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USE OF SEQUENTIAL SAMPLING OF AMPHIPOD ABUNDANCE TO CLASSIFY THE BIOTIC INTEGRITY OF ACID-SENSITIVE LAKES

R. A. C. PROJECT NO. 470C

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ABSTRACT

A sequential sampling program is described for ubiquitous, cost-effective biomonitoring of the effects of lake acidification. Spring densities of the littoral amphipod *Hyalella azteca* are quantitatively sorted into 5 abundance categories. Stratification of proportional densities per unit macrophyte biomass in relation to the aqueous total phosphorus concentration is an essential step in the a priori definition of decision criteria. Density rankings were related to lake acidity and to detailed lake-specific information on patterns of *Hyalella* life history and acid tolerance. Incorporation of *Hyalella* abundance as a potential metric for the assessment of the biological integrity of acid-sensitive waters is recommended. The temporal integration of transient changes in spring meltwater chemistry is an important rationale for the development of such biomonitoring procedures.

Key words - *Hyalella azteca* density, lake acidification, quantitative biomonitoring, sequential sampling, biological integrity.

INTRODUCTION

Environmental scientists greatly err in assuming that measures of aqueous chemical imbalance alone are sufficient to gauge ecosystem stress (Karr 1987). Living organisms provide convenient full-time monitors of transient variability in environmental quality, an important consideration when assessing the effects of anthropogenic acidification on lakes and rivers in north-temperate climates. Because the soil in such regions may still be partially frozen in the spring, much of the acidic snowmelt can quickly run off with little neutralization taking place within the watershed. For example, typically between 36 to 77% of the annual export of H^+ from watersheds over much of central Ontario occurs during this short interval (Jeffries *et al.* 1979). Due to logistic difficulties, acidic pulses are rarely monitored throughout the littoral zone over the melting period. More frequently, if the spring episodes are sampled at all, they are normally estimated from midlake stations or inferred from the more accessible outflows which are assumed representative of the lake whole (e.g. Goldstein et al. 1984). Such measures are at best, however, but an imprecise reflection of the actual environment to which littoral organisms are exposed, and misleading conclusions regarding the severity of such pulses can arise (France and LaZerte 1987). Obviously, the function of biomonitoring as a temporal integrator of cumulative stress would he valuable in such circumstances.

Considerable attention has been devoted to study of the effects of acidification on the littoral amphipod *Hyalella azteca* (Saussure) in Canadian Shield lakes. Such work is has been motivated through recognition of the demonstrable biomass dominance of *Hyalella* in the littoral zones of such lakes (e.g. Dermott 1988), as well as the acknowledged importance of this species to the diet and production of several commercially important fishes (e.g. Cooper 1965; Hall *et al.* 1970), some of whose abundances are known to be correlated with lake pH (e.g. Minns *et al.* 1985; Wales and Beggs 1986). Laboratory experiments (de March 1979; France and Stokes 1987a, 1987b; Mackie 1989), *in-situ* transplantations (Grapentine 1987), empirical modelling (France and LaZerte 1987), synoptic surveys (Stephenson and Mackie 1986; Fiske 1987; Peterson 1987), and detailed life history/production studies (Stephenson 1986; France and Stokes in prep), have all demonstrated *Hyalella* to be quite

susceptible to low pH. *Hyalella* is therefore believed to be able to provide a sensitive biological assessment of lake acidification stress.

Stephenson and Mackie (1986) concluded that the absence of *Hyalella* "from a lake sensitive to acidification is sufficient evidence to suggest that acidification may be having a substantial biological impact. *Hyalella azteca* can be collected without specialized equipment (waders and fine sieve are all that is necessary), and it is easily identified (with the aid of a dissecting microscope). It should find application as an indicator organism in regional surveys of lake acidification status and as an indicator of deterioration of sensitive lakes over a period of years". As a result of the aforementioned studies, a recent EPA-sponsored meeting on the screening of potential candidates for biomonitoring concluded that benthic invertebrates (including *Hyalella*) were appropriate sentinels of the degradative influences of acidification upon the integrity of aquatic environments (Marmorek *et al.* 1988). Nevertheless, to date, a protocol for use of *Hyalella* in such biomonitoring programs has not been developed. This is the subject addressed in the present study. Such consistency in design considerations of acid biomonitoring programs ensures data comparability among sites and/or times and thereby reduces the risk that revealed differences merely reflect artifacts of methodological inconsistencies (Arnason 1988).

HYALELLA SAMPLING STRATEGIES

The need to characterize both the temporal and spatial variability of parameters is crucial to the rational planning and implementation of any biomonitoring program (Arnason 1988). These considerations are particularly requisite for the design of sequential sampling programs (Sevacherian and Stern 1972; Ives and Prentice 1958).

Time

The life history of *Hyalella* in softwater lakes of central Ontario (France and Stokes in prep) is very similar to that described by Lindeman and Momot (1983) and Biette (1969) for populations near Thunder Bay, northwestern Ontario and Duck Mountains Prov. Park,

southwestern Manitoba, respectively. Because these populations are predominately univoltine, it is essential for survival that the cohort of young amphipods produced in midsummer exists through the period of snowmelt to mature and produce young the next year. As a result, Cooper (1965) considered mortality to be a more important demographic variable than either growth or reproduction in regulating *Hyalella* population dynamics.

Onset of reproductive activity, recruitment of young, and seasonal rate of population increase, are strongly influenced by lake temperature and alkalinity in the Haliburton-Muskoka region (France 1987a; Stephenson and Mackie 1986; France and Stokes in prep). Different lakes, even within a localized area, may have dissimilar thermal regimes, being influenced by among other things, basin morphometry. Because of this, it is extremely difficult to standardize among lakes with respect to the stage of the population density cycle being sampled at any particular time during the summer. Interlake comparisons of *Hyalella* density are therefore best performed before recruitment begins as soon as is logistically feasible in the spring. The animals are largest at this time so that efficiency and speed in sorting is maximized. Such a sampling period is consistent with respect to the predictions made concerning impacts of anthropogenic lake acidification through vernal pH depressions (France and LaZerte 1987).

Location

Hyalella density decreases rapidly with depth due to the attenuation of macrophytes in relation to light transmittance (France and Stokes 1988). Population abundance can therefore be estimated at wading depths (Lindeman and Momot 1983; Stephenson 1986; France 1987a), which of course encompasses that area most affected by pH fluctuations (France and LaZerte 1987).

The dramatic effect of macrophyte biomass on the density of littoral invertebrates has received recent study (Downing and Cyr 1985; Downing 1986; Cyr and Downing 1988). Because macrophyte distribution (Hitchin *et al.* 1984) and biomass (France 1988) varies considerably within individual Haliburton-Muskoka lakes, *Hyalella* are aggregatively

distributed (France 1987b) in relation to the localized pattern of plant biomass (France and Stokes 1988). Unless careful attention is paid to this relationship and some standardization applied to the data, the validity of interlake comparisons of *Hyalella* abundance expressed on a simple per meter squared basis (e.g. Stephenson 1986) may be questionable. This was recognized by Strong (1971) in the first interpopulation investigation of *Hyalella* life history:

> "In the initial phases of the work, densities of the natural populations were estimated. These proved valueless owing to the extremely patchy distributions of Hyalella within habitats, and the differences between the substrates of the habitats of the various populations".

