

French Greeting:

Pour la Commission Mixte Internationale, je vous souhais le bienvenu à l'atelier pour échantillonner la biologie de la Rivière Rouge du Nord. J'espère que vous vous amusez. Merci.

SETTING THE TONE FOR THE BIOSAMPLING WORKSHOP FOR UNWADEABLE PORTIONS OF THE RED RIVER SYSTEM:

ELEVEN COMMANDMENTS FOR BIOMONITORING AND BIOASSESSMENT

Joel L Fisher, PhD
Environmental Advisor and Senior Scientist
United States Section
International Joint Commission, US & Canada

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Workshop for Bioassessment of the Red River

This talk is about one-third science, one-third metaphysics, and one-third war stories. John Cairns inspired this particular combination. He originally had hoped to be part of our discussions, and to welcome us by a live videoconferencing connection from Virginia Tech. Medical problems prevented this, but his spirit, I hope hovers over us today. I will call him after this presentation to check in on him, since he is a friend, colleague, mentor and advisor of forty years. Also, I spent part of last week at the Academy of Natural Sciences of Philadelphia with my personal mentor of nearly fifty years, Dr. Ruth Patrick. She is clearly the "The Queen of River Ecology," and I am pleased to extend her ecological blessing over the activities to be accomplished at this workshop.

This workshop is about the sampling of unwadeable portions of the Red River. Nobody expects us to solve that suite of problems. In setting the tone for this workshop, I have decided that my job is to give you sufficient inspiration and things to consider in your deliberations to make a serious inroad, and hopefully generate some creative juices in your thinking. I also wish to give you a rousing send off as you go about your work. It is about time that this suite of problems was attacked systematically and purposefully at a workshop. On behalf of my own organization, the International Joint Commission, which has provided major funding for this workshop, I welcome you and wish you every success in your efforts.

Some background:

For those of us, this author included, whose limnology training focused mainly on rivers rather than lakes, the program emphasized biosampling of the wadeable portions of rivers. We all knew what to do, and how to do it because we could “touch the environment,” and we had a “hands-on” comfort in our work. Most of our problems materialized only after we returned to the laboratory, and discovered such things as: our collections were so contaminated with debris to be almost useless, or what we thought we found we had not.

The unwadeable portions and reaches were a challenge then, and remain a challenge now. Sampling the unwadeable portions meant using remote tools, including vessels. Very few of us had received training as graduate students with boats. We did not study the unwadeable portions of rivers then, and much of the work I have reviewed over the past forty years shows this same deficiency now. Ironically, the methods I learned about in those years might still work, but often remain untested.

The organizers of this workshop have included among the materials developed for attendees, a document related to a bioassessment program for the Red River. That document reviewed much of the work on establishing “reference watersheds” and the discussions of multivariate statistical methods, and the use of various biotic indices. Its bibliography updated much of the recent world literature on the study of rivers, but it did not update information on field methods and sampling.

My earliest training was in chemistry. Dr. Henry Hope, my professor of analytical chemistry once remarked to us “sampling was an art, not a science.” *How to sample, was the basic question*, and it still is. The word *how* has some semantic baggage for this workshop. In many ways what Julia Child’s book, *The Way to Cook*, did for cooking, parallels what we need now: a volume called *The Way to Sample*. That should be a future product of this workshop.

What is the difference between the *how* and the *way*? The answer is mainly philosophical, in fact, almost liturgical/ontological. The *how* tends to be prescriptive, sterile, and devoid of insight. The *way* implies that the *how* has a philosophical and/or in this case, scientific basis which can be recognized as truthful, guiding, and rewarding.

As previously noted, the major problem on studying unwadeable stream reaches is the need to do this remotely. There are no “hands-on” areas for sampling. The limitations of remote access apply especially to sampling of biota. Existing remote sampling methods seem satisfactory for most chemical and physical parameters. Sampling of biota adds a dimension of complexity not found in abiotic sampling.

With these introductory remarks, I now introduce my Eleven Commandments:

1. Thou Shalt Know Thy Taxon!

Among the first questions to ask with respect to the study of biota, is: Which Biota?

