Morgan, M. D., S. T. Threlkeld, and C. R. Goldman. 1978. Impact of the introduction of kokanee (Oncorhynchus nerka) and opossum shrimp (Mysis relicta) on a subalpine lake. J. Fish. Res. Board Can. 35: 1572-1579.

Reynolds, J. B. and G. M. DeGraeve. 1972. Seasonal population characteristics of the opossum shrimp, Mysis relicta, in southeastern Lake Michigan, 1970-71. Proc. 15th Conf. Great Lakes Res. (Internat. Assoc. Great Lakes Res.), p. 117-131.

Schindler, D. W. 1980. Experimental acidification of a whole lake: a test of the oligotrophication hypothesis. Proc. Int. Conf. Ecol. Impact Acid Precip., Norway 1980, SNSF project.

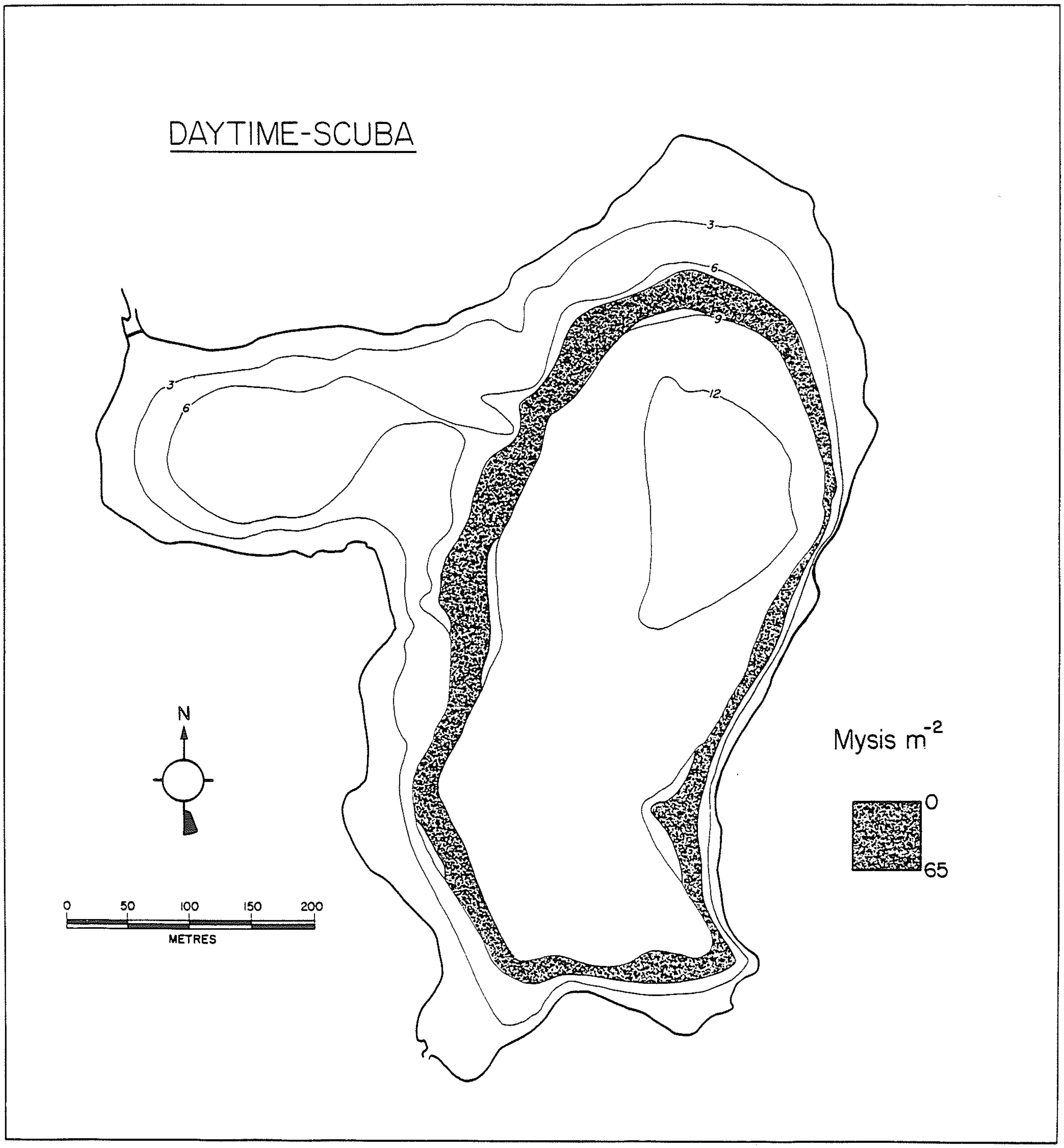
Schindler, D. W., R. Wagemann, R. B. Cook, T. Ruszczynski, and J. Prokopowich. 1980. Experimental acidification of Lake 223, Experimental Lakes Area: background data and the first three years of acidification. Can. J. Fish. Aquat. Sci. 37: 342-354.

Table 1. Total catch of *Mysis relicta* from a standard vertical tow net and a downward sampling-bottom closing net from 8 trials in Lake 239 during summer 1979.

	Depth (m)	Standard Net	Downward Net
June 24	13.8	98	75
July 1	5.9	123	160
Aug 15	7.0	41	36
Aug 21	13.7	108	124
Aug 26	9.4	94	97
Sept 2	9.6	176	126
Sept 2	29.9	104	72
Sept 3	13.6	150	181