Interlake comparisons should be based on small "community-type" vegetative strata (Nichols 1982) common among lakes, rather than on entire lake-magnified, and therefore, -approximated, values (France and Stokes 1988). The isoetid *Eriocaulon septangulare* (With.) is the most ubiquitous and abundant macrophyte in Canadian Shield lakes (Yan *et al.* 1985a; France and Stokes 1988) and was selected as the habitat template from which to make all comparative assessments of *Hyalella* abundance. Densities of *Hyalella* were 3 to 6 times higher here than in other habitats such as sand with sparse macrophytes, allochtonous detritus, or organic sediments (France, unpubl. data). Use of ratios of *Hyalella* density per gram Eriocaulon dry weight (both roots and shoots included) as a correction for sampling variability in macrophyte biomass, have previously proven successful (France and LaZerte 1987; France and Stokes 1988). The dangers and efficacy in use of such ratios is discussed in France (1990a).

Eriocaulon covers an average of 13% (range 4 - 42%) of the total shoreline length in Haliburton-Muskoka lakes, with biomasses usually being highest near the mouths of inflows (Hitchin *et al.* 1984; France and LaZerte 1987). *Hyalella* samples should be collected from several different, randomly selected locations around the lake deemed representative of Eriocaulon beds, and these data then pooled to achieve an integrated value for this habitat type for the lake (France and Stokes in prep). If tradeoffs in effort must be made, Arnason (1988) warns that multiple samples per station cannot compensate for inadequate replication of stations (i.e. this is pseudo-replication). From the average variance function

presented in France (1987b), the requisite sample number needed to obtain a confidence of ± 20% of mean *Hyalella* density estimates for any single station, is between 6 and 12 (based on the levels of density encountered during the spring, and the use of a 7.62 cm diameter corer) A total of 40 to 60 samples per visit for the entire lake, maintaining at least 10-15 for each of 3 or 4 stations (France and Stokes in prep; Stephenson 1986), is recommended. Each sample takes less than 3 min to collect, sieve and store.

The sampling apparatus is a circular corer of PVC pipe which can be forced into the macrophyte bed with a handle. This sampler has been used to enumerate *Hyalella* in these lakes (Stephenson and Mackie 1986; France 1987a; France and Stokes 1988), and is described in detail in Stephenson (1986). The companion sieve (0.4 mm mesh) and mason jar storage technique was developed by G. Mackie (Univ. of Guelph, Guelph, Ontario; see Stephenson 1986) and has gained widespread use in a number of zoobenthos studies in Haliburton-Muskoka lakes.

SEQUENTIAL SAMPLING DESIGN AND METHODS

The desire for cost-effective sampling programs for benthic macroinvertebrates has become increasingly recognized (e.g. Cuff and Coleman 1979; Downing and Cyr 1985). One procedure with a long established tradition of use in applied entomological surveys is sequential sampling (Morris 1954; Reeks 1956; Ives and Prentice 1958; Shepard and Brown 1971; Allen *et al.* 1972; Sevarchrian and Stern 1972). Recently, Resh and Price (1984), Resh *et al.* (1988) and Jackson and Resh (1988,1989) have advocated the use of sequential sampling in the design of zoobenthcs biomonitoring programs.

The great strength of sequential sampling is in reducing the total number of samples needed to catagorize a population compared with the sample requirements for fixed sample-size procedures. As Jackson and Resh (1988) elaborated, for fixed sample-size designs, all collected samples are examined, statistical parameters estimated, and data then classified based on hypothesis testing. With sequential sampling, in contrast, decisions relating to a priori specified tests of hypotheses with known error risks, are iteratively performed after each sample is examined until the minimum information needed to make a classification is obtained. Data can be interpreted in a graphical format as a pair of decision lines enclosing a band of no-decision from which, with sequential sample assessment, the cumulative plotted points must "escape" before a density classification is satisfactorily defined (Morris 1954). Statistical parameters of the threshold classifications are calculated only once from baseline information. The advantage is that no time is wasted in analyzing superfluous samples in situations where the population density falls quickly and distinctly into a particular classification category. As long as the plotted points remain between the decision lines, sampling continues until some predetermined maximum is reached.

A related use of sequential sampling and the one that is the primary mandate of the present analysis, is in the combination of several independent tests to sort data into broad

categories with which one has considerable confidence in assigning densities and basing conclusions upon (Green 1979; Morris 1954; Waters 1955; Ives and Prentice 1958; Cole 1962). Armitage (1950) provided formulae for a combined sequential test involving 3 binomial distributions. However, he concluded that such a test was not very different from the method of a series of paired tests in parallel such as used in the present study.

Finally, sequential decision plans are cost-effective when the time spent collecting a zoobenthos sample in the field is minor relative to the time needed to examine that sample in the laboratory (Resh and Price 1984; Jackson and Resh 1989).

Three essential elements must be defined a priori in the creation of a sequential sampling program. First, the classification thresholds or class limits (which describe the decision lines) must be set using available ecological information. Data from a sampling program of 24 lakes ranging in average annual pH from 5.2 to 7.3 over 5 years (1984-1988) were used in this study (France 1987a, 1987b; France and Stokes 1988, in prep; France and LaZerte 1987; France, unpubl. data). As previously mentioned, only those data collected during the early spring at depths between 0.1 to 0.7 m depth and over regions of extensive Eriocaulon growth were considered. Reeks (1956) also expressed his animal densities per unit vegetative substrate. In addition, because acidification effects on benthic invertebrates cannot be examined in isolation (Yan *et al.* 1985b; France 1990a), lakes were stratified in relation to their system productivity.

Concentrations of average ice-free seasonal total phosphorus (TP) have been shown to be correlated with spring *Hyalella* densities (France and LaZerte 1987) and integrated biannual averages of biomass (France and Stokes, in prep). Lakes of higher productivity therefore support a higher density of *Hyalella* per unit macrophyte biomass. Study lakes ranged in TP from 5 to 21 mg m^{-3}, and were divided into the following divisions: < 7 mg m^{-3} (5 lakes), 7-11 mg m^{-3} (14 lakes), and > 11 mg m^{-3} (5 lakes). Density classification thresholds were therefore adjusted depending on the system productivity of each lake. To

my knowledge, only Sheperd and Brown (1971) have used such sliding class limits to enhance the accuracy of density prediction with sequential sampling.

There is no doubt that *Hyalella* contribute substantially to the diets of many centrachid and salmonid fishes (e.g. Cooper 1965; Strong 1971). Experimental manipulations also indicate to varying degrees, a reciprocal relationship between fish and *Hyalella* abundances (Ball and Hayne 1952; Hall *et al.* 1970; Milstead and Threlkeld 1986). None of these predation impacts have, however, been demonstrated to be permanent (see Thorp 1986). Similarly, evidence for a top-down control of *Hyalella* demography in Canadian Shield lakes is weak (France 1987a; France and Stokes in prep). This no doubt results from the complex heterogeneity of substrates, very low fish abundances, and establishment of long-term stability within these environments. Differences in *Hyalella* density among Canadian Shield lakes occur therefore for the most part, irrespective of variability in fish abundance.

Some lakes were visited only once during the spring pre-recruitment period while others were sampled up to 7 times during this interval over the years (France and Stokes 1988). Sample numbers ranged from 8 to 48 cores per visit. The total number of observations per lake ranged from 10 to 88. Serial classification thresholds were defined as being greater than 2SEs to achieve non-overlapping confidence intervals as a conservative adjustment for the effects of sampling error on the mean (Jackson and Resh 1988).

