Aquatic macroinvertebrates; their potential application in monitoring long term river ecosystem change in Denali National Park, Alaska.

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INTRODUCTION

In recent years there has been a shift in approaches to assess aquatic ecosystem health and integrity from chemical and physical monitoring to an ecosystem approach that addresses the complexity of ecological interactions, the intrinsic importance of humans within the ecosystems and the need for a balanced view to resource management (Karr 1991, Calow 1992). Karr (1995 in Scrimgeour and Wicklum 1996) suggests that ecosystem health and integrity are different in that health describes the preferred condition considered acceptable by humans (but where anthropogenic modification may have occurred) whereas integrity describes a condition where the organisms at any particular site are products of evolutionary and biogeographical processes with little or no influence from humans. Steedman (1994) considers that the concept of ecosystem health is now becoming central to natural resource management, particularly where there has been a history of anthropogenic disturbances.

What are the appropriate indicators of ecosystem health and integrity in rivers? Structural and functional attributes of biotic communties are important indicators; structural components are considered more responsive to early ecosystem stress than functional ones (Scrimgeour and Wicklum 1996). Structural attributes include density, taxa richness, diversity and indicator taxa while functional attributes include species and trophic equilibrium and production (Milner 1996). However functional attributes are important to overall ecosystem health. Candidate structural components of the aquatic community for monitoring include diatoms, macrophytes, bryophytes, protozoa, meiofauna, macroinvertebrates and fish.

Macroinvertebrates have recently become a popular tool for assessing aquatic ecosystem health and integrity, because communities are relatively sedentary, comprise a range of species with varying tolerances to pollutants, and occupy a variety of niches involved in ecosystem functioning (Burton 1991). Being ubiquitous, stream invertebrates can potentially respond to a wide range of stream disturbances including sedimentation, heat pollution, organic loading, as well as toxic chemicals. Their lifecycles permit temporal examination of the effects of intermittent disturbance events that may have occurred prior to sampling. Resh and Jackson (1993) describe the use of rapid bioassessment approaches in water quality monitoring as analogous to the use of thermometers to assess the general well being of human patients; values are compared with a "normal condition" and the larger the deviations from this value, the more serious the condition is likely to be. In addition because river macroinvertebrates integrate processes occuring at the watershed scale they are important indicators of natural processes such as gradient, discharge fluctuations, water chemistry and geology, riparian zone characteristics

Macroinvertebrate monitoring

The principal goal of the Rock Creek study was to ascertain whether bioassessment using macroinvertebrates was a feasible tool in the Denali I & M project to detect long term changes in water quality within watersheds of interest. Sampling commenced in June of 1992 when two sites were examined and has been continued on throughout 1996.

MONITORING DESIGN CONSIDERATIONS

Sampling Device

Macroinvertebrates can be collected from streams by a number of methods which generally involve disturbing the bottom substrate with the foot or the hand and catching the organisms in a net held downstream. Variations depend upon the collection device and whether the area disturbed is quantitively delineated. One of the simplest devices is a simple kick net made of two wooden dowls about 1.25 m long which support a 1 m^2 screen of 500 µm mesh Nitex. The top of each stick is held in the stream and dislodged organisms are retained on the net. A modification of the kick net is the D-frame net where there is a single pole attached to a frame with a net bag attached. The Surber and Hess samplers are where an area of approx 0.1 m^2 is delineated within which the substrate is disturbed thereby providing a quantitative sample. The kick net can be semi-quantified by placing a frame in front of the net or disturbing a known area. In a survey of 90 macroinvertebrate studies, Winterbourn (1985) found that 60% used the Surber or the Hess sampler while 28% used the kick net. Comparisions of taxa richness among streams are difficult unless standard sampling areas are used and a series of pooled 0.1 m^2 samples is a better approach than a sample from a larger area (Vinson and Hawkins 1996).

Mesh size

This is very important consideration in macroinvertebrate sampling as it determines the size of organisms retained by the net. In a survey of bioassessment techniques Resh and McElravy (1993) found that 50% of the 45 bioassessment studies used a mesh size in the range 301-500 µm mesh. This mesh size may not retain first or second instars of certain taxa although finer mesh sizes requires a disportionate time to sort organisms from the sediment and organic matter.