Sampling for particular biota presumes knowledge of the organisms involved. Thus, the first rule should be: Regardless of the kind of water body which is being studied, an investigator must know thoroughly the physiology, behavior, and ecology of the taxa to be studied. That basic information enables one to decide where to sample, when to sample, and ultimately how to sample, and how often to sample. The greatest limnologists have/had taxonomic specialties: G. E. Hutchinson (corixidae), Ruth Patrick (diatoms), John Cairns (protozoa), Edward Deevey (calanoid copepods), Ralph Brinkhurst (oligochaeta), W. T. Edmondson (rotifera), John Brooks and Clyde Goulden (cladocerans, mainly *Daphnia*), Henry Van der Schaale (unionidae), etc.

2. Thou Shalt Know Thy Equipment!

Fisheries biologists use nets, and a variety of techniques with them, including electric shock, rotenone, etc. The benthos biologists use dredges, scoops, finer nets, and artificial substrates. The meiofauna and pelagic invertebrate specialists often use special traps. All of these tools share some common features which are important in formulating sampling protocols:

(a) All equipment and methods are *selective* with respect to the organisms sampled. This selectivity includes types of organism, their size, motility, avoidance and escape skills (ability to not be sampled), hardiness (will the equipment or method injure or damage the sample).

(b) All equipment needs continuous calibration and maintenance. Nets need cleaning and often repairing. Scoops and dredges need cleaning. Items which are towed need calibration of what they collect as a function of towing speed and direction.

(c) All equipment needs categorization with respect to special circumstances. I will discuss an example of this later.

3. Thou Shalt Learn Remote Sampling Methods from Marine Biologists!

I have found that the marine biologists and estuarine specialists seem to do remote biotic sampling the best. Every successful graduate program in marine biology that I examined for its applicability to this workshop used research vessels. The programs included the training of students to work on research vessels, as well as to develop in their students scuba diving skills. Biologists who use research vessels to study open lake waters confront a different set of problems from those who study rivers, but for the most part, except when using row boats with winches to attach nets or drag lines, the vessel experiences of lake biologists are often minimal.

The marine and estuarine specialists also have two tools which river biologists rarely use: divers and submersibles. The police routinely use these tools when investigating crimes, in which evidence is discarded into a waterway, but one does not see biologists routinely using these tools to study rivers; yet, these tools may offer some special advantages for the Red River, especially for the deep and unwadeable portions.

Dr. Pierre Beland, a former IJC Commissioner and mammalian physiologist, has studied the effects of pollution on the populations of beluga whales in the Gulf of St. Lawrence. He has used submersibles to study these animals. Another friend, Dr. Will Davis, of the EPA Laboratory at Gulf Breeze (Florida), studies reef systems created in various waterbodies to encourage the development of diverse habitats. He dives to see the bottom structure, the reef structure, and observe and collect organisms from these habitats. A diver might be a useful component of a biosampling plan for the Red River, simply to scout the bottom habitats in unwadeable portions, and assess their biosampling potential. All of us understand that muddy streams create a problem of low or virtually no visibility. Divers do can carry lamps, and may become useful in determining if the area is a biological desert, and thus unsuitable for benthic biological sampling.

Presently, one must guess the value of a location for sampling by using a scoop or dredge, and inferring from the collection the value of the location for sampling. This guessing approach may cost less to use than the services of a diver, but a diver gives that additional and often priceless piece of information: a first-hand look at what is really there.

4. Thou Shalt Sample Biotic Communities, Not Just Biota

There is a major difference between sampling of biota communities and sampling only biota. For work on the Red River, the emphasis should be on sampling communities.

David Arscott, a presenter at this workshop, asked me to discuss this topic at some length. First note that sampling of biota rather than communities produces a sterile list of species collected, and to a limited degree, their relative populations.

Much of the biotic sampling in waterbodies within the US aims to meet requirements of the Clean Water Act. One such requirement is to establish a baseline, and somehow to assess the “biological integrity of an aquatic system.”

