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Environmental biomarkers as indicators of chemical exposure*

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Abstract

Biomarkers are anatomical, physiological, and biochemical responses of an organism which indicate exposure to and/or effects of a stressor. Research on environmental biomarkers has focused on responses in fish and birds to xenobiotics such as heavy metals and organic pollutants. The research objective for biomarkers is to develop "early warning" indicators at the individual level that can predict population or ecosystem level impacts. Biomarkers may play an important role in future ecological risk assessments, especially if suites of markers responsive to general and specific stressors are developed, validated, and employed. Towards this goal, we designed a field study to evaluate biologically treated, bleached kraft mill effluents (BKME) in Northern Canada by integrating traditional fisheries approaches with biomarker and chemical analyses (metals, organochlorines) in individual fish. Cytochrome P4501A was induced differentially in two fish species, while histopathology, blood chemistry, and population parameters appeared normal compared to values from a reference river system. Thus, P4501A induction appears to be an indicator of BKME exposure, but induction at this site does not predict individual health or population level effects.

1. Introduction

Although environmental biomarkers have been in development and use for decades, albeit under different terminologies, their use in ecological risk assessment is in its infancy. This article focuses on the potential application of biomarkers in environmental risk assessment and is based on a presentation at

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the August 1992 American Chemical Society Symposium "The Role of Environmental (Ecological) Assessments in the Management of Chemical Pollution." First, the concept of environmental biomarkers is briefly developed. Second, a case study is used to highlight: (1) some advantages and disadvantages of the current use of biomarkers; and (2) what is needed for practical ecological risk assessment applications in the future. Some of the concepts are drawn from a NATO workshop on the application of environmental biomarkers [1]. The case study — an examination of biomarkers in Northern Canadian fish exposed to bleached kraft pulp effluent discharges — has been described in detail elsewhere [2, 3].

2. Background

The term "biomarker" is perhaps most familiar in a biomedical context. For example, the presence of environmental agents in human tissue samples is used to quantitate exposure to compounds. This information can be used by epidemiologists to assess any relationship between dose and effect. In a more general sense, the routine clinical tests performed on human blood and urine samples can be considered biomarkers. Ratios of high and low density lipoproteins and high cholesterol levels are used as indicators of potential heart disease or stroke, and numbers and condition of white blood cells are used to indicate the status of the immune system. As a result, an extensive human database is available to indicate normal and abnormal values. Even when the clinical significance of "out-of-range" values is unknown, such values are easily identified and further testing can be undertaken.

It has been difficult to obtain a consensus on a definition for "environmental" biomarkers. For example, what levels of biological organization — subcellular, cell, tissue, organ, individual, population, community, and/or ecosystem — should be included? While there is general agreement that the focus of biomarkers is suborganismal, the exact cutoff may depend on an individual investigator's technical background, research or regulatory area of interest, and perhaps even philosophy. One definition states:

'. . . any contaminant-induced physiological and/or biochemical change in a not-too-sensitive organism which leads to the formation of altered structure (a lesion) in the cells, tissues, or organs of that individual" [4].

This is an excellent, but quite focused definition — it should not be surprising that the lead author is a histopathologist and thus most interested in structural problems.

An "environmental biomarker" is defined in this article as a biological indicator of change, an early, sensitive, quantifiable measure of altered function in an organism in response to a stressor. Stressors may be physical (e.g., low dissolved oxygen levels) and/or chemical (e.g., natural or man-made compounds) factors which drive the organism outside of its normal "homeostatic"

range of responses [5]. This definition is broad and flexible enough to accommodate site- or study-specific requirements.

Current interest in biomarkers is focused upon their potential to define exposure and/or to predict higher order effects from foreign compounds, generally of anthropogenic origin [6]. Field use of biomarkers has been primarily with avian species and fish, in situations of known pesticide, heavy metal, or polycyclic aromatic hydrocarbon exposures. One specific interest is in natural populations exposed to combinations of "normal" stressors (e.g., changes in temperature, water flow, species density, prey availability, etc.) and the additional foreign stressor(s). These realistic exposure situations cannot be adequately reproduced in controlled laboratory experiments. Despite the difficulty inherent in interpretation, the advantages, such as integration of actual exposures to multiple stressors, make the effort to identify and utilize biomarkers quite attractive. One eventual goal is to fully integrate biomarker measures into an ecological risk assessment framework by assisting in risk characterization in field situations.