Number of replicates

The number of replicate samples collected in benthic macroinvertebrate surveys is an important concern in experimental design. The principal issue is that bioassessment studies differ from surveys to determine absolute density numbers for say production estimates. In most cases bioassessment metrics involve some form of ratio measure which is less sensitive to changes in the number of sample replicates. In the majority of lotic studies between three and five replicates are collected (Voshell et al. 1989). Russek (1993) in evaluating the number of replicates for bioassessment studies within the Municipality of Anchorage found no significant difference in metric values between three and ten replicates for the following metrics; number of EPT taxa, EPT/Total Individuals ratio and Hilsenoff's Family Biotic Index. Three replicate samples is the minimium number necessary for ANOVA comparisons between sites.

Habitat stratification

Stratification involves dividing the selected reach into habitat types (e.g. riffle, pool, run etc.) and taking samples in each of these habitat types depending upon their relative contribution to the reach. In many bioassessment studies stratification involves simply restricting sampling to the riffle areas as this is the habitat type that supports the greatest diversity and abundance of taxa and the goal of assessment is to ascertain what clean water fauna can be supported at that site. A problem with this approach is if riffle habitat is not affected by an impact. Although proportionally sampling each subhabitat provides a better characterization of a site, it can make comparison with reference sites more difficult because of intersite differences in habitat proportions that will always exist (Resh and Jackson 1993). Parsons and Norris (1996) support the "riffle only" model as it reduces inter-habitat variations and thus comparisons are made between equivalent environmental units.

Sample sorting and level of taxa identification

This is another important issue in the bioassessment of water quality. Some techniques sort only a sub-sample of 50, 100 or 250 individuals while others sometimes specify a proportion of the entire sample e.g. 3/8. The RBP's developed by the Environmental Protection Agency (Plafkin et al. 1989) recomend the use of a D net and a 100 organism sub-sample, i.e. calculating the metrics from the first 100 organisms picked from the net in the field. Courtemanch (1996) argues that a fixed count of organisms results in inaccurate estimates of taxa richness (e.g. EPT index, the number of EPT genera) and does not consider the value of rare taxa to an assessment of ecosystem integrity. However Barbour and

Gerritsen (1996) defend the fixed sub-sample method as providing a cost effective unbiased representation of a larger sample since the assessment of biological condition at a site is based on a multi-metric approach which incorporates the diversity and relative abundance of taxa, together with other metrics, to represent surrogate measures of ecological processes. Sub-sampling has to be consistent between sites however; i.e. you cannot compare a 100 organism sub-sample with a 250 organism sub-sample. Richness estimates from 5 x 0.1 m^2 samples pooled before sorting and then sub-sampled will be different from those estimates of richness by (1) the mean of 5 independently sorted samples or (2) pooling the taxa counted in 5 separate samples (Vinson and Hawkins 1996). These authors conclude that statistical tests based on as few as 100 individuals (i.e. as in a sub-sample) are nearly as sensitive as analyses based on a larger collection of individuals in estimating richness but they caution that fixed number subsampling may have reduced power to discriminate differences between communities when certain metrics (e.g. indicator taxa and community similarity measures) are used.

The choice of taxonomic level represents a compromise between the desire for increased information from species level identification with the cost in time and required expertise to achieve it (Resh and Jackson 1993). The time taken to identify certain families (e.g. Chironomidae) to genera and species would be excessive and would require expert taxonomic assistance . However lack of species-level information can decrease senstivity and reduce the ability of a study to detect more subtle changes (Resh and McElravy 1993) that are apparent from some stressors. However in Alaska, we have a limited knowledge of detailed invertebrate taxonomy and frequently identification past the genera level is not feasible in any case.

Frequency of sampling

Clearly the more frequent the sampling the more resolution that will be achieved but again costs can be prohibitive. In Alaska the window of sampling opportunity is restricted to the ice-free season which decreases with increasing latitude. Many insect taxa will be absent from the stream during the summer months as they emerge and reproduce (or are present as very small instars). Similarly to selecting riffle areas to sample, the goal in bioassessment is to estimate the maximum number of insect taxa that a stream can support. Sampling in the spring after the ice has gone out and again in the fall before freeze-up are the most appropriate times. If only one sampling period is possible then the spring is probably the most appropriate.