In the nearly forty years of the Clean Water Act Amendments of 1977, baseline assessment of “biological integrity” has become somewhat standardized by USEPA. I remember my own tenure in EPA, and the aim of much of the research was to make it “idiot-proof” so that technical people in regional offices could advise local governments and groups, about biological assessment tools to meet the needs of permit requirements, environmental impact statements, etc. The idea of standardization now permeates not only EPA, but the US Army Corps of Engineers, and other agencies.

Many State agencies follow these approaches because of the NPDES (discharge permit) system and State responsibilities under the Clean Water Act. EPA has worked over the years to produce quality control criteria for field sampling and analysis of environmental samples. I am relatively familiar with the early history of “round robin testing” of environmental samples, and auditing by EPA of laboratories, but I have not kept up with its development and implementation. I do know, however, that whatever system ultimately evolved, has become relatively uniform system throughout the United States. Methods are tested for “equivalency” (whatever that is), and the maintenance of quality/quality control is codified in regulations. In many respects the work has been extended into Canada because of the various international agreements and groups in which both United States and Canadian regulatory and research people participate.

In the entire history of the Clean Water Act, there has been no satisfactory definition or protocol that determines the meaning of biological integrity. As far as I am concerned, there still is not a definition, although there may be a protocol. Regardless, when the biological samples have met some kind of standard of reproducibility and level of acceptable “precision” for some measure of biological integrity, the work still does not achieve its goal. Also, the sampling may be very precise, and also completely biologically inaccurate.

Species lists provide a very limited picture of the structure of the biotic community. They do not describe evolutionary processes and phenomena, and do not address such special problems of sampling biota as sampling “efficiency” of what is measured compared to what one expects to find versus what was actually found. Because all sampling methods for biota have selectivity biases, a species list gives no clue as to the uncertainties associated with these biases.

That problem is compounded by the fact that the identification of species has not had much progress in the aquatic community in many years. I did my graduate work in a museum, and thus I am personally biased toward and supportive of work in taxonomy. However, the recent trends in taxonomy are mainly theoretical, emphasizing such things as computerized methods of taxonomy and the basis of theories of cladistics. New or revised taxonomic keys to important groups of river species have not been forthcoming since the late 1970’s. I understand that the EPA has supported the development of regional taxonomic keys for biological surveys, but I am not aware that the amount of work suggested in these projects reflects new data on organism physiology, form and function, and improved measures of identification. This is especially true for larval insects. Further, the ecological roles of certain taxa often found in collections, are unknown, and thus there is no incentive to work on taxonomic projects for these groups. Examples: ostracods, certain amphipods, and worm species.

Taxonomy is not a skill taught in graduate schools, or of interest to environmental science curricula. I was informed through some cynical hearsay that biotic collections from river surveys are often sent to a commercial lab, and their people “work up” the material. The output is a “consultant” report which states that one has a “good community, or a bad community.” I am not sure what that means. Basically, if what one finds does not appear in the latest edition Pennak’s book on freshwater species, it is probably unknown. Unfortunately, I am not sure that the most recent edition of Pennak’s book (about four years ago) really updates taxonomic keys. His original keys still seem to be best, but then those investigators were my teachers and colleagues, so again I give my bias.

Sampling of communities aims to establish the structure of the community and make judgments on its function, and possibly its health. When one samples communities, one should take advantage of the selectivity bias of equipment and methods by using *several* different methods, cross correlating the results, and thus discussing where the individual methodological biases occur. One now has the basis to correct these biases when evaluating data from population distributions of specific taxa within the samples and using knowledge of them to describe in more realistic terms the structure and function of the biotic communities.

5. Thou Shalt Be Particularly Aware of Rare, Unexpected, or Absent Species.

When sampling for biotic communities, three special sampling problems arise: how does one deal with rare species? How does one deal with unexpected species (*i.e.*, species that do not seem to belong because they are away from their normal habitats, or outside of their known range of occurrence, etc.)? How does one deal with “absent species” (species that are known to occur in the area but have not shown up in the collected material)?