In applying biomarkers to field situations, multiple stressors make it likely that multiple endpoints will be chosen. There are currently "non-specific" biomarkers that may change in response to a variety of different stressors (e.g., increased blood levels of tissue enzymes which indicate hepatotoxicity or damage to other organs) as well as "specific" biomarkers that may change due to exposure to a narrow class of stressors (e.g., pesticide inhibition of acetylcholinesterase). Thus, depending upon the character of the suspected stressor(s), different — and multiple — biomarkers are likely to be chosen for analysis of a given field exposure situation.

A fundamental principle of toxicology is the dose–response relationship, whereby a biological response will increase with increased dose (sometimes up to a point of overt toxicity, at which point the response declines). One set of biomarkers may respond in "traditional" fashion (with individual linear, S-shaped, or bell-shaped dose–response curves) but only indicate exposure to and biological response to a compound. Another set of biomarkers (each again with its own dose–response pattern) may be predictive of, or directly linked to, significant biological effects (e.g., mortality, growth rate changes, reproductive impairment). As exposure to a toxic substance increases, some biomarkers may return to, or even fall below, normal values, before or while the threshold of response of others is reached (see also [5], Fig. 2.1). The duration, as well as the magnitude, of exposure will determine the overall response pattern. Thus, a highly complex series of response relationships may exist in the field. Substantial fundamental research into the relationships between different biomarker responses will greatly aid in the interpretation of field measurements and in the extrapolation from laboratory to field situations.

Biomarkers are being developed and applied in a regulatory context in the field. For example, the U.S. Environmental Protection Agency is currently developing a new Ecological Risk Assessment Framework [7]. Details about this program are provided in other presentations at this Symposium. Several

countries have already included biological indicators in field monitoring guidelines, focusing on pulp and paper industry discharges to the aquatic environment [8, 9]. These markers include assessment of cytochrome P4501A enzyme activity, levels of circulating reproductive hormones, and histopathology of exposed animals. There will be growing interest in the application of these and other sensitive techniques in a variety of field situations. A case study describing the measurement of a suite of markers at a specific study site and the study's key conclusions are outlined below.

3. Case study

3.1 Background

Biomarkers have been utilized in field situations for many years, although seldom within a broad framework of population-level measures to place them in context. The most frequent application has been at sites with point-source (e.g., oil spills) or known non-point source (e.g., pesticide application) pollution. One of the first aquatic studies to employ an extensive suite of biological markers occurred at a bleached kraft pulp mill site at Norrsundet, Sweden, where obvious physical, chemical, and biological degradation of the ecosystem had occurred [10]. The levels of several biomarkers in fish collected near the mill also changed significantly from those measured at a reference site [11].

In designing a comprehensive study of the environmental fate and potential effects on fish populations of bleached kraft mill effluent (BKME) in a river system in Western Canada, we drew upon the biomarker list from the Norrsundet study and included most of those which showed significant differences. Our objectives were: (1) to determine whether biomarker responses occurred as a consequence of BKME exposure at this site and, more importantly; (2) to better understand potential relationships between biomarker responses, contaminant exposures, and fish population "health" at this site. Thus, key components included: (1) relevant fish population parameters (Table 1); (2) a suite of suspected contaminants in all relevant aquatic compartments, particularly individual fish (Table 2); and (3) a suite of biomarkers in the *same* individuals (Table 3). With this dataset, one-to-one correspondence between exposure measures and biomarker responses could be examined, as well as relationships among biomarker responses. In addition, the general health of these populations could be assessed and tied to the biomarker responses.