The degree to which sequential decision plans can be extended into new geographic areas or slightly different situations is an important consideration (Allen *et al.* 1972). *Hyalella* is the most ubiquitous freshwater amphipod in the Western Hemisphere, being distributed from the Northwestern Territories of Canada to Central America. In North America, *Hyalella* inhabits most permanent bodies of water that have mean monthly temperatures exceeding 10 °C (Bousfield 1958). The use of this species for biomonitoring purposes is therefore very appealing. As Morris (1954) indicated, knowledge of local

conditions should govern the application of defined decision limits, or in turn, the establishment of alternative limits. For example, inter-regional differences exist in *Hyalella* demography due to climatic influences (Lindeman and Momot 1983; France in prep). As a result, the threshold criteria in Fig. 1 should apply to only north-temperate populations (< 90 d above 20 °C water temperature) inhabiting oligo-mesotrophic, softwater lakes.

The second step in designing a sequential plan is to establish the mathematical distribution of the data. Considering the aggregated pattern of distribution for both *Hyalella* density (France 1987a; France and Stokes 1988) and Eriocaulon biomass (France 1988), it is not surprising that proportional densities (number of amphipods per gram macrophyte) were found to conform to a negative binomial distribution. Estimates of the dispersion index, k, ranged from 0.3 to 2.6. Because the mean proportional density varied with 1/k, it was not possible to calculate a common k_c for all the data (Elliott 1979); i.e. there is no constant and characteristic level of aggregation for *Hyalella* populations (France 1987a). Dispersion indices did stabilize, however, within the following groupings of density (Fig. 1): $k_c = 1.4$ for Categories 1 and 2; $k_c = 1.0$ for Categories 3 and 4; and $k_c = 0.5$ for Categories 5 to 7.

Finally, the probability of classification errors (Type I and II) in sequential sampling plans (see Jackson and Resh 1988) were set at 0.05. Together, the classification thresholds, α, β, and k, are combined to calculate the decision lines (Fig. 1) using the formulae presented in Oakland (1950), Morris (1954) or Jackson and Resh (1988, 1989).

The theoretical minimum number of samples required to make a decision (intersection of lower of the pair of decision lines with the X-axis on Fig. 1) ranged from 24 to 1 with increasing density. Due to the overlap in the decision lines, in reality the minimum sample number was always about 20. The maximum average sample number (ASN), calculated from the decision lines and error risks from the operating characteristic curve (see Waters 1955; Jackson and Resh 1988, 1989), ranged from 36 for the first pair of decision lines

(between Categories 1 and 2) to 13-18 for the second to sixth pairs of decision lines.

Twenty simulations were run based on a sample size of 48 (i.e. designed to reflect sampling 4 different locations in a lake with 12 samples in each) to obtain a modal density rank. In those situations where the total number of sample units taken were less than 48 (the "B" lakes identified in France and Stokes 1988), simulations were run with replacement; in the other situations, without. If the cumulative total remained within the no-decision category after 48 samples, the population was assigned to one of the two adjacent categories which it was closest to based on the truncated decision method (Jackson and Resh 1988).

RESULTS

Despite some intrapopulation variability in density ranking, an obvious modal rank was always apparent (Fig. 2). The percentage of no-decisions in which classification was determined through truncation, ranged from < 15% for populations at the extremes of density (e.g. Plastic and Glen), 15-25% for those populations in which the simulations indicated 3 density rankings (e.g. Leonard and Heney), and from 20-45% for those populations in which the simulations indicated densities spread over 4 rankings (e.g. Red Chalk Main and Little Clear).

The modal rankings of density determined from sequential sampling were found to be related to lake pH (Fig. 3). Lakes with "Nil" or "Present" rankings ranged in pH from 5.2 to 5.8; lakes with "Rare" or "Common" rankings ranged in pH from 5.7 to 6.4; and lakes with "Abundant" and "Very Abundant" rankings ranged in pH from 6.1 to 7.3. The density ranking of lakes specifically identified in Fig. 3 corresponds to detailed information on life history and tolerance patterns of *Hyalella* in these lakes (see Discussion).

DISCUSSION

Biomonitor Sampling

A reciprocity exists between depth and breadth in scientific research involving tradeoffs between precision and generality. Often methods used for intensive population studies are not suitable for applied surveys (Ives and Prentice 1958). Because sequential sampling serves to classify populations rather than to provide estimates of population parameters, it is particularly amenable to survey mandates (Waters 1955). The widespread extent of acidification damage to Canadian aquatic resources (Dillon *et al.* 1987; Neary and Dillon 1988) dictates that certain sacrifices in descriptive detail must be made to increase the frame of reference from which general conclusions about the severity of these impacts can be drawn. The danger lies in trading off too much scientific acumen in the pursuit of "band-aid, fire-fighting efforts" (Vallentyne 1978). Abandonment of quantitative power for logistic ease is, in the end, self-defeating, and the reason why some synoptic work (i.e. impact assessment) has borne the brunt of much harsh criticism in the past (Schindler 1987, 1976; Larkin 1984).

Stephenson and Mackie (1986), Fiske (1987) and Grapentine (1987) all used a scoop-like sieve or a D-frame sweep-net raked across the surface of the substrate to assess the presence or absence of *Hyalella* along gradients of lake alkalinity. Both Stephenson and Mackie (1986) and Fiske (1987), correctly recognizing the strictly qualitative nature of such sampling methods (*see* Lenat 1988; Marmorek *et al.* 1988), supported their conclusions with additional independent quantitative estimates of *Hyalella* density as did Peterson (1987). An extensive survey program is currently being initiated by the Ontario Ministry of the Environment (Mierle in Marmorek *et al.* 1988) patterned after the similar programs by the Canadian Dept. of Fisheries and Oceans (Davies in Marmorek *et al.* 1988) and the U.S. EPA's TIME (Temporally Integrated Monitoring of Ecosystems) Project (Thorton *et al.* in Marmorek *et al.* 1988).

One design of this program is, through use of similar sweep techniques, to apply an ordinal scale to zoobenthos (including *Hyalella*) abundance in attempt to increase the conclusive power of the data set over an appraisal of simple (either all or none) presence or absence (G. Mierle, Dorset Research Centre, Ontario Min. Environ., pers. comm.). However, the final catches from such procedures are influenced by, among other things, the arc, angle and speed of each sweep, the depth of penetration of the scraping edge into the sediment or macrophyte bed, questions as to whether each successive sweep covers a completely new or previously sampled area, and the resulting organism escape response due to cumulative habitat disturbance.

Unless methods can be developed to quantify these errors and biases (simply timing the sweep period for a CPUE is not sufficient, Lenat 1988), ordinal abundance rankings generated from sweep techniques cannot be related in a defensible way to interlake differences in water quality. Bias distorts probability statements and some biased estimates, although logistically easier than unbiased ones, may cause incorrect management decisions (Fowler and Witter 1952). Such sub-optimal plans are often less sensitive and interpretable than those which might have been possible for the given level of effort (Arnason 1988).