Some thirty years ago, I reviewed for an administrative law judge at EPA, a biological survey of the Brunswick Estuary in Georgia. The study’s investigators noted a large number of stomatopods in their collections, and from this, the investigators concluded that the benthos was dominated by a rather unusual and rare fauna. I was not so sure about their conclusion. I discussed this issue with my friend, noted marine biologist, Dr. Joel Hedgpeth. He indicated that most investigators had great difficulty in finding stomatopods but that stomatopods are not necessarily rare. They burrow into the sediments and emerge at night to feed. They are also very adept at avoiding moving objects, such as motorboats and trawled nets.

Based on Dr. Hedgpeth’s comments, I concluded that unless one sampled an area of low light penetration, using nets of a relatively fine mesh trawled from a vessel moving at relatively high velocities, and the nets were near the benthic boundary layer, there was little likelihood of a collection containing any, no less dominated by, stomatopods. The pollution tolerance or sensitivity, depending on your view, for stomatopods was not ascertained from the study, and to me that environmental property remains unknown – I haven’t bothered to inquire about that situation in the years since. Nevertheless, I thought at the time that there was good reason to question the interpretation given from the biotic sampling.

6. Thou Shalt Learn a New Song for Biosampling: “Night and Day, You are the Two”!

The refrain lyrics of Cole Porter’s classic song, “Night and Day,” go “Night and Day, you are the One,” but the stomatopod incident just describes indicates that when it comes to biosampling, the song lyrics should be: “Night and Day, you are the Two.” Recall, that I had indicated a need to consider sampling with equipment that has been categorized for special uses. One such special use of equipment is night sampling on a river. Sometimes one must dedicate separate or special equipment, or extra equipment for night sampling.

What about night sampling in general? I have not seen many sampling studies (maybe four or five in a forty-odd year career) in which night sampling was undertaken. Many biosampling plans simply do not include this under the implicit assumption that a day collection accounts for whatever is collected by day or night. Night collections are also more expensive because of the use of vessels at odd hours, and costs of crews.

There is no inherent reason to believe that distribution of organisms in a night collection, even if all the taxa collected are identical to those in a day collection, will be identical to a day collection. Some taxa move vertically in the water column on diel or other cyclic schedules, and night sampling may collect them in different proportions than day sampling.

What does that say about community structure and sampling to describe it? It might be useful to consider at some point a night sampling of the Red River if only to remove the doubts one would have about its usefulness and contribution to the assessment of biotic community structure.

One special requirement of night sampling where vessels are involved, are that the velocities of the vessels must be more precisely controlled. Certain vessel velocities may be detrimental to sampling at night, while these same velocities are essential to successful sampling by day. One reason is a noise factor of the vessels. This may disturb organisms, and they move to avoid the capture. The noise issue may not be as serious during the day, and often one can use different velocities and net meshes to compensate here. This latter statement means that trawl velocities and net types need separate calibrations for day and night sampling.

7. Thou Shalt Know about “The Substantive Velocity Field” and “Frames of Reference” in Hydrological Studies

An important aspect of sampling (especially from vessels, but also in general) is whether the sampling will involve transects, or sampling at locations in some hydraulically pre-determined way to capture the continuous behavior of a particular water mass as it traverses the river course. Civil and chemical engineers refer to two kinds of velocity distributions in a fluid: one in which sampling is fixed in space, and the other in which sampling follows a given mass of the fluid. The latter velocity field is referred to as the “substantive velocity field,” and the calculus term for the total derivative of velocity in this particular velocity field is the “substantive derivative.” The physicists call this a “Frame of Reference” question, and it enters into their discussions on Relativity Theory. For biosampling, the two descriptors of velocity in a “frame of reference” context can produce different results in the biotic collections.

When Stephen Groves was the director of the State of Maine's environmental agency (during the early 1990's), he instituted biomonitoring using special artificial substrates. He allowed them to float down the river, The organisms that these substrates collected over their course reflected biotic sampling to mimic the substantive velocity field. In discussions with David Arscott, he noted that for some rivers which have fast currents and great widths, when artificial substrates were released into the river, they were quickly lost, or difficult to recover. If one were to recover such materials, they would have sampled the substantive velocity field, because they move with a given parcel of water.

