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Biomarker/bioindicator response profiles of organisms can help differentiate between sources of anthropogenic stressors in aquatic ecosystems

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Aquatic ecosystems can be chronically stressed by multiple environmental factors which originate from a variety of point and non-point sources. In addition, these stressors may vary both spatially and temporally, and, combined with synergestic and cumulative interactions of these stressors, complicate the interpretation and evaluation of stress responses in organisms. To help identify and differentiate between sources of anthropogenic stressors in aquatic systems, a diagnostic approach based on exposure–response profiles in sentinel organisms was developed from the known effects of various anthropogenic activities on biological systems. 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, and agricultural activities. Biomarkers of exposure to environmental stressors varied widely depending on the type of anthropogenic activity involved. Bioindicator effects, however, including histopathological lesions, bioenergetic status, growth, reproductive impairment, and community-level endpoints were similar among several of the major anthropogenic activities because responses at these higher levels are less specific to stressors than are biomarkers. This approach appears useful for helping to identify and diagnose sources of stress in environments impacted by multiple stressors. By identifying the types and sources of environmental stressors impacting key components of biological systems, aquatic ecosystems can be more effectively protected, regulated, and managed to help improve and maintain environmental quality and ecosystem fitness.

Keywords: biomarkers, bioindicators, multiple stressors, diagnostic approach.

Introduction

Environmental science is continually confronted with issues related to assessing and evaluating the effects of stressors on the health of aquatic ecosystems. Some of the more challenging issues which have proven to be 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 (i.e. biomarkers), (3) determining the importance of temporal and spatial variability of physicochemical and biological factors in modifying responses to stress, and (4) establishing cause and effect relationships between specific stressors and the types and levels of environmental damage.

Laboratory studies, employing standard toxicological testing, have traditionally been one of the primary approaches used to assess the effects of contaminants on 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,

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growth, or reproductive potential. However, organisms usually cease to function normally long before these critical endpoints are reached (Larsson *et al.* 1985). Furthermore, the test conditions during laboratory studies seldom reflect the environment of natural populations (Cairns 1981) lacking ecological realism (NRCC 1985, Lagadic *et al.* 1994). Thus, much of the progress in aquatic toxicology to date has been due to an increase in data rather than an increase in the knowledge necessary to adequately assess ecotoxicological effects on aquatic ecosystems (Wester and Vos 1994).

Over the past two decades there have been many sensitive 'early-warning' indicators (biomarkers) developed and measured on sentinel organisms exposed 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 (Jimenez and Stegeman 1990, Stegeman *et al.* 1992), heat stress (HSP70 stress proteins) (Bradley 1993, Pyza *et al.* 1997), antioxidant enzymes (DiGiulio 1992, Doyotte *et al.* 1997), and various measures of DNA damage (Shugart *et al.* 1992, Theodorakis *et al.* 1992). In several cases, these biomolecular and biochemical measures have been validated by laboratory and field studies and have proven to be excellent biomarkers of exposure to a variety of environmental stressors. In most situations, however, these exposure biomarkers do not serve as ecologically relevant indicators of stress effects at higher levels of biological organization (Calow 1994). Biomarkers are important in field studies, however, to help understand the mechanistic relationships between stressors and ecologically relevant endpoints.

Notwithstanding these first two issues related to the biological relevance of laboratory studies and the ecological significance of early warning biomarkers of stress, perhaps the most important challenge for environmental science is establishing causal relationships between stressors and ecological relevant effects (Attrill and Depledge 1997, Adams *et al.* 2000). Relating stressors to biomolecular and biochemical markers of exposure and ultimately to some relevant ecological endpoint 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 (McCarty and Munkittrick 1996, Wolfe 1996), the orders of magnitude involved in extrapolation over both spatial and temporal scales (Holdway 1996), and compensatory mechanisms such as density-dependent responses that operate in nature populations (Power 1997).