Monitoring Design suitable for Denali National Park

With all these considerations in mind what approach should be adopted for monitoring aquatic ecosystem health in Denali National Park?   As discussed, the adoption of a procedure that uses samples from a known area of streambed and is therefore quantitative is important for comparisons of richness between studies and for certain statistical analyses. Thus the use of a Surber sampler is recommended which samples an area of approximately 0.1 m^2. A mesh size of between 300 and 350 µ m is appropriate - certain small instars will be missed but since absolute densities and production are not being measured this is acceptable. Confining sampling to riffle areas is justifiable given that this is overwhelmingly the predominant habitat type in Denali streams and it is more appropriate for intersite comparisons within the Park.

Two other points are important to consider in designing a monitoring protocol; (1) how is the data going to be analyzed and (2) the typical densities of invertebrates in samples from streams within the Park. Data analysis will be addressed later but clearly the monitoring protocol should allow for the greatest flexibility in allowing for the analysis of data. Hence simple measures like ANOVA require a minimium of three separate values (e.g. number of taxa) to test for significance between sites while multivariate techniques can use average data of these samples. Because the most flexibility in data analysis is achieved through independent samples, between three and five separate samples are recomended to be collected at each site for each sampling period. In a large number of streams within the Park the number of invertebrates in any one sample frequently does not exceed 100 individuals (unless moss or extensive amounts of filamentous algae are present) and thus sub-sampling would not be an issue given the justification for maintaining separate samples.

MONITORING PROTOCOL

Sampling

Basic equipment required;

Surber sampler with a 300 to 350 µm mesh net and detachable collecting bucket
Whirlpak bags
Rite in the Rain notepaper
Tape Measure
Random number tables
Current Meter
Sorting tray and magnifying light
Glass vials for sample and specimen storage

Number of Stations

The number of stations along a stream should be sufficient to reflect the different characteristics of the stream course and potential disturbances to aquatic integrity. One station should always be selected above any suspected sources of water quality degradation to act as a control station. In certain cases where point source pollutants are suspected, an upstream and downstream comparison of the input might be appropriate.

Time of Sampling

It is recommended that sampling be carried out at two time periods during the year: (1) in the spring after the ice has gone out and (2) in September before freeze-up. As spring is typically the time of the year when the diversity of the invertebrate fauna will be the greatest and reflect the fauna that a station could potentially support this is the recomended sampling time if budgets allow only one sampling period. Before freeze-up in September is the next optimum time period to sample, but insect instars are typically small at this time of year and there is frequently the problem of large numbers of leaves to contend with. However, it is preferable to sample at two different time periods to cover the possible emergence of any taxa.

Station Selection

As riffle habitat is being used to minimize variation in certain parameters that may significantly influence macroinvertebrate distribution, stations will have a predominant substrate size within the 2.5- to 15-cm range (gravel to medium cobble), current velocities of 30 to 80 cm/s, depths of less than 40 cm, and degree of embeddness of the gravel and cobbles in silt and sands of less than 30%.

Sampling Techniques

Stream macroinvertebrates should be sampled using a modified Surber sampler with a 340-360 μm mesh net and a detachable collecting bucket. The sampler is modified by having a closed 3- x 3-inch foam surrounding the square foot sampling area to provide a better adhesion to the cobbles and gravels of the streambed and reduce the possible loss of organisms.

At the station selected, a 30-foot tape (using English units as Surber samples are 1 ft^2) should be stretched along the bank with 0 at the lower end of the section under study. Three to five samples should be collected at each station using random numbers selected before arrival using appropriate tables. Two numbers are chosen for each sample: the first from 0 to 30 gives the position along the bank and the second from 0 to 10 gives the position across the width of the stream. This splits the stream width into tenths; 0 would place the sampler next to the right bank, 5 would be the middle, and 10 would be next to the left bank (looking upstream). This procedure eliminates having to know the exact width of a station for the selection of random numbers. The sampler is placed at the intercept of the two coordinates. The first sample should be taken at the lowest most downstream bank co-ordinate so that samples are collected working upstream. In this way, dislodgment of organisms will not affect subsequent samples.