Most artificial substrates placed in the water, notably the Catherwood Diatometer (pioneered by Ruth Patrick), and the special sponges for protozoa (pioneered by John Cairns), are kept at fixed locations. They receive continuously whatever biota comes by them. They sample a fixed point over time. The interpretations of the data obtained from the two kinds of sampling depend on two different kinds of models and may give two different kinds of information. It may be important to compare the two kinds of biological sampling for locations in the Red River in order to assess the structure of the river's biotic community.

8. Thou Shalt Understand the Models which Underpin Various Sampling Strategies

This is a very difficult thing for most people. Many field biologists often have only the most rudimentary understanding of the statistical models which underlie certain sampling strategies. The mathematics can quickly become onerous, but failure to understand the conceptual basis for these models often leads to erroneous sampling strategies, and scientifically unsupportable interpretations of the resulting data.

First, forget about random sampling of biota. Organisms do not distribute themselves randomly on the landscape. If one were sampling biota based on random distributions, one would likely sample more areas without biota than areas with significant biotic collections. One must sample knowing where one is likely to find the required biota (Know thy taxon). This is a special problem when one studies biotic communities in "patchy" environments.

Next, consider the differences in sampling under the "Frame of Reference" concerns. For artificial substrates fixed in location, a substrate sample reflects the following sequential processes:

(a) immigration rate of organisms. This is the rate at which organisms are carried downstream to the substrate. As a gross measure of immigration, one can use the velocity of the carrying current, since most organism will move passively.

(b) the attachment or colonization rate. Since substrates are selective, the colonization rate will be some fraction of the immigration rate because not all organisms will attach.

(c) the growth rate of organisms on the substrate. After a period of time those organisms that can grow and reproduce on the substrate will do so. The assumption is that sufficient is also carried down by the currents and will contact the organisms on the substrate.

(d) the attainment of a steady state, or if it occurs earlier, the sloughing off of the organisms from the substrate.

(e) predation of organisms on the substrate. Some taxa will act as predators for organisms on the substrate. These predator taxa may also be resident of the substrate, or contact it through currents, or by deliberate independent movement and motility.

Now look at these processes in a context of biological sampling. It is usually not possible to examine each of the substrates continuously without removing them. Consequently, a typical procedure uses several identical substrates at the same location and removes one of them periodically for study according to some protocol. The time at which a substrate is removed for study freezes the processes which have occurred up to that time. If the sample substrate is cleared of its biota, and returned to the original sampling location, it begins the colonization process all over again, and thus can be used as part of a study to follow succession patterns.

Through systematic removal and examination of substrate, one obtains a picture of when a steady state is achieved on the substrate. But more important, one obtains a picture of how colonization of the substrate is monitoring the immigration of species, and one can now estimate the maximum holding capacity of a substrate in terms of both number of species and populations within species. Further, by correlating the velocity of the river with the extent of colonization of a substrate at a given time, it is now possible to estimate the immigration and colonization rates for specific taxa.

By careful examination of the attached taxa, for example by passing a light beam through them to estimate the thickness of a biofilm which they form, it is possible to quantify the adhesion rate and build-up rates of the film. Note that biofilm is formed by a secretion of materials which allows the organisms to attach (actually glue) themselves and remain on the substrate, to attach other organisms to them (epiphytic and parasitic colonization) and act as a trapping medium for food carried by the current. Each layer of film will itself have a maximum population. In this way, one estimates the population build-up of taxa.

Using population models, one can often estimate reproductive and growth rates of the attached taxa. Also, once one characterizes the carrying capacity of the substrate, one now has a time period during which it is likely that organisms will slough off the substrate because this carrying capacity is exceeded. The sloughing off process can be induced by currents or gravity effects within the biofilm. The data will also yield those threshold values of current velocity that will lead to scour at various biofilm depths. By noting the selective changes of taxa population on the substrate at different times, and the presence of a predator species, it is possible to quantify the rates of selective predation on the substrate.