LIST OF FIGURES

1 A



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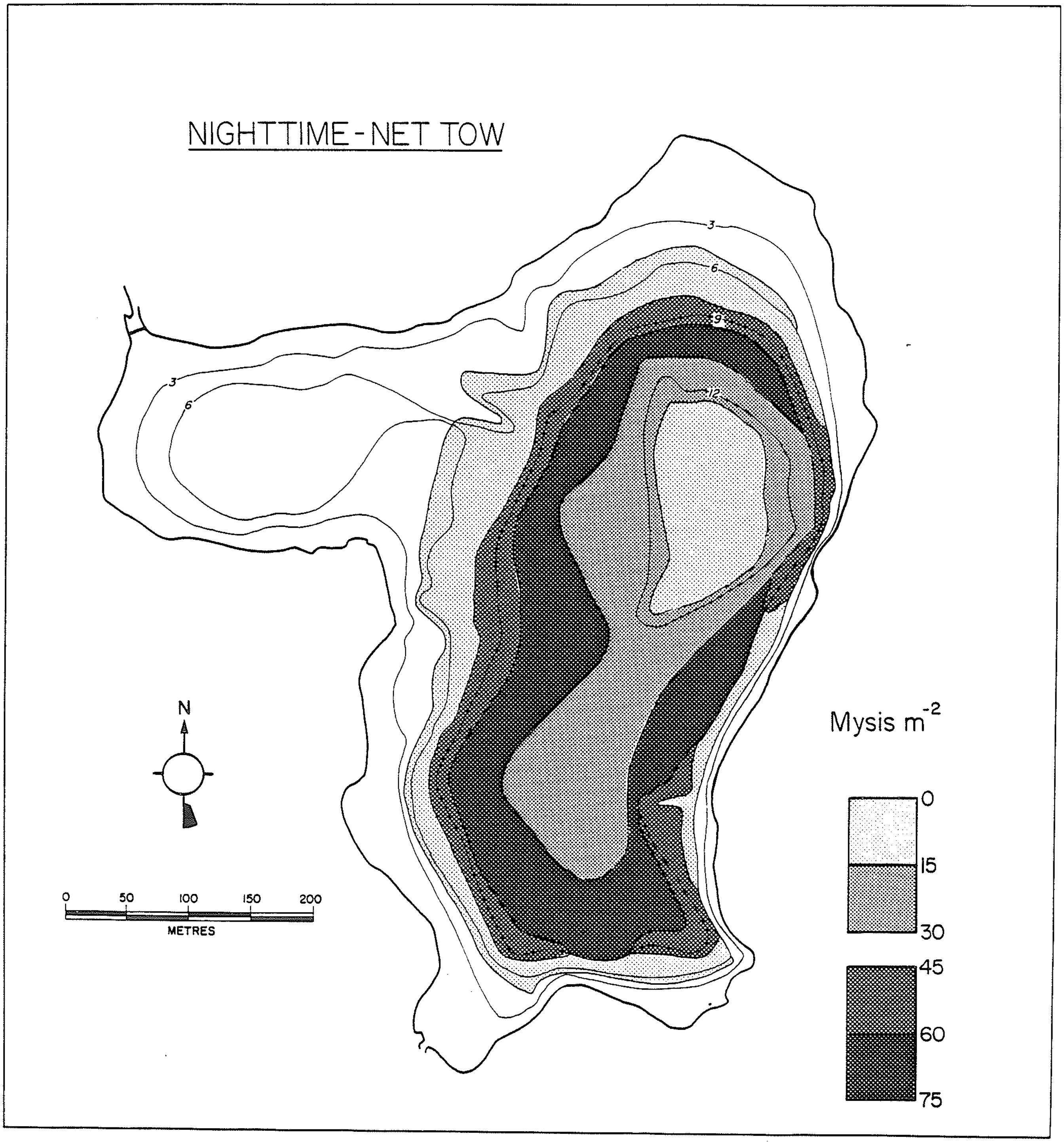
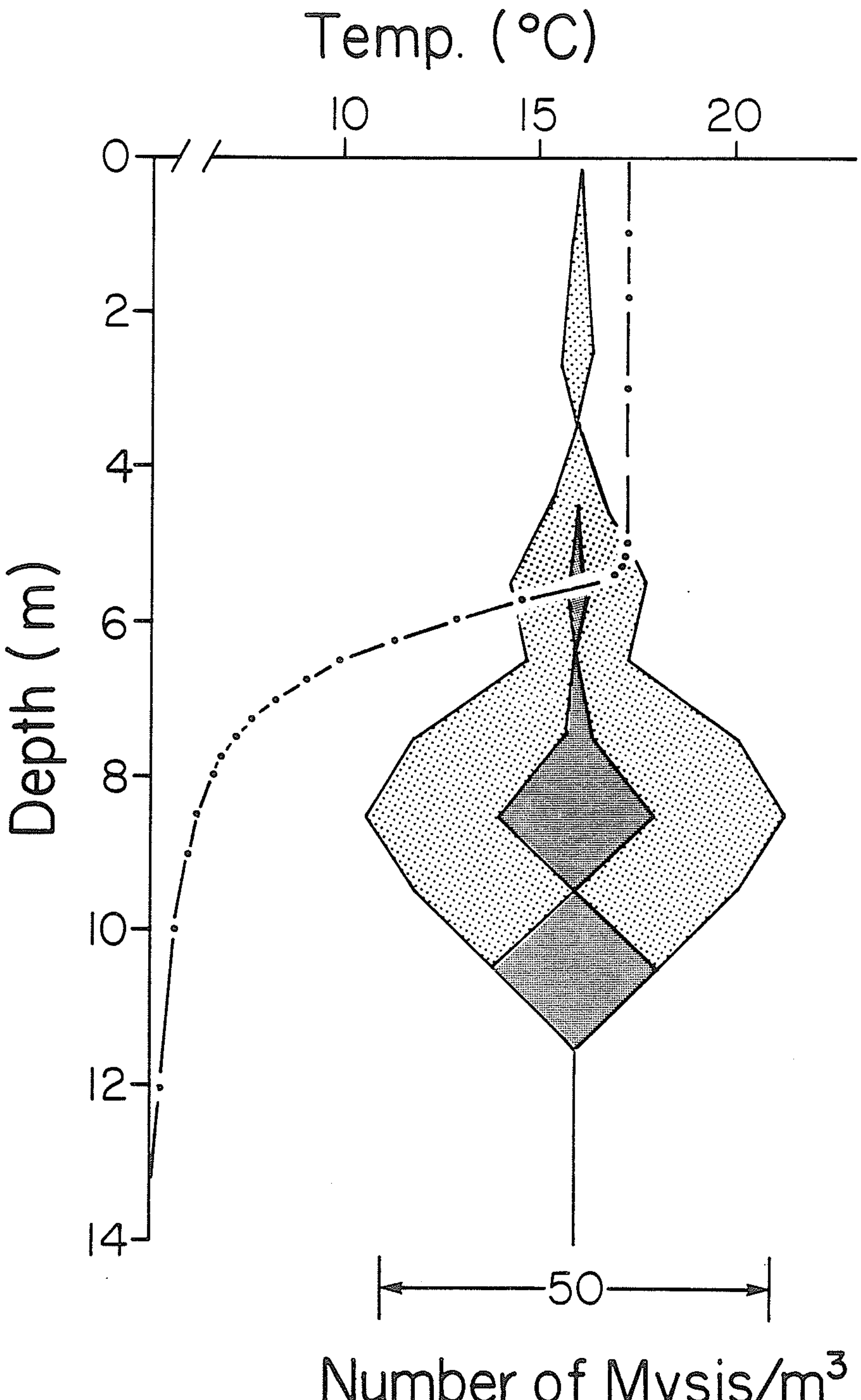