In contrast, with little or no additional effort, a truly quantitative ordinal ranking of *Hyalella* density can be obtained through adoption of a sequential sampling design based on core samples not sweeps. Stephenson and Mackie (1986) believed it necessary to spend up to 60 min of search time with a scoop-sieve at a single site to adequately assess the presence or absence of *Hyalella*. During this same interval one could collect up to 30 quantitative cores. The effort required to sort and enumerate adult *Hyalella* from a pooled sweep collection is only about a third less in terms of time than that required to do the same for an identical number of *Hyalella* spread over a series of independent core samples. Criticisms leveled against conclusions reached in synoptic monitoring of lake acidification (e.g. Howells 1982, 1984; EPRI 1986) could often be circumvented through increased rigor in sampling design and execution. Similar attention paid to the methodology of zoobenthos

sampling programs (e.g. Downing 1979; Resh 1979) would likewise increase the scientific defensibility of such research (France 1990b). Decisions based on quantitative sequential sampling from core data will prove more profitable toward such an end than merely relying upon qualitative sweep-net techniques. Relative abundance estimates, erroneously perceived by Marmorek *et al.* (1988) "not [to] require quantitative sampling methods", are at best but weak substitutes (see Mozley 1974).

Sequential sampling

Jackson and Resh (1988) correctly identified an important caveat in acceptance of sequential decisions; this involved consideration of the effects of sampling error and spatial and temporal variation on the suitability of classification thresholds. Despite narrowing the time window of sampling to only the spring pre-recruitment period and selecting thresholds separated by 2SE, the present simulations showed that ordinal rankings still displayed variability for any single *Hyalella* population. This no doubt resulted from the highly skewed density distribution of *Hyalella* in these lakes (France 1987b; France and Stokes 1988). In all cases, however, a definite modal rank was always discernable following 20 iterations. For example, France and LaZerte (1987) calculated the mean proportional density ratio to be 2.49 ± 0.45 (SE) (n = 12) for Blue Chalk Lake, a result consistent with the present ascription of the modal rank of "Abundant" to that population.

Allen *et al.* (1972) also used frequency distributions in their sequential decision assessments. Some of the present ambiguity could of course be removed by collapsing several of the decision categories. Resh and Price (1984) and Jackson and Resh (1988) used only two divisions - "impacted" and "unimpacted". Alternatively, once a decision has been made using a division of multiple categories, and sample examination ceased, simulation iterations could be undertaken as in the present study to achieve a modal abundance rank (i.e. in a sense, a simplified form of bootstrapping). Similarly, Fowler (1983) used Monte Carlo simulations to reduce the number of samples needed in field surveys.

The final, modally-ranked, assessment of *Hyalella* density (stratified for lake productivity) correctly identified those populations which, through more labor-intensive research efforts on life history and tolerance patterns, are known to experience acidification stress. Amphipods in Glen Lake are extremely abundant (France, unpubl. data) and have a high acid sensitivity as a result of never being exposed to extensive littoral pH depressions in the spring (France and Stokes 1987a).

Hyalella from Harp and Blue Chalk lakes are abundant and have a moderate acid sensitivity (France and Stokes in prep; 1987a; Stephenson and Mackie 1986; Mackie 1989). *Hyalella* inhabiting Crosson and Heney lakes have greater acid tolerances relative to animals from the other lakes (France and Stokes 1987a; Mackie 1989), perhaps due to heightened mortality during the springmelt period (France and Stokes in prep) which may have induced adaptation (France 1987c). Estimates of secondary production are low for these latter populations (France and Stokes in prep; Stephenson 1986). *Hyalella* in Plastic Lake are at an extremely low level of abundance (Stephenson and Mackie 1986), possibly due to increased mortality from acid snowmelt (France and LaZerte 1987).

Peterson's (1987) data although based on only a limited sampling effort (5 Birge-Ekman hauls) of 145 maritime Canadian lakes (with no error terms provided), indicated that "the abundance of *Hyalella* exhibited a graded decrease with decreasing lake pH". This is consistent with the present findings.

Modal density ranks are best calculated from between 40 to 60 independent samples but can be approximated through simulations (at least 20 such) on fewer individual samples by using replacement. Important for the design of synoptic surveys is the finding that there were no differences in the ascription of density ranks to either intensively or sporadically sampled lakes in relation to their pH values (Fig. 3). A sequential sampling program can therefore be quite effective in ordinal description of *Hyalella* abundance with only a moderate effort.

The maximum ASN value has been suggested as a truncation point to cease sampling (Waters in Resh and Price 1984; Jackson and Resh 1988). Characteristically, in sequential sampling programs designed to detect reductions of > 40% in macroinvertebrate densities, ASN's range from 7 to 16 (Resh and Price 1984; Jackson and Resh 1988, 1989). Attempting to identify smaller density differences results in maximum ASN's that are well above this range (in the present case, exceeding 20 to 30 samples in certain situations). Also, as Waters (1955) explains, the ASH is directly related to the gap of no-decision and the desired degree of reliability. Narrowing the gap (as done here through using multiple tests) or lowering the risks of error will increase the average amount of sampling required.

Morris (1954) cautioned against strong adherence to ASN truncation decisions because he found this sample number had to be "considerably exceeded at certain sampling points". Due to the nested overlapping nature of the decision lines near the origin, the absolute minimum number of samples needed to discriminate density ranks is 20, regardless of ASN's calculated for individual pairs of decision lines. Increasing the sampling effort to 40-60 if possible, thereby avoiding theoretical difficulties in using replacement in the simulation analysis, is recommended. Such a program entails about 1-2 hr of sampling time per lake. Although the final number of samples enumerated in the laboratory is frequently less than this, in several situations truncation was still necessary at the present arbitrary termination point of 48 samples. This is no cause for alarm as classifications using truncated decisions (based on smaller termination sample sizes or ASN's than those here) have been found to be correct in 71 to 95% of the cases (Jackson and Resh 1988; Connola *et al.* 1959).

In the present analysis, enumeration of 48 samples was occasionally close to the sample number required for fixed-end point parametric tests to determine whether lakes had significantly different *Hyalella* densities. My use of sequential sampling was not, however, primarily motivated by the common desire to reduce the number of samples needed to compare populations. Instead, my purpose was to design an objective procedure whereby populations could be quantitatively classified into a series of density categories for

development of more comprehensive biomonitoring methods (see below). In this respect, design of the present sequential 19 decision plan for amphipod density should be viewed as a means, not an end to itself.

Biological Integrity

Due to recognition that no single organism is capable of satisfactorily reflecting all the manifestations of cultural stress in aquatic environments (Ryder and Edwards 1985), there is a pressing need for biomonitoring to embrace an ecosystem approach, and as a result, to give more consideration to integrative and holistic interpretations of "health" (sensu Schaffer *et al.* 1988) at the community level of organization. Recently, the term "integrity" has become ensconed as a directive concept in environmental decision making, in addition to being used as an operationally defined managerial tool in biomonitoring protocols (e.g. France 1990b; Steedman 1988).

The most effective approach for maintenance of biological integrity as dictated in several water quality mandates (Regier and France 1990; NRC 1985), is to ensure that a suite of representative species are monitored at various trophic levels. In this respect, it is erroneous, if not dangerous, to perceive some ranking of *Hyalella* abundance in isolation, as a sort of panacea approach for judging the extent of biological degradation resulting from lake acidification. Instead, *Hyalella* density rankings should be viewed as only one of a suite of metrics (see Steedman 1988) which together provide an agglomerative description of biological integrity. Examples of other potential acidification metrics might include diatom stratigraphy scores (Earle *et al.* 1988), metaphyton biomass ranks (France and Stokes under review), crayfish carapace rigidity (France 1987d), and mysid or gastropod densities (Nero and Schindler 1983; Peterson 1987).