Biomolecular or biochemical responses such as induction of the P450 system from PAH exposure or inhibition of acetylcholinesterase from pesticide exposure will not necessarily be manifested as measurable biological effects at higher levels of organization. For effects to be realized at increasingly higher levels, the stressor(s) must be of sufficient magnitude and/or 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 or when the rate of toxicant damage exceeds repair capacity, pathological damage can occur to tissues and organs (Depledge 1989). Structural damage to liver tissue can, for example, compromise the ability of this organ to produce vitellogenin, a critical component of egg development, which in turn ultimately compromises reproductive success of individuals. Continued exposure to contaminants and other stressors results in a progressive deterioration in

organism health which may ultimately compromise the success of populations and communities (figure 1). Departures from the healthy state in organisms are associated with the initiation of compensatory responses with little change in disability (zone 1, figure 1). Additional impairment beyond this compensation limit may become associated with increased disability and overt disease (zone 2, figure 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 (Depledge 1989) (zone 3, figure 1). Provided, however, that environmental conditions improve sufficiently and rapidly enough, an organism may be able to recover somewhat and repair damaged systems including restoration of compensatory responses. Monitoring impairment of biochemical, physiological, and behavioural responses should provide early warning signals related to the onset of disabilities (Depledge 1989). On the disability scale (figure 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 (Soimasuo *et al.* 1995, Adams *et al.* 1996, 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 endpoints. 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, the ultimate effects of which occur 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 and differentiate among sources of anthropogenic stress in aquatic systems 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 can produce specific environmental stressors such as contaminants which characterize that activity. These characteristic sets 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

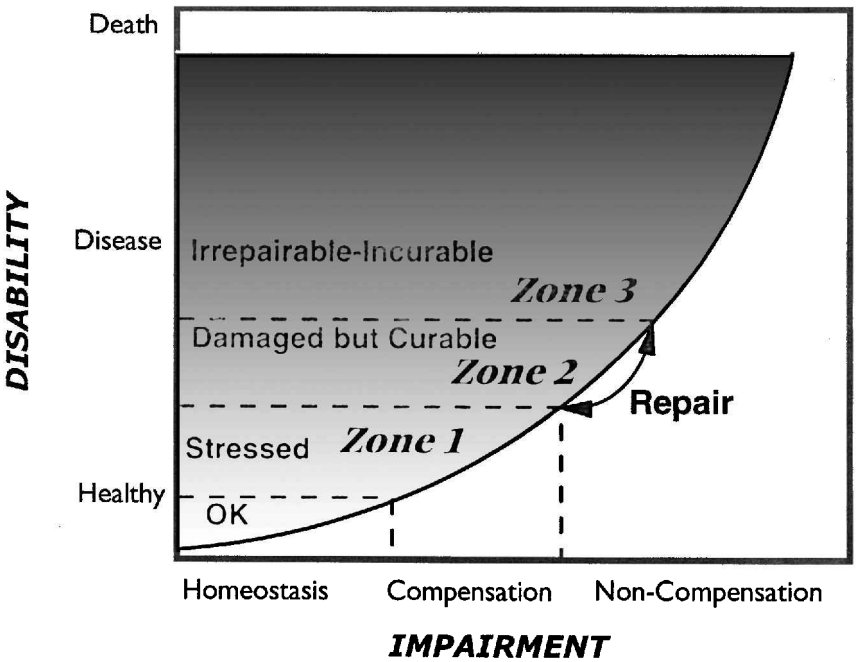

Figure 1. The responses of aquatic organisms to environmental stressors is characterized by three major stress-response zones which correspond to the level of environment damage incurred relative to the disability–impairment curve (modified from Depledge 1989).

activities, can also produce characteristic sets or profiles of biomolecular, biochemical, or physiological responses in organisms which are generally referred to as biomarkers of exposure to environmental stressors (McCarthy and Shugart 1990, Huggett *et al.* 1992). The primary advantage of biomarkers is that they generally respond rapidly to stressors and can serve, therefore, as 'early-warning' indicators of effects which could be ultimately manifested at higher levels of biological organization. The principal biomarkers generally used as sensitive early-warning indicators and which characterize zone 1 of the disability–impairment curve (figure 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 biomarkers of exposure (i.e. biochemical, biomolecular markers). For example, the principal types of exposure responses elicited by PAH-type compounds originating from petrochemical activities are generally high inductions of the P450 system and production of fluorescent aromatic bilary metabolites. 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 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. For the purposes of this study, responses to environmental stressors at these more

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		

ecologically relevant endpoints are defined as bioindicators of effects (Adams 1990, McCarty and Munkittrick 1996).