Wastewaters from the Grande Prairie mill are treated by a primary clarifier and secondary aerated lagoon, then discharged to the Wapiti River, a free-flowing stream (Fig. 1). Major reductions in effluent color, AOX, dioxin/furan, and chlorophenolics were achieved by spring 1991 following major process modifications [12]. At the point of discharge, effluent comprised from less than 0.1% at high flow to 3% at low winter flow of the receiving water. The mill has been in operation since 1972, thus providing "chronic" exposure to BKME for several generations of fish. Fish were collected by boat electroshocking in

TABLE 1

Population parameters examined in the case study

Parameter	Parameter
Group characteristics	*Fish movement*
Species composition	Tag/recapture
Relative species abundance	Radiotelemetry
CPUE	
Age distribution	*Reproduction*
Recruitment (young-of-year)	Age to maturity
Mortality	Fecundity
	Relative gonad size
Habitat surveys	Egg diameter
Channel form	
Substrate characteristics	*Condition*
Bank characteristics	Length/weight relationships
Water depth and flow	Fate content
Flow type	Growth rate
Stream gradient	Stomach contents
Vegetation cover (banks, substrate)	
Qualitative invertebrate density	

reaches upstream and downstream of the discharge, as well as on a reference river without BKME inputs (Figs. 1A and B). Two major species, the longnose sucker (*Catostomus catostomus*) and the Rocky Mountain whitefish (*Prosopium williamsoni*), were selected due to their size and abundance, plus the presence of detectable organochlorine residues in both species [3]. Sampling occurred repeatedly over several seasons from summer 1990 to spring 1992. Details of chemistry [13], fish population findings [3], and individual biomarker responses [2, 14] are provided elsewhere.

3.2 Summary

Radiotelemetry confirmed that both species could be highly mobile, moving kilometers upstream or downstream of the mill discharge area within a few days time [3]. Thus, chemical analysis of bile, liver, and muscle tissue from individual fish was necessary for direct exposure assessment. Whitefish were found to have greater body burdens of lipophilic compounds than suckers. However, higher lipid content in whitefish can account for only some of this elevation. Diet is likely to play an important role, although both whitefish and suckers feed upon benthic organisms. Stomach content analysis indicated whitefish prefer benthic invertebrates which themselves accumulate lipophilic compounds by feeding upon contaminant-bearing suspended sediment. In contrast, suckers prefer benthic invertebrates which burrow into the cleaner bottom sediments. Analysis of various environmental compartments, from the

TABLE 2

Chemical parameters[a] and environmental compartments examined in the case study[b]

Parameter	Compartment						
	Water	Bottom sediment	Suspended sediment	Benthic invert.	Fish muscle	Fish liver	Fish bile
Conventional parameters							
Major ions	✓						
Nutrients	✓	✓					
BOD, Color	✓						
Chlorate	✓						
Texture		✓	✓				
% OC		✓	✓				
Metals							
ICP Scan, 25 metals	✓	✓		✓	✓		
Mercury	✓	✓		✓	✓		
Mill effluent organics							
AOX	✓						
EOCl		✓	✓	✓	✓	✓	✓
Dioxins (7 congeners)	✓	✓	✓	✓	✓	✓	✓
Furans (10 congeners)	✓	✓	✓	✓	✓	✓	✓
Chlorinated phenolics	✓	✓	✓	✓	✓	✓	
Chlorinated phenolic metabolites							✓
Resin and fatty acids	✓	✓	✓	✓			✓
Chlorinated resin acids	✓	✓	✓	✓			✓

[a] BOD–biological oxygen demand, OC–organic carbon, ICP–ion-coupled plasma, AOX–adsorbable organic halide, EOCl–extractable organic chlorine. Chlorinated phenolics include phenols, catechols, guaiacols, veritroles, syringols, vanillins.
[b] Table revised from [21]. For details of compounds and test methodologies, see [13].

water column, to suspended and bottom sediments, to invertebrates and fish [15], was necessary to assemble this "fate" pathway.

Few differences were observed among biomarker measurements in suckers and whitefish from the Wapiti and the reference river systems. For example, of 23 blood chemistry/hematology parameters examined in each species (Table 3), only one was consistently different. Blood hemoglobin content was lower in Wapiti River longnose suckers. There were no significant reductions in