NIGHTTIME - NET TOW
3
6
9
12
N
0 50 100 150 200
METRES
Mysis m^{-2}
0
15
30
45
60
75

2 A



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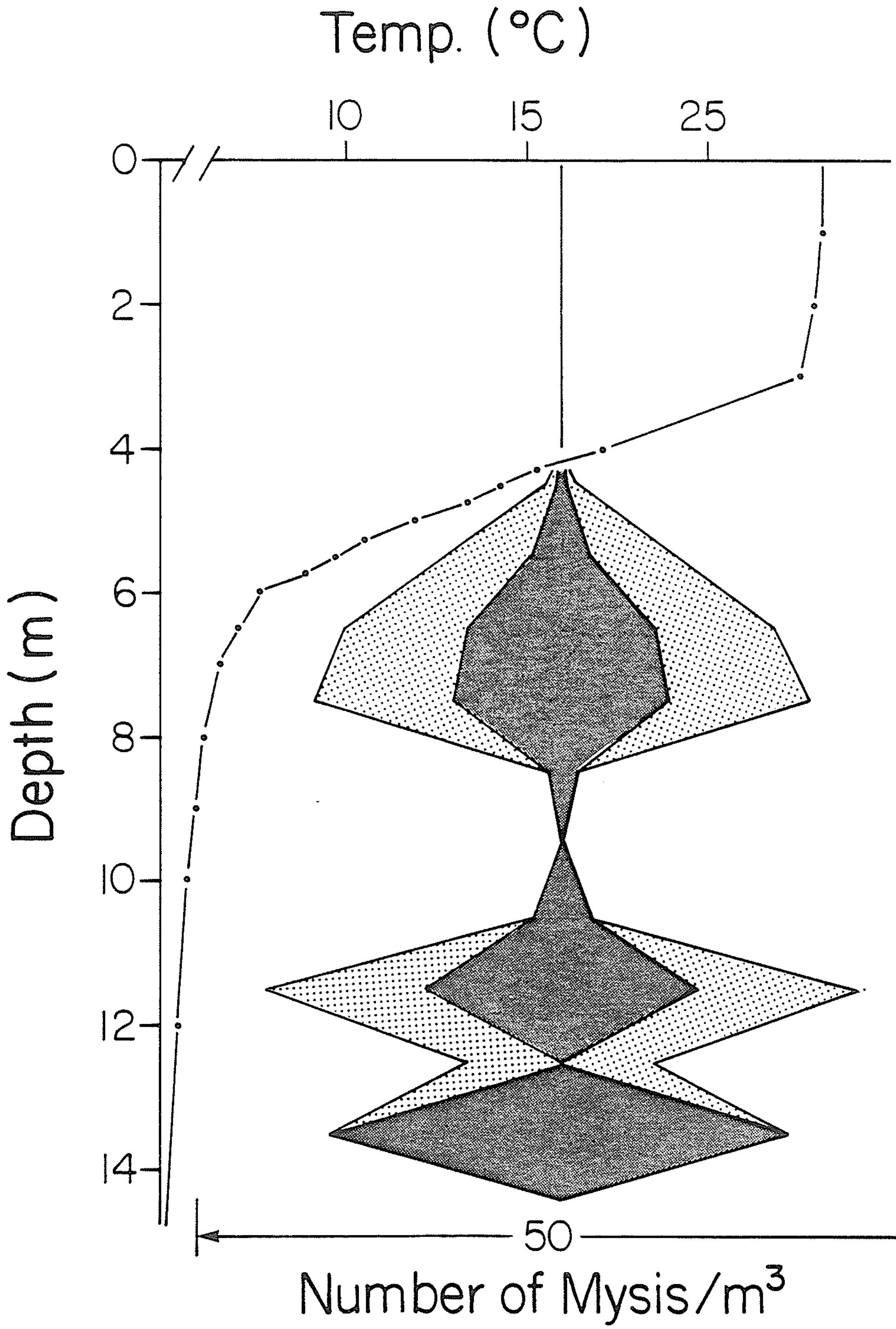
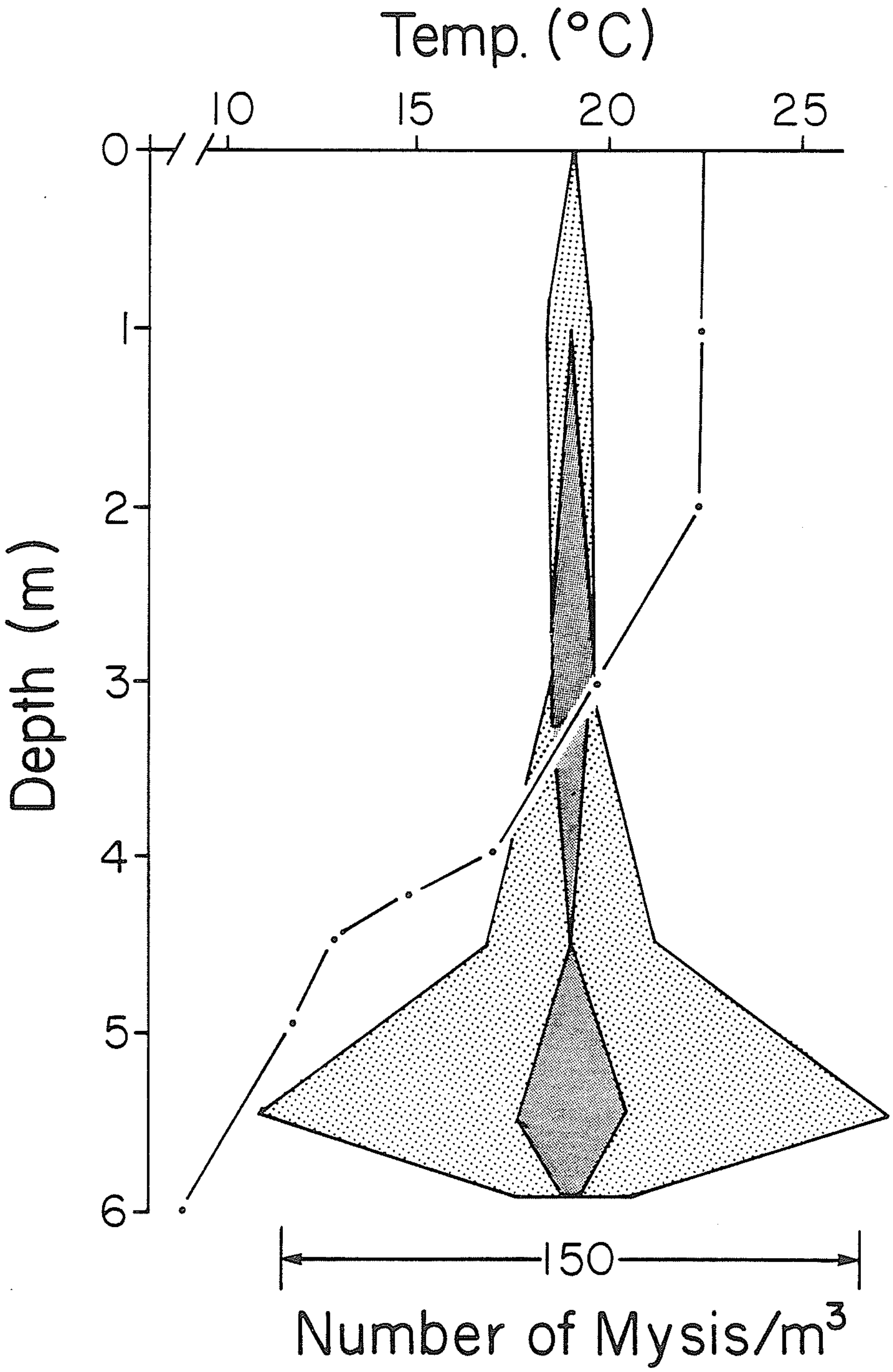


