CHAPTER 6

IMPACT OF ANTHROPOGENIC ALTERATIONS IN THE AQUATIC HABITAT AND THE PREVALENCE OF CHYTRID FUNGUS ON THE POPULATION OF AMPHIBIANS IN VADODARA

6.1 INTRODUCTION

Amphibians are considered to be good indicators of 'environmental health' especially in detecting damage to local environments. Most of the amphibians are exposed to terrestrial and aquatic habitats at different stages of their life cycles, and because they have highly permeable skin, they may be more sensitive to environmental toxins than are other terrestrial vertebrate groups (Blaustein and Wake, 1990; Vitt *et al.*, 1990; Wake, 1991).

This unique group of vertebrate, containing over 6,000 known species, is threatened worldwide. A recent report from the IUCN's Global Amphibian Assessment (2006) indicates that nearly one third (32%) of the world's amphibian species are threatened, representing 1,896 species. By comparison, just 12% of all bird species and 23% of all mammal species are threatened. Amphibians have existed on earth for a very long time (~350 million years), yet very recently (the last several decades) nearly 168 species are believed to have gone extinct and at least 43% of the species are declining in population, indicating that the number of threatened species will probably continue to rise (Stuart *et al.*, 2004; Global Amphibian Assessment, 2006). Nevertheless, the largest numbers of threatened species occur in Latin American countries such as Colombia (209), Mexico (198), and Ecuador (163). The highest levels of threat however are in the Caribbean, where more than 80% of amphibians are threatened in the Dominican Republic, Cuba and Jamaica, and a staggering 92% are categorized threatened in Haiti (Global Amphibian Assessment, 2006).

Amphibian declines were first presented as a conservation issue in 1989 at the First World Congress of Herpetology held at Kent University in Canterbury, UK. However, with an initial skepticism (that this change in population is natural as it is known to vary through time), biologists have come to a consensus that declines in amphibian populations are a real and severe threat to biodiversity (Barinaga, 1990; Wake, 1991, 1998). Though the decline of this group of vertebrate have various likely causes, there has been a wide spread controversy about their significance (Pechmann *et al.*, 1991; Pechmann and Wilbur, 1994; Alford and Richards, 1999; Blaustein and Keisecker, 2002). The possible causes of amphibian declines are

6.1.1 Introduced Species

Declines and local extinctions have been variously ascribed to introduced fish like the trout species (*Oncorhynchus mykiss, Salvelinus fontinalis*), bluegill sunfish (*Lepomis gibbosus*) and amphibians such as *Rana catesbeiana*, (Bradford, 1989; Bronmark and Edenhamn, 1994; Kats and Ferrer, 2003). The introduction of trout, for sporting purposes, into mountain lakes in the Californian Sierra Nevada range resulted in the major decline in the population of mountain yellow legged frog *R. muscosa* (Knapp and Mathews, 2000). Colonization of normally fish-free water bodies by predatory fish can result in rapid extinction of amphibian assemblages (Fisher and Shaffer, 1996). Mosquitofish (*Gambusia affinis*) have been introduced throughout the world to control mosquito populations and these introductions have been reported to have negative effects on amphibians. In experimental studies, mosquitofish reduced the survival of larval pacific treefrogs (*Hyla regilla*) (Goodsell and Kats, 1999). *R. catesbiana*, which is an introduced species in many parts of the world, is considered to be an effective carrier of chytridiomycosis, a major fungal disease in amphibians (Daszak *et al.*, 2003).

6.1.2. Climate Change

The more complex and elusive mechanisms potentially underlying declines also includes climate change (Pounds *et al.*, 1999; Kiesecker *et al.*, 2001 and Carey and Alexander 2003). Amphibians are extremely sensitive to small changes in temperature and moisture and therefore the change in global weather patterns (e.g. global warming) can alter breeding behavior, affect reproductive success, decrease immune functions and increase their sensitivity to chemical contaminants (Young *et al.*, 2001). Studies by Pounds and Crump (1994) suggest that the disappearance of the golden toads, *Bufo periglenes* in the rainforests of Monteverde, Costa Rica due to the change in the climate of this region. Recent changes in the global climate might impact adversely on amphibian populations. Global mean temperature rose by about 0.6^0 C over the past 100 years with an accelerating trend since the 1970s and there are increasing evidences for multiple effects of climate change on wildlife and ecosystems (Parmesan and Yohe, 2003). However there is no strong evidence that recent climate patterns have led to amphibian declines (Alexander and Eischeid, 2001). It is therefore ambiguous, whether the recent climatic changes play any significant role in decrease of amphibian populations.

6.1.3. UV-B Radiation

Anthropogenic depletion of ozone and resultant seasonal increases in ultraviolet B (UV-B) radiation at the Earth's surface have stimulated interest in the probable relationship between resistance of amphibian embryos to UV-B damage and population declines (Kerr and McElroy, 1993). A number of studies, mostly in mountainous regions of North America have demonstrated that ambient or enhanced UV-B radiation reduces survival or hatching success of amphibian embryos (Ovaska *et al.*, 1997; Lizana and Pedraza, 1998). Nevertheless, the significance of increased UV-B radiation in amphibian declines remains uncertain, and on going conflicts over methodologies, analysis and interpretation of available data show no sign of warning (Kats *et al.*, 2002; Blaustein *et al.*, 2003; Heyer, 2003)

6.1.4. Habitat Modification and Pollution

Habitat modification is the best understood and documented cause of amphibian population declines. Habitat loss certainly reduces amphibian abundance and diversity, in the areas that are directly affected (Hecnar and M'Closkey, 1996; Hecnar, 1996). Removal or modification of vegetation has a rapid and severe impact on some amphibian populations (Ash, 1988). Urbanization is perhaps the major reason for change in habitat of the amphibians and also the major reason for pollution of many wetlands (Chapin *et al.*, 2000). Urban development primarily affects the species richness of ecosystems via the loss or fragmentation of habitat (McKinney, 2002). Wetlands are very sensitive and are largely influenced by urban development. As a result of human development (e.g. agriculture, industrialization, and urbanization), wetlands have been lost at levels exceeding 50% during the last 200 years (Ehrenfeld, 2000). Urbanization is also associated, however, with increased exposure to contaminants, eutrophication and alterations in hydrology (Ehrenfeld, 2000; McKinney, 2002).

Amphibians are considered reliable indicators of environmental quality (Dunson *et al.*, 1992). The early life stages (egg and larval) of many species are restricted to the aquatic environment, and many adults respire through moist skin (Duellman and Trueb, 1986). Consequently, all life stages of amphibians are susceptible to dermal absorption of toxicants in water. Ingestion of contaminated prey is also a potential pathway for toxicants to enter amphibians (Duellman and Trueb, 1986). Modification of terrestrial and aquatic habitats for urban development can reduce or eliminate amphibian populations. Draining wetlands directly affects frog populations by removing breeding sites (Elmberg, 1993; Semlitsch and Bodie, 1998) and therefore increasing the regional probability of extinction (Corn and Bury, 1989). Water contamination and poor water quality in general have escalated in recent past (Smith *et al.*, 1987). Water quality needs of wildlife have often been neglected; this neglect is particularly true for amphibians (Hall and Henry, 1992). Elevated pH, low dissolved oxygen, high water temperatures, and elevated un-ionized ammonia levels which characterize water in many of the agro ecosystem, may singly or in combination have significant detrimental effect on the developing embryos of frogs (Boyer and Grue, 1995). Johnson and Chase (2004) provide evidence that connects the prevalence of the parasite to the growing number of ponds swamped by nutrients, which may explain why the incidence of deformities has increased to more than 90% in some frog populations. **The first objective of this chapter therefore, was to evaluate the water bodies in the Vadodara for their physicochemical characteristics and to find how these variables influence species richness and abundance of the inhabitant amphibians.**

Concordant with habitat destruction, there has been a wide spread use of fertilizers and pesticides that also have deleterious consequences. The environmental contamination due to organic and inorganic compounds derived from agricultural and industrial development has received considerable attention (Mora and Anderson, 1995; Loumbourdis and Wray, 1998). Environmental toxicants act directly to kill animals, or indirectly by impairing reproduction, reducing growth rates, disrupting normal development and also increasing susceptibility to disease by immunosuppression or inhibition of immune system development (Carey and Bryant, 1995). The presence of elevated levels of heavy elements in drain waters has been connected with teratogenetic, reproductive, and other anomalies in vertebrates. For example, heavy metal like nickel (Ni) is immunotoxic and induces skin sensitization in human (Menne, 1994). Chromium (Cr) can be absorbed by the respiratory tract and also to a certain extent by the intact skin (Lauwerys and Hoet, 1993). Cadmium (Cd) is frequently found at contaminated sites and has the potential to bioaccumulate and alters food chains (James, 2005).

Aquatic habitats become polluted with these heavy metals from terrestrial runoff, aerial deposition, and the release of effluent directly into water bodies. Frogs and their larvae exhibit discernible deformities following exposure to chemicals used in insecticides and herbicides (Power *et al.*, 1989) and heavy metals (Nebeker *et al.,* 1994), and, as a consequence, have been repeatedly used as bioindicators of contaminants.

The discharge of effluents from the industries situated around the major cities of Gujarat (India) into nearby rivers or open land is of great concern with respect to soil-plant-water pollution (Ramani *et al.*, 2004). Bioaccumulation of metals in macrophytes at Nalsarovar, a Ramsar site in Gujarat, revealed a high accumulation of metals like Co, Ni and Cu in the macrophytes (Kumar *et al.*, 2006). There have been apparently no or very few studies in Gujarat, evaluating the bioaccumulation and impact of heavy metals on freshwater organisms. Studies by earlier workers have shown that among the aquatic organisms, amphibians are most sensitive to aquatic contaminants (Loumbourdis and Wray, 1998, Nebeker *et al.,* 1994). **Hence in the present chapter an attempt was made to evaluate the role of heavy metals *viz.* Nickel (Ni), Cadmium (Cd) and Chromium (Cr) in population decline in this part of the world by analyzing the concentrations of heavy metals in tissues of an aquatic species of amphibian, *Euphlyctis cyanophlyctis.*** Frogs and other aquatic organisms provide a relatively long-term measure of aquatic pollution, unaffected by short-term, intermittent changes in water chemistry

6.1.5. Diseases

Dramatic mass mortalities have been observed in some of the amphibian populations suggesting that diseases may play a significant role in the decline of some of the species (Berger *et al.*, 1998; Lips, 1999; Daszak *et al.*, 2003). Declines in populations of *Bufo boreas* between 1974 and 1982 were associated with *Aeromonas hydrophila* infection, but Carey (1993) suggested that environmental factor(s) caused sublethal stress in these populations, directly or indirectly suppressing their immune systems. *Rana viruses* (Iridoviridae) cause high levels of mortality in tiger salamanders (*Ambystoma tigrinum*) but populations usually recover afterwards (Jancovich *et al.*, 1997). *Ribeiroia ondatraeis* is a trematode worm that causes leg deformities in frogs (Johnson and Chase, 2004).

Undoubtedly the most worrying pathogen so far discovered is a chytrid fungus *Batrachochytrium dendrobatidis.* This has been implicated in mass mortalities and population decline of amphibians in America, Europe, Australia and New Zealand (Berger *et al.*, 1998). It is proposed that Africa is the origin of the amphibian chytrid and that the international trade in *Xenopus laevis* that began in the mid-1930s was the means of dissemination (Weldon *et al.*, 2004). Amphibian chytridiomycosis was first described from carcasses collected by wildlife biologists at sites of mass mortality in the montane rain forests of Australia, Costa Rica and Panama (Berger *et al.,* 1998). Chytridiomycosis has a wide host range including urodeles, aquatic amphibians such as *Xenopus*, terrestrial and semi-terrestrial frogs and toads (Daszak *et al.*, 1999). Chytridiomycosis is a fatal disease of post-metamorphic frogs and can be carried by healthy tadpoles. The chytrid fungus is the most common cause of death in Australian frogs (Berger *et al.,* 1999a) and has also been found in a small proportion of apparently healthy frogs and tadpoles (Berger *et al.*, 1999b). The disease is present in North America, causing mass mortalities and population declines in a range of ranid and bufonid species, notably *Rana yavapaiensis*, *R. chiricahuensis*, *Bufo boreas* and *B. canorus* (Daszak *et al.*, 1999).

Members of the phylum Chytridiomycota are heterotrophic fungi that are ubiquitous and cosmopolitan (Sparrow, 1960). They are found primarily in soil and water where they usually act as primary degraders or saprobes. The chytrid reported is the first member of the phylum Chytridiomycota to be recognized as a parasite to the phylum Vertebrata (Barr, 1990). It attacks the skin and causes death by impairing cutaneous respiration and osmoregulation. Disease out break may follow either 1) a weakened immune response in the amphibians caused, perhaps, by another stressor; or 2) an increased virulence of the pathogen (Beebee and Griffith, 2005). Evidently there is substantial interspecific and intraspecific variation in susceptibility to this pathogen, and interaction with other factors may be crucial to its epidemiology. Chytrid fungi might therefore be responsible for some amphibian population declines, but it remains uncertain as to whether they are primary or secondary cause (Beebee and Griffith, 2005). Through histological analyses of amphibian skin, Berger and coworkers (1999) reported the presence of the disease causing fungus in a large number of species from diverse geographical regions *viz.* Austaralia, USA, Panama and Ecuador. Through surveys of extant and archived specimens, *Batrachochytrium* has been found in every continent that has amphibians, except Asia (Bosh *et al.*, 2000; Weldon, 2002; Speare and Berger, 2000).

The disease chytridiomycosis, or frog chytrid fungus, which claims the lives of frogs worldwide, is so far not reported from Asia (Speare and Berger, 2000). One of the major reasons for this could be that no studies related to this disease have yet been undertaken in this part of the world. In India also no such work has been carried out. **Therefore, the focus of the present study was also extended to understand the prevalence of the cutaneous disease the chytridiomycosis in the amphibian community at the selected study sites in Vadodara by analyzing the histologic profile of the amphibian skins.**

6.2. STUDY SITES

Intensive study areas selected to evaluate the impact of pollution on the amphibians were based on the different objectives. This study was conducted during the year 2005.

6.2.1 Study Sites for first objective

Nine water bodies selected within the Vadodara city limits to study the influence of physicochemical variables on the species richness and abundance were as follows:

SITES	POND	Geographical Position
Warasia pond	A	22°18'27"N 73°13'41"E
Danteshwar small pond	B	22°16'41"N 73°12'35"E
Danteshwar main pond	C	22°16'34"N 73°12'38"E
Harni main pond	D	22°20'18"N 73°13'9"E
Harni temple pond	E	22°20'41"N 73°13'5"E
Sama	F	22°20'31"N 73°12'6"E
Lalbaug	G	22°16'59"N 73°11'44"E
Kashivishwanath temple Pond	H	22°17'14"N 73°11'40"E
Ajwa pond	I	22° 19’12” N 73 °14’53” E

6.2.2. Study Sites for second objective

Vadodara urban agglomeration is an industrial node of national importance. There are a number of industrial establishments related to textiles and auxillaries, chemical, petrochemicals, fertilizers, pharmaceuticals, glass and ceramics, synthetic fibre and rubber, dye materials, electronics, light engineering, tobacco, vegetable oil processing and so on.

In the present study, metal burdens in the body tissues of the skittering frog *(E. cyanophlyctis)*, an aquatic amphibian present in abundance throughout the industrially disturbed area within Vadodara district was investigated. They were collected from three different sites which included:

1. Sardar estate (Position: 22^{0}18’54” N and 73^{0}14’04” E). This site is within the Vadodara city limits and was directly affected by discharges coming from various industrial sources like electroplating unit, plastic industries, chemical producing factories, automobile manufacturing industries and metal fabricating unit.

2. Waghodia (Position: 22^{0}17’25” N and 73^{0}22’02” E). The second polluted site is 10 km east of Vadodara city and the water body at this site was also directly affected by the industrial effluent discharged from only one industrial unit which was involved in the manufacturing of tyres and production of machinery tools.

3. MSU Campus (Position: 22^{0}19’34” N and 73^{0}11’16” E). This site was considered as a control site for comparison, as the water body in this area was unaffected from any of the industrial pollution.

6.2.3. Study Sites selected for the third objective

In order to know prevalence of chytridiomycosis in the amphibians, different species were selected, from the polluted sites (ibid, p.107) and nonpolluted sites (Chapter 4), during the study period.

6.3 MATERIALS AND METHODS

6.3.1 Physicochemical Analysis of the Water Body and Amphibian Survey

During the study period (2005), permanent water bodies were also surveyed and the entire perimeter of the pond out to about 4-5 m from the shore was searched for amphibians. To check the presence of amphibians and their larvae we dipnetted through the submerged vegetation. Amphibians were identified visually and also by their breeding calls. If any life stage was detected on any visit at a pond during the study period we considered that species to be present and conversely, absent if no life stage was detected. For each pond the physicochemical variable was also studied.

Water contamination and poor water quality in general have escalated in near years (Smith *et al.*, 1987). During the preliminary survey, larger permanent water bodies within the Vadodara city were identified and were studied. Water from the study sites, were collected and analyzed for various physicochemical parameters as per the treatise, "Standard Methods for the Examination of Water and Wastewater", prepared and published jointly by the American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF). These include temperature, pH, dissolved oxygen (DO), chemical oxygen demand (COD), phosphate-phosphorous ($PO_4^{3-}-P$) and nitrate-nitrogen ($NO_3^{-}-N$) (Chapter 3).

Visual interpretation of the premonsoon satellite images of the studied water bodies were done and digitized with Coral Draw Graphics Suite 12, Macrovision Europe Ltd., Macrovision Corporation. The map produced were verified with ground data using Geographical Positioning System (Garmin, GPS 12XL).

6.3.2 Method for Heavy Metal Analysis

A total of 15 Adult *Euphlyctis cyanophlyctis* of both sexes were collected from each study sites, so as to comprehend the bioaccumulation of selected heavy metal in these animals. The frogs were then sexed and anaesthetized with ether, individually weighed, and snout to vent length measured. After anesthetization, the belly was excised and the tissues to be analyzed were removed, digested and heavy metals were assessed using atomic absorption spectrophotometer. The metals analysed in the current study were Nickel (Ni), Cadmium (Cd) and Chromium (Cr) (Refer materials and methods for detail).

6.3.3 Method for Diagnosing Chytridiomycosis

6.3.3.1 Collection of Specimen

Anurans belonging to different families were collected during monitoring programme of amphibian population in Vadodara, Gujarat.

6.3.3.2. Histology

Strips of skin from the pelvic region were undertaken for the study of chytridiomycosis, as the chytrid fungus invades only the stratum corneum and stratum granulosum (Berger *et al.*, 1999). Tissues were preserved in 10% formalin. These were then embedded in paraffin, sectioned at 6µm, stained with haematoxylin and eosin and observed under Leica DMRB photo microscope.

6.3.4. Statistical Analysis

All the parameters characterized by continuous data were analyzed to give group means and standard error. Independent group Analysis of Variance (One Way ANOVA) was used to test the difference between the means. Pearson's Correlation Coefficient (r) was calculated to know the strength of the linear relationship between two variables. All statistical analyses were done using a statistical program SPSS, 11.5; (SPSS Inc. Chicago, IL, USA).

6.4. RESULTS

6.4.1. Water Quality and Amphibian Population

6.4.1.1. Physicochemical Parameters

The investigated water bodies are permanent and though they were filled with water in all the seasons, a periodical fluctuation in the water levels throughout the study period was observed. In the present study the sampling sites reflect a large variation in physicochemical properties. Data concerning the pH, temperature, dissolved oxygen, chemical oxygen demand, phosphate-phosphorous and nitrate-nitrogen are presented in Table 6.1, 6.2, 6.3.

Mean water temperature showed fluctuation at different study sites. Minimum temperature recorded was 18°C during winters whereas maximum temperature went up to 32.4°C during the summers. Pond E showed the highest mean summer temperature and the lowest temperature values were recorded during the winter at Pond F. The temperature during the monsoon was moderate and ranged between the summer and the winter values. Majority of the water bodies were alkaline with higher pH value. The mean pH values ranged from 7.43 -10.29 during the study period. More than 75% of the water bodies were having pH value high than 8. The highest pH value of 10.29 was recorded from Pond H during the summers, however the lowest value of 7.28 was observed during monsoon at Pond E. The summer values in general were high at all the study sites and the monsoon values were low. A significant negative correlation was observed between pH and COD ($r=-998$, $p<0.05$) at Pond E.

The COD values ranged from 12.8 to 135.2 mg/L, while the DO ranged from 0.43 to 7.5 mg/L. Low values for COD was documented during the monsoon, whereas these values were high during the summers. COD values higher than 120 mg/L was recorded from Pond A, C and Pond H. In Pond C, a significant negative correlation was observed between the COD values and the species abundance($r=-998$, $p<0.05$). Contradictory to the above result, the DO values were low during the summers, whereas high value was observed during winters. Pond D and E had high DO value, while low DO value were recorded from Pond B and Pond A during winters.

The $PO_4^{3-}-P$ level fluctuated significantly during the entire study period. Higher $PO_4^{3-}-P$ value was recorded during the summer and during this period, out of nine water bodies, eight of them showed the $PO_4^{3-}-P$ concentration more than 0.2mg/L. Mean values of $NO_3^{-}-N$ at most sampling sites were higher than 0.1 mg/L for subject to eutrophication. In order to understand the (inter)relations between the species abundance and the measured parameters, the Pearson correlation test has been used. Species abundance showed statistically significant bivariate negative correlation with $PO_4^{3-}-P$ ($r=-999$, $p<0.05$) in Pond C, but the correlation between amphibian abundance and $NO_3^{-}-N$ was weak and statistically non significant.

6.4.1.2. Species Richness and Species Presence/ Absence.

The species richness was very low for the water bodies studied. Only two species were recorded during the present study *viz. E. cyanophlyctis* and *H. tigerinus. E. cyanophlyctis* is an aquatic species while *H. tigerinus* is a semiaquatic species. Pond C, D, E, F, G, and I showed the presence of these species. The historical data gathered by distributing pictorial questionnaire to locals however, differs from the present study.

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6.4.1.3. Breeding of Amphibians

In total two frog species have been recorded in these water bodies namely the Skittering frog, *E. cyanophlyictis* and the Bull frog, *H. tigerinus*. Only 33% (Pond D, G and I) of the water bodies showed the presence of breeding amphibians. The remaining water bodies (Pond A, B, C, E, F, and H) showed no signs of breeding by either of the species.

6.4.1.4. Eutrophication

The pH and nutrients ($NO_3^- - N$ and $PO_4^{3-} - P$) exhibit a significant correlation. Therefore, these parameters may be regarded as key factors for determining the quality of water. All the water bodies were eutrophied with varying degree of eutrophication (Figure 6.2 to 6.16) Nutrient like $NO_3^- - N$ and $PO_4^{3-} - P$ was found high in all the ponds except. This nutrient excess could be the possible contributing factor for the eutrophication observed in the majority of the studied aquatic habitats.

6.4.2 Heavy Metal Study

In order to assess the extent of accumulation of heavy metals in *Euphylctis cyanophylictis* from the areas experiencing direct pollution from industries, mean liver and kidney metal content of the frog from the polluted sites *viz.* Waghodia and Sardar estate was compared with the mean values for frogs collected from the non-polluted sites, namely, the Campus area. The mean and standard error of the metals *viz.* nickel (Ni), cadmium (Cd), chromium (Cr) measured in the liver and kidney is presented in Table 6.4 and Table 6.5. The heavy metal concentration of the respective water bodies is given in Table 6.6. The lack of any statistically significant difference in any of the heavy metals analyzed between males and females tissues enabled pooling of the results of both sexes. Further, the metals studied were not detected in all the animals analyzed.

The results demonstrated that Ni content in frog liver and kidney from the polluted site *viz.* Sardar estate was significantly higher compared to the non-polluted sites ($p \leq 0.001$)(Figure 6.1). Nickel content in liver and kidney was significantly greater ($p \leq 0.001$ and $p \leq 0.01$ respectively) for the Sardar estate samples when corelated with Waghodia. The relatively higher level of Ni in the liver and kidney of *Euphylictis cyanophylictis* may be directly related to the contamination of this water body that is located in the industrial area. Examination of heavy metal content of the water bodies from this area have indicated unusually high amount of Ni and slightly elevated amount of Cd. Liver and kidney of frogs collected from the polluted sites *viz.* Waghodia contained significantly higher concentrations of cadmium ($p \leq 0.05$ and $p \leq 0.001$) as compared to the non-polluted sites. Cd content in the kidney of the animals collected from Waghodia was significantly higher ($p \leq 0.001$) compared to the Sardar estate samples. No significant difference was observed between the contents of Cr in the liver and kidney of *E. cyanophylctis* collected from polluted sites and the control site of comparison. The frogs collected from the polluted sites revealed the presence of only low concentration of chromium in the studied tissues. There is no data available for the content of heavy metals in other aquatic organisms found in these water bodies.

Of the three metals evaluated in the tissues of animals collected from the polluted sites, Ni indicated the greater degree of accumulation where the mean Ni levels in the liver and kidney were increased by a factor of 16 to 30 fold over specimen collected from MSU campus site, while tissue Cd levels were enhanced by a factor of 7 to 16 fold. Tissue concentrations of Ni and Cd were found to be significantly correlated in liver ($r=0.994$; $p< 0.05$) at Waghodia but no such correlation was observed for Sardar estate. Nickel and Cadmium content in both the tissue *viz.* liver and kidney had no significant correlation at any of the polluted sites. However, there was a significant positive correlation between the metal content in the water and in the tissues at the study sites (Table 6.7).

6.4.3. Chytridiomycosis Study

Stratum granulosum [SG] and stratum corneum [SC] (epidermis/keratin layers) in amphibians is much thinner than that found in the human skin. The stratum corneum in the studied amphibians is rarely more than a couple of cells thick, and these keratinised cells usually appear flattened. In the present study, diagnosis of the skin of amphibians for chytridiomycosis showed a negative result, in all the samples of the skin examined. Further confirmations of the above result were done through peer reviewing of the images by two independent experts from the field-Rick Speare and Diana Mendez (James Cook University, Queensland). Usually the SC in the infected animals is loosely adhered to the SG (Figure 6.24) and full as well as empty zoosporangia are embedded in this layer. There are no such features observed in the SC of the epidermal layer in the studied anurans (Figure 6.17 to 6.23)The normal thickness of SC is 2-5µm thick while the infected SC measure about 60µm (Berger *et al.,* 1998). In the current study, SC and SG of the epidermal layer of the anurans collected from various sites were measured and recorded (Table 6.8). None of the skin samples studied showed the thickness of SC layer more than 8µm. The thickness of SC ranged from 4-8 µm, while that of SG ranged from 20 µm to 41.25 µm (Table 6.8). There is no evidence of epidermal fungal infection in adult anurans based on the analysis of histological profile. Thus, it could be logical to surmise that the anurans from the present study site might be free of *Batrachochytrium dendrobatidis* zoosporangia.

6.5. DISCUSSION

The general principle regarding the water pollution and frog abundance holds true in the present study also "if there are plenty of frogs present at a freshwater site, the water quality is likely to be good; if frogs are absent or scarce, be wary of the water". The growing urbanization, scarcity of potable water and ever increasing anthropogenic influences have been constantly exerting pressure on surface water bodies. Not surprisingly, water quality needs of aquatic organisms including amphibians have often been neglected (Boyer and Grue, 1995). Recently widespread decline in amphibian population has been reported from world over. Several reasons for the perceived frog decline have been suggested. The most widely known and recognized reason is the habitat loss. Status of the nine water bodies studied within the Vadodara city revealed that more than 90% of them fall in the category of polluted based on the physicochemical parameters. Majority of the studied water bodies in Vadodara are eutrophicated. More than 90% of the water bodies are rich in nutrients as evidenced by elevated levels of $PO_4^{3-} - P$ and $NO_3^- - N$. The major factors influencing the concentrations of N and P might be terrestrial runoff and release from domestic sewage.

Concentration in excess of 0.2 mg/L nitrates tend to stimulate algal growth and indicate possible eutrophic conditions (Kumar and Ravindranath, 1998). The $NO_3^- - N$ concentration was high for many of the water bodies studied during the present investigation. Environmental concentrations of $NO_3^- - N$ in watersheds in the present study ranged from 0.19 to 2.71 mg/L. This increased level of nitrate concentration can be one of the major reasons for the eutrophic condition of the aquatic body. However, of all the water samples collected from study areas, none of them showed the nitrate concentrations exceeding the limits which can cause lethal effects in amphibians. Rouse *et al.* (1999) showed that the lethal effects of nitrate for a variety of anurans ranged from 14 to 385 mg/L, while sublethal developmental effects on larvae ranged from 2.5 to 10 mg/L nitrate. These responses were species and life-stage specific, with early life stages being more sensitive than adults. Despite the uncertainty of causal mechanisms, it is clear from many other studies that nitrogenous compounds have potent negative effects on amphibian development, growth, and survival (Huey and Beitinger, 1980; Baker and Waights, 1994; Marco and Blaustein, 1999).

In most of the natural surface waters, it has been documented, phosphorous ranges from 0.005 to 0.020 mg/L (Kumar and Ravindranath, 1998). The present study one observed

$PO_4^{3-}-P$ level ranging from 0.1 to 1.8 mg/L. The high concentration of $PO_4^{3-}-P$ might be the reason for the high eutrophic condition in the water bodies. In addition, nutrient stimulated invasion of the margins by weeds could eventually displace the free water in the pond and make it unsuitable for the populations of *E. cyanophlyctis* and *H. tigerinus* breeding there. Experimental evidences suggest that the elevated nitrate and phosphate concentrations in water bodies contributed to the decline of *Litoria aurea* (Hamer *et al.*, 2004). Study by Bishop *et al*, (1999) documented nutrient run-off as a causal or contributing factor in lowering the diversity, density and reproductive success of American toads and green frogs in Ontario.

Johnson and Chase (2004) observed high incidences of parasite infestation in the frogs that reside in water having elevated levels of phosphorous. They also proposed that the cause of outbreaks of frog deformities can be traced to the increased frequency of eutrophication of waters where tadpoles hatch and then metamorphose into frogs. According to their study eutrophication favors expansion of the populations of a family of snails, Planorbidae, which are hosts to a parasitic trematode. The trematode has been shown to cause deformities in frogs by forming cysts around developing limbs of the tadpole. Results of other studies also support the notion of eutrophic condition induced frog embryo mortality and malformations (Boyer and Grue, 1995). Moreover, natural history information indicates that several species may prefer breeding sites with moderate or low amounts of vegetation rather than heavily vegetated sites (Laurila, 1998).

Pond A, B, C and H were subjected to high levels of sewage pollution resulting in high levels of COD and reduced levels of dissolved oxygen. Nutrients from the sewage promoted severe eutrophication leading to significant reduction in the levels of dissolved oxygen which attains fall below detectable level. High COD values in the study sites may be due to contamination, either by the inflow of wastes from terrestrial runoff or of anthropogenic in origin, and therefore is a cause of concern. Local pollution from sewage discharges as well as from the industrial effluent can be considered as the major pollutants that are seriously degrading this surface water. All the water bodies studied were subject to pollution by sewage or animal excrement or by washing. These reached high levels during summer with consequent high rise in COD values and perceivable reduction in the level of dissolved oxygen. The only two species found in all the water bodies were *E. cyanophlyctis* and *H. tigerinus* and thus contributing to very low species richness. Both *H. tigerinus* and *E.cyanophlyctis* appear to be adapted to take advantage of man- made or degraded sites unsuitable for many other frog species. They are versatile and adaptable animal, able to inhabit small and degraded sites. Species such as *Limnodynastes peronii* and *Litoria aurea* have also adapted to such changes in its environment (Hengl and Burgin, 2002). Rubbo and Kiesecker (2005) hypothesized that certain species of amphibians are more sensitive to the adverse effects of urban development.

Amphibians have been found in wastewater treatment wetlands, farm ponds, mining sites, and other contaminated habitats. However, they are unlikely to escape environmental degradation because they have high breeding-site fidelity and low mobility. Contamination may act both directly on larval amphibians through dermal and oral uptake, and indirectly by altering the aquatic community on which they survive (Mills and Semlitsch, 2004). Few researchers have worked on heavy metal contamination in amphibians (Lee and Stuebing, 1990; Nebeker *et al.* 1994; Read and Tyler 1994; Nebeker *et al.*, 1995). These studies suggest that there is a high impact of heavy metal on the survival of amphibians.

Euphlyctis cyanophlyctis is one of the common frog encountered during the study period and is widely distributed. It can apparently tolerate substantial levels of pollution and physical site degradation (ibid, p-12). The results of the present study showed that Vadodara city, in which both the polluted areas studied belonged, could be considered polluted with metals such as nickel, cadmium and chromium. One of the possible reasons for the heavy metal contamination could be the run off from industries carrying heavy metal in their effluent. However, another source of pollution could be the urban runoff. The amount of bioaccumulations of heavy metals in tissues may vary depending on length and weight of samples (Barghigiani and Ranieri, 1992; Zyadah, 1999). Therefore, the samples chosen were of the same weight for each study sites. The Ni concentration was significantly high in both the tissues of frogs collected from Sardar estate and Waghodia. The elevated level of Ni in the tissue indicates heavy metal pollution in these areas. The main source of this pollution originates from the effluent resulting from the scrap metal reprocessing units in this area. The World Health Organization (WHO) classifies nickel compounds in Group 1 (human carcinogens) and metallic nickel in group 2B (possible human carcinogen) [U. S. Public Health Service (USPHS), 1993]. Human activities that contribute to nickel loadings in aquatic and terrestrial ecosystems include mining, smelting, refining, alloy processing; scrap metal reprocessing, fossil fuel combustion, and waste incineration (Chau and Kulikovsky-Cordeiro, 1995). High amount of nickel in the environment may pose threat to the amphibians. Population abundance of adult treefrogs declined with increasing atmospheric deposition of nickel, and abundance of toad tadpoles declined as nickel concentrations in pond water rose from 3.3 μg Ni/L at more distant sites to 19.5 μg Ni/L at sites near Sudbury (Glooschenko *et al.*, 1992). Thus it is important to check the amount of nickel from the polluted sites as these may result in the total elimination of the population of *Euphlyctis cyanophlyctis* from the area due to its carcinogenic effect.

The current study also reports a significantly high concentration of cadmium in the kidney and liver which may adversely affect the amphibians in the long run. Cadmium (Cd) is an ubiquitous trace metal, biochemically classified as a nonessential element. *Rana ridibunda,* which was exposed to 200 ppm aqueous solutions of cadmium showed major histological and histochemical alterations in the liver and kidney. In liver there was an increase in the area occupied by Kupffer cells whereas in kidney, the hyaline globules (HG) and apoptotic bodies occurred at a higher rate resulting into progressive nephropathy, or progressive glomerulonephropathy (Loumbourdis, 2005). In the present study, kidney of the frogs collected from Waghodia had an elevated content of cadmium compared to the liver. The kidney is a common target organ for toxic metals since it forms a major pathway for excretion of metals from the body and has a high metabolic activity with a number of sensitive metabolic processes (Vogiatzis and Loumbourdis, 1998). Laboratory studies and field investigations in polluted environment have reported localized cadmium accumulation in kidney and liver tissue for most species including man (Schroeder and Balassa, 1961; Roberts *et al.*, 1976; Shah and Dubale, 1983), while age accumulation of the element in renal tissue is a well established phenomenon associated with the formation of stable cadmium-metallothionein complexes (Nilsson, 1950). Significantly high amount of cadmium in the kidney and liver of the frog can be correlated to its extremely long biological half-life (about 30 years) in both human and animals bodies and its ability to accumulate in tissues, and in particular in the kidneys (Friberg *et al.*, 1974; Vogiatzis and Loumbourdis, 1998).

Correlation analysis clearly indicated that in both liver and kidney, tissue accumulations of Cd and Ni were positively correlated in Waghodia but not in Sardar estate. While the dynamics of this Ni-Cd interaction presently remain unclear, the observed correlation supports the view of a common exposure source, as well as route of uptake for these two elements at Waghodia. Water at this water body receives industrial effluent from only one source. Comparable trends have been similarly reported in the tissue uptake and accumulation of Ni and Cd by Muskrat in Ontario, Canada (Parker, 2004). Hillis and Parker (1993) reported a significant positive correlation in the accumulation of Ni and Pb by Beaver residing within the influence of the Sudbury- area ore smelters in Ontario. The non significant correlation between these metals in Sardar estate could be due to the different sources and different industrial units through which the effluents are released.

Animals analysed in the present study showed very low level of chromium in both the tissues evaluated. This could be due to the low level of this metal in the water, where this animal resided. Our observations suggest a significant bioaccumulation of heavy metal concentration on the frog population studied. This characteristic, make the frog *Euphlyctis cyanophylictis* a good bioindicator species of heavy metals. High levels of observed heavy metal contamination warrant studying other persistent pollutants. Further it is also suggested that there should be regular monitoring of heavy metals along with possible reclamation of the area.

Typical clinical signs in Australian frogs with chytridiomycosis were lethargy, inappetence, skin discolouration, presence of excessive sloughed skin, and sitting unprotected during the day with hind legs held loosely to the body (Berger *et al.,* 2000). Frogs became moribund in terminal stages with loss of righting reflex, and death usually occurred a few days after the onset of clinical signs (Berger *et al.*, 1999a). Diagnosis of skin of the amphibians, using histological techniques is an effective method for identifying the chytrid fungus (Nichols *et al.*, 1998; Berger *et al.*, 1999a; Carey *et al.*, 1999). Since the fungus invades only the *stratum corneum* and *stratum granulosum*, in particular the subsurface layer, skin is only the organ used for diagnosis (Berger *et al.*, 1999b). During the current study, skin samples of amphibians collected from different study sites were diagnosed for the chytrid infection. These skin specimens of the amphibians studied showed non appearance of any chytrid fungus *viz. Batrachochytrium dendrobatidis.* Stratum corneum may attain a thickness of 60 µm during infection (Berger *et al.*, 1999b). The zoosporangia may have a diameter ranging usually between 6 and 15 µm, this size helps in distinguishing zoosporangia from other bodies of stratum corneum (Berger *et al.*, 1999b). In the present study the size of stratum corneum layer ranged from 4.50 -7.81 µm which is comparatively less than those seen in the infected animals. Zoosporangia were not observed in the SC layer. Thus, screening of amphibians for chytridiomycosis, confirms a negative result (no chytrids fungus, *Batrachochytrium dendrobatidis* found). However, during the current study the histological sections of the skin taken from selected amphibian species revealed that this pathogen has not yet infected the amphibians at least in the selected study sites. Therefore, it can be inferred that the much hyped chytridiomycosis is not contributing to the decline of amphibian population if any, in Gujarat.

Currently a total of 2 amphibian orders (Anura and Caudata), 14 families and 93 species have been diagnosed infected with *Batrachochytrium dendrobatidis.* Australia has the most species infected (46) of any country. There is no report of this fungus infecting amphibians in Asia (Speare and Berger, 2000, 2002). This study has to be extended further into other regions of Gujarat state as well as of India. Work on amphibian chytridiomycosis must continue to confirm or reject the hypothesis that it is the primary cause of the declines, to determine how the epidemic began, to find ways to manage the problem in areas where the fungus is established, and to prevent it occurring in new regions. Knowledge gained from these investigations will be useful in preventing population crashes, occurring in frogs if any and in developing management strategies for other wildlife species.

TABLE 6.1 Physicochemical parameters of the water bodies during summer – 2005

	Temp	pH	NO_3-N	PO_4	DO	COD
Pond A	29.53± 1.67	9.67 ± 0.35	1.98 ± 0.17	0.26 ± 0.07	0.68 ± 0.22	127.47 ± 6.47
Pond B	30.93± 1.90	9.08 ± 0.17	1.79 ± 0.05	0.59 ± 0.14	0.61 ± 0.22	95.47 ± 26.92
Pond C	29.10±0.95	8.64 ± 0.42	0.38 ±0.15	1.39 ± 0.39	2.11 ± 0.41	126.67 ±
Pond D	28.17±1.04	8.28 ±0.46	0.35 ± 0.04	0.38 ± 0.15	3.55 ±0.28	28.59 ± 5.51
Pond E	31.81±0.52	9.71 ± 1.27	0.55 ± 0.05	0.12 ± 0.02	3.87 ± 0.11	19.07 ± 5.43
Pond F	30.30±1.48	7.60 ± 0.26	0.36 ± 0.04	0.25 ± 0.13	4.77 ± 0.22	59.47 ± 8.21
Pond G	30.77±0.76	9.59 ± 0.45	0.96 ± 0.08	0.48 ± 0.09	1.15 ± 0.18	80.00 ± 11.84
Pond H	29.40±1.12	10.29± 0.52	1.17 ± 0.05	1.44 ± 0.32	0.65 ± 0.22	124.80 ± 5.77
Pond I	28.99±0.46	7.43 ± 0.19	0.50 ± 0.04	0.51 ± 0.16	1.76 ± 0.16	80.15± 0.98

TABLE 6.2 Physicochemical parameters of the water bodies during winter – 2005

	Temp	pH	NO_3-N	PO_4	DO	COD
Pond A	21.75±1.77	9.07 ± 0.45	2.54 ± 0.25	0.16 ± 0.02	1.58 ± 0.22	121.60 ± 14.47
Pond B	23.30±1.70	9.22 ± 0.21	1.88 ± 0.07	0.59 ± 0.10	0.94 ± 0.24	93.60 ± 6.55
Pond C	20.80±0.28	8.41 ± 0.16	1.06 ± 0.07	1.48 ± 0.38	1.79 ± 0.06	81.87 ± 21.87
Pond D	20.65±1.91	8.35 ± 0.59	0.42 ±0.04	0.34 ± 0.19	6.74 ± 1.18	19.07 ± 7.66
Pond E	22.25±1.06	9.65 ± 1.37	0.21± 0.05	0.14 ± 0.01	7.13 ± 0.35	23.20 ± 8.47
Pond F	18.80±1.41	7.77 ± 0.15	0.27 ± 0.05	0.13 ± 0.05	5.68 ± 0.13	56.27 ± 6.90
Pond G	22.25±0.35	8.42 ± 0.27	0.40 ± 0.07	0.15 ± 0.06	2.53 ± 0.35	32 ± 4.53
Pond H	22.40±0.57	9.71 ± 0.13	0.78 ± 0.06	0.80 ± 0.03	1.73 ± 0.30	127.40 ± 12.16
Pond I	19.90±1.56	7.72 ± 0.14	0.29 ± 0.03	0.24 ± 0.05	3.60 ± 0.31	55.20 ± 4.53

TABLE 6.3 Physicochemical parameters of the water bodies during monsoon – 2005

	Temp	pH	NO_3-N	PO_4	DO	COD
Pond A	25.8± 1.74	8.67 ± 0.46	1.18± 0.08	0.14 ± 0.01	1.07 ± 0.10	114.67±14.54
Pond B	26.37±1.55	8.09 ± 0.12	1.09± 0.11	0.55 ± 0.09	1.27 ± 0.17	84.80 ±10.02
Pond C	24.93±1.21	7.68 ± 0.12	1.11± 0.05	0.37 ± 0.16	1.19 ± 0.13	98.13 ± 8.40
Pond D	26.97±1.29	7.57 ± 0.32	0.22± 0.03	0.13 ± 0.04	5.09 ± 0.66	20.53 ± 6.99
Pond E	25.97±1.80	7.28 ± 0.14	0.32± 0.11	0.11 ± 0.03	4.26 ± 0.53	19.87 ± 6.74
Pond F	27.1±0.66	7.37 ± 0.37	0.30± 0.05	0.15 ± 0.06	5.09 ± 0.66	35.87 ± 6.42
Pond G	29.45±0.81	8.01 ± 0.07	0.48± 0.04	0.23 ± 0.09	1.61 ± 0.11	31.73 ± 6.42
Pond H	28.53±0.87	8.73 ± 0.56	0.76± 0.13	0.52 ± 0.04	0.62 ± 0.11	106.93 ± 6.42
Pond I	25.16±0.96	7.74 ± 0.16	0.30± 0.07	0.13 ± 0.06	4.16 ± 0.25	52.53 ± 16.63

TABLE 6.4 Heavy metal content (µg g^{-1} dry wt) in the liver of the frog, *Euphlyctis cyanophlyctis*

SITES	Ni	Cd	Cr
MSU campus (control)	2.55 ± 0.38$^{@}$	0.24 ± 0.13	0.11 ± 0.06
Waghodia	^{a}1.16 ± 0.10	4.05± 0.25**	0.24 ± 0.05
Sardar estate	^{b}73.73 ± 3.8***	4.3 ± 0.7*	0.80 ± 0.6

$^{@}$ Values are expressed as Mean ± SE, $^{***}p \leq 0.001$, $^{**} p \leq 0.05$, $^{*} p \leq 0.01$

Data shown with different letters are statistically significant at the $p \leq 0.001$

TABLE 6.5 Heavy metal content (µg g^{-1} dry wt) in the kidney of the frog, *Euphlyctis cyanophlyctis*

SITES	Ni	Cd	Cr
MSU campus (control)	4.60 ± 0.70$^{@}$	1.11 ± 0.06	0.14 ± 0.09
Waghodia	^{a}25.37 ± 3.68*	^{a}10.80± 0.88**	0.42 ± 0.10
Sardar estate	^{b}77.83 ± 5.73**	^{b}2.67 ± 0.27*	0.22± 0.05

$^{@}$ Values are expressed as Mean ± SE, $^{*}\,^{*}p \leq 0.001$, $^{*} p \leq 0.05$

Data shown with different letters are statistically significant at the $p \leq 0.001$

TABLE 6.6 Heavy metal content in the water bodies at the study sites (µg/ml)

SITES	Ni	Cd	Cr
MSU campus (control)	0.61 ± 0.16	ND	ND
Waghodia	0.60 ± 0.11	0.91 ± 0.21	ND
Sardar estate	50.05 ± 3.5	1.72 ± 0.3	0.34 ± 0.2

$^{@}$ Values are expressed as Mean ± SE; ND- Not Detected

TABLE 6.7 Correlation coefficients assessing the relationships between nickel and cadmium concentrations in the liver and kidney of *E. cyanophlyctis* at the polluted sites

Variables	Pearsons Correlation coefficient	
	Waghodia	Sardar estate
Ni_{liver} with Cd_{liver}	0.995*	-0.345
Ni_{kidney} with Cd_{kidney}	0.543^{NS}	0.817^{NS}
Ni_{liver} with Ni_{kidney}	0.267^{NS}	0.269^{NS}
Cd_{liver} with Cd_{kidney}	0.589^{NS}	-0.861^{NS}
Ni_{liver} with Ni_{water}	.656^{NS}	0.998*
Ni_{kidney} with Ni_{water}	0.902^{NS}	0.990*
Cd_{liver} with Cd_{water}	0.486^{NS}	0.988*
Cd_{kidney} with Cd_{water}	0.993*	0.929^{NS}

* correlation is significant at $p \leq 0.05$; NS- Non significant

TABLE 6.8 Relative size of stratum corneum and stratum granulosum in selected anuran species.

SPECIES	Thickness of SC	Thickness of SG
Euphylctis cyanophlyctis	5.00 ± 1.02	28.13 ± 6.25
Hoplobatrachus tigerinus	5.63 ± 0.72	20.94 ± 1.20
Bufo stomaticus	7.19 ± 1.20	30.00 ± 2.70
Duttaphrynus melanosticus	7.81 ± 0.63	25.63 ± 2.98
Fejervarya limnocharis	6.25 ± 1.02	2.37 ± 35.31
Polypaedates maculatus	4.50 ± 0.58	40.31± 2.13
Uperodon systoma	5.00 ± 1.02	24.06 ± 2.37

Values are expressed as Mean ± SD

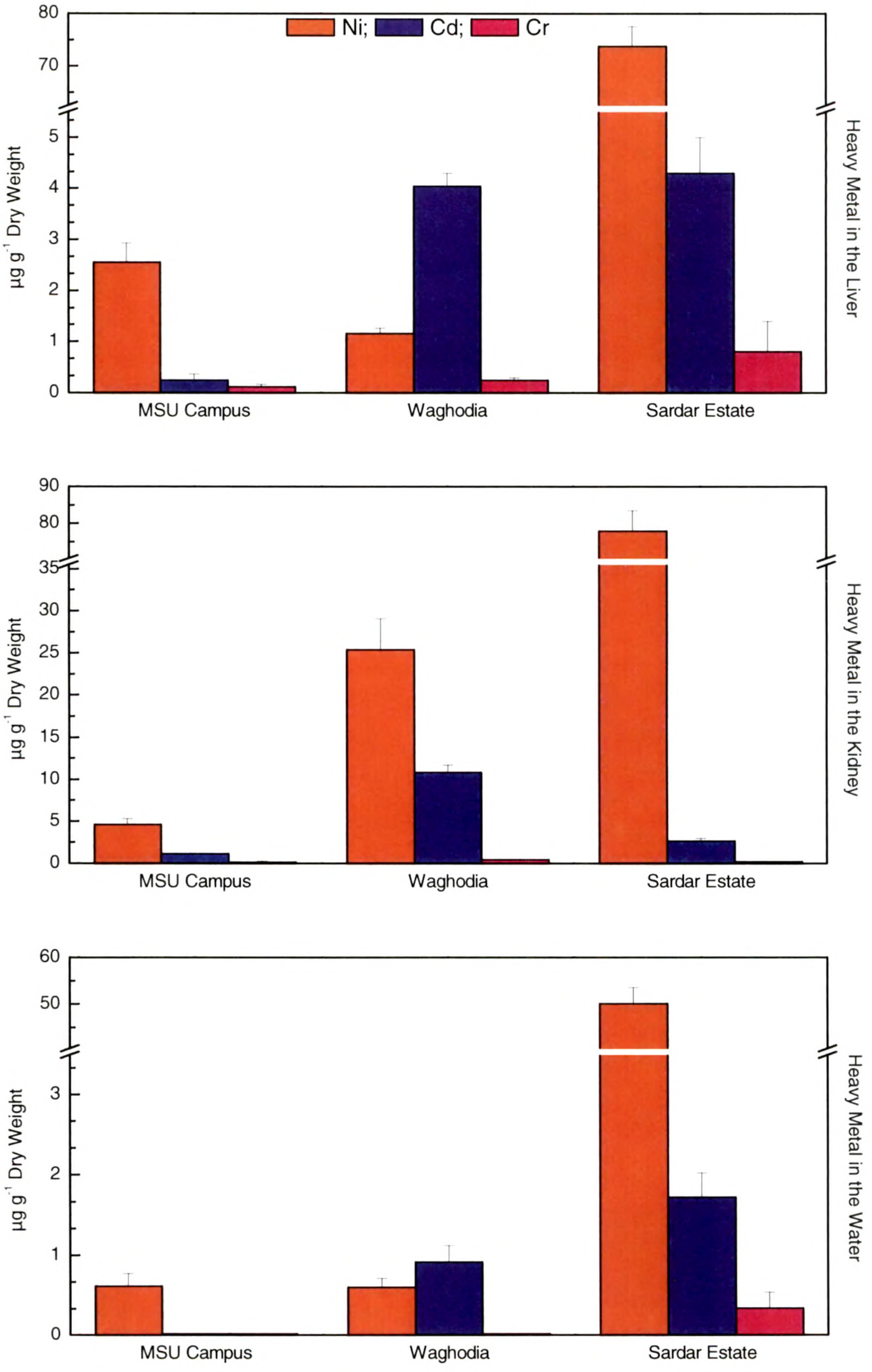

FIGURE 6.1. Heavy metal content (µg g^{-1} dry weight) in the liver and kidney of *Euphlyctis cyanophlyctis* as well as in the water bodies.

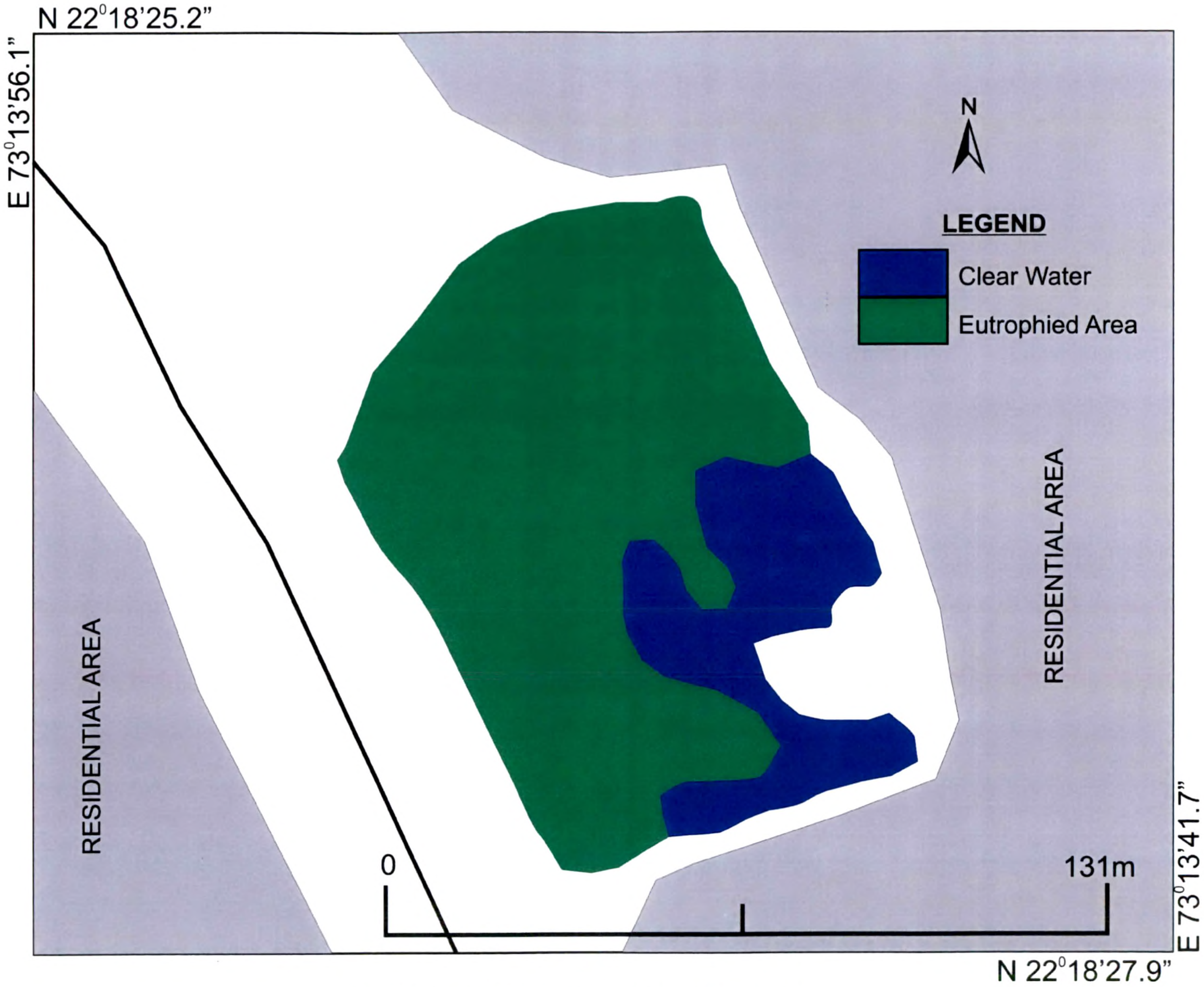

FIGURE 6.2 Eutrophication at Pond A

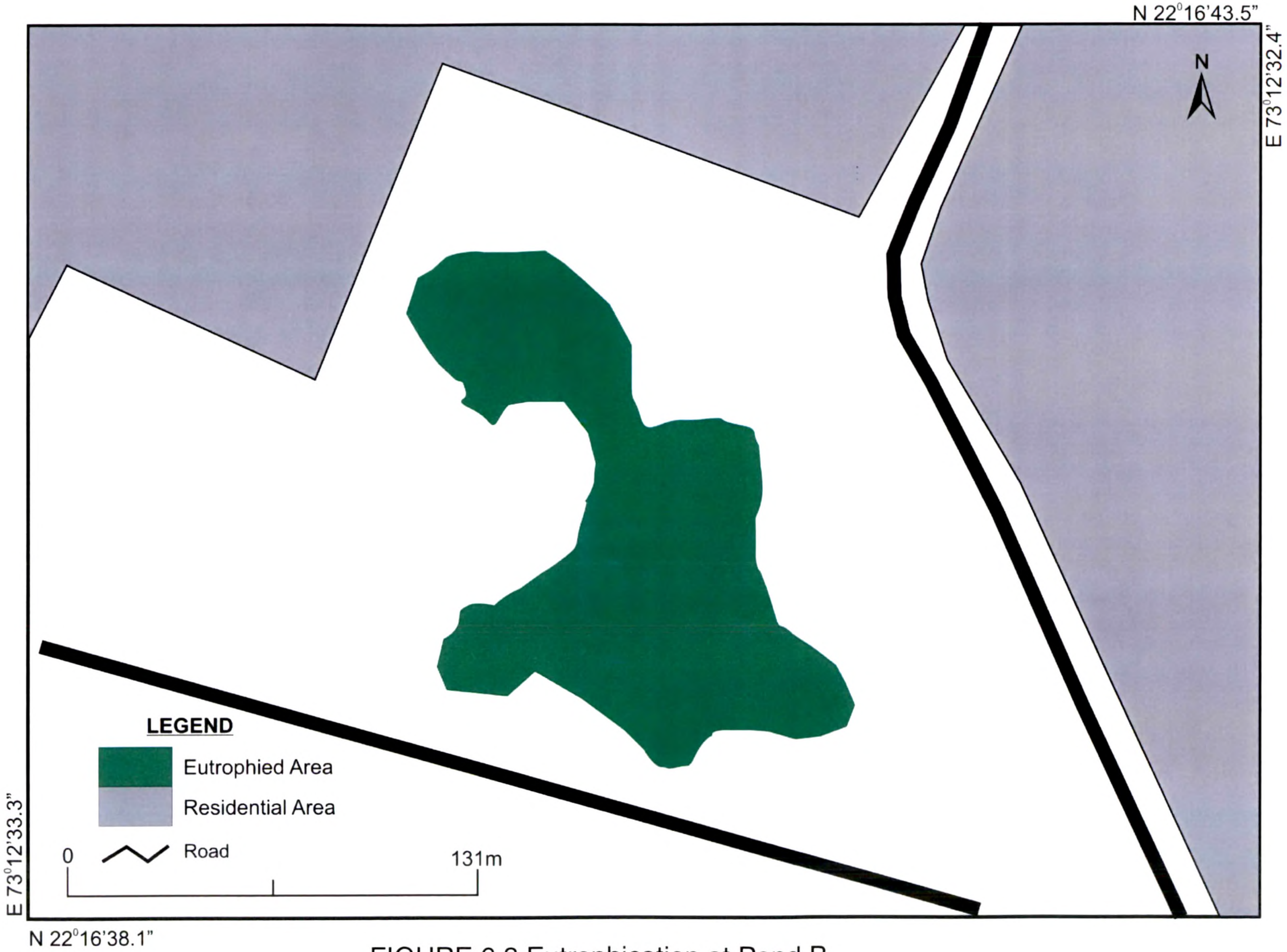

FIGURE 6.3 Eutrophication at Pond B

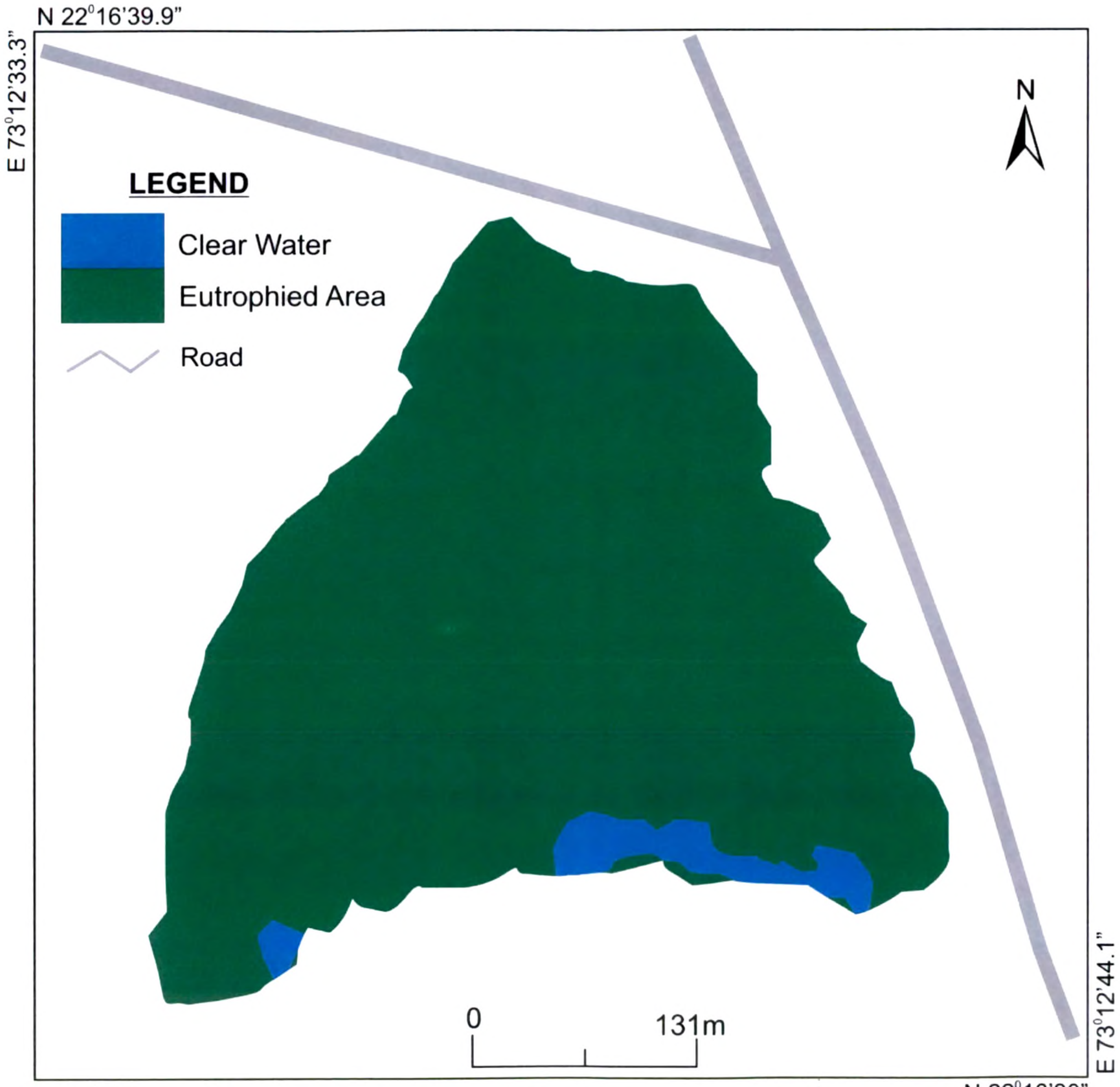

FIGURE 6.4 Extensive eutrophication at Pond C

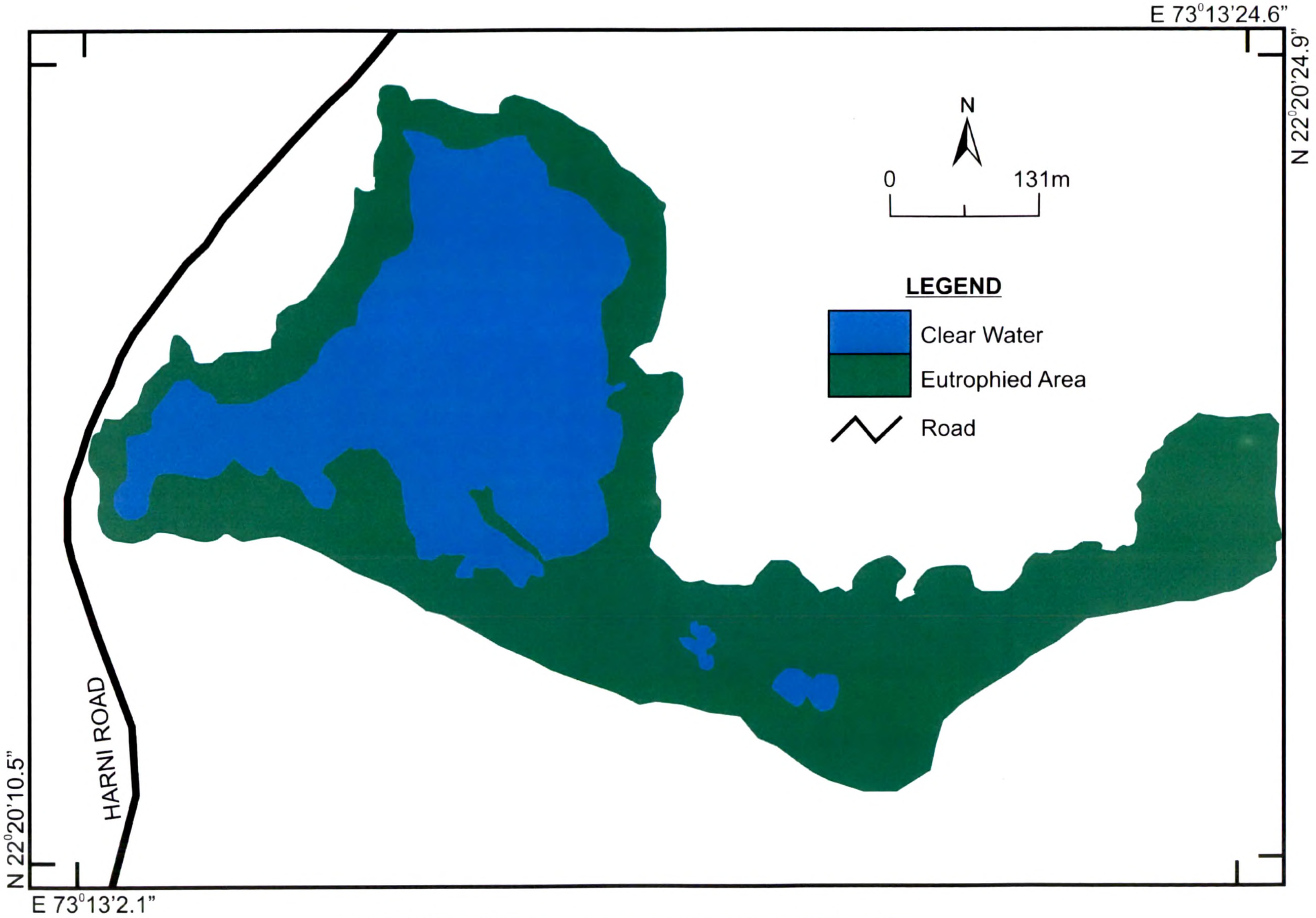

FIGURE 6.5 Extensive eutrophication at Pond D

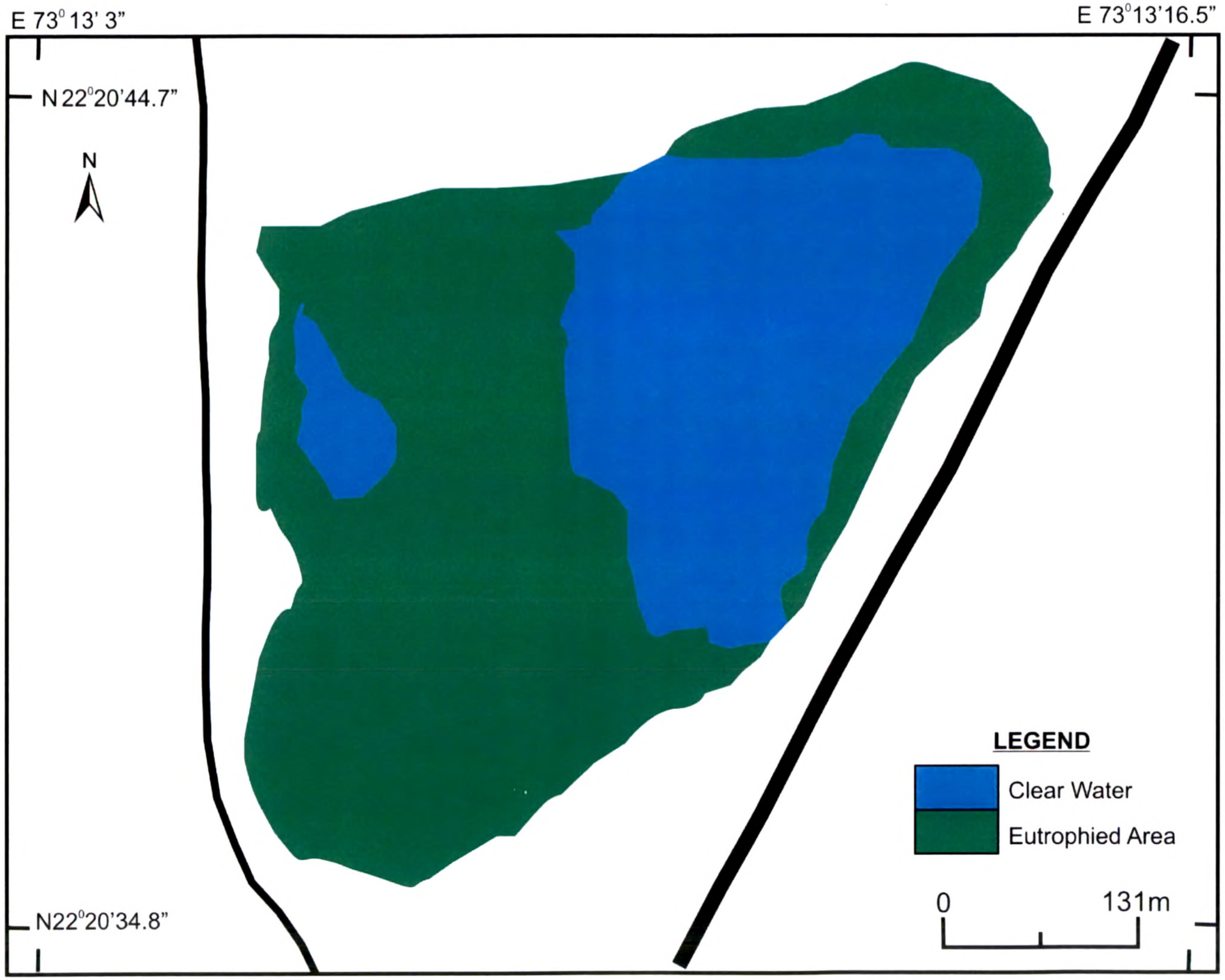

FIGURE 6.6 Eutrophication at Pond E

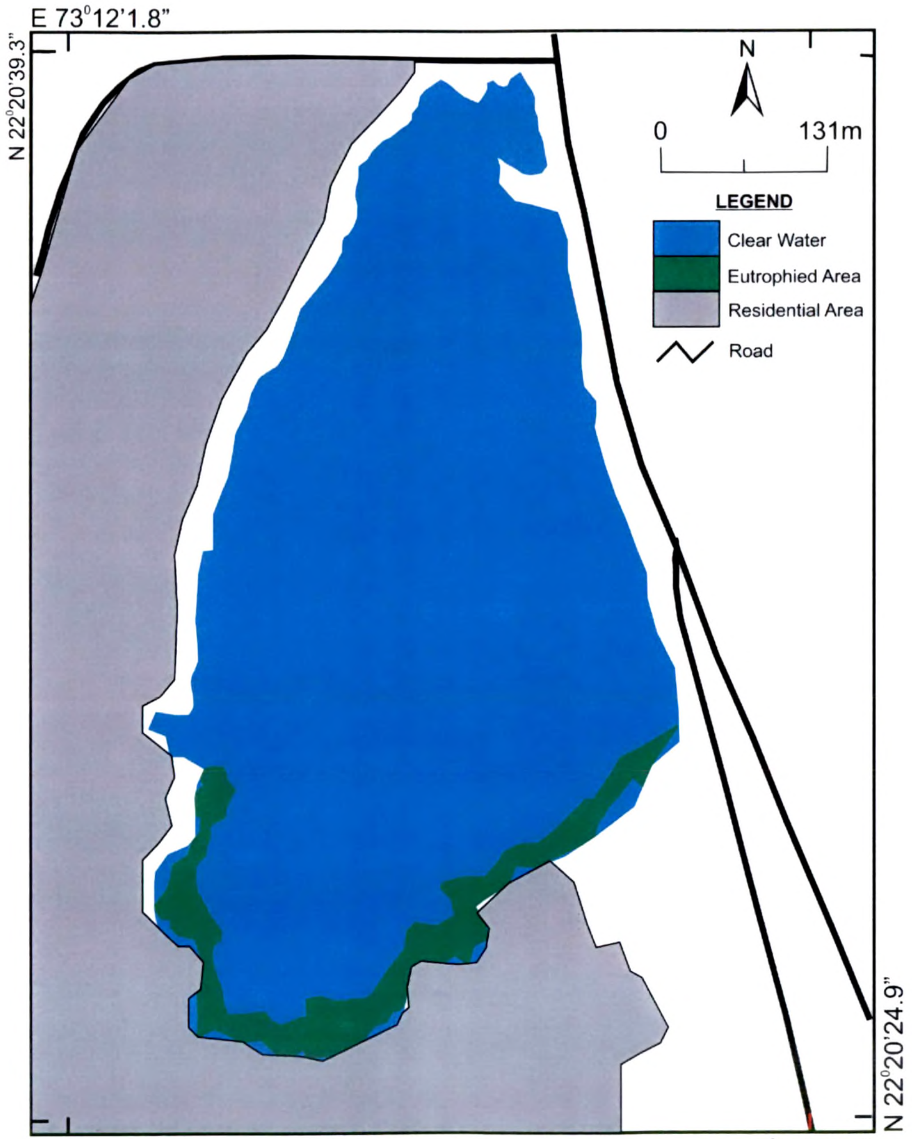

FIGURE 6.7 Less eutrophication at Pond F

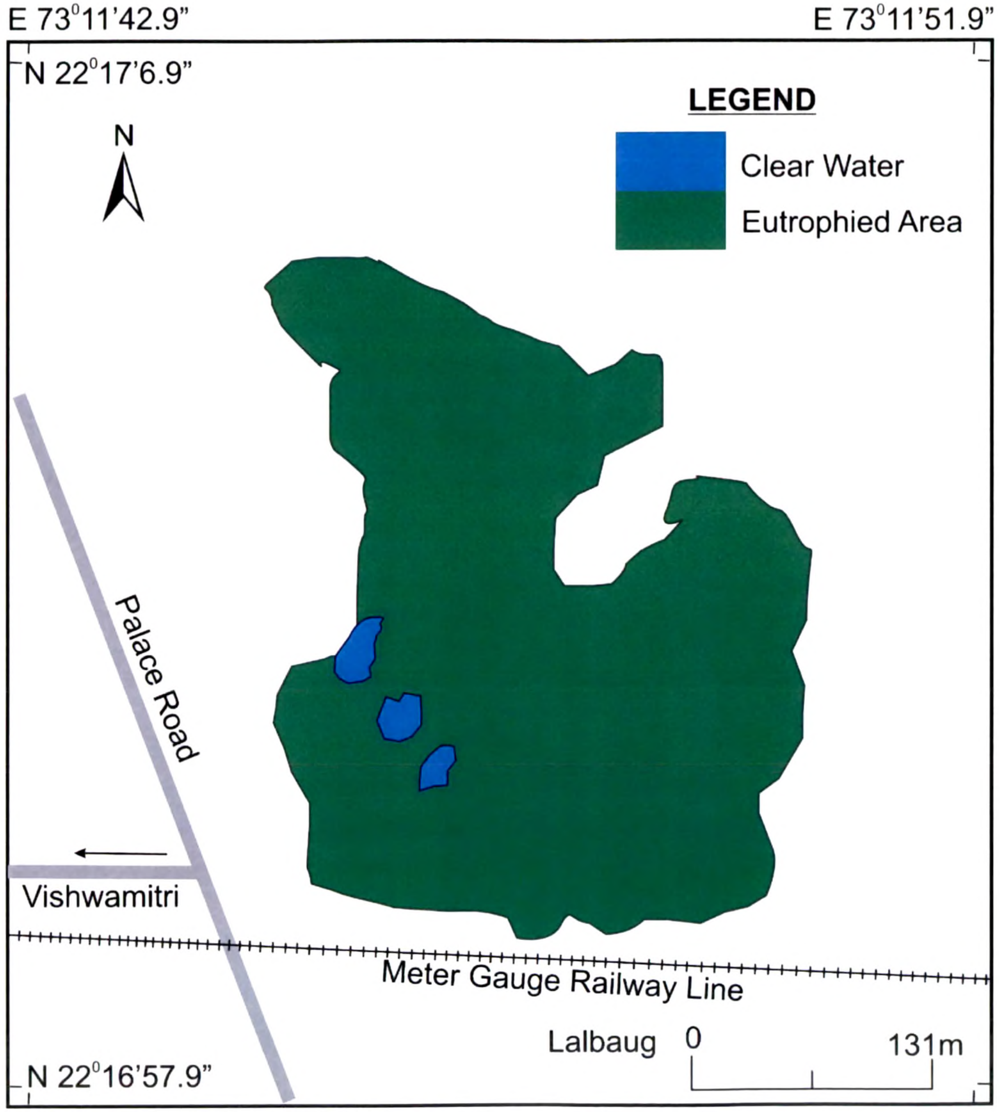

FIGURE 6.8 Extensive eutrophication at Pond G

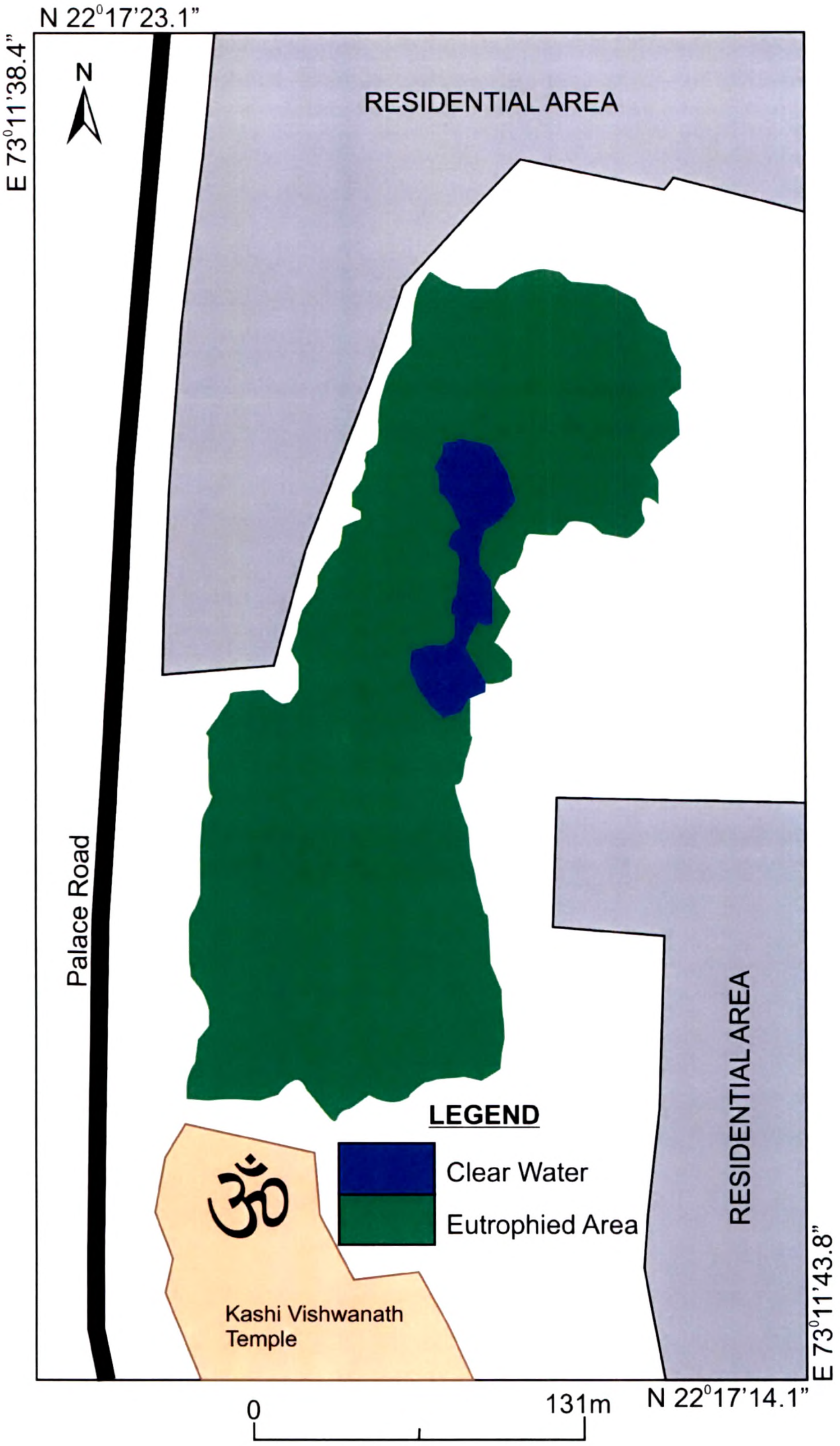

FIGURE 6.9 Eutrophication at Pond H

FIGURE 6.10 Eutrophication at Pond I

FIGURE 6.11 Heavy eutrophication at Danteshwar small pond (Pond B)

FIGURE 6.12 Heavy eutrophication at Danteshwar big pond (Pond C)

FIGURE 6.13 Eutrophication at Harni main pond (Pond D)

FIGURE 6.14 Eutrophication at Harni temple pond (Pond E)

FIGURE 6.15 Heavy eutrophication at Lalbaug pond (Pond G)

FIGURE 6.16 Heavy eutrophication at Kashivishwnath pond (Pond H)

FIGURE 6.17 Histological skin section of *Duttaphrynus melanostictus*

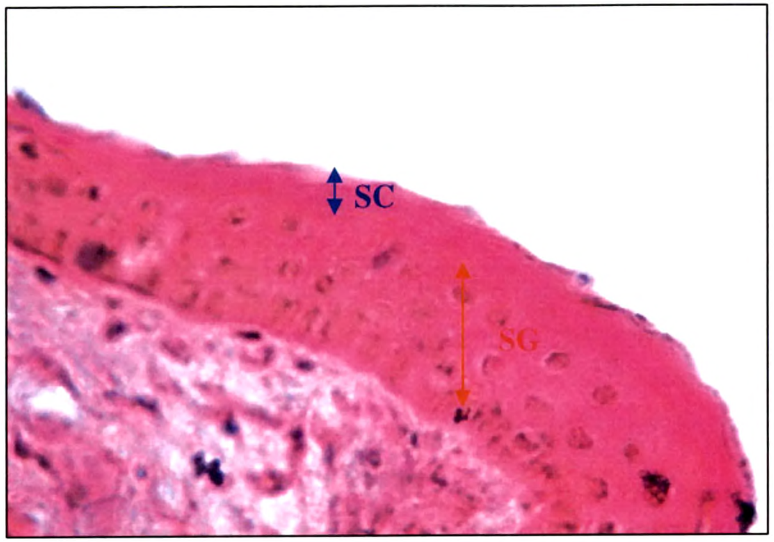

FIGURE 6.18 Histological skin section of *Bufo stomaticus*

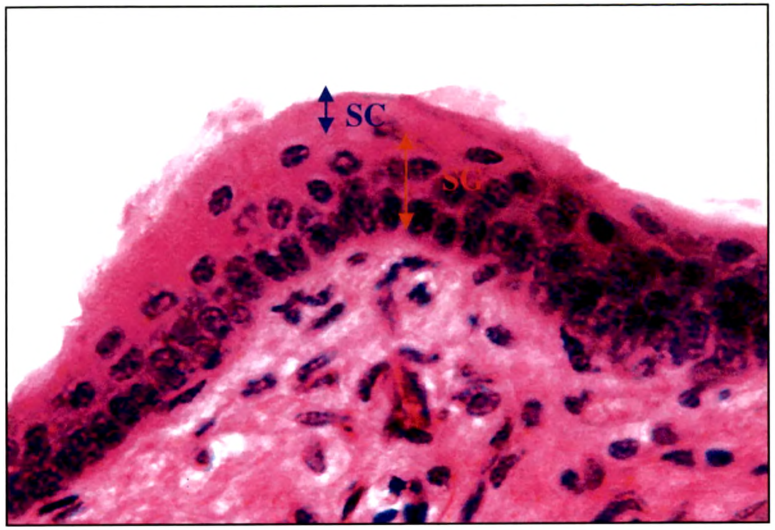

FIGURE 6.19 Histological skin section of *Hoplobatrachus tigerinus*

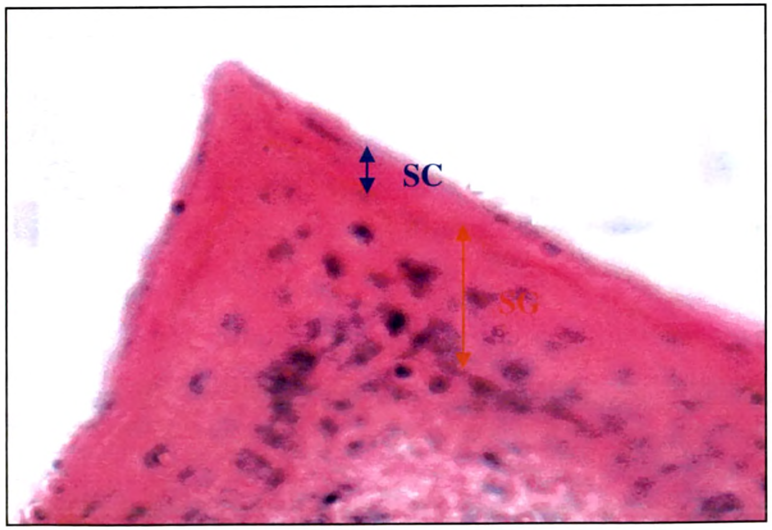

FIGURE 6.20 Histological skin section of *Euphlyctis cyanophlyctis*

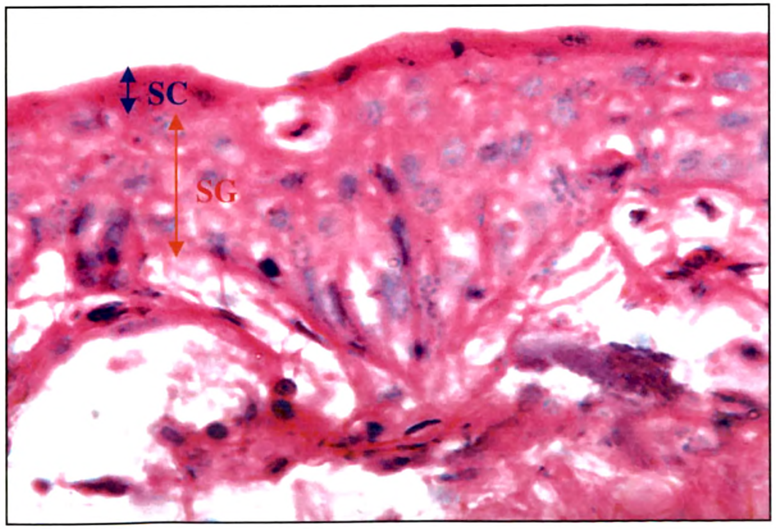

FIGURE 6.21 Histological skin section of *Fejervarya limnocharis*

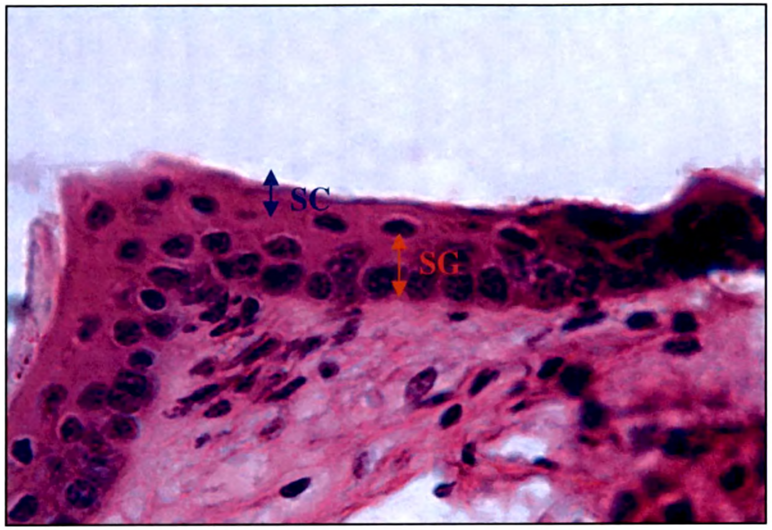

FIGURE 6.22 Histological skin section of *Polypedates maculatus*

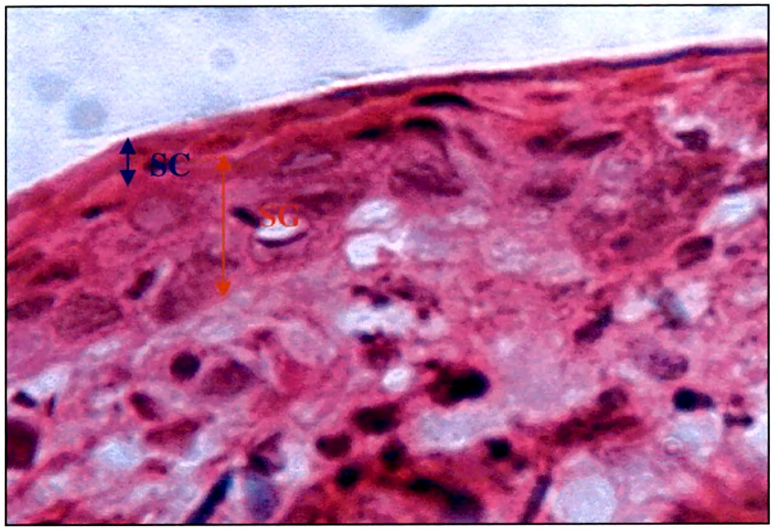

FIGURE 6.23 Histological skin section of *Uperodon systoma*