TABLE 3

Biological indicators examined in case study

Tissue	Parameter	Tissue	Parameter
Whole body	Internal pathology		
	External pathology	Gonad	Histopathology:
	Condition factor	Gill	Structure of tissue,
Viscera	Visceral fat stores	Liver	
		Heart	e.g., lesions, foci
Otolith/Scale/	Age (age to maturity;	Kidney	
fin ray	age distribution)	Spleen	
Blood	White blood cell (WBC) counts		
	Red blood cell counts	Liver	Total cytochrome P-450
	Hemoglobin		P4501A content
	Packed cell volume		EROD activity
	WBC Differentials (% cell type)		Microsomal yield
	Sodium		
	Potassium	Liver	Liver somatic index
	Chloride		
	Calcium	Ovary	Fecundity (eggs/female)
	Phosphorus		Egg diameter
	Magnesium		
	Urea	Ovary/Testis	Gonadosomatic index
	Creatinine		
	Glucose	Blood	Testosterone
	Cholesterol		17-β-Estradiol
	Alkaline phosphatase		17,20-Dihydroprogesterone
	Creatinine kinase		
	Aspartate aminotransferase		
	Alanine aminotransferase		
	Gamma globulin		
	Total protein		
	Albumin		
	Albumin/globulin ratio		

circulating hormone levels. (Levels of sex steroids have been lower at some BKME sites, such as Jackfish Bay [16] and the St. Maurice River [17].) Further work on biologically active ranges of sex steroids in these little-studied species will aid in the interpretation of such results.

The major consistent difference observed in Wapiti River fish was an increase in the content and consequent activity of a detoxification enzyme, cytochrome P4501A. Content of this enzyme increases when vertebrates are exposed to lipophilic compounds with similar structural and physico-chemical properties. The catalytic activity of the enzyme appears to be the rate limiting step in the eventual elimination of such compounds. Activity, as measured by

ethoxyresorufin O-deethylase (EROD) activity, was increased two- to threefold in suckers and an average of 30-fold in whitefish during the period of highest whitefish EROD activity [2, 18]. Subsequent sampling after the mill process changes indicated that whitefish EROD activities, while still elevated, had declined [19]. EROD activity correlated well with some chemical measures of chronic exposure in whitefish, but not suckers.

Fisheries analysis [3] indicated that the Wapiti/Smoky river system supports a more diverse community of fishes than does the North Saskatchewan River. Sucker and whitefish populations appear to be viable in both systems. There were no significant differences in growth rates and age distributions between the systems for either species. Gross measures of reproduction (e.g., secondary sex characteristics, relative gonad size, recruitment, age to

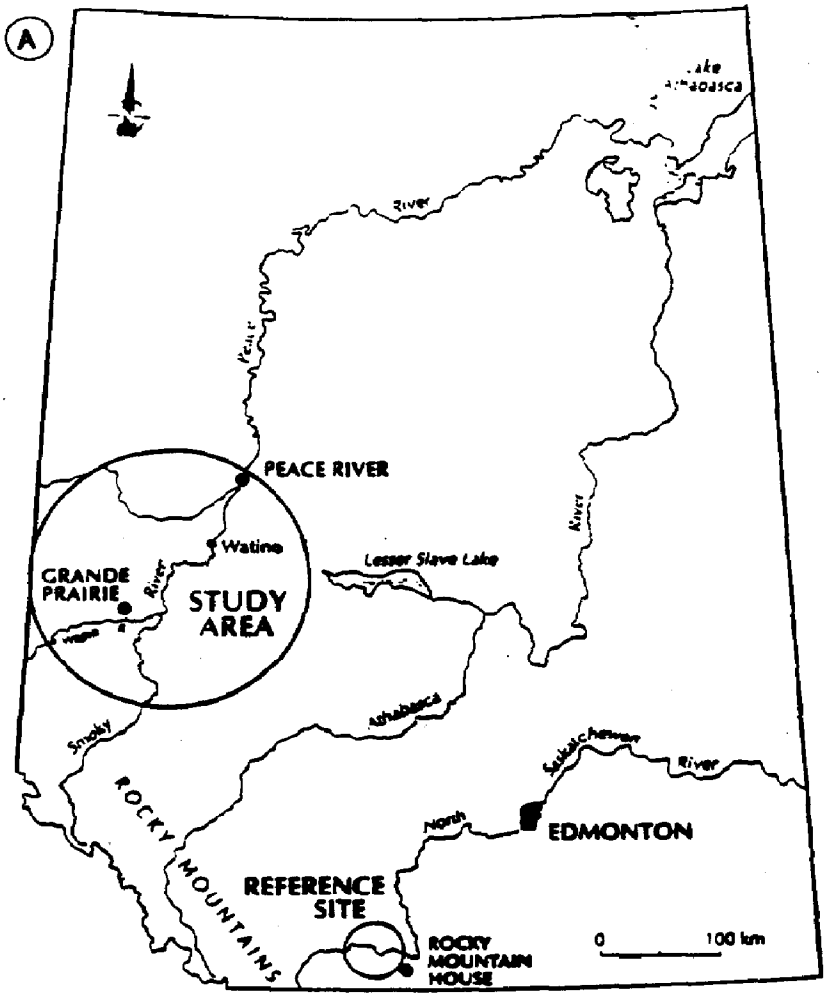

Fig. 1. Study sites in Northern Canada.