Combining field monitoring and laboratory toxicity testing in concert is essential to understand whether legislative criteria are productive in mitigating environmental

disturbance (France 1986). Finally, use of indicator species such as *Hyalella* should never be advanced as a surrogate for direct measures of water chemistry whenever the latter are possible. Together both techniques - chemical and biological surveillance/monitoring, comprise the most realistic means we have of achieving a comprehensive assessment of ecosystem integrity (Karr 1987; Ryder and Edwards 1985).

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LIST OF FIGURES

Fig. 1. Sequential sampling plan for monitoring the impact of anthropogenic acidification on the density of *Hyalella azteca* in Canadian Shield lakes. Classification thresholds (average number of amphipods per gram macrophyte), decision line regression equations (= B = 0.5; d = cumulative amphipod count; see text for K_c values), and categorization "windows" (1 - 7) are indicated. For lakes with total phosphorus (TP) concentrations < 7 mg m^{-3}, the categorization windows correspond to density ranks as follows:
1 = Present (P),
2 = Rare (R),
3 = Common (C),
4 = Abundant (A), and
5+ = Very Abundant (VA);
for lakes with 7-11 mg m^{-3} : 1 - 2 = P, 3 = R, 4 = C, 5 = A, and 6 - 7 = VA; and for lakes with > 11 mg m^{-3} : 1 - 3 = P, 4 = R, 5 = C, 6 = A, and 7 = VA.

FIGURE NEXT PAGE

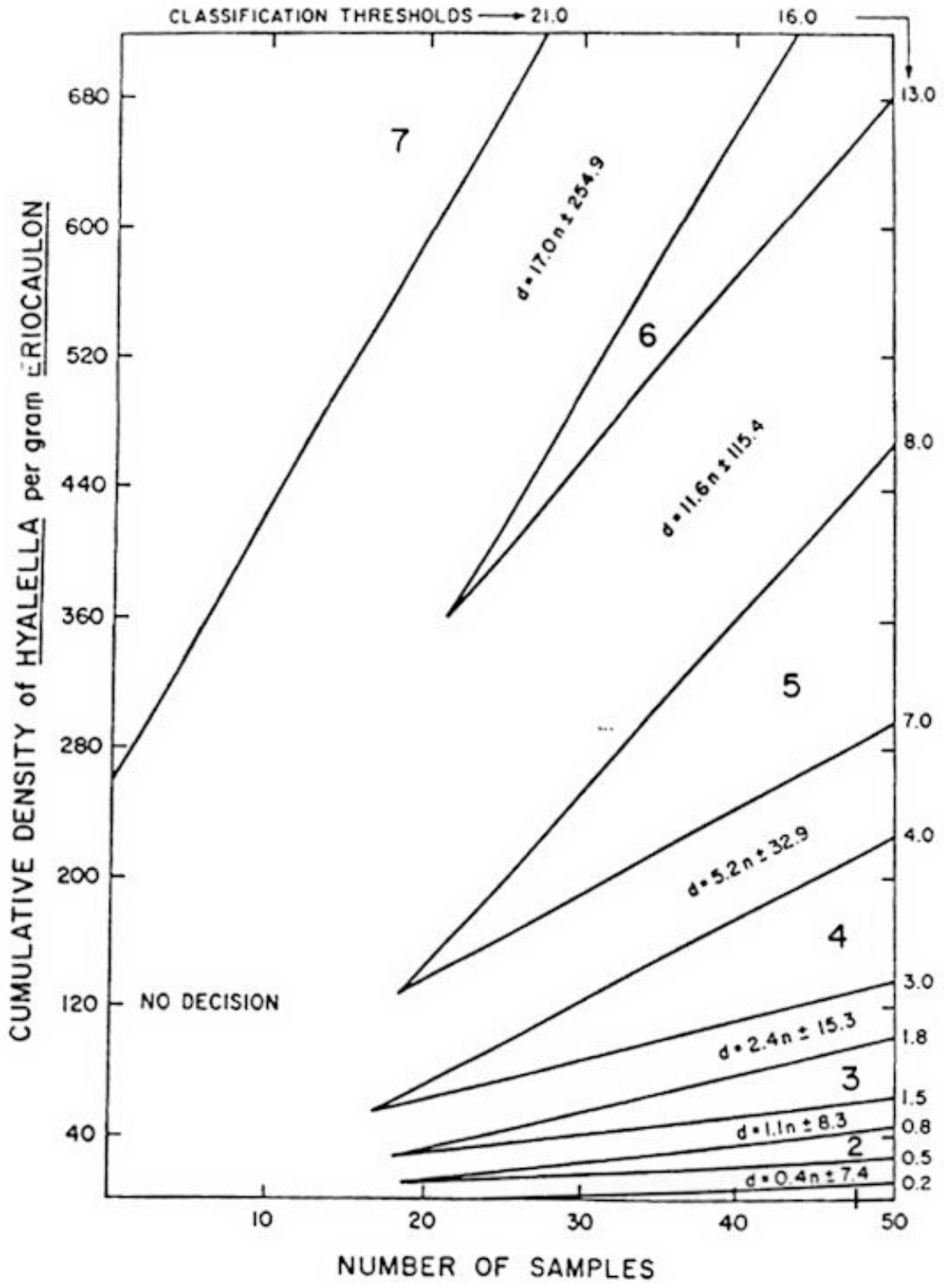

CLASSIFICATION THRESHOLDS → 21.0
16.0
13.0
8.0
7.0
4.0
3.0
1.8
1.5
0.8
0.5
0.2
7
6
5
4
3
2
d = 17.0n ± 254.9
d = 11.6n ± 115.4
d = 5.2n ± 32.9
d = 2.4n ± 15.3
d = 1.1n ± 8.3
d = 0.4n ± 7.4
NO DECISION
CUMULATIVE DENSITY of HYALELLA per gram ERIOCAULON
680
600
520
440
360
280
200
120
40
10
20
30
40
50
NUMBER OF SAMPLES

Fig. 2. Distribution of density ranks from 20 simulations of sequential sampling for selected amphipod populations in the Haliburton-Muskoka region of central Ontario. Density ranks abbreviated as in Fig. 1.