To construct diagnostic exposure–response profiles for various stressor exposure–effects relationships corresponding to each major type of anthropogenic activity, the principal 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 their corresponding 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 exposure biomarkers on one axis and bioindicator responses 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 (figure 1) and are listed in table 1.

Results and discussion

In figure 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 principal biomarkers of exposure for petrochemical and pulp and paper activities are induction of the P450 enzymes 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, decreases under petrochemical exposure and actually increases in systems receiving paper mill effluents due primarily to nutrient enrichment and increased productivity of receiving waters. In addition, organisms

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	Principal references
Agriculture	Pesticides, herbicides, nutrients, sediments	Inhibition of acetylcholinesterase	Reproductive impairment, increased growth, disease	Bass *et al*. 1977 Kaur and Dhawan 1996 Coulliard *et al*. 1997 Sanchez *et al*. 1997
Pulp and paper	Nutrients, dioxins, resin acids, color, chlorophenolics	Mild MFO induction, bile metabolites,	Reproductive impairment, increased growth, gill histopathology	Owens 1991 Sandstrom 1996 Axelsson and Norrgren 1991 Andersson *et al*. 1988
Petrochemical	PAHs, heavy metals	High MFO induction, aromatic bile metabolites	Impaired reproduction, decreased growth, liver tumors	Spies *et al*. 1996 Baumann *et al*. 1991 Moles and Norcross 1998 Vetemaa *et al*. 1997
Mining	Heavy metals, sediments	Metallothiones, antioxidant enzymes, DNA damage	Reduced growth, gill histopathology, metabolic impairment, genetic diversity	Larsson *et al*. 1985 Farag *et al*. 1995 Mallatt 1985 Woodward *et al*. 1995
Domestic sewage	Chorine, nutrients, detergents	Mild MFO induction, antioxidant enzymes	Behavioural changes, gill and liver histopath, bioenergetic impair	Mitz and Giesy 1985 Bass *et al*. 1977 Osborne *et al*. 1981
Land development, clearcutting	Sediment and temperature increases, altered hydrodynamic regimes	Stress proteins	Decreased growth, gill abnormalities, changes in feeding guilds	Newcombe and McDonald 1991 Alabaster and Lloyd 1982 Bergstedt and Bergersen 1997 Bradley 1993
Power plants	Temperature increases, chlorine	Stress proteins, antioxidant enzymes	Bioenergetic impairment, behavioural changes	Coutant 1997