Samples should be taken by a two-person team who approach the sampling coordinates from a downstream direction. One person should place the sampler on the streambed at the desired location with the net opening facing upstream and stand on the metal sides so that the foam compresses around the stones. The other person, with arm length rubber gloves, should stand to one side (not in front of the net as this blocks flow) and check that the seal is satisfactory. If a large stone is incompletely covered by the Surber sampler, then the sampler should be moved to exclude the stone or if the habitat is not a riffle area the sampler should be moved to the nearest riffle area.

The person then picks up the stones and larger gravel and rubs their fingers over the rock surfaces to remove organisms which are then carried into the net. Once cleaned, the stones are placed to one side. The remaining small gravel, silt, and sand is stirred to a depth of approximately six inches over the entire square foot of the sampling area. The net is then removed from the sampling area and placed in the water and quickly raised a number of times so as to wash material into the collecting bucket. On the bank, the collecting bucket is detached and backwashed through the mesh with 90% ethyl alcohol (ethanol) into a Whirlpak plastic bag. A label of Write-in-the-Rain notepaper that identifies the station, date, and sample number is added to the Whirlpak bag. The samples are double bagged for transport and storage.

Depth and current velocity should be recorded at each point a sample is collected, together with an estimate of the predominant size of substrate and its extent of embeddness among any sands or silts.

LABORATORY SORTING AND IDENTIFICATION OF SAMPLES

In the laboratory organisms should be sorted from detritus and small material using a magnifying light and placed in individual vials. Seives may be used to assist with the separation Although this approach underestimates the total number of individuals as inevitably some small instars will be missed, it will significantly shorten the sorting time. However it should not significantly alter the value of ratio measures, much in the same way as sub-sampling does not (see earlier discussion).

Invertebrates should be enumerated and identified to genera level for the Ephemeroptera, Plecoptera and Trichoptera (EPT) so that the important metric, number of EPT genera, can be calculated. Diptera should be identified to the family level because further identification would be very time consuming. Voucher specimens should be maintained of each genera/family identified and one set fowarded to the Institute of Arctic Biology, University of Alaska, Fairbanks c/o Milner/Oswood for reference and archival with other similar material.

DATA STORAGE

The numbers of each taxa should be entered on a spreadsheet (Excel or equivalent) under order and families. Total numbers per m^2 should be calculated on the spreadsheet for each individual taxa and for each sample. For each sample replicate the measures calculated (see data analysis) can be entered appropriately at the bottom of each column - see Appendix Table 1 for example. These data can then be used for the appropriate multi-variate analyses.

DATA ANALYSIS

Historically there have been many ways to analyzing macroinvertebrate data to evaluate river ecosystem health and integrity. Many of these approaches involve comparison with unstressed reference sites in different streams or with an upstream control site in the same stream. In the case of Denali National Park the initial stream monitoring was focused within the I & M program on Rock Creek. This stream has been sampled since 1992 and measures would seem to indicate that the macroinvertebrate community is stressed. The number of EPT genera are low (typically less than 2) and this influences such ratios as

the EPT/Total Individuals ratio. This stream is significantly influenced by natural variables which limit the macroinvertebrate community that is able to colonize and sustain a community - these variables include high gradient, large substrate, unstable flows and potentially low nutrient levels limiting primary productivity. Consequently although these protocols can be applied to Rock Creek, this type of system is probably not suitable for long term ecological monitoring to detect anthropogenic stressors that impinge on river ecosystems within Denali National Park.

In a scaling up excercise from Rock Creek, Roberts and Milner (1995) examined macroinvertebrate communities in 26 streams along the Park road and used TWINSPAN classification to ascertain how many different stream types existed with similar benthic communities. Five stream groups were apparent including one group which included the two sites sampled in Rock Creek. Another group contained the predominantly glacier-fed rivers which again are "naturally stressed" due to the overriding influence of low temperature, sediment and unstable flows - hence the macroinvertebrate community is low in diversity being composed of specialist taxa able to survive the conditions. This work is ongoing and is being extended to other regions of the Park (Roberts and Milner 1996). Nevertheless, the TWINSPAN analysis has identified streams within certain groups that would potentially be excellent monitoring sites with which to monitor long term ecological change and possible changes to ecosystem integrity. The type of stressors in a watershed that could be detected by this long term monitoring are discussed in the next section.