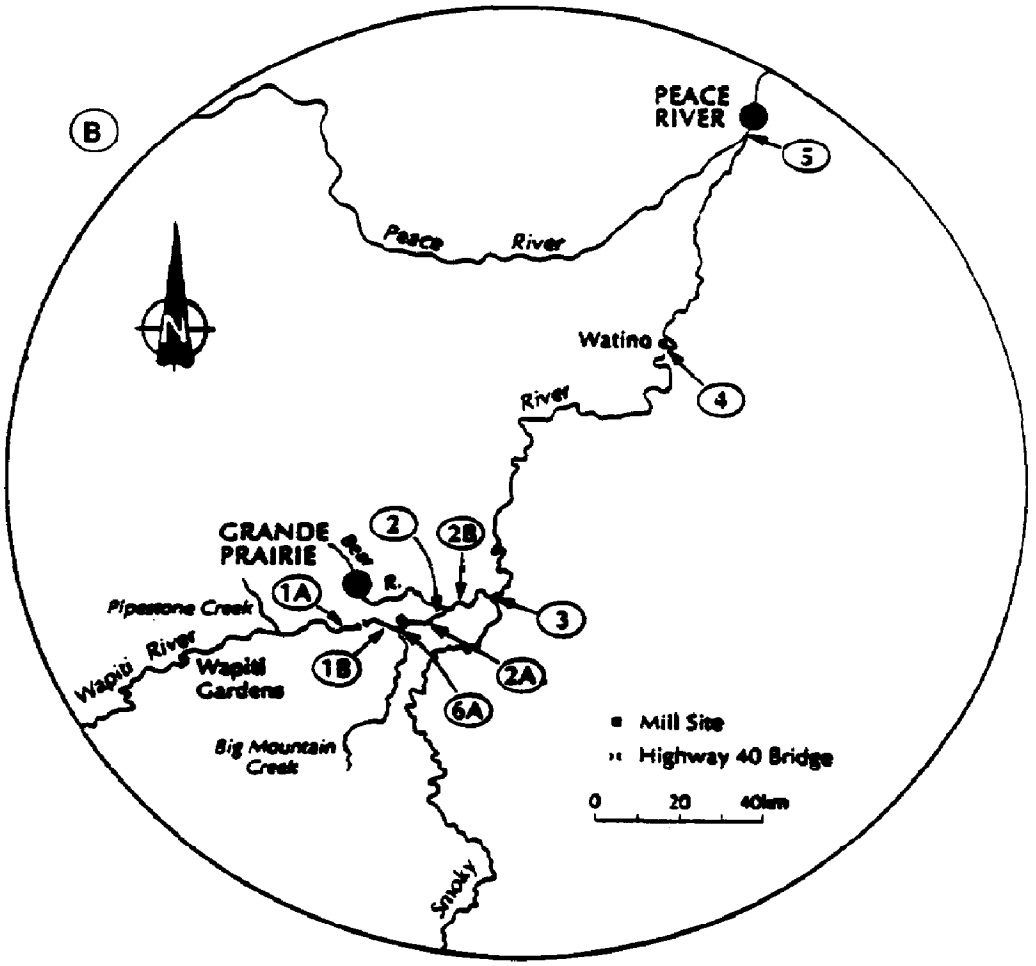

Fig. 1. Continued.

maturity) were similar for BKME-exposed and reference suckers and for BKME-exposed and reference whitefish.