2 C



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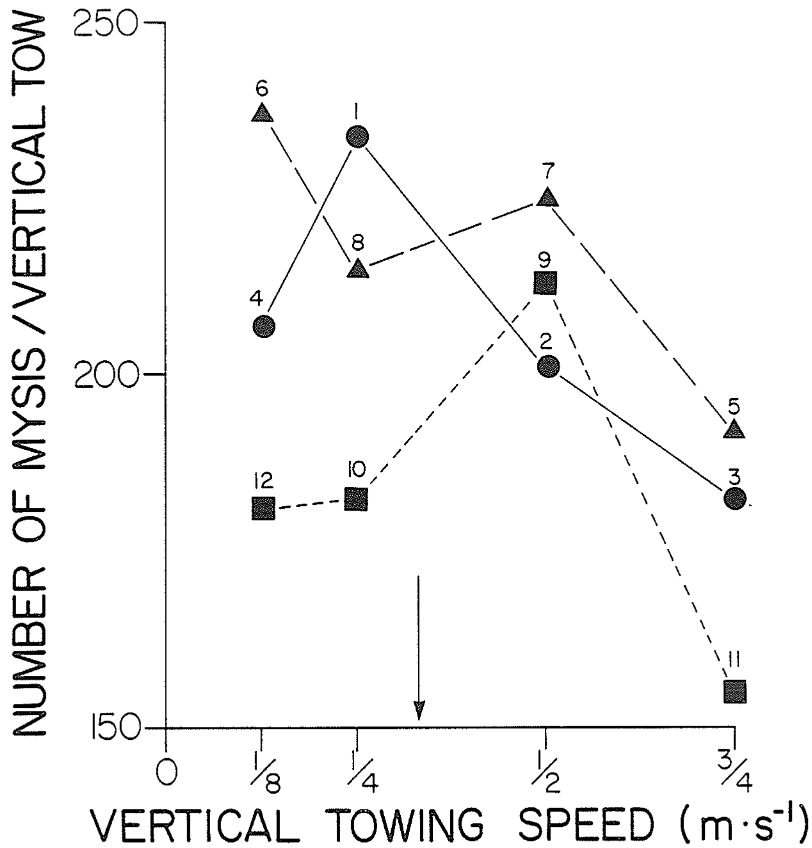
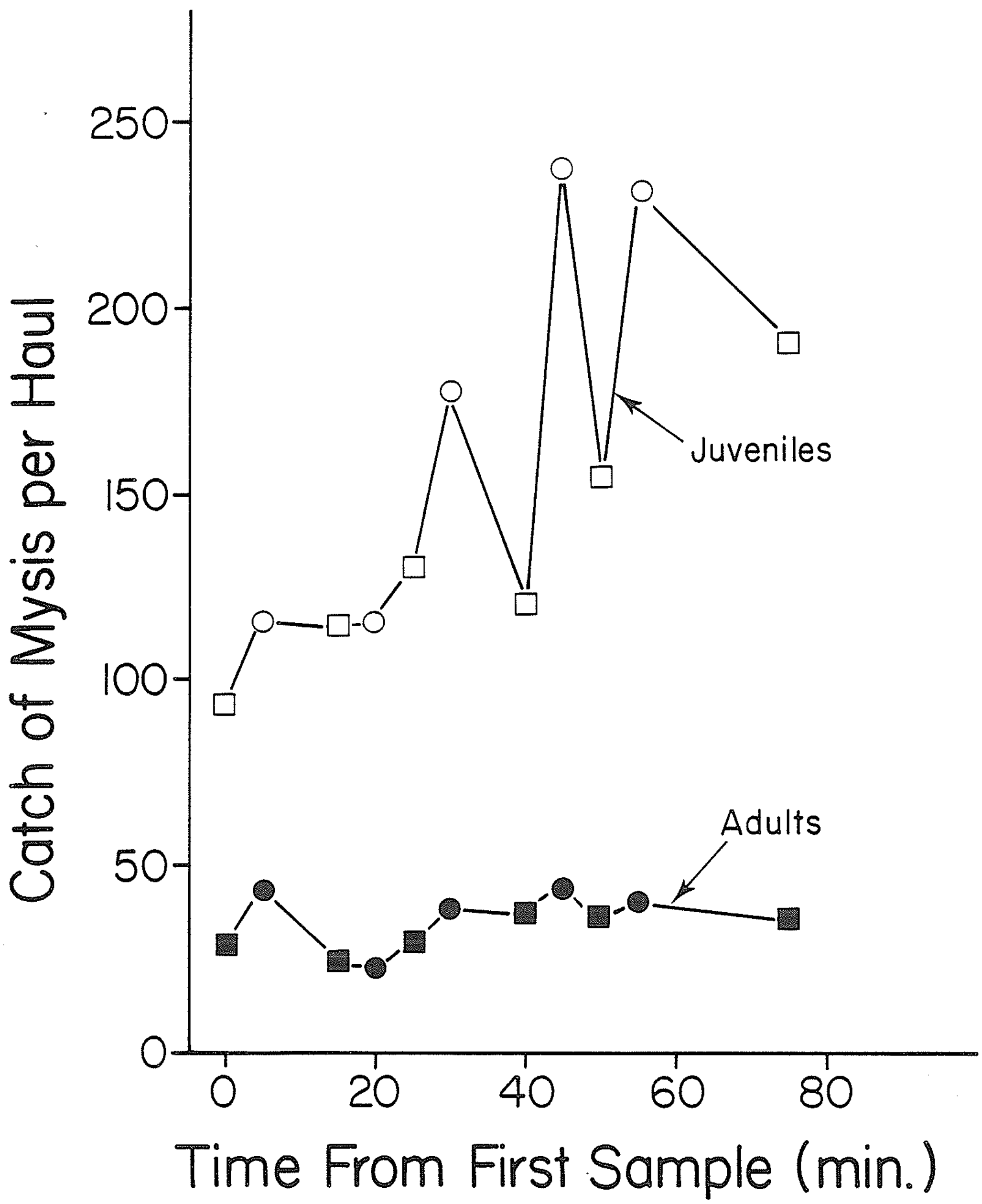

NUMBER OF MYSIS / VERTICAL TOW
250
200
150
6
1
7
8
9
4
2
5
12
10
3
11
0
1/8
1/4
1/2
3/4
VERTICAL TOWING SPEED (m·s-1)