The choice of RBA measures and the data analyses used to apply to detect possible changes to aquatic ecosystem health and integrity are varied and an extended discussion is outside the scope of this preliminary protocol concept. Nevertheless a short summary is provided;

Many undisturbed streams are characterized by a high richness (number of taxa) with a relatively even distribution of individuals among the species; this attribute is less applicable at higher latitudes. Taxa richness is a common measure of health and integrity. Water quality degradation typically causes a reduction in taxanomic richness because sensitive organisms (e.g., most Ephemeroptera, Plecoptera and Trichoptera; the EPT taxa) are lost and a decrease in evenness of the community also occurs. The most commonly used diversity index is the Shannon Diversity Index (H¢). Diversity indices have the advantage of being independent of the tolerances of individual species. Conversely, disadvantages of diversity indices exist which may outweigh their advantages and reduce their overall applicability to water quality assessment. These disadvantages include:

1.   Wide variations in diversity index values in pristine waters as some unpolluted communities naturally have low diversity (e.g., under nutrient-poor conditions).

2.   Some diversity indices, such as the Shannon Index, are more affected by changes in evenness than species richness, and so may give "false negatives." For example, natural sediment accumulation on the streambed may result in an increase in the abundance of certain species, but no reduction in species number.

3.   Many studies have indicated that diversity indices are insensitive and inadequately discriminate between sites affected by moderate ranges of such pollutants as metals and pesticides (Metcalfe 1988).

The biotic score or index is an approach which integrates taxa diversity/abundance with pollution tolerance of these taxa into a single value. The range of these values are then interpreted on a scale indicating water quality. The Biotic Condition Index (BCI), was developed in Utah by Winget and Magnum (1979) using Predicted Tolerance Quotients calculated for each site based on four physico-chemical variables: total alkalinity, sulfate, substrate size, and stream gradient. The BCI is then determined by comparing the predicted tolerance quotient with an actual tolerance quotient calculated from the sum of tolerance values for invertebrate families and/or genera found at that site divided by the number of the taxa. Although it has been applied to a number of Alaskan streams and rivers (including a number in Denali) the database to support its use is not extensive in Alaska. The biotic index of Hilsenoff (1987) was developed for evaluating organic pollution in Wisconsin streams using insect species. Hilsenoff (1988) adapted the BI to create a family biotic index (FBI) by using the tolerance values of insect families, a taxonomic level more appropriate for rapid bioassessment. The FBI involves using family abundances rather than presence (e.g., as in the U.K. Biological Monitoring Working Party scores) and has been applied successfully to forms of water quality degradation other than organic pollution (Resh and Jackson 1993).

In the late 1980s the U.S. Environmental Protection Agency published a handbook entitled *Rapid bioassessment protocols for use in streams and rivers* by Plafkin et al. (1989), which was subsequently followed by a manual entitled *Macroinvertebrate field and laboratory methods for evaluating the biological integrity of surface waters* by Klemm et al. (1990). Plafkin et al. (1989) propose a Rapid Bioassessment Protocol (RBP) based on percent similarity with reference sites for the following eight biotic metrics: (1) taxa richness, (2) FBI, (3) ratio of scrapers to collector-filters, (4) ratio of EPT to chironomid abundance, (5) percent contribution of dominant taxa, (6) number of EPT taxa, (7) coefficient of community loss, and (8) ratio of shredders to total number of individuals. Three protocol levels are recommended depending upon the sampling technique and the level of taxonomy used. This approach has been widely applied and variations tested in a number of states. The use of functional feeding group measures (e.g. ratio of

scrapers to collector-filters and ratio of shredders to total number of individuals) requires generic level identifications which has only been recomended for the EPT taxa - hence these measures are not viable for Alaska.

Milner and Oswood (1995), in an extensive study of the effects of urbanization on stream water quality within the Municipality of Anchorage, recomended the use of four metrics that provide the greatest ability to discriminate among metrics based on the work of Fuller (1995). These metrics were;

NUMBER OF EPT GENERA (EPT GEN)

The number of EPT genera averaged for the number of replicate samples.

