Introduction

Environmental monitoring is performed to detect changes over time and space in indicators of ecological integrity or environmental quality. When these changes are detected in biota or their environment, question-oriented research is used to investigate the causes of observed changes and to better understand consequences, especially of the ecological effects of stress and disturbances (Freedman, 1989). Ecological indicators are those species that are chained to the habitat in one way or other and hence compelled to adapt to changing environment and survive or resist to adapt and perish. Amphibians are one such group. As predators and prey, they constitute the functionally important elements in most terrestrial and many freshwater ecosystems and thus form a significant component of the world's biota. Therefore, the impact of ecological stress or disturbance upon this class of animals is relevant to an understanding of ecosystem health.

Since 1989, there has been growing realization that amphibian populations have been declining at an alarming rate (Balustein and Wake, 1990; Wake, 1991; Pechmann *et al.*, 1991; Bishop and Pettit, 1992). For many years before that, direct habitat loss was acknowledged as the major causative factor of a general loss of global biodiversity. In 1989, Species Survival Commission (SSC) established with `Declining Amphibian Population Task Force' (DAPTF) to identify critical habitats and priorities of research. In the workshop of DAPTF held in 1990 at Irvine, California, it was reported that large scale destruction and transformation of habitats, pollution and direct harvest by man are the main factors contributing to the decline of amphibians (Vial, 1991a,b). However, some declines in amphibian populations are currently unexplained. Certainly, the habitat loss and degradation are the most important and best understood causes of species decline, but the simple connection between habitat loss and species decline has become difficult to explain. The crash of some populations in pristine environments where there is no perceived change in the quality of habitats confounds the issue. Whether these declines are merely normal extirpations due to stochastic events or actually hastened by anthropogenic factors defines the issue of declining amphibian populations (Johnson, 1992). Since it has been a global phenomenon, it was decided in the Irvine workshop to identify the courses of action to deal with the problems of amphibian decline. These include (1) studies on selected populations to identify and monitor both abiotic and biotic factors that could potentially contribute to the declines (2) a comparison of historical records of geographical ranges with current ranges, and (3) developing the means for assessing local species richness and decline. The accumulation of genetic information was also a suggested plan of action at several DAPTF workshops.

The impact of environmental contaminants on amphibians is a subject that has received limited attention. Relative to the amount of toxicological studies devoted to the invertebrates, fishes and birds very little information exists on amphibians (Power *et al.*, 1989). Apart from the direct concern for the health of amphibians, amphibian toxicological studies have another important aspect - the use of amphibians as indicators of environmental quality - an aspect that has received the attention only during the recent years. Both the above aspects of wildlife toxicology need controlled laboratory experiments as well as field studies (Power *et al.*, 1989).

Environmental Pollution and Bioindicators

Environmental monitoring is accomplished using indicators, which are surrogates for a complex of related characteristics or processes (Freedman and Shackell, 1992). Recently, there has been a considerable awareness among the ecotoxicologists to develop a framework and common language for the future to predict (toxicity tests), assess (bioassays), recognize (bioindication) and monitor (biomonitoring) pollution impacts on freshwater ecosystems (Lovett Doust *et al.*, 1994).

Current inputs of pollutants into the environment are from both non-point and point sources. Non-point sources (NPS) are diffuse inputs of contaminants from diverse points of origin that may include landfills, atmospheric deposition, ground water and agricultural run-off (IJC, 1988). Urban run-off principally contribute metals, which in likelihood, are present as a result of automotive activities and the corrosion of metallic surfaces (Pollman and Denek, 1988). Most of the contaminants in NPS pollution are associated with suspended particles; therefore it is generally believed that most of the effects attributable to such pollutants tend to be localized in wetlands (Baker, 1992; Hammer, 1992). In Gujarat State, the point source pollution is the most identifiable source of contaminants into the aquatic environment. There are several industries in the State, releasing the untreated or semi-treated effluents into the nearby water bodies and thereby insulting the aquatic ecosystems.