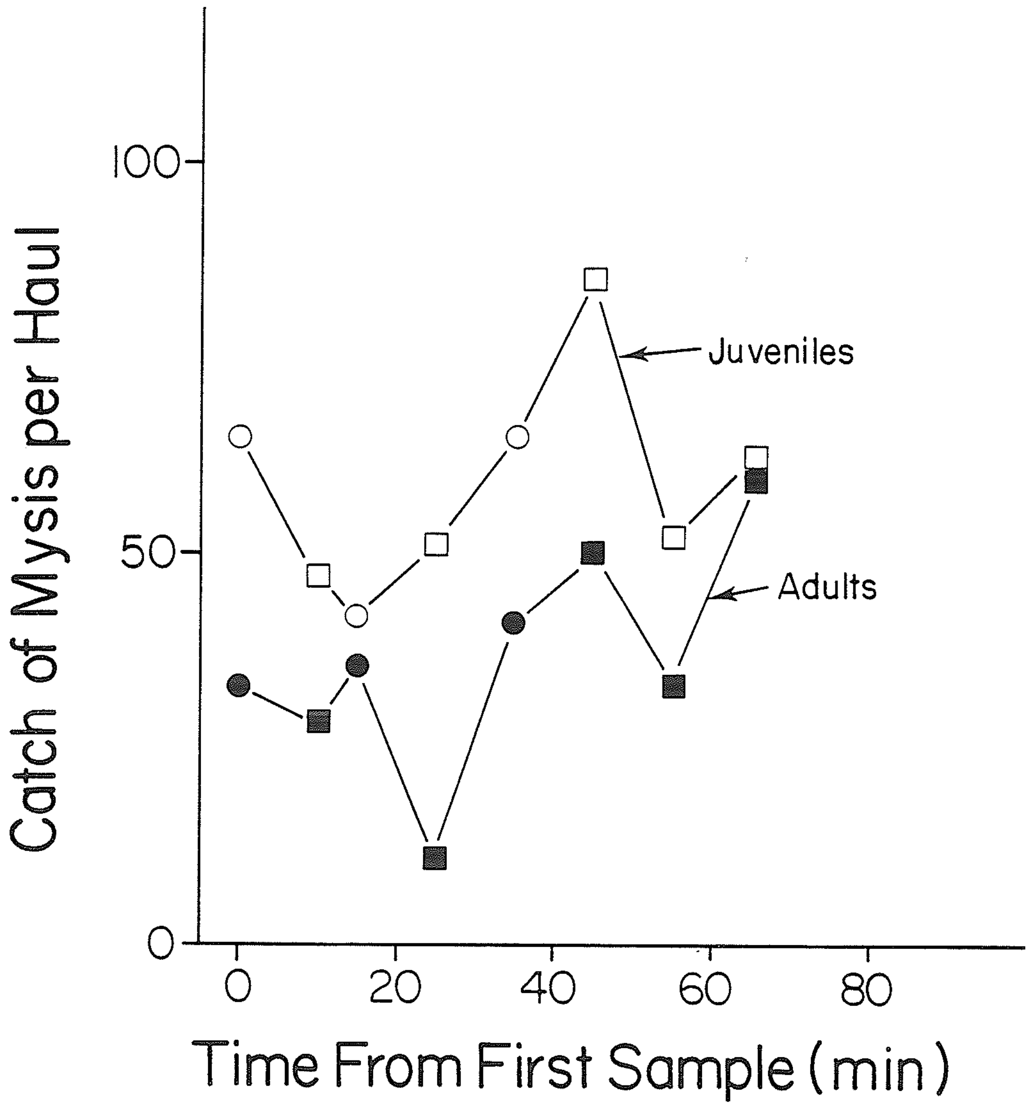
4 A



250
200
150
100
50
0
Catch of Mysis per Haul
Juveniles
Adults
0
20
40
60
80
Time From First Sample (min.)

4 B

1542



Catch of Mysis per Haul
100
50
0
Juveniles
Adults
0
20
40
60
80
Time From First Sample (min)

Appendix C

SEX AND MATURITY STAGES OF *MYSIS*

This classification is identicle to that used by Reynolds and DeGraeve (1972), except for the addition of two immature stages for males and females.

Sex	Description	Code	
Juvenile	- No distinguishing sex characteristics. .	0	1
Male - Oostegites lacking.			
Immature			
Immature I	- Fourth pleopods short not extending beyond the fifth tail segment	1	2
Immature II	- Fourth pleopods not extending beyond the base of the telson.	1	3
Adult			
Mature	- Fourth pleopods extending beyond the the base of the telson; exposed fully developed with a delicate extension on its last segment. Male process on antennular peduncle well developed and setiferous.	1	4
Breeding	- Possessing all the characteristics of a mature male, but also having bundles of sperm extruding from the copulatory organs or adhering to them.	1	5
Female - Oostegites present.			
Immature			
Immature I	- No contact or loose contact between oostegites.	2	2
Immature II	- Oostegite plates have firm contact with no pigmentation.	2	3
Adult			
Mature	- Oostegites well developed, posterior pair larger than and overlapping the anterior pair and mottled with contracted chromatophores. Brood pouch well shaped and tightly enclosed (not expanded). No brood visible.	2	4
Brooding	- Oostegites hyaline, fully developed and having chromatophores expanded arborescently over the lateral surfaces. Brood pouch firm and distended and filled with		

either eggs or embryos (unhatched young) or larvae (hatched young). 2 6

Spent - Oostegites fully developed, but thin, hyaline and either distended or collapsed. Brood pouch empty. 2 7

Disturbed brood - Possessing all the characteristics of a brooding female, but showing evidence that the brood was aborted (brood severely reduced; at least one embryo, egg, or larva must remain as proof that brood was present. 2 9

Appendix D

A COMPARISON OF THREE MEASURES OF THE SIZE OF MYSIS RELICTA.

Holmquist (1959) used total length as a measure of the size of *Mysis relicta*. Animals were measured from the apex of the rostrum to the apices of the telson (Holmquist 1959). More recently Morgan and Beeton (1978) have used the diameter of the dissected statolith as a measure of size. Grossnickle and Beeton (1979) used antennal scale length as a measure of mysid size and found that it correlated well with total length. In this study of the three measures of size, 599 animals from L239 were measured. Antennal scale was measured at 50X magnification with a resolution of .025mm. Statolith diameter was measured at 500X magnification with a resolution of .0025mm. Total length was measured under a dissecting scope giving a .1mm resolution.

Antennal scale length and total length were found to be linearly related with only 5% of the variation about the linear relationship unexplained (Fig. 22). The relationship is identical to that found by Grossnickle and Beeton (1979) except for a change in the relationship at 9mm total length (Fig. 22). Antennal scale lengths at this point appear to increase out of proportion to the total length measures and probably represents a small change in the relative growth between these two body measurements (Hartnoll 1978). The relationships between statolith diameter and total length as well as statolith diameter and antennal scale length were not as good as antennal scale and total length (Table 7).