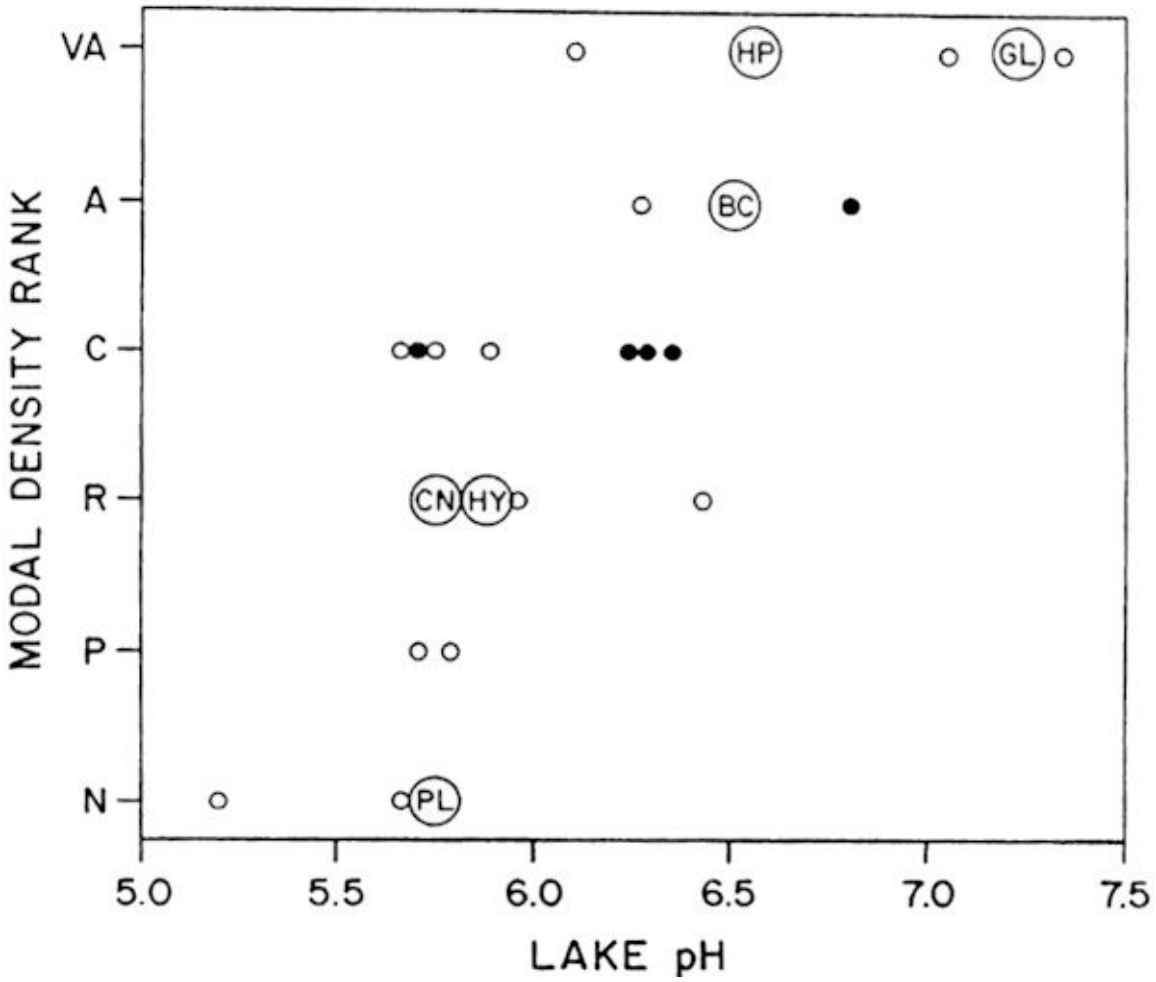
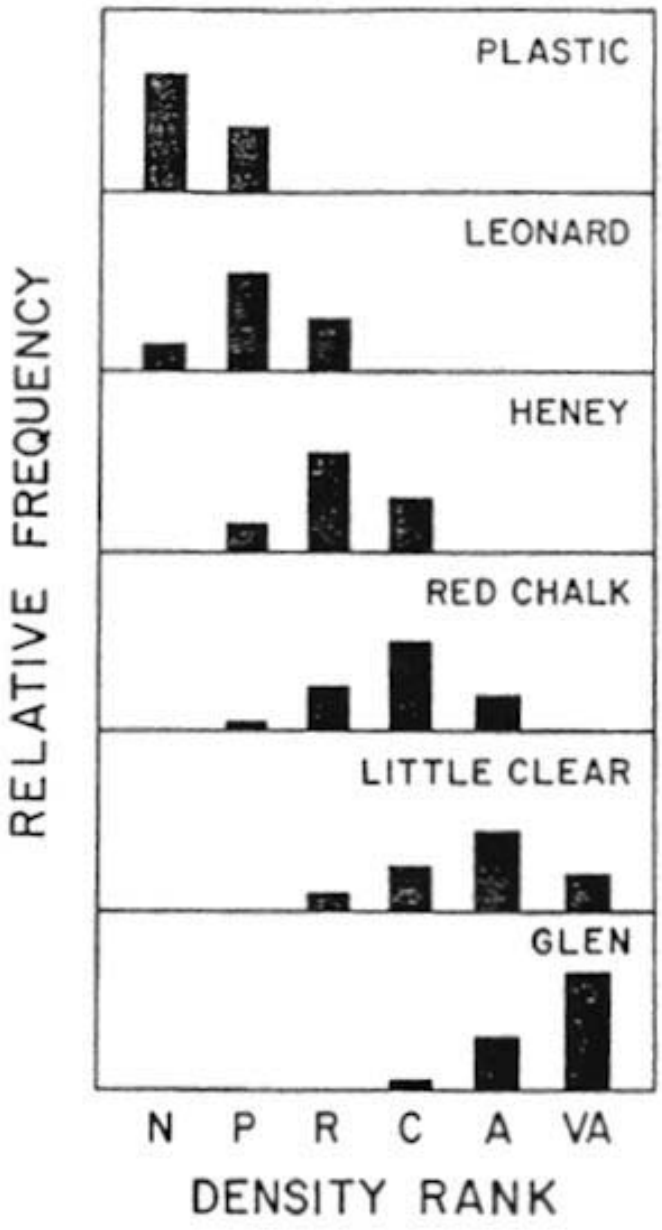


Fig. 3. Relationship of modal density rank and lake pH. Identified lakes - Harp (HP), Glen (GL), Blue Chalk (BC), Crosson (CIS), Heney (HY), and Plastic (PL) contain *Hyalella* populations which have been previously described in the literature (see text for references). Closed and open circles denote intensively and sporadically sampled populations, respectively. Density ranks abbreviated as in Fig. 1.



REFERENCES

Allen J., Gonzaliz D. and Gokhale D.V. 1972. Sequential sampling plan for the worm, *Heliothis zea*. Environmental Entomology 1:771-780.

Arnason A.N. 1988. Some perceived needs in sampling methodology for biomonitoring. *In* Marmorek D.R., Bernard D.P. and Ford J. Biological monitoring for acidification effects: U.S. - Canadian Workshop. March 21-23, 1988, Burlington, Ontario. U.S. EPA, Environ. Res. Lab., Corvallis, Oregon.

Armitage P. 1950. Sequential sampling with more than two alternative hypotheses, and its relation to discriminant function analysis. Journal Royal Statistical Society Series B 12:137-144.

Ball P.C. and Hayne D.W. 1952. Effects of the removal of the fish population on the fish food organisms of a lake. Ecology 33:41-48.

Biette P.M. 1969. Life history and habitat differences between *Gammarus lacustris* (Sara) and *Hyalella azteca* (Saussure) in West Blue Lake, Manitoba MSc. Thesis, Univ. of Manitoba, Winnipeg, Manitoba.

Bousfield E.L. 1958. Fresh-water amphipod crustaceans of glaciated North America. Canadian Field-Naturalist 72:55-113.

Cole L.C. 1962. A closed sequential test design for tolerance experiments. Ecology 38:226-233.

Cooper W.E. 1965. Dynamics and production of a natural population of a freshwater amphipod, *Hyalella* azteca. Ecological Monographs 35:377-394.

Cyr H. and Downing J. 1988. Empirical relationships of phytomacrofaunal abundance to plant biomass and macrophyte bed characteristics. Canadian Journal Fisheries Aquatic Science 45:976-984.