The wide range of contaminants discharged into the waterways has historically been detected and monitored using the methodology of analytical chemistry. Despite several recent advances in direct chemical assay techniques, chemical monitoring systems are severely limited in their capability to monitor water for suspected pollutants. Many hazardous organic compounds have adverse biological effects at concentrations below existing analytical detection capabilities (Warwick, 1988). Further, chemical techniques are incapable of predicting the detrimental effects some pollutants can have on ecosystems because several environmental-quality parameters mediate their toxic response. Chemical testing alone cannot predict the cumulative impact of mixed pollutants upon the biotic components of the environments (Lambou and Williams, 1980; Kozhurao, 1985). Such synergistic attributes of contaminants can only be determined by exposing living organisms to combinations of pollutants and observing impairments or mortality rather than by merely detecting the presence of individual compounds at particular concentrations in water samples (Kovacs and Podani, 1986).

Toxicity tests involve exposing a well-defined organism to a dilution of series of a suspected toxicant under laboratory conditions while bioassays are used to assess the toxic effects of mixtures on biota by exposing test organisms to naturally contaminated water or sediment samples (Biard, 1992; Calow, 1992) Theoretically, toxicity tests performed on the most sensitive species should be able to indicate impending contaminant impacts on the entire environment. However, it is impossible to predict which species will be the most responsive towards a particular toxicant (Hertz, 1991; Cooper and Barmuta, 1993).

Ideally an indicator species should be both representative of a specific trophic level within the ecosystem and capable of being used at a renewable cost under laboratory conditions (Power *et al.*, 1989). Fishes are used as indicator species in various toxicological assays. Amphibians could be used to broaden the approach and might be particularly valuable for assessing the impact on temporary water bodies. (Power *et al.*, 1989).

The sensitivity of amphibians to metallic contaminants or other organic or inorganic compounds may allow environmental monitoring of these compounds or local changes in the environment using amphibians as indicators once standardized techniques and representative species have been established (Birge *et al.*, 1979; Niethammer *et al.*, 1985; Vinod and Naik, 1992). On the other hand, the diversity and distribution of amphibian species could also provide a picture on the quality of our environment (Naik and Vinod, 1994) as the environmental pollution has been considered as a major threat on the declining amphibian population (Naik and Vinod, in press).

Based on a series of studies on the effects of metal contamination on amphibians, Birge *et al.* (1977, 1979) have suggested the possibility of using amphibians as bioindicator species. Dumpaert and Zietz (1984) have also suggested that *Xenopus laevis* could be used as an indicator species for determining the embryotoxic effects of environmental chemicals. In the present work an anuran amphibian *Rana tigerina* commonly known as the Indian Bullfrog, has been selected as an indicator species to determine the toxicological effects of heavy metal contaminants on amphibian population.

There are multitudes of organismal responses that could be observed as endpoints in toxicity tests. The most frequently used is survivorship (Moriarity, 1990). Other frequently used end points include biochemical and genomic perturbations. However, it has been argued that ecosystem level endpoints are most important from the applied perspective (Lovett Doust *et al.*, 1994). Survivorship is generally considered not to be a sufficiently early warning signal of impending environment damage through contamination because drastic perturbations will already have occurred in wildlife populations before any changes are noticed in survivorship (Moriarity, 1990).

Biochemical endpoint as organismal response, has several draw backs in toxicity tests. The concept of such toxicity tests is that the presence of certain toxins may induce a physiological response in an organism, often involving a heightened production of enzymes that are capable of metabolizing and/or degrading the toxicant in question (Giesy *et al.*, 1988). However, due to the specific activity of the enzymes, the quantity of one particular enzyme will only indicate the presence of one compound or at the most, one class of compounds at a time and will not generally able to indicate the presence of other toxicants. Thus no single biochemical indicator will suffice to assess the extent of contamination present in an environment, but numerous indicators would need to be used. Apart from these, the factors such as sex, age and reproductive state, will also affect the level of enzymes within organisms (Lowet Doust *et al.*, 1994). This is especially true in the case of amphibians. Thus the perturbations at the genomic level are more practicable to deal with than the biochemical perturbations in a toxicity test. The first part of the present study deals with some bioassays in which the genomic perturbations have been considered as endpoint.