Scatter plots were produced for combinations of antennal scale length, total length, and statolith diameter for Lakes 223 and 239 for juvenile, and adult male and adult female Mysis. The only plot which shows a definite pattern for the five instars as found by Teraguchi (1969) and Morgan (1976) is that for statolith diameter versus antennal scale length for L239 juveniles and females (Fig. 23).

The seperation of these instars by size categories is much less clear than for Mysis found in Green Lake Wisconsin (Teraguchi 1969), and Lake Michigan (Morgan 1976). Resolution of the measures is equal to that used in Teraguchi (1969) and Morgan (1976). Plots of statolith diameter versus antennal scale length and total length, which are not shown here, indicate that most of the variability is inherent in the statolith diameters. This variability may be due to the low Ca^{++} concentrations found in ELA waters resulting in less Ca^{++} uptake and a less definitive relationship between statolith diameter and instar. Therefore, the measurement of statolith diameter was discontinued and the grouping of Mysis into instar was not used.

Due to the results of the above tests, antennal scale length was the only length measurement used for the remainder of the study.

159

Figure 22: The linear relationship between antennal scale length and total length for L239 *Mysis relicta*. The dot size indicates the number of individuals represented by each dot.

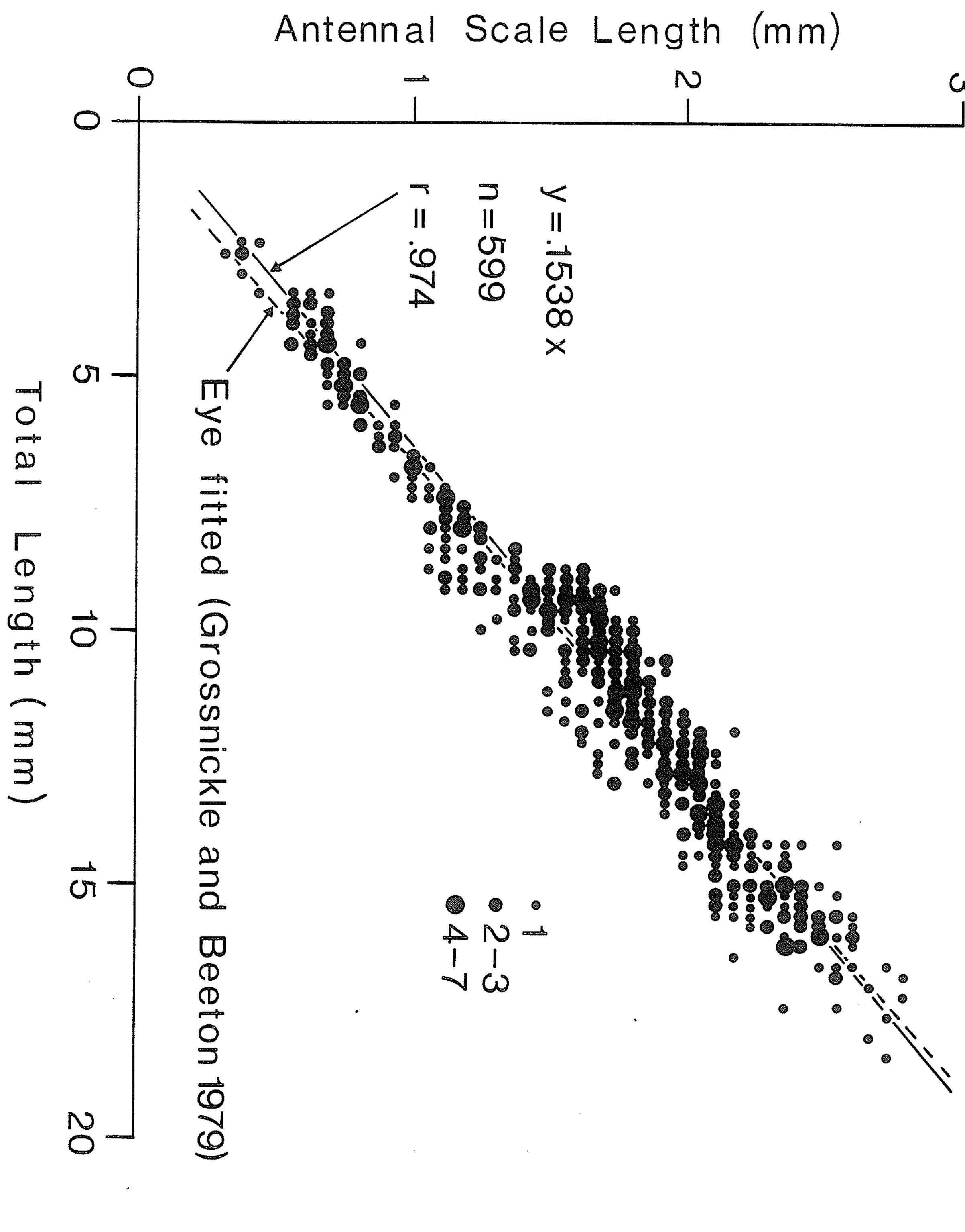

Antennal Scale Length (mm)
0
1
2
3
0
5
10
15
20
Total Length (mm)
y = .1538 x
n = 599
r = .974
Eye fitted (Grossnickle and Beeton 1979)
1
2–3
4–7