Cuff W. and Coleman N. 1979. Optimal survey design: lessons from a stratified random sample of macrobenthos. Journal Fisheries Research Board Canada 36:351-361.

de March B.G.E. 1978. The effects of constant and variable temperature on size, growth, and reproduction of the freshwater amphipod *Hyalella azteca* (Saussure). Canadian Journal Zoology 56:1801-1806.

Dermott R.M. 1988. Zoobenthos distribution and biomass in the Turkey Lakes. Canadian Journal Fisheries Aquatic Science 45(Suppl. 1):107-114.

Dillon P.J., Reid R.A. and de Grobois E. 1987. The rate of acidification of aquatic ecosystems in Ontario. Nature (london) 329:45-48.

Downing J.A. 1979. Aggregation, transformation and the design of benthos sampling programs. Journal Fisheries Research Board Canada 36:1454-1463.

Downing J.A. 1986. A regression technique for the estimation of epiphytic invertebrate populations. Freshwater Biology 16:161-173.

Downing J.A. and Cyr H. 1985. Quantitative estimation of epiphytic invertebrate populations. Canadian Journal Fisheries Research Board Canada 42:1570-1579.

EPRI 1986. An evaluation and compilation of the effects of acidification on aquatic biota. Vol. 2. Evaluation forms. Electric Power Research Institute. EA - 4825, Vol. 2. 465 p.

Earle J.C., Duthie H.C., Glooschenko W.A. and Hamilton P.B. 1988. Factors affecting the spatial distribution of diatoms on the surface sediments of three Precambrian Shield lakes. Canadian Journal Fisheries Aquatic Science 45:469-478.

Elliott J.M. 1979. Some methods for the statistical analysis of samples of benthic invertebrates. Sci. Publ. Freshwater Biological Association 25:156p.

Fiske S. 1987. Macroinvertebrate survey of 25 soft water-pH sensitive lakes in Vermont. Vermont Acid Ppt. Prog., Long-term Lake Monitoring. 34p.

Fowler G.W. 1983. Accuracy of sequential sampling plans based on Wald's sequential probability ratio test. Canadian Journal Forestry Research 13:1197-1203.

Fowler G.W. and Witter J.A. 1982. Accuracy and precision of insect density and impact estimates. Great Lakes Entomology 15:103-117.

France R.L. 1986. Current status of methods of toxicological research on freshwater crayfish. Canadian Technical Report Fisheries Aquatic Science 1404: iv + 20p.

France R.L. 1987a. Test of biotic and abiotic environmental determinants of amphipod (*Hyalella azteca*) preamplexus. Canadian Journal Fisheries Aquatic Science 44:478-482.

France R.L. 1987b. Aggregation in littoral amphipod populations: transformation controversies revisited. Canadian Journal Fisheries Aquatic Science 45:1510-1515.

France R.L. 1987c. Differences in H-ion sensitivity among *Hyalella azteca* populations: an illative hypothesis invoking natural selection. Ecophysiology of Acid Stress in Aquatic Organisms. Annals Society Royal Zoology Belgium 117 (Suppl. 1):129-137.

France R.L. 1987d. Calcium and trace metal composition of the crayfish *Orconectes virilis* in relation to experimental lake acidification. Canadian Journal Fisheries Aquatic Science 44(Suppl. 1):107-113.

France R.L. 1988. Biomass variance function for aquatic macrophytes in Ontario (Canada) Shield lakes. Aquatic Botany 32:99-114.

France R.L. 1990a. Littoral zoobenthos density, biomass and mean body weight within low alkalinity, oligotrophic lakes on the Canadian Shield. Archives Hydrobiologica 118:477-499.

France R.L. 1990b. Theoretical framework for developing and operationalizing zoobenthos community integrity as a continuous monitoring tool for the Great Lakes Areas of Concern. p. 169-194, *In:* C.J. Edwards and H.A. Regier [eds.] An ecosystem approach to the integrity of the Great Lakes in turbulent times. Great Lakes Fisheries Commission Special Publication 90-4.

France R.L. and LaZerte B. 1987. Empirical hypothesis to explain the restricted distribution of *Hyalella azteca* (Amphipoda) in anthropogenically acidified lakes. Canadian Journal Fisheries Aquatic Science 44:1112-1121.

France R.L. and Stokes P.M. 1987a. Life stage and population variation in resistance and tolerance of *Hyalella azteca* (Amphipoda) to low pH. Canadian Journal Fisheries Aquatic Science 44:1102-1111.

France R.L. and Stokes P.M. 1987b. Influence of manganese, calcium, and aluminum on hydrogen ion toxicity to the amphipod *Hyalella azteca*. Canadian Journal Zoology 65:3071-3078.

France R.L. and Stokes P.M. 1988. Isoetid-zoobenthos associations in acid-sensitive Ontario, Canada lakes. Aquatic Botany 32:99-114.

Grapentine L.C. 1987. Consequences of environmental acidification to the freshwater amphipod *Hyalella azteca*. MSc. Thesis, Univ. of Manitoba, Winnipeg, Manitoba. 173pp.

Green R.H. 1979. Sampling design and statistical methods for the environmental biology. Wiley, New York, New York. 257p.

Hall D.J., Cooper W.E. and Werner E.E. 1970. An experimental approach to the production dynamics and structure of freshwater animal communities. Limnology and Oceanography 15:839-928.

Hitchin G.G., Wile I., Miller G.E. and Yan N.D. 1984. Macrophyte data from 46 south Ontario soft-water lakes of varying pH. Ont. Min. Environ., Data Rep. DR 84/1.

Howells G.D. 1982. Interpretation of aquatic pH trends. Transactions American Fisheries Society 111:779-781.

Howells G.D. 1984. Fishery decline: mechanisms and predictions. Philosophical Transactions Royal Society London B 305:529-547.

Ives V.G.H. and Prentice R.M. 1958. A sequential sampling technique for surveys of the larch sawfly. The Canadian Entomologist 90:331-338.

Jackson J.K. and Resh V.H. 1988. Sequential decision plans in monitoring benthic macroinvertebrates: cost savings, classification accuracy, and development of plans. Canadian Journal Fisheries Aquatic Science 45:280-286.

Jackson, J.K. and V.H. Resh. 1989. Sequential decision plans, benthic macroinvertebrates, and biological monitoring programs. Environmental Management 13:455-468.

Jeffries D.S., Cox C.M. and Dillon P.J. 1979. Depression of pH in lakes and streams in central

Ontario during snowmelt. Journal Fisheries Research Board Canada 36:640-666.

Karr J.R. 1987. Biological monitoring and environmental assessment: a conceptual framework. Environmental Management 11:249-256.

Larkin P.A. 1984. A commentary on environmental impact assessment for large projects affecting lakes and streams. Canadian Journal Fisheries Aquatic Science 41:1121-1127.

Lindeman D.H. and Momot W.T. 1983. Production of the amphipod *Hyalella acteca* (Saussure) in a northern Ontario lake. Canadian Journal Zoology 61:2051-2059.

Mackie, G.L. 1989. Tolerances of five benthic invertebrates to hydrogen ions and metals (Cd, Pb, Al). Archives Environmental Contamination Toxicology 18:215-223.

Marmorek D.R., Bernard D.P. and Ford J. 1q88. Biological monitoring for acidification effects: U.S. - Canadian Workshop, March 21-23, 1988, Burlington, Ontario. U.S. EPA, Environ. Res. Lab., Corvallis, Oregon.

Milstead B. and Threlkeld S.T. 1986. An experimental analysis of darter predation on *Hyalella* azteca using semipermeable enclosures. Journal North American Benthological Society 5:311-318.