Genomic Perturbation as End Point

When a pollutant enters the environment it is expected that its initial effect on an exposed organism will be a suborganismal one - either biochemical or genetic (Giesy *et al.*, 1988). Genotoxicity assays the damage that has occurred at the genetic material of organisms as a result of the action of contaminants. There are two major kinds of alteration that may occur in DNA as a consequence of toxicants being present in an organism's habitat: mutation and chromosome breakages (Legator and Harper, 1987).

Gene activities change during cell development, cell differentiation and cell maturation. It is highly probable that some of these changes are expressed at chromosomal level (Hsu, 1981). Chromosomal aberrations, involving a great many bases resulting in drastic rearrangement of the base sequences in the genetic code. The structural anomaly in DNA is of sufficient degree to produce microscopically detectable alteration in the structure/number of chromosomes (De Bruin, 1976). Further, the genetic perturbation caused by some chemicals cannot be detected by gene mutation studies alone while chromosomal analysis can be done in such cases (Bilgrami, 1988; Legator and Harper, 1987).

Analysis of chromosomal aberration has been the most common method of detecting DNA damage. It is known that virtually all chemical and physical agents that are found to produce specific-locus mutation will also produce chromosomal aberration under the proper experimental conditions. The correlation is also high enough for induced chromosome aberration to be very useful as a presumptive test for gene mutation. Moreover, chromosome aberration tests can identify agents, which cause chromosome breakage and is useful for comparisons of a series of agents in determining risk-benefit relationships (Hollstein *et al.*, 1979).

Usually the mutagenic studies are carried out in mammalian systems. There are many situations involving environmental exposure, or some kinds of testing in which mammalian systems are not suitable for evaluating genetic hazards. Such is the case of determining the genotoxic effects of environmental pollutant on species in the wild. Nonmammalian systems for evaluating the genetic hazards of manufactured chemicals are needed, because there are many compounds used directly on organisms as in the case for the application of pesticides and use of antibiotics and additives. The assessment of genetic toxicity in such cases would determine the risk of breeding stock and signal potential danger of mutagenicity to mammalian species including man.

Genotoxic studies on amphibians are very rare and a late entrant. A decade back, some studies (Chakrabarti *et al.* 1984; Geard and Soutter, 1986) have suggested that amphibian can be used as a suitable *in vivo* cytogenetic system to study the chromosomal aberration and sister chromatid exchanges. More recently, Zakhidov *et al.* (1993) reported the possibility of using amphibian hemopoietic cells as a reliable test system for the detection of cyto- and genotoxic compounds as well as for the genetic monitoring of aquatic environment. Amphibian system has two main advantages over the fish in mutagenic studies; the larger metaphase complements and less 2n numbers. It is believed that use of amphibian systems in biomonitoring of the toxic level of watersources or of a particular atmosphere will be more convenient than the existing systems (Chakrabarti *et al.*, 1984)

A great many cytogenetic methods have been described that permit the study of somatic or germinal chromosomes *in vivo* or *in vitro* following acute or chronic exposure in a variety of species (Sharma and Sharma, 1980). *In vivo* studies have some advantage over the *in vitro* studies. *In vivo* analysis accounts for host mediation of the drug and thereby afford several advantage for mutagenic trials. For example, the genetic effects from both direct and indirect acting agents that undergo metabolic activation or deactivation in the intact animal may be examined. Also, multiple tissue analyses allow assessment of tissue specific level of risk for genetic damage. In addition, the *in vivo* systems permit analyses, which are complementary to *in vitro* trials and often provide information which is otherwise unobtainable (Hollstein and Mc Cann, 1979).