The mathematical models of these processes are highly complex. Even when linear and density-dependent rates for immigration, growth, reproduction, predation, emigration and sloughing of the organisms from the substrate are used, the models are difficult, although the descriptive differential equations can often be solved in a closed mathematical form. One deals with a combination of adsorption with chemical reaction, diffusion, and purging. One interesting physical model of this process is the B.E.T. (Brunauer-Emmet-Teller) adsorption isotherm combined with a density-dependent chemical reaction to represent the growth rate or reproductive rate of organisms. The model can be applied at two biological levels: species number, and populations within species. (For more on this, see the speaker personally.)

How does all of this affect biosampling? A critical variable for sampling is the frequency of removal and study of individual substrates. Also important are the care, efficiency, and accuracy of the evaluation of the taxa on the substrates. A trial and error approach permits zeroing in on the rates of achievement of a steady state. This steady state will very likely indicate relative completeness of the census of different species or taxa which a substrate selectively collects, and allows one to begin the analysis of the structure of the biotic community.

Such studies must be performed year around to reflect seasonal succession of species and capture the rare or infrequently observed biotic components of a given taxonomic group which the substrate selectively collects. That means that sampling plans cannot assume a summer short season, or a spring collection, and call it quits. Ouch! Most groups I know want a simple one-shot sampling season, say early spring, or mid-summer, and forget about the problem of succession.

9. Thou Shalt Not Misuse and Misinterpret Diversity Indices

A concern of considerable importance in assessing biotic communities is in the use of diversity indices. The calculation of diversity indices from sampled biota has both theoretical and practical components. Volumes have been written on them, but for me the most authoritative works were the books and papers of E.C. Pielou (Christine to some of her colleagues). I make this statement for some rather unusual reasons which should become clear shortly.

I say up front: I do not and will not recommend a particular diversity index for community structure assessment. I do not believe that one should legislatively encode or entrench a particular diversity index in a sampling plan, such that the use of that index becomes “gospel.” I have been told that current biological monitoring plans by USEPA call for these things, because they have a history of use and understanding. If that is EPA policy, it may be practical and expedient, but I am not sure that it is scientifically supportable.

I was one of the original six Executive Secretaries when the EPA Science Advisory Board was created in 1972. Despite the fact that individual members of the Science Advisory Board of EPA felt that highly diversified biological communities, as reflected in the values of particular diversity indices, were likely to be more “healthy” or “stable” compared to communities with lower values of their appropriate indices, the EPA Science Advisory Board never to my knowledge issued either a pronouncement or endorsement of that “wisdom” as EPA science policy. There are very good scientific reasons why one should not regulate a diversity index.

E.C. Pielou began her work by noting that the statistical analysis of biotic assemblies using diversity indices must consider whether the biotic sample is a complete collection, or itself a sample of a larger collection or assembly. In looking at the properties of complete collections, she noted that an appropriate measure of diversity is the Shannon-Wiener metric from communications theory. There is a school of environmental analysis which, originating in the 1960's with the work of Ramon Margalef, looked at fish community diversity using this index, and noted certain characteristics of the community structure. Much of the success of Margalef's work rested in the fact that he measured and observed a considerable number of related parameters and systematically collected huge amounts of collateral information to do these correlations.

In the numerical limit of large collections, essentially “infinite” numbers of species and “infinite” populations within species, the evaluation of the Shannon-Wiener metric can use the well-known mathematical formula, Stirling’s approximation of the logarithm of factorial. This approximation rapidly evolves the Shannon-Wiener formula to one that is known in communications theory as the “statistical entropy.” This is the formula which Boltzman used in his work.

What is numerically a suitable “infinite” number of species and a suitable “infinite” population within a species? For some systems, as few as twenty species, and as few as thirty individuals within a species qualifies for the use of the approximation. For lower numbers, one must use calculators and evaluate the factorials.