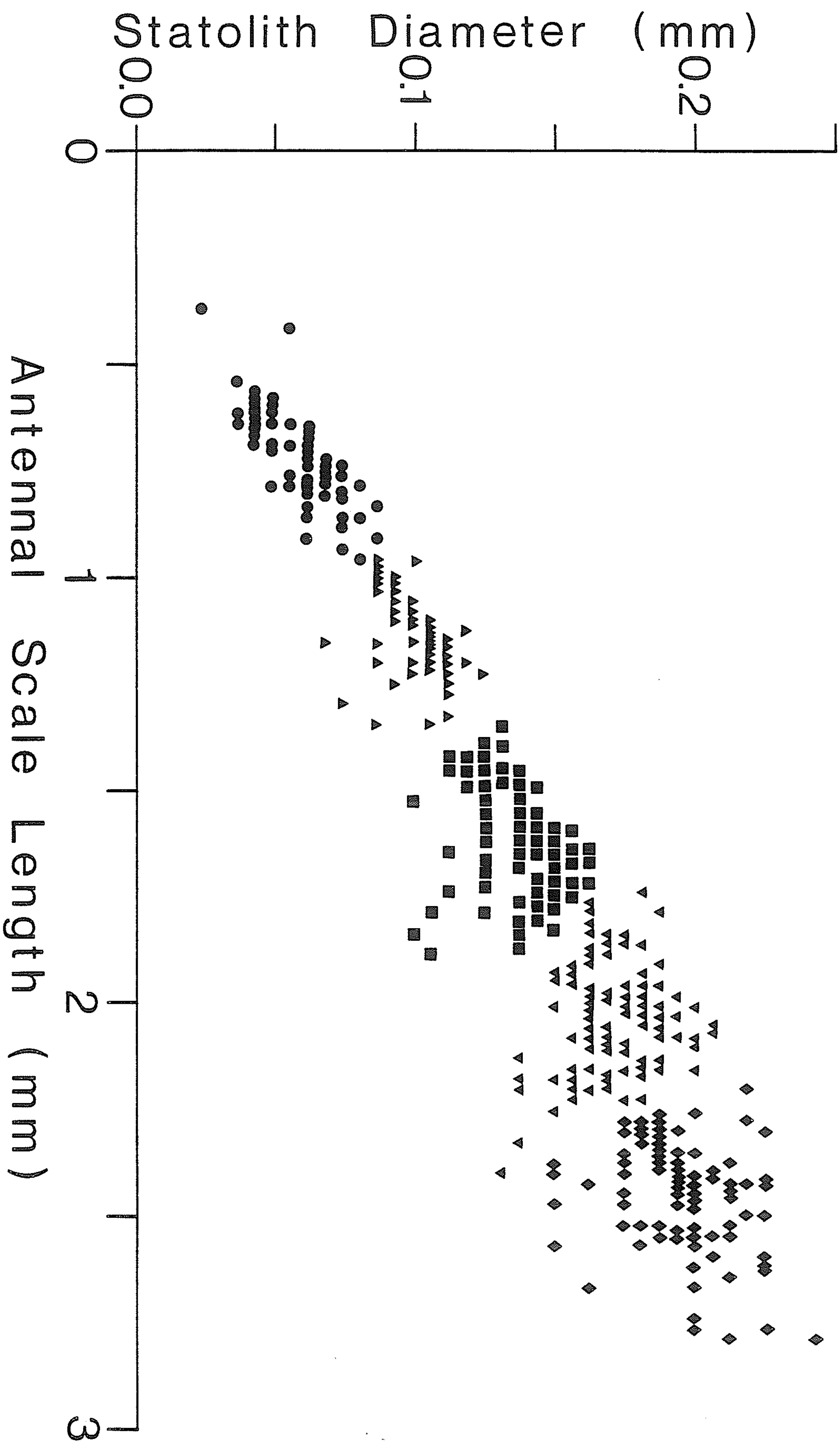
TABLE 7

Percent of variation of 3 measures of size.

A comparison of 3 measures of size used in a life history analysis of <u>Mysis relicta</u> and the relative merits of each measure based on the unexplained variation.

Length Measure	V.S.	Length Measure	R^2	Unexplained Variation
Antennal		Total	.949	5%
Statolith		Total	.797	20%
Statolith		Antennal	.856	14%

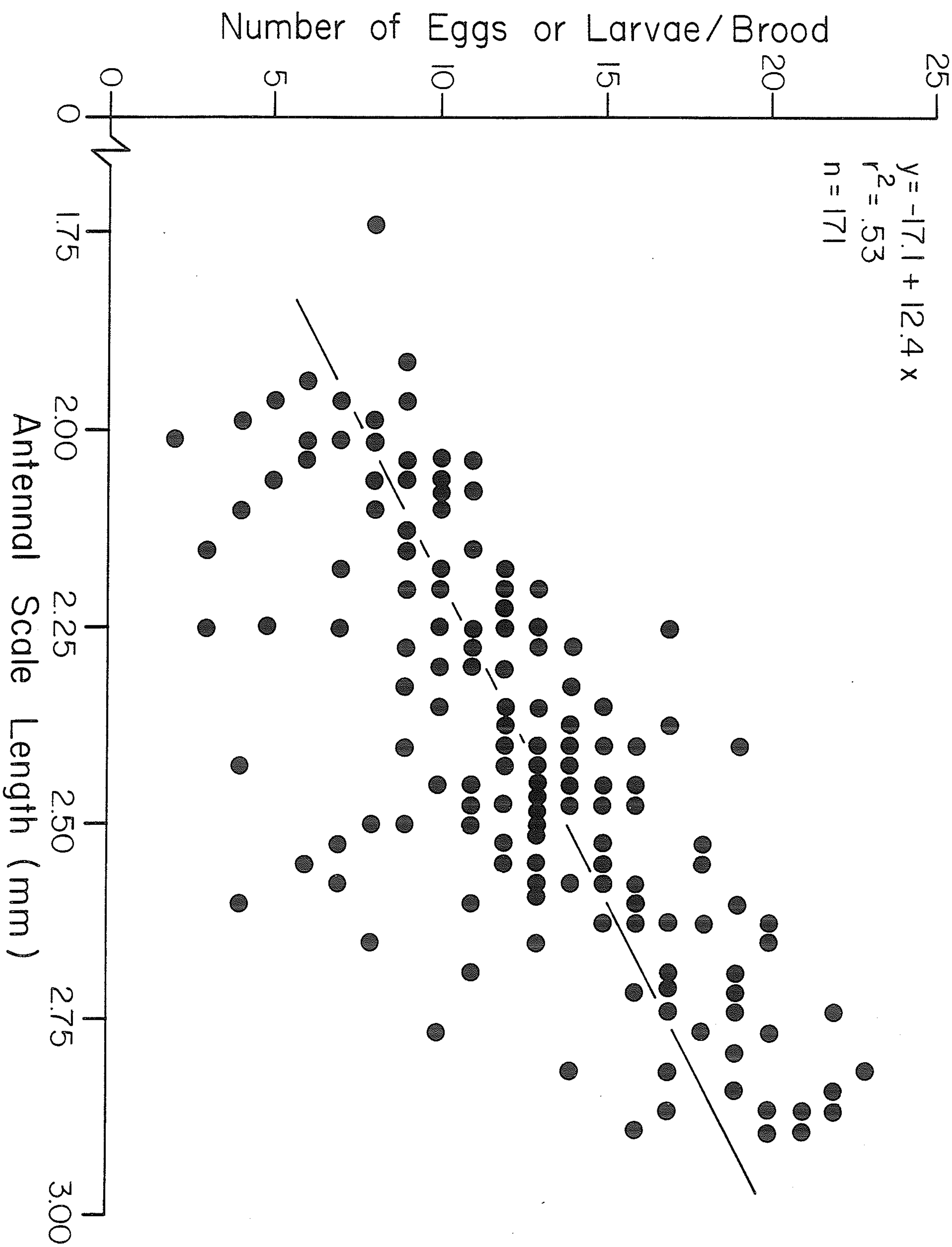
Figure 23: Plot of statolith diameter versus antennal scale length for L239 *Mysis relicta*. The 5 instars are; circles, 1st instar; triangles, 2nd instar; squares, 3rd instar; reversed triangles, 4th instar; and diamonds, 5th instar (instars have been previously defined by Morgan and Beeton 1978).