In vivo studies, using the whole animal allow more than one type of tissue to be analyzed, although the bone marrow has been proven to be the most practical. In the present study, the bone marrow was used for the analysis of the metaphase cells. Bone marrow has been considered as the best tissue for routine chromosome analysis primarily because of its high mitotic index. This is generally true, especially in the case of mammals. Mammals have an active hemopoietic bone marrow round the year. In contrast, in most amphibians, the activity of bone marrow has strict seasonal variation. Availability of a considerable number of metaphase cells for the chromosome analysis is a primary requisite in the clastogenic assays. Considering this aspect, in Chapter 2, an evaluation has been made on the suitability of the species *Rana tigerina* in mutagenic assays.

Heavy metals and their salts take part in the genetic and cellular function and also play a vital rôle in our daily life. Thus any alteration in their balance in the environment might cause the health hazards to man and animals. Metals and their salts adversely affect the activity of a living organism. They may exert toxic effects on living tissue at different levels - cellular, subcellular and/or molecular. At the molecular level, they may bind to nucleic acids, resulting in irreversible conformational changes; interaction with DNA may cause mutation and even carcinogenesis. At the microscopic level these activities may be recorded as alteration in chromosomal configuration and cell and chromosomal division. A good correlation has also been drawn between the mutagenic properties of metals and their ability to cause cancer (Leonard, 1981).

The hazards caused by metallic compounds are different in different organisms. Most of them involve some form of genetic toxicity (De Bruin, 1976). A review of literature on amphibian toxicology shows that the effect of heavy metals on the chromosome of amphibians is hardly known (Power *et al.*, 1989). Therefore, in the present study some salts of heavy metals, which are known to occur in common industrial pollutants, have been selected in order to assess the clastogenicity of these chemicals on the mitotic chromosome of the frog, *Rana tigerina*. Chapter 3 deals with the effect of three metallic (Mercury, Cadmium, Nickel) chloride salts on the bone marrow chromosomes of the test animal. In Chapter 4, the effect of Lead acetate on the chromosomes was assessed after administration of the chemical intraperitoneally as well as exposing the animals to the lead solution. The clastogenic effect of effluent contaminated river water, which contains high concentration of cobalt, was evaluated in Chapter 5; a separate series of experiments was carried out (in the same chapter) to assess the clastogenic effect of cobalt alone. An overall appraisal of these experiments has been done in the section, General Consideration.

Ecosystem Level Analysis

It is doubtful whether the laboratory-based toxicity tests alone can predict the potential effects of contamination on natural environments primarily because the responses of large-scale system cannot be accurately predicted from the analysis of the response of its individual components (Kimball and Levin, 1985). Hence, it is expected that toxicity tests performed on individual organisms, regardless of the end points used, will simply be not able to predict accurately the effects toxicants have on an ecosystem. In addition, ecosystem level toxicity tests should be performed because indirect effects of toxicants may at times be more important than direct ones (Kimball and Levin, 1985).

The health of an aquatic ecosystem can be assessed by noting alterations in the organizations of biota at the population, or at the community level (Kovacs and Podani, 1986; Maltby and Calow, 1989). Bioindication consists of comparing species composition and/or other indices of diversity at various sites in order to estimate the pollution loading present in the locations (Moriarity, 1990; Smith, 1991). Presence or absence of certain organisms can be used as indication of the type and level of pollution that is present in various environments (Calow, 1992; Smith, 1991). However, biotic indices of pollution that merely note the presence/absence of a species in an area are problematic, in that the absence of a species, in itself, could be correlated with numerous other ecological phenomena besides the pollutant loading present in an environment (Moriarity, 1990). Other potential explanations of a species' absence include various biological as well as physical attributes (Kovacs and Podani, 1986).

Considering the above aspects, a three-year field study has been carried out to analyze the distribution pattern of amphibian fauna of the Narmada valley in Gujarat State, in relation to various biological and physical attributes. Even though the study has mainly been carried out in the Shoolpaneshwar sanctuary, situated on the left bank of Narmada, the specimens obtained from various parts of Gujarat were also examined. The previous records were also compiled to get an updated image of the distribution (Naik and Vinod, 1993). Several factors such as the physiography, agroclimatic divisions, climate and vegetation have also analyzed in relation to the distribution and diversity.

The major conclusions derived from both the parts (Part I and II) have been discussed and appreciated in detail as the general consideration at the end of text narration.