One of the proposals was that scaled values of this diversity index were like the barometers of community health or stability. When investigators noted that the diversity index was an entropy function, they concluded that this meant somehow that the diversity of a biotic community had a thermodynamic meaning or significance. From there to “health and stability” was a very short trip.

I have taught thermodynamics to chemical engineers, molecular biologists, and other groups. My own doctoral dissertation used non-linear thermodynamics to evaluate certain thermal physiological properties of fishes. Much of the current theory in molecular biology derives from non-equilibrium, non-linear thermodynamics, as pioneered by Ilya Prigogine, Elliott Montroll, John Ross, Manfred Eigen, Joel Liebowitz and others. Statistical entropy plays a very important role in this theory, and it is a *particular time derivative of statistical entropy* which has the mathematical properties of a measure of stability in the classical Lyapunov sense. When that was noted, some biologists felt that this diversity index was a theoretically justified measure of stability. A further axiom of ecology in this school held that “stable communities are healthy communities.”

E.C. Pielou showed that diversity indices, even as functions of time, for a collection or community, although they may resemble thermodynamic functions, have no thermodynamic meaning. The mathematical advantages are that the metrics behave like “functions of state,” in that if one can characterize a community as in State I, and another community in State II, the combined community will be in a state represented as State I + State II. What that means ecologically is unknown. It is all mathematical coincidence in evaluating diversity.

I have used these mathematical properties in my own work to look at the structure of models of selected biotic communities in which one is using “indicator biota.” Here one notes that the population distribution of the indicator biota over time fit a particular statistical distribution, a pdf (probability density function). Given a pdf, one can directly calculate an entropy related to this pdf. That number can be combined with the entropies from other species in the area to develop an entropy measure of a community, but one has a particular statistical/mathematical model of that community as a starting point. The resulting numbers have no interpretation with respect to ecological health, but can point out instability properties of the model. If these properties are actually in found nature, then one has an independent assessment of a kind of stability. The particular stability is resilience to a perturbation or disturbance that may remove one or more species, add one or more species, change one or more species, or some combination thereof.

The particular entropy calculated in the Shannon-Wiener metric is known in thermodynamics as the “configurational” entropy. This is entropy related to the contribution from molecular symmetry, arrangements, etc., and is a non-thermal (non-temperature related) entropy.

How does “configurational entropy” measure community structure? Joel Hedgpeth once noted the conceptual difficulty in considering taxa as analogized to the symbols in a message, and the community structure visualized as a particular kind of message. In chemical physics, many molecular arrangements, including the arrangements of redundant quantum states, will produce the same numerical value of configurational entropy. In a biotic community, the substitution of taxon A for taxon B in the collection, while maintaining the same population distributions of all taxa, will give the same result. Other situations related to changes in the number of taxa in a collection and their relative distribution of populations, will also produce similar or identical indices. From a community assessment standpoint, the effects of collection bias and method or equipment sensitivity and selectivity tend to work against assigning any meaning to these numerical results.

My advice is E.C. Pielou’s advice: Measure diversity. Use the metrics to examine the structure of different collections which belong to a given community to evaluate community content, succession, selectivity of bias in collecting method or equipment, and to give other insights into community structure. Then with other information on rare or unusual species, absence of expected species, and comments on the “efficiency” of completeness of a particular sampling method, one can assess community structure without resorting to some speculative pronouncements on health and stability.

10. Thou Shalt Include Ground Truth in Your Protocols

Ground truth is just that: showing that what one observes remotely is indeed what exists at the observed site. One does this by going to the observed site, and simultaneous with the remote approach, does an at-hand approach. It may be observational, or it may be instrumental, or it may be on-site collections and measurements with subsequent laboratory work.

Establishing ground truth is a complicated situation when only the remote tools are easily available. The diver, who describes the bottom of the river to establish reasonable sampling locations by remote equipment, is an example of a ground truth technique.

Ground truth prevents conclusions based on possible artifact situations. Many of the sampling techniques, computer models and methods of analysis, generate possible artifact situations. These artifacts arise from the selectivity biases of equipment and methods, the mathematical nature of inherently non-linear processes which are approximated by linear functions, and unforeseen circumstances.