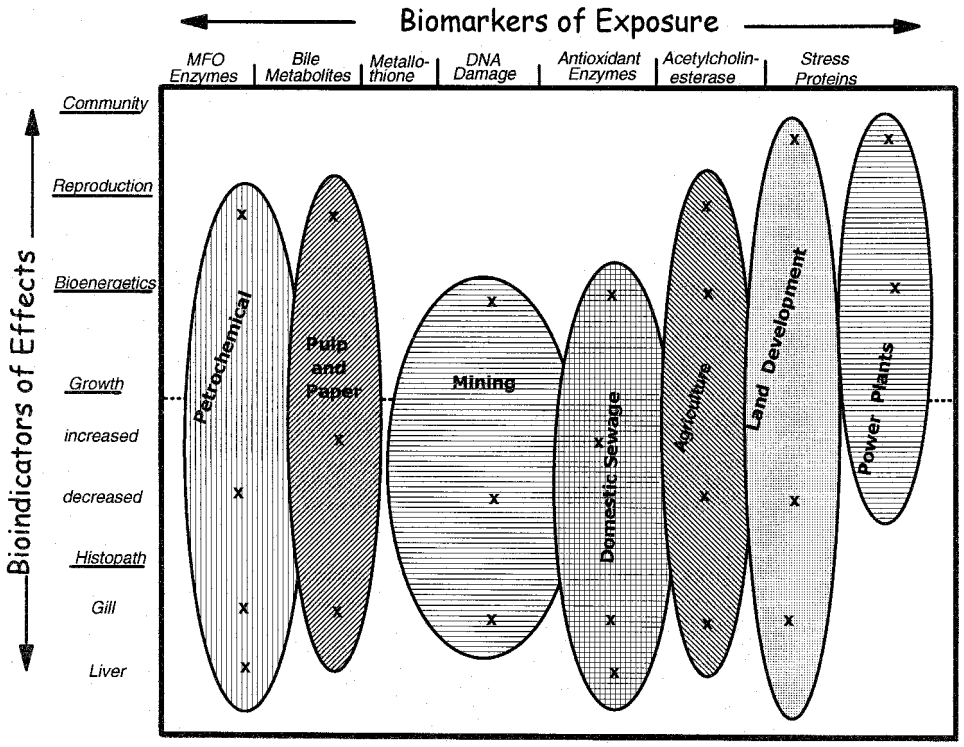

Figure 2. Biomarker (exposure)–bioindicators (effects) profiles characteristic of each major type of anthropogenic activity which can be use to help identify major sources of stress in aquatic environments impacted by multiple stressors.

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 the resulting heavy metal contamination of the environment, are the metallothione-type protein compounds, antioxidant enzymes, and DNA damage (genotoxicants). With this particular 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 principal 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 contaminants are usually not involved in these types of activities, increased temperatures can trigger induction of the stress protein system which is a biomarker of increased temperature exposure. Bioindicators of environmental effects characteristic of this type of activity are gill damage (from suspended sediment), decreased growth (due to impact of siltation on food supplies), and changes in community richness and diversity which result from the ecological effects (primarily habitat modification) 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 (Coutant 1997).

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 (e.g. 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 glutathione peroxidase are well known indicators of exposure for several types of stressors including heavy metals, and even supersaturated oxygen levels can trigger the antioxidant defense system. Acetylcholinesterase (AChE) is very specific to organochlorine insecticides even though there is now 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 higher levels of biological organization were similar for some of the major anthropogenic activities. This is not surprising because many biological responses at the population, community, and ecosystem levels are generally non-specific to environmental stressors. For higher level responses, this non-specificity to stressors results from the considerable time lags that usually occur between the periods that organisms are exposed to a stress and when effects are ultimately manifested at ecological relevant levels. During these time lags, a variety of other biotic and abiotic factors can influence, modify, and/or modulate the overall stress response of biological systems.

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 shown in figure 2 cannot also affect 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 dysfunction, bioenergetic pathways, or directly through detrimental effects on eggs and larvae and spawning habitat modification. 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 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 occurring in systems perturbed by petrochemical, mining, agricultural, and land development activities. Decreased growth in these cases has been attributed directly to increases in metabolic demands when organisms are under environmental stress, and indirectly to effects on the food chain which alters both the quality and quality of available prey for 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 many types of environmental stressors. All the anthropogenic activities shown in figure 2 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 with 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 figure 2 were generated based on the major biomarkers of exposure and bioindicators of effects reported in the scientific literature. Each of these activities may be also associated with other biomarkers of exposure and bioindicators of effects, but for the basic purpose of generating these simplistic exposure–response profiles, only the principal biomarkers and bioindicators reported for each activity were utilized in this presentation. The primary objective of this exercise was to demonstrate the practical use of a diagnostic approach that can help identify and differentiate among 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 disturbance in environments impacted by multiple stressors. For example, San Diego 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 environmental monitoring, assessment, and clean-up. Since the Navy is not the only source of environmental contaminants and other stressors present in San Diego Bay, this method would help in not only identifying the types and sources of stress to the Bay ecosystem, but also help in differentiating or separating out which of the observed effects at the individual, population, or community levels may be due to specific anthropogenic activities. By

identifying the types and sources of environmental stressors, aquatic ecosystems can be more effectively managed and regulated to improve and maintain environmental quality and ecosystem fitness.

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