Major disturbances in physiological function, morphology, or reproduction which have been observed at other selected pulp mill sites [10], were not observed at the Grande Prairie mill (see Table 4 for comparison of biomarker findings). Grande Prairie effluents have been biologically treated since mill start-up and the health of the benthic invertebrate community has been monitored routinely. Some of the effects observed in other, earlier studies may have been due directly to toxic effects or indirectly to major ecosystem (e.g., habitat) disturbances. Even at the Norrsundet site, conditions have improved since the initial sampling periods, as evidenced by fewer significant changes in perch biomarker responses in recent years (Table 4). In our study, induction of P4501A was an excellent marker of exposure to *Ah*-receptor active compounds [20]. Organ size, blood electrolytes, condition indices, reproductive capacity, and population level endpoints were not affected.

TABLE 4

Biochemical and physiological responses in perch (*Perca fluviatilis*) and whitefish (*Prosopium williamsoni*) collected near pulp mills

Measure	Perch[1]			Whitefish[2]
	1984/85	1989	1990	1990–1992
LSI	+ +	0	0	0
GSI	+ + +	+	+(+)	0
EROD	+ + +	+	+	+ + +
Glycogen	+	0	+	nd
Lactate	+ + +	0	0	0
Cl^-	+ +	+	+	0
Ht	+ +	+	+	0
WBC	+ +	0	nd	0
ALAD	+ + +	0	nd	nd
Histopath	nd	nd	nd	0

[1] Perch from the collection site nearest a conventional pulp mill, Norrsundet, Sweden. The mill has undergone extensive process modifications, with resultant changes in effluent characteristics. Data are from [22].

[2] Rocky Mountain whitefish collected near Grande Prairie, Alberta over several seasons. Data are summarized from [2] and [14].

Legend: + = significantly different from reference site, 0 = not significantly different from reference site, and nd = not determined. LSI – liver somatic index, GSI – gonadosomatic index, EROD – ethoxyresorufin *O*-deethylase activity, glycogen – muscle glycogen, lactate – blood lactate, Cl^- – plasma chloride, Ht – hematocrit, WBC – white blood cells, ALAD – δ-aminolevulinic acid dehydratase, histopath – histopathological analysis of key tissues (e.g., liver, kidney, spleen, heart, gill).

4. Conclusions

(1) Earlier field studies at pulp mill sites generally lacked a comprehensive dataset of both biological and chemical observations. This necessitated assumptions about the relationships between exposure, body burdens, and biomarker- and population-level effects. In the case study presented, chemical data for individual fish were essential for assessing BKME exposure. First, these species were highly mobile. Second, the species apparently incorporated contaminants via different primary routes despite the same water borne exposure and the same general bottom-feeding strategy.

(2) Coordination of population-level indices and biomarker measurements provided a means to assess the potential cause and effect relationships of endpoints measured at different levels of biological organization. Although relatively costly and time-consuming, such an approach is necessary given our current limited understanding. This is due to both the interaction of organisms with foreign chemicals in their environment and the complexity of pulp mill effluents. Our results indicate that release of BKME from the Grande Prairie

mill is having no adverse effect on key fish populations in the Wapiti/Smoky, even at biochemical, physiological, and anatomical levels.

(3) Biomarkers can provide valuable insight into the initial responses of an organism to exposure, as evidenced by this investigation, and may also predict higher level effects. Some biomarkers may quantify transient changes in fish physiology or important acclimation responses of the organism to exposure to stressor(s). Other markers may directly indicate or predict deleterious effects. As mentioned above, an ultimate goal is to fully integrate biomarker responses into an ecological risk assessment framework. Thus, two items must be clarified in order to make accurate assessments: (a) is a biomarker an indicator of exposure or of effect; and (b) where on the continuum of dose–response curves the biomarker value is found.

(4) Because of this knowledge gap, continued laboratory and field research is needed to fully exploit the potential of biomarkers as tools for environmental monitoring and risk assessment. Importantly, improved understanding of the normal fluctuations in both biomarker and higher-level parameters is necessary for appropriate interpretation of field data. In addition, the "expected" patterns of responses must be validated for a given stressor type. (For example, BKME-exposed sucker hemoglobin levels were lower than reference levels, while those for Norrsundet perch were elevated.) In the future, it is likely that nondestructive biomarker sampling techniques will be further developed and that new emphasis will be given to biomarkers more easily linked to higher order effects (e.g., reproduction, growth, energy utilization). Research efforts are also needed to expand environmental biomarkers beyond vertebrates to aquatic and terrestrial invertebrates and plants, given the growing realization that in order to assess the ecosystem, monitoring the health of target species in fundamentally different functional niches will be required.

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