An element of ground truth unique to biological sampling is assuring the identity of what is found in certain biological samples. In my training in limnology, I studied entomology at the hand of the great aquatic chironomid specialist, Dr. Selwyn (Sam) Roback. He impressed the need to run simultaneous culturing facilities for insects, because many biological samples were dominated by unknown larval forms.

The taxonomic keys at the time covered adult chironomids only. Guess what? Most of them still do, but some inroads have been into this problem. Unless one samples at the end of a growth season, the chances are not great that the insect collection from a river is dominated by adult chironomids rather than larval forms.

Sam's purposes in culturing were three-fold: first, to see what adult species emerged from basically unidentifiable larvae, second, to improve the taxonomic keys for future investigators based on laboratory information from growth studies of the larval forms, and third, to obtain additional information on life support needs, organism physiology, and the ability to recognize species out of their normal range, especially for rare and unusual forms. Along the way, one obtained insights into collecting techniques and sampling methods, when one had to decide whether to sample an area that had mainly immature insect collections versus areas which had mature insect collections.

For bioassessment purposes, if one is looking at the structure of insect communities, and trying to draw inferences about this structure from the presence or absence, abundance or scarcity of specific taxa, it is absolutely essential to know what species are present. The statement “chironomids” alone does not indicate much about the community, since in rivers chironomids often dominate the insect fauna without special significance. At other times, this group implies organic enrichment, or an ability to tolerate certain kinds of pollutants. In this latter regard, Sam noted over the years that iron enrichment was often present in the water when certain chironomids dominated, and his study of rivers subject to acid mine drainage, had such special chironomids.

Chironomids are not the only groups which benefit from a ground truth laboratory confirmation. My friend Ralph Brinkhurst, whose mantle probably has moved to another person I have known for many years, Trefor Reynoldson, always cultured the oligochaete worms from his biological collections as a laboratory confirmation. In fact, except for hirudinea (leeches), and possibly some platyhelminthes (mainly species of *Planaria*), almost all vermiform species could benefit from laboratory culturing and confirmation. Another group of organisms, the freshwater sponges, sometimes require such confirmation, especially if only the certain resting stages of these organisms are in the collection. And then there was the king of culturing, the late Prince Luigi Provasoli, who to my amazement, could culture almost anything, and in most cases, could do it axenically.

At some point, all sampling plans should have a protocol to include the ground truth. It may not apply to all situations, but failure to include provision to assess ground truth may lead to erroneous conclusions from observed and calculated situations.

11. Thou Shalt Use Common Sense

My final remarks relate to **common sense**. All of the previous advice can go for naught if one does not use some common sense in approaching these problems. Unfortunately, the process of establishing bioassessment and biomonitoring plans has sometimes become inextricably linked with some pseudo-ecological and bizarre statistical thinking. Recall at the beginning I quoted from my former professor of analytical chemistry: “sampling is an art.” If it were a science, then these final remarks would be less urgent. Science would prescribe what to do, and here one uses the common sense understanding that there are: no perpetual motion machines, and therefore, do not propose something that violates the Second Law of Thermodynamics.

In looking at sampling as an art, I caution against becoming transfixed by the “artistic” components. Aesthetic components of monitoring and modeling are nice, but represent luxuries one can ill afford. Rather, I recognize that art implies “empiricism,” and empiricism implies “trial and error.”

My colleague, Dr. Ursula Cowgill (who at one time was G. Evelyn Hutchinson’s analytical chemist) struggled with field sampling problems for years. Her constant testing and Bayesian learning approach to the sampling problems were remarkable. In one study she even compared sampling plans which involved sampling from different positions and sides on a vessel to see if it mattered where one trawled a net or used pump or trap device. Even the use of Bayesian learning/statistics becomes a common sense question because of the philosophical aspects of buying into a Bayesian statistical framework.

With these remarks, I wish all of you success in your deliberations and discussions. I can entertain a few questions, but for the most part, it would probably be more fruitful to address your questions in the various panel discussions.