Minns C.K., Kelso J.R.M. and Johnson M.G. 1986. Large-scale risk assessment of acid rain impacts on fisheries: models and lessons. Canadian Journal Fisheries Aquatic Science 43:900-921.

Morris R.F. 1954. A sequential sampling technique for spruce budworm egg surveys. Canadian Journal Zoology 32:302-313.

Mozley, S.C. 1974. Preoperational distribution of benthic macroinvertebrates in Lake Michigan near the Cook nuclear power plant. p. 5-138 in Seibel, E. and J. Ayers [eds.] The biological, chemical, and physical character of Lake Michigan in the vicinity of the Donald C. Cook nuclear plant. Univ. Michigan Great Lakes Research Division Special Report 51.

NRC 1985. The Great Lakes Water Quality Agreement. An evolving instrument for ecosystem management. National Academy Press. Wash., D.C.

Neary B.P. and P.J. Dillon. 1988. Effects of sulphur deposition on lake-water chemistry in Ontario, Canada. Nature 333:340-343.

Nero R.W. and D.W. Schindler. 1983. Decline of *Nysis relicta* during the acidification of Lake 223. Canadian Journal Fisheries Aquatic Science 40:1905-1911.

Nichols S.A. 1982. Sampling characteristics of macrophyte biomass. Water Research Bulletin 18:521-523.

Oakland G.B. 1950. An application of sequential analysis to whitefish sampling. Biometrics 6:59-67.

Peterson, R.H. 1987. Influence of water pH on frequency of collection of certain invertebrates during lake and stream surveys in New Brunswick and Nova Scotia. Canadian Technical Report Fisheries Aquatic Science No. 1523. 7p.

Reeks V.A. 1956. Sequential sampling for larvae of the winter moth *Opprophtera brumata* (Linn.) (Lepidoptera: Glometridae). The Canadian Entomologist 88:241-246.

Regier H.A. and France R.L. 1990. Perspectives on the meaning of ecosystem integrity: 1975. p. 1-16, *In*: C.J. Edwards and H.A. Regier [eds.] An ecosystem approach to the integrity of the Great Lakes in turbulent times. Great Lakes Fisheries Commission Special Publication 90-4.

Resh V.H. 1979. Sampling variability and life history features: basic considerations in the design of aquatic insect studies. Journal Fisheries Research Board Canada 36:290-311.

Resh V.H. and Price D.G. 1984. Sequential sampling: a cost-effective approach for monitoring benthic macroinvertebrates in environmental impact assessments. Environmental Management 8:75-80.

Resh V.H., Jackson J.H. and McElravy E.P. 1988. The use of long-term ecological data and sequential decision plans in monitoring the impact of geothermal energy development on benthic invertebrates. Verh. Internat. Verein. Limnol. 23:1142-1146.

Ryder R.A. and Edwards C.J. 1985. A conceptual approach for the application of biological

indicators of ecosystem quality in the Great Lakes Basin. International Joint Commission, Windsor, Ontario.

Schaffer D.J., Herricks E.E. and Kerster H.W. 1988. Ecosystem health: 1. Measuring ecosystem health. Environmental Management 12:445-455.

Schindler D.W. 1976. The impact statement boondoggle. Science 192:509.

Schindler, D.W. 1987. Detecting ecosystem responses to anthropogenic stress. Canadian Journal Fisheries Aquatic Science 44(Suppl. 1): 6-25.

Sevacherian V. and V.M. Stern. 1972. Sequential sampling plans for Lygus bugs in California cotton fields. Environmental Entomology 1:704-710.

Shepard P.R. and Brown C.E. 1971. Sequential egg-band sampling and probability methods of predicting defoliation by Malacosoma disstria (Lasiocompidae: Lepidoptera). The Canadian Entomologist 103:1371-1379.

Steedman R.J. 1988. Modification and assessment of an index of biotic integrity to quantify stream quality in southern Ontario. Canadian Journal Fisheries Aquatic Science 45:492-501.

Stephenson M. 1986. Distribution, production and cadmium concentrations of *Hyalella azteca* (Crusteacea: Amphipoda) in central Ontario softwater lakes. Ph. D. Thesis. Univ. of Guelph, Guelph, Ontario. 212p.

Stephenson M. and Mackie G.L. 1986. Lake acidification as a limiting factor in the distribution of the freshwater amphipod *Hyalella azteca*. Can. J. Fish. Aquat. Sci. 43:288-292.

Strong Jr. D.R. 1971. Differentiation of reproductive characteristics among populations of the fresh-water amphipod *Hyalella azteca*. Ph. D. Thesis. Univ. of Oregon. 79p.

Thorp J.H. 1986. Two distinct roles for predators in freshwater assemblages. Oikos 47:75-82.

Vallentyne J.R. 1978. Facing the long-term: an inquiry into opportunity to improve the climate for research with reference to limnology in Canada. Journal Fisheries Research

Board Canada 35:350-369.

Wales D.L. and Beggs G.L. 1986. Fish species distribution in relation to lake acidity in Ontario. Water, Air and Soil Pollution 30:601-609.

Yan N.D., Miller G.E., Wile I. and Hitchin G.G. 1985a. Richness of aquatic macrophyte flora of soft water lakes of differing pH and trace metal content in Ontario, Canada. Aquatic Botany 23:27-40.

Yan N.D., Nero R.W., Keller W. and Lasenby D.C. 1985b. Are Chaoborus larvae more abundant in acidified than in non-acidified lakes in central Canada? Holarctic Ecology 8:93-99.

Waters W.E. 1955. Sequential sampling in forest insect surveys. Forestry Science 1:68-79.

APPENDIX

Decision plan formulae for negative binominal data (see Jackson and Resh 1988, 1989 for more detailed presentation).

(1) y - intercepts (h_1, h_2), slope (b) of decision lines:

$$h_1 = \frac{\log [B/(1 - \alpha)]}{\log [p_2 q_1 / p_1 q_2]} \; ; \quad h_2 = \frac{\log [(1 - \beta)/\alpha]}{\log [p_2 q_1 / p_1 q_2]} \; ; \quad b - k = \frac{\log [q_2 / q_1]}{\log [p_2 q_1 / p_1 q_2]}$$

where $p_1 = m_1 / k$, $q_1 = 1 + p_1$, $p_2 = m_2 / k$, $q_2 = 1 + p_2$, m_1 is the lower classification threshold, m_2 is the upper classification threshold, k is the distribution index, α and β are risks of mis-classification.

The equation for the lower decision line is $d_1 = b(n) + h_1$ and the upper decision line is $d_2 = b(n) + h_2$, where d is the cumulative total when n samples are examined (see Fig. 1).

(2) the OC curve is derived with various values of X from 10.0 to-10.0 (see Jackson and Resh 1988) from plotting L(m) (the probability of accepting H_1) versus various means (m) where

$$L(m) = [A^x - 1] / [A^x - B^x] \quad \text{where} \quad A = [1 - \beta] / \alpha \; ; \quad B = \beta / [1 - \alpha]$$

$$m = Kp \quad \text{with} \quad p = \frac{1 - (q_1 / q_2)}{[p_2 q_1 / p_1 q_2]^x - 1}$$

The ASN curve is derived by plotting E(n) versus various means (m) where

$$E(n) = \frac{h_2 + (h_1 - h_2) L(m)}{m - b}$$