EPT INDIVIDUALS/TOTAL INDIVIDUALS (EPT/TOT)

Calculated from the number of EPT individuals in a sample divided by the number of individuals of all the taxa. The mean is then calculated for the number of replicate samples collected. A high EPT/TOT value would generally indicate an unimpaired site, although it rarely reaches 1 as some non-EPT taxa will be found at even the most pristine sites. This ratio is similar to the EPT individuals/Chironomidae individuals found in the RBP protocols but whose value is variable (Barbour et al. 1992).

PERCENT DOMINANT TAXA (DOM TAX)

Percent dominant taxa is a metric that evaluates community evenness and is calculated as the percent that the most numerically abundant taxon contributes to the total organisms in the sample. Dominance of the community by one taxon may indicate environmental stress, although it will depend upon the taxon that dominates. For example, chironomid dominance of a community will likely indicate different water quality conditions than if the mayfly *Baetis* dominates the community. The recomendation was use non-EPT dominant taxa in the Municipality of Anchorage protocol.

FAMILY BIOTIC INDEX (FBI)

The FBI was developed to assess the impacts from organic pollution based on the relative abundance of macroinvertebrate families with varying tolerance to organic pollution (Hilsenoff 1988), but has been

shown to be sensitive to other forms of water quality degradation (Resh and Jackson 1993). The tolerance ratings are given in Appendix Table 2. A value of 0 indicates a family extremely sensitive to water quality degradation, while a value of 10 indicates a family very tolerant of water quality degradation. Oligochaeta (worms) were not included in Hilsenhoff's original index, but have been given a tolerance rating of 8 because they are known to be generally tolerant of poor water quality conditions. The total number of individuals in each family is multiplied by the score for that family and these values are summed across the total number of families collected. The FBI is then calculated by dividing by the total number of individuals in the sample.

SUMMARY

Using a number of different measures is preferable than a single metric or biotic score since it allows for one measure to detect a change where another measure is not sensitive. Until existing data analysis from the Denali study is undertaken it is suggested that these four measures be used to monitor long term changes to ecosystem processes in candidate streams. Others can be added or some deleted as the analysis becomes more complete. In addition this discussion has focused on macroinvertebrate communities but other attributes of the biotic community should be considered for incorporation into the protocol e.g. chlorophyll a as an indicator of primary productivity, coarse particulate organic matter. It is important to first categorize the stream types and then determine a range of metric values for these streams with which comparisons can be made.

The other major question is how using a monitoring protocol is change going to be detected and the level of impact assessed. What is an acceptable level of impact? Can the candidate sites be classified as unstressed now and used as reference sites for future change? If this is the case a powerful approach is Before-After-Control-Impact (BACI) technique (Cooper and Barmuta 1993) which involves examining trends over time that occur both in the potentially impacted and in the control sites.

A potential approach that can be developed from the work of Roberts and Milner is the use of classification and discriminant functions using the macroinvertebrate community structure patterns to provide a predictive model for Denali National Park whereby stream sites can initially be classified with physicochemical variables. Subsequently for any particular group an expected unstressed invertebrate community (or surrogate metric values) can be predicted with which the actual community can then be compared and the degree of change assessed and quantified. The development of this approach is ongoing and involved fieldwork in 1997 at sites not previously sampled to see if correct group prediction was possible using a small number of key physicochemical varaibles.

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Scale	Plecoptera (Stoneflies)	Ephemeroptera (Mayflies)	Trichoptera (Caddisflies)	Diptera (True flies)
0	Leuctridae		Glossosomatidae Rhyacophilidae	
1	Chloroperlidae Perlidae Capniidae	Ephemerellidae	Brachycentridae	
2	Nemouridae Perlodidae Taeniopterygidae			
3				Tipulidae
4		Baetidae Heptageniidae	Limnephilidae	
5				
6				Chironomidae Simuliidae Empididae Ceratopogonidae
7				
8				Oligochaeta (not Diptera)
9				
10				Psychodidae

Appendix Table 2   Different tolerances of invertebrate families to changes in water quality on a scale of 0 to 10. [0 = least tolerant, 10 = most tolerant.] (Adapted from Hilsenoff 1988). See text for application.