Statolith Diameter (mm)
0.0
0.1
0.2
Antennal Scale Length (mm)
0
1
2
3

Appendix E

BROOD SIZE OF ELA *MYSIS*

Figure 24: Brood size of *Mysis relicta* in 5 ELA lakes. A significant linear relationship was found between antennal scale length and the number of young per brood ($P \geq .0001$). 30 observations in the plot are masked by subsequent observations.



Number of Eggs or Larvae/Brood
0
5
10
15
20
25
y = -17.1 + 12.4 x
r^2 = .53
n = 171
0
1.75
2.00
2.25
2.50
2.75
3.00
Antennal Scale Length (mm)

Appendix F

METAL AND SE CONCENTRATIONS IN ELA *MYSIS RELICTA*

TABLE 8

Metal and Se Concentrations in *Mysis relicta* (µg/g dry wt.).

LAKE=223

N	n	Zn	Al	Cd	Cu	Ni	Hg	Se	Mn	Fe	Rank
1	1	52	52	0.2	42	0.5	0.38	2.1	37	120	6
2	2	56	44	0.1	68	0.5	0.39	2.3	44	170	4
3	3	48	37	0.1	60	0.5	0.34	2.8	40	290	1
4	4	51	50	0.1	50	0.5	0.46	2.4	46	220	2
5	5	46	46	0.1	65	0.5	0.42	1.8	33	220	3
6	6	42	37	0.1	46	0.5	0.31	1.8	29	130	5
7	7	53	58	0.2	76	0.5	0.35	2.4	36	210	7

LAKE=224

N	n	Zn	Al	Cd	Cu	Ni	Hg	Se	Mn	Fe	Rank
8	1	96	95	0.4	77	0.6	0.34	3.5	110	630	1
9	2	112	126	0.7	88	1.0	0.44	7.0	170	730	6
10	3	102	107	0.4	58	0.5	0.65	5.2	52	300	2
11	4	84	54	0.5	64	0.5	0.60	6.5	26	200	5
12	5	79	51	0.5	60	0.7	0.64	4.7	20	120	3
13	6	69	55	0.7	32	0.5	0.51	4.3	21	130	4
14	7	78	64	0.4	46	0.5	0.49	3.9	30	200	7

LAKE=228

N	n	Zn	Al	Cd	Cu	Ni	Hg	Se	Mn	Fe	Rank
15	1	105	187	0.3	.	.	0.21	4.4	3000	860	7
16	2	84	76	0.2	.	.	0.13	4.2	94	110	2
17	3	107	115	0.3	.	.	0.16	3.5	.	.	1
18	4	101	120	0.4	.	.	0.20	4.3	550	230	6
19	5	84	61	0.2	.	.	0.13	3.8	21	48	3
20	6	81	59	0.3	.	.	0.09	3.4	37	68	4
21	7	65	56	0.0	.	.	0.14	3.0	97	130	5

LAKE=239

N	n	Zn	Al	Cd	Cu	Ni	Hg	Se	Mn	Fe	Rank
22	1	88	156	0.4	.	.	0.41	3.6	67	320	6
23	2	96	129	0.1	.	.	0.40	3.5	49	250	4
24	3	82	82	0.2	.	.	0.36	3.3	64	320	7
25	4	64	74	0.1	.	.	0.28	2.3	34	150	1
26	5	92	89	0.2	.	.	0.40	3.4	66	240	3

27	6	74	99	0.1	.	.	0.29	2.7	34	160	2
28	7	154	123	0.4	.	.	0.30	2.8	140	280	5

------------------------- LAKE=305 -------------------------

N	n	Zn	Al	Cd	Cu	Ni	Hg	Se	Mn	Fe	Rank
29	1	91	152	0.5	75	1.2	1.06	5.5	93	290	7
30	2	80	38	0.5	52	0.5	0.82	4.6	24	63	6
31	3	81	60	0.3	40	0.5	0.73	5.1	38	100	5
32	4	74	52	0.4	36	0.5	0.71	4.8	21	72	3
33	5	84	55	0.4	46	0.5	0.56	4.6	26	71	4
34	6	79	48	0.3	38	0.5	0.46	4.1	23	64	2
35	7	84	62	0.4	26	0.5	0.45	4.6	37	120	1

------------------------- LAKE=468 -------------------------

N	n	Zn	Al	Cd	Cu	Ni	Hg	Se	Mn	Fe	Rank
36	1	75	29	0.3	48	0.5	0.45	3.7	69	81	7
37	2	59	99	0.1	32	0.5	0.39	3.0	47	100	6
38	3	62	41	0.2	28	0.5	0.44	2.3	49	160	5
39	4	73	30	0.2	29	0.5	0.55	3.5	39	45	2
40	5	66	31	0.1	34	0.5	0.39	2.6	37	110	4
41	6	75	27	0.3	52	3.9	0.81	3.7	70	87	3
42	7	80	73	0.3	81	0.7	0.55	3.3	120	250	1

Appendix G

MEAN METAL AND SE CONCENTRATIONS IN *MYSIS RELICTA*

TABLE 9

Mean metal and Se concentrations in ELA *Mysis relicta*

The mean concentrations of the various metals and Se in tissue samples of *Mysis relicta* from the 6 study lakes. Sample sizes of 7.

Lake	Mean Metal Concentrations (µg/gm dry weight)						
	Zn	Al	Cd	Cu	Ni	Hg	Se
223	49.7	46.3	.129	58.1	.50	.379	2.23
224	88.6	78.9	.514	60.7	0.61	.537	5.01
228	89.6	96.3	.243			.151	3.80
239	92.9	107.4	.214			.349	3.09
305	81.9	66.7	.400	44.7	0.60	.684	4.76
468	70.0	47.1	.214	43.4	1.01	.511	3.16

Appendix H

CALCIUM CONCENTRATIONS OF ELA WATERS

TABLE 10

Calcium concentrations of ELA waters

Four measurements of calcium concentration in the six study lakes are: Epi M., denotes the mean epilimnion concentrations from the open water season; 3m, a single determination at 3m during stratification; Turnover, the range of values at spring and fall turnover; and January, the range of values from a profile obtained in January.

Lake	Year	Ca^{++} Concentration ($mg.l^{-1}$)			
		Epi M.(1)	3m(2)	Turnover(3)	January(3)
223	78-79	2.17	2.3	2.3-2.5	2.3-3.0
224	74-78	1.75	1.7	1.6-2.0	1.5-2.3
228	71	2.14	2.2	2.1-2.3	
239	73-79	2.39	2.9	2.3-2.9	2.5-3.0
305	71,74	2.17	2.3	2.1-2.4	
468	81		2.2		2.3-2.5

1 - Prokopowich (1979)
2 - Beamish et al. (1976)
3 - ELA unpublished data.