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Assessing Sources of Stress to Aquatic Ecosystems: Using Biomarkers and Bioindicators to Characterize Exposure-Response Profiles of Anthropogenic Activities

S. Marshall Adams

Environmental Sciences Division
*Oak Ridge National Laboratory**
Oak Ridge, Tennessee 37830 USA

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Abstract

Establishing causal relationships between sources of environmental stressors and aquatic ecosystem health is difficult because of the many biotic and abiotic factors which can influence or modify responses of biological systems to stress, the orders of magnitude involved in extrapolation over both spatial and temporal scales, and compensatory mechanisms such as density-dependent responses that operate in populations. To address the problem of establishing causality between stressors and effects on aquatic systems, a diagnostic approach, based on exposure-response profiles for various anthropogenic activities, was developed to help identify sources of stress responsible for effects on aquatic systems at ecological significant levels of biological organization (individual, population, community). To generate these exposure-effects profiles, biomarkers of exposure were plotted against bioindicators of corresponding effects for several major anthropogenic activities including petrochemical, pulp and paper, domestic sewage, mining operations, land-development activities, and agricultural activities. Biomarkers of exposure to environmental stressors varied depending on the type of anthropogenic activity involved. Bioindicator effects, however, including histopathological lesions, bioenergetic status, individual growth, reproductive impairment, and community-level responses were similar among many of the major anthropogenic activities. This approach is valuable to help identify and diagnose sources of stressors in environments impacted by multiple stressors. By identifying the types and sources of environmental stressors, aquatic ecosystems can be more effectively protected and managed to maintain acceptable levels of environmental quality and ecosystem fitness.

Introduction

Environmental scientists are continually confronted with issues related to assessing and evaluating the effects of environmental stressors on the health of aquatic ecosystems. Some of the more challenging issues which have proven to the most problematic are (1) evaluating the relevance of laboratory studies, particularly toxicity testing, for application to field situations, (2) assessing the ecological significance of the sensitive early-warning indicators of ecosystem health, (3) importance of temporal and spatial variability in assessing aquatic ecosystem health, and (4) establishing cause and effect relationships between specific stressors and environmental damage.

Laboratory studies, employing standard toxicological testing, have been one of the primary approaches by which the effects of stressors has been assessed on the health of aquatic organisms. Laboratory studies typically involve short-term exposures of one or more contaminants on standard test organisms, and the effects of these contaminants are evaluated using lethal or other simple endpoints such as survival, growth, or reproductive potential. However, it is evident that an organism ceases to function normally long before these critical endpoints are reached (Larsson *et al.* 1985). Furthermore, the test conditions during lab studies seldom reflect the environment of natural populations (Cairns 1981) lacking ecological realism (NRCC 1985, Lagadic *et al.* 1994). Many of these laboratory studies have focused on short-term exposures using lethal and other nonspecific endpoints. Thus, much of the progress in aquatic toxicology had been due to an increase in data rather than an increase in knowledge (Wester and Vos 1994).

Over the past two decades there have been many sensitive "early-warning" indicators (biomarkers) measured on sentinel organisms collected at field sites which have been subjected to pollutants and other environmental stressors. Some of the more well known and sensitive biomarkers of pollutant exposure are the mixed function oxidase (MFO) P450 detoxification enzymes (Stegeman *et al.* 1992; Jimenez and Stegeman 1990), heat shock (HSP70 stress proteins) (Pyza *et al.* 1997, Bradley 1993), antioxidant enzymes (DiGiulio 1992, Doyotte *et al.*

1997), and various measures of DNA damage (Shugart *et al.* 1992, Theodorakis *et al.* 1992). In many cases these biomolecular and biochemical measures have proven to be excellent biomarkers of exposure to a variety of environmental stressors. These exposure biomarkers cannot serve, however, as ecologically relevant indicators of stress effects at higher levels of biological organization. Studies which include biomarkers of exposure must also be conducted, however, to help understand the mechanisms or relationships between stressors and ecologically relevant endpoints.

Notwithstanding these first two issues related to the relevance of laboratory studies to field applications and the ecological significance of early warning biomarkers of stress, perhaps the most important challenge facing environmental scientists is establishing causal relationships between environmental stressors and ecological relevant effects (Adams et al. in press). Relating stressors to biomolecular and biochemical markers of exposure and ultimately to some relevant ecological effect is particularly problematic in natural field situations primarily because of the many biotic and abiotic factors which can influence or modify responses of biological systems to environmental stressors (McCarthy and Munkittrick 1996; Wolfe 1996), the orders of magnitude involved in extrapolation over both spatial and temporal scales, and compensatory mechanisms such as density-dependent responses that operate in nature populations (Power 1997).

An observable biomolecular or biochemical response such as induction of the P450 system from PAH exposure or inhibition of acethycholinerase from pesticide exposure will not necessarily be manifested as measurable biological effects at higher levels of organization. For effects to be realized at increasing higher levels, the stressor (s) must be of sufficient magnitude and duration to overwhelm the normal homeostatic capacity of specific biological systems (Schlenk *et al.* 1996). For example, when the capacity of protein systems such as the HSP70 stress proteins are exceeded, pathological damage can occur to important tissues or organs such as the liver, gill, or kidney. Structural damage to liver tissue can, in turn, compromise the ability of this organ to produce vitellogenin, a critical component of egg development and ultimately compromise reproductive success of individuals. Starting with normal individuals, exposure to contaminants

and other stressors results in a progressive deterioration in organism health which may ultimately compromise the success of populations and communities (Fig. 1). Departures from the healthy state in organisms are associated with the initiation of compensatory responses with little change in disability (zone 1, Fig. 1). Additional impairment beyond the compensation limit may become associated with increased disability and overt disease (zone 2, Fig. 1). With additional environmental challenges, the survival potential of organisms declines because of their decreased ability to respond to increased challenges. Beyond the limit of compensation, it is unlikely that organisms could successfully mount any response at all to additional environmental challenges (zone 3, Fig. 1). Provided, however, that environmental conditions improve sufficiently and rapidly enough, an organism may be able to recover somewhat and repair damaged systems and restore compensatory responses. Monitoring impairment of biochemical, physiological, and behavioral responses, however, should provide early warning of the onset of disabilities (Depledge 1989). On the disability scale (Fig. 1), measures of stress effects are usually not detected until after the loss of compensation, while on the impairment scale effects of stressors are detected much earlier and can be reversible and curable.

Several approaches have been applied to the problem of establishing causal relationships between stressors and biological effects. Except for field studies that have attempted to relate spatial patterns in contaminant loading with spatial patterns in biological responses over several levels of organization usually from point sources of contamination (Adams *et al.* 1996; Soimasuo *et al.* 1995; Ericson *et al.* 1998), there are no reliable and proven techniques that have been applied in field studies to address the issue of establishing causal relationships between stressors and ecological significant effects. Because it is difficult to establish causality between environmental stressors and ecologically relevant effects in field situations, approaches are needed which can help identify sources and causes of environmental damage which at higher levels of biological organization. The primary objective of this study, therefore, is to develop and demonstrate a diagnostic approach that can help identify sources of anthropogenic stress in aquatic systems which are impacted by multiple stressors.

Approach

The health of aquatic ecosystems can be compromised by a variety of stressors which are related to anthropogenic activities including domestic sewage; atmospheric deposition; agricultural activities; mining operations; land -use activities including urban development, logging, and clear cutting; heavy industry including pulp and paper mill discharges; and petrochemical operations including drilling, refining, and exploration. Each of these activities produces a specific suite of environmental stressors such as contaminants which characterize that activity. These characteristic set of stressors can be used to separate or identify these activities from each other. For example, point-source discharges from paper mill operations are typically characterized by chlorophenolic and resin acid compounds, dioxin-type contaminants, and high nutrient loading. In contrast, non-point source agricultural activities can contribute pesticides, nutrients, and sediment to receiving aquatic ecosystems. At the lower levels of biological organization, these various environmental stressors, which are related to specific anthropogenic activities, can also produce a characteristic set or profile of biomolecular, biochemical, or physiological responses in organisms which are generally referred to as biomarkers of exposure to environmental stressors (Huggett *et al.* 1992). The primary advantage of biomarkers is that they can generally respond rapidly and directly to stressors and can serve, therefore, as "early-warning" indicators of effects at higher levels of biological organization. The principle biomarkers generally used as sensitive early-warning indicators and which characterize zone 1 of the disability-impairment curve (Fig. 1) are shown in Table 1.

Because certain stressors can cause specific types of biological responses, it is relatively straightforward to relate cause (the particular stressor) to lower level responses or to these biomarkers of exposure (i.e, biochemical, biomolecular markers). For example, the principle types of exposure responses elicited by PAH-type compounds originating from petrochemical activities are generally high inductions of the P450 system and fluorescent aromatic metabolites in the bile. Agricultural activities, however, may result in inhibition of acetylcholinesterase activity from pesticide exposure. Relating biological responses at higher levels of organization such as at the individual, population, or community level to specific environmental stressors

from specific anthropogenic activities, however, is much more difficult because of the modifying effects of biotic and abiotic factors in the environment, the high temporal and spatial variability of natural systems, and compensatory mechanisms that operate in populations and communities. Responses to environmental stressors at these more ecologically relevant endpoints are typically referred to as bioindicators of effects (Adams 1990).

To construct diagnostic exposure-response profiles for the various stressor exposure-biological effects relationships corresponding to each major type of anthropogenic activity, the principle types of stressors produced or characterized by each activity were first determined and then matched with the corresponding biomarker exposure response (Table 2). Since certain stressors, and in particular various types of contaminants, are associated with specific responses at the biomolecular, biochemical, or physiological levels, this analysis matched each major type of stressor to responses at these lower levels of biological organization. Once the major exposure responses (biomarkers) associated with each anthropogenic activity were identified, a stress exposure-biological response profile was generated for each of these activities by plotting these exposure biomarkers on one axis and bioindicator responses at higher levels of organization on the other axis. A literature review was conducted in order to identify which major types of biological responses at the higher levels of organization (bioindicators) were typically associated with each major type of anthropogenic activity. Those bioindicators which reflect effects at the individual, population, and community levels are generally used to characterize zones 2 and 3 of the disability-impairment curve (Fig. 1) and are listed in Table 1.

Results and Discussion

In Fig. 2, biomarkers of exposure are plotted against bioindicators of corresponding effects to generate biomarker-bioindicator response profiles which are characteristic of each major type of anthropogenic activity. Cross marks within the exposure-response profile for each activity indicate those specific biomarkers of exposure that are associated with various bioindicators of effects based on both field and laboratory studies. The principle biomarkers of exposure for petrochemical and pulp and paper activities are induction of the P450 mixed function oxidase

(MFO) system and production of aromatic and chlorophenolic biliary metabolites, respectively. Effluent discharges from both petrochemical and paper mills have been reported to cause various gill lesions in fish and impair reproductive function in aquatic organisms. Growth, however has been found to decrease under petrochemical exposure and actually increase in systems receiving paper mill effluents due primarily to nutrient enrichment and increased productivity of receiving waters. In addition, organisms inhabiting systems impacted by PAH compounds typically have relatively high incidences of liver tumors, a situation not normally observed in aquatic systems receiving paper mill effluents. The exposure biomarkers related to mining activities and resulting heavy metal contamination of the environment, are the metallothione -type protein compounds, antioxidant enzymes, and DNA damage (genotoxicants). With this activity, decreased growth of fish has been observed along with severe gill damage and bioenergetic impairment. The indicators of exposure for domestic sewage are primarily the antioxidant enzymes which are produced from exposure to chlorine and detergents and some genotoxic indicators of DNA integrity. Organisms living downstream of municipal sewage outfalls have been reported to have increased growth (due to nutrient enrichment), both gill and liver damage, and various levels of bioenergetic impairment. The major environmental stressors associated with agricultural activities are pesticide and herbicides, nutrient additions, and sediment inputs into aquatic systems. The principle indicator of exposure in this situation is usually acetylcholinesterase inhibition. The major bioindicators of effects reported for agricultural activities are reproductive dysfunction, decreased growth, gill damage, and bioenergetic impairment. Land use and development activities resulting from urban development, logging, and clear cutting can result in a variety of stressors to aquatic systems including increased loading of sediment and nutrients, destruction of spawning and feeding habitat for aquatic organisms, and increased temperature regimes of aquatic systems. Even though there might not be contaminants involved in this type of activity, increased temperatures can trigger induction of the heat shock (stress proteins) which is a biomarker of increased temperature exposure. Bioindictors of environmental effects characteristic of this type of activity are gill damage (from suspended sediment), decreased growth, and changes in community richness and diversity which result from the ecological effects of sedimentation and increased water temperature. Because of

elevated water temperatures, stress proteins may also be a characteristic biomarker of exposure in systems subjected to power plant thermal discharges. Even though many organisms can avoid thermally enriched areas, both bioenergetic impairment and changes in community structure and diversity have been reported for this activity.

Biomarkers of exposure to environmental stressors vary depending on the type of anthropogenic activity (stressor) involved. The MFO enzymes and bile metabolites are typically related to petrochemical and pulp and paper activities even though a few other chlorinated organic compounds such as PCBs and insecticides are known to induce the P450 system. Metallothione-type protein compounds are usually induced only under heavy metal exposure (i.e. mining activities) while DNA damage is typically caused by carcinogenic and genotoxic compounds such as heavy metals and PAH-type compounds. Antioxidant enzymes such as superoxide dismutase and gutathione peroxidase are well known indicators of exposure for several types of stressors including heavy metals, and even supersaturated oxygen levels in the environment can trigger the antioxidant defense system. Acetylcholinesterase (AChE) is very specific to organochlorine insecticides even though there is some evidence that levels of AChE may be mildly influenced by other environmental pollutants. There are no specific biomarkers of exposure for land-use and development activities and power plant operations because these two activities typically involve non-specific stressors such as elevated temperature regimes and increased sediment and nutrient loading to aquatic systems in the case of land development. The stress proteins may be induced when the normal thermal tolerance of aquatic organisms is exceeded, but induction of these proteins are also relatively non-specific to environmental stressors.

In several cases, bioindicator responses at the higher levels were similar for some of the major anthropogenic activities. This of course is not surprising because biological responses at the population, community, and ecosystem levels are generally non-specific to environmental stressors, and by the time stressor effects can be manifested at these ecological relevant levels, a variety of biotic and abiotic factors may have influenced or modified the overall stress response.

For example reproductive success of aquatic organisms was found to be impaired by petrochemical , pulp and paper, and agricultural activities. This is not to imply that the other anthropogenic activities in Fig. 2 cannot also effect the reproductive integrity of organisms, but just that reproductive competence was frequently found to be one of the more significant biological effects caused by these three activities. Also, different stressors may affect reproductive success in organisms by different mechanisms such as through hormonal imbalance, bioenergetic pathways, or directly through detrimental effects on eggs and larvae. Community level effects were frequently noted in the literature for activities related to land-use and development (logging, urban development, clear cutting). Environmental effects of power plant operation can be manifested either through direct pathways from siltation and temperature, or through indirect pathways which impair metabolic and feeding mechanisms and influence bioenergetic homeostasis of organisms. Growth was found to generally increase in organisms exposed to pulp and paper and domestic sewage discharges due to increased nutrient loading and increases in system-wide primary and secondary productivity. Conversely, decrease growth appears to be a major affect in systems perturbed by petrochemical, mining, agricultural, and land development activities. Decreased growth has been attributed directly to increases in metabolic demands when organisms are under environmental stress, and indirectly due to effects on the food chain which alters both the quality and quality of available prey for higher-level consumers. Structural changes in tissues and organs as determined by histopathological analysis has consistently identified gill lesions as one of the most common individual-organism level responses to environmental stressors. All the anthropogenic activities shown in Fig. 1 except power plant operations were commonly associated with gill pathologies (and thus metabolic and respiratory stress). Liver pathologies were also noted with domestic sewage and in particular petrochemical activities where effluents from the latter are widely known to cause hepatic tumors.

Conclusions and Synthesis

The exposure-response profiles shown for the various anthropogenic activities in Fig. 2 were generated based on the major biomarkers of exposure and bioindicators of effects reported in the

scientific literature. Each of these activities may have additional biomarkers of exposure and bioindicators of effects also associated with them, but for purpose of generating these simplistic exposure-response profiles, only the principle biomarkers of exposure and bioindicators of effects reported for each activity were utilized in this presentation. The primary purpose of this exercise was to demonstrate the practical use of a diagnostic approach that can help identify sources of stress responsible for causing ecologically-relevant responses in aquatic systems. Because it is difficult to establish causal relationships between environmental stressors and significant ecological endpoints, such an approach is valuable to help identify and diagnose sources of stress in environments which may be impacted by multiple stressors. For example, Chesapeake Bay is impacted a number and variety of stressors including non-point source agricultural inputs, domestic and industrial sewage, and point-source inputs from Naval activities such as heavy metals and hydrocarbon (PAH) compounds. Various state and federal environmental laws require that the Navy, for example, comply with these regulatory statutes in the form of environmetnal monitoring, assessment, and cleanup. Since the Navy is not the only source of environmental contaminants and other stressors present in Cheseapeake Bay, this method would help in not only identifying the types and sources of stress to the Bay ecosystem, but also help in determining or separating out which of the observed effects at the population, community, or ecosystem level may be due to specific anthropogenic activities. By identifying the types and sources of environmental stressors, aquatic ecosystems can be more effectively protected and managed to maintain acceptable levels of environmental quality and ecosystem fitness.

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Table 1. Biomarkers of exposure (Zone 1) which respond relatively rapidly to environment stressors and bioindicators of higher-level biological effects which correspond to Zones 2 and 3 in Figure 1.

ZONE 1 (rapid responses)	ZONE 2 (intermediate responses)	ZONE 3 (population-community responses)
Detoxification enzymes	Selected histopathologies	Size-frequency distributions
DNA damage	Immune system dysfunction	Altered sex ratios
Biliary metabolites	Bioenergetic impairment	Food-web alterations
Antioxidant enzymes	Reproductive integrity	Trophic-level relationships
Acetylcholinesterase	Growth	Community diversity, richness
Stress proteins		

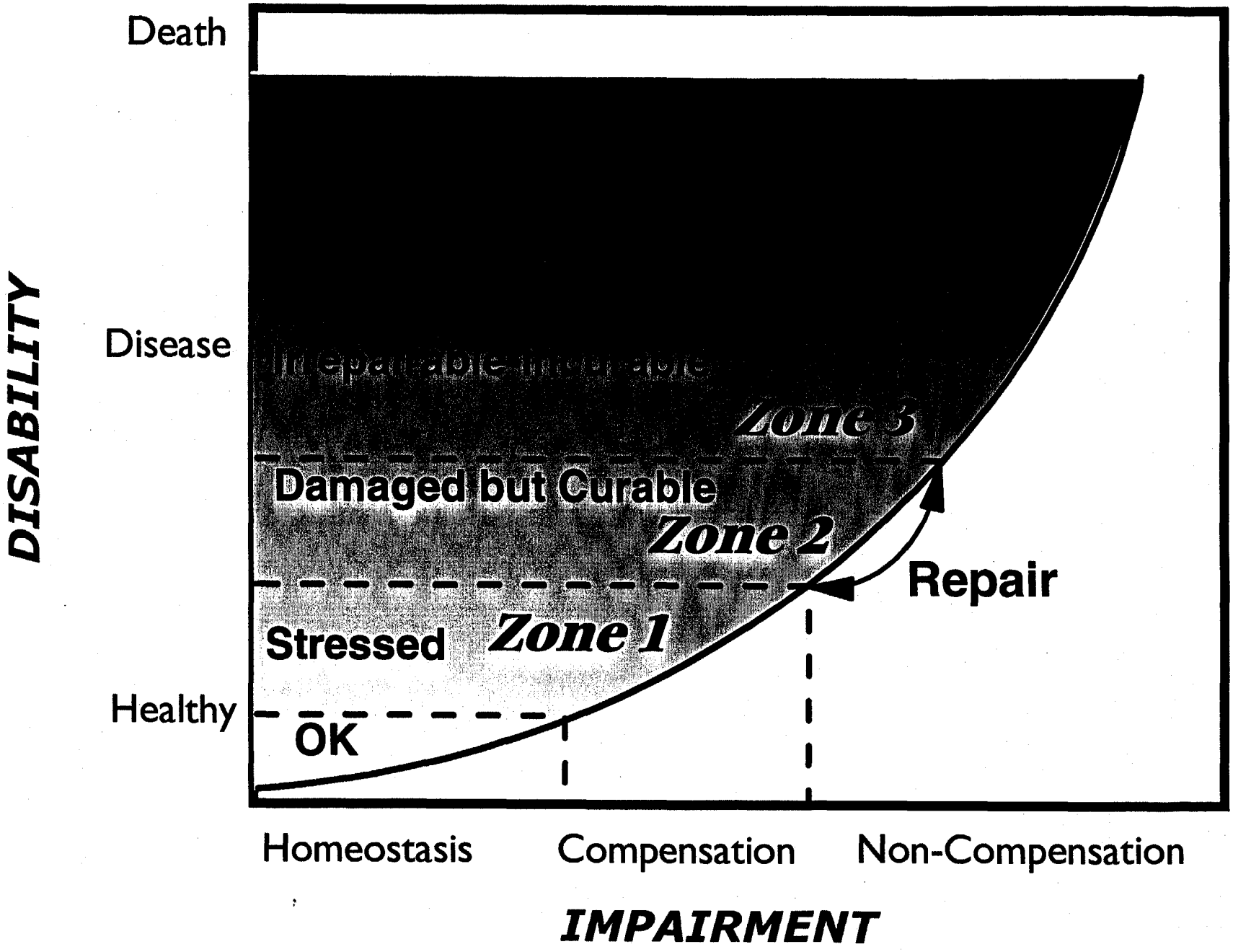
Table 2. Major types of anthropogenic activities and stressors that can compromise the integrity of aquatic systems and their corresponding biomarkers of exposure and bioindicators of significant ecological effects.

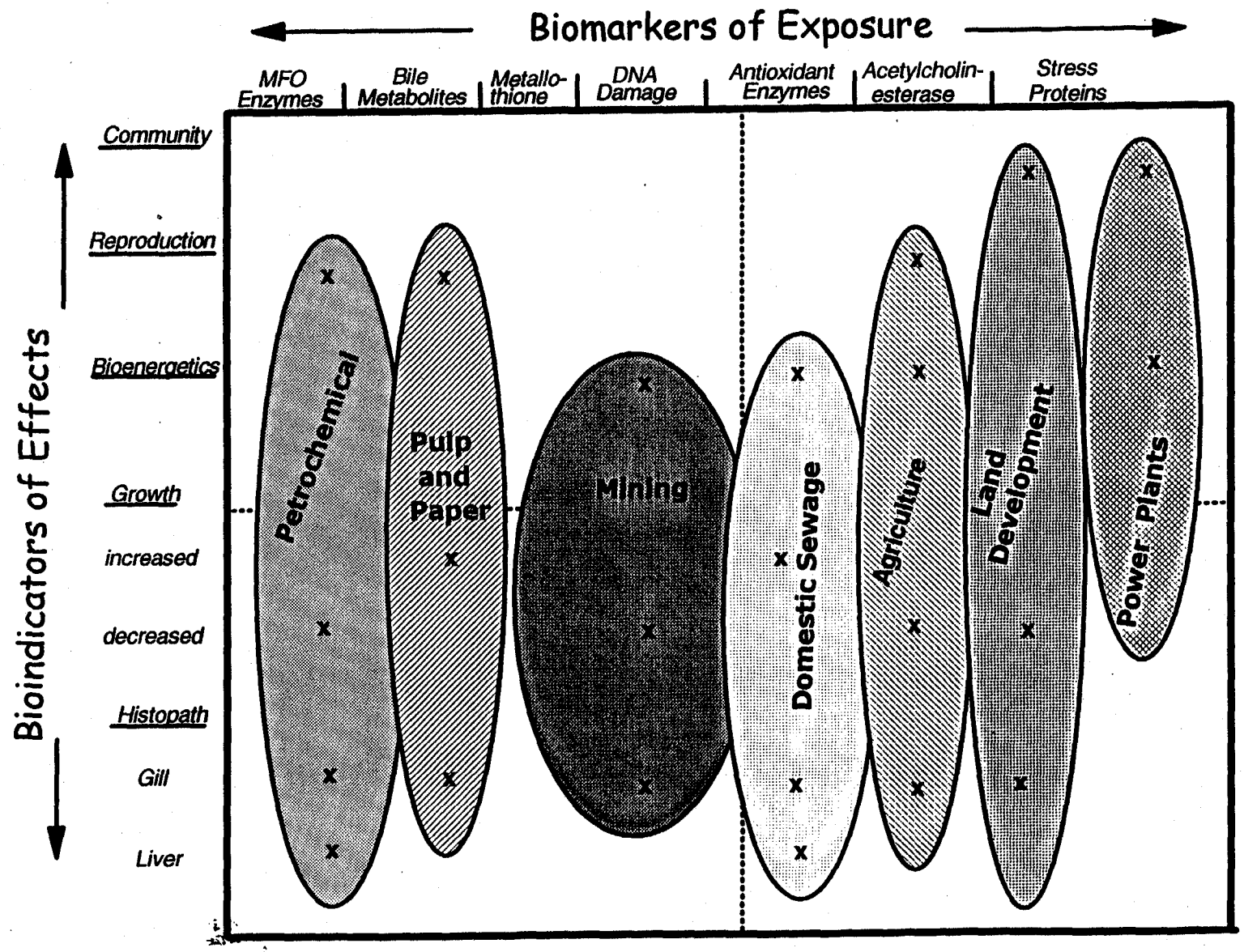
Anthropogenic activity	Major types of stressors	Representative exposure responses	Representative ecological relevant responses	Principle references
Agriculture	Pesticides, herbicides, nutrients, sediments	Inhibition of acelylcholinesterase	Reproductive impairment, increased growth, disease	Bass et al. 77 Kaur & Dhawan 96 Coulliard et al. 97 Sanchez et al. 97
Pulp and Paper	Nutrients, dioxins, resin acids, color, chlorophenolics	Mild MFO induction, bile metabolites,	Reproductive impairment, increased growth, gill histopathology	Owens 91 Sandstrom 96 Axelsson & Norrgren 91 Andersson et al. 88
Petrochemical	PAHs, heavy metals	High MFO induction, aromatic bile metabolites	Impaired reproduction, decreased growth, liver tumors	Spies et al. 96 Baumann et al. 91 Moles & Norcross 98 Vetemaa et al. 97
Mining	Heavy metals, sediments	Metallothiones, antioxidant enzymes, DNA damage	Reduced growth, gill histopathology, metabolic impairment, genetic diversity	Larsson et al. 85 Farag et al. 95 Mallatt 85 Woodward et al. 95
Domestic Sewage	Chorine, nutrients, detergents	Mild MFO induction, antioxidant enzymes	Behavior changes, gill & liver histopath, bioenergetic impair	Mitz & Giesy 85 Bass et al. 77 Osborne et al. 81
Land Development, Clearcutting	Sediment & temperature increases, altered hydraulics	Stress proteins	Decreased growth, gill abnormalities, changes in feeding guilds	Newcombe & McDonald 91 Alabaster & Lloyd 82 Bergstedt & Bergersen 97 Bradley 93
Power Plants	Temperature increases, chlorine ,	Stress proteins, antioxidant enzymes	Bioenergetic impairment, behavioral changes	Coutant 97

Figure Legends

Fig. 1. The responses of aquatic organisms to environmental stressors is characterized by three major stress-response zones which correspond to the level of environment damage incurred relative to the disability- impairment curve.

Fig. 2. Biomarker (exposure) -Bioindicators (effects) profiles characteristic of each major type of anthropogenic activity which can be use to help identify major sources of stress in aquatic environments impacted by multiple stressors.


Death
Disease
Healthy
DISABILITY
Irreparable Incurable
Zone 3
Damaged but Curable
Zone 2
Repair
Stressed
Zone 1
OK
Homeostasis
Compensation
Non-Compensation
IMPAIRMENT


Biomarkers of Exposure
MFO Enzymes
Bile Metabolites
Metallo-thione
DNA Damage
Antioxidant Enzymes
Acetylcholin-esterase
Stress Proteins
Bioindicators of Effects
Community
Reproduction
Bioenergetics
Growth
increased
decreased
Histopath
Gill
Liver
Petrochemical
Pulp and Paper
Mining
Domestic Sewage
Agriculture
Land Development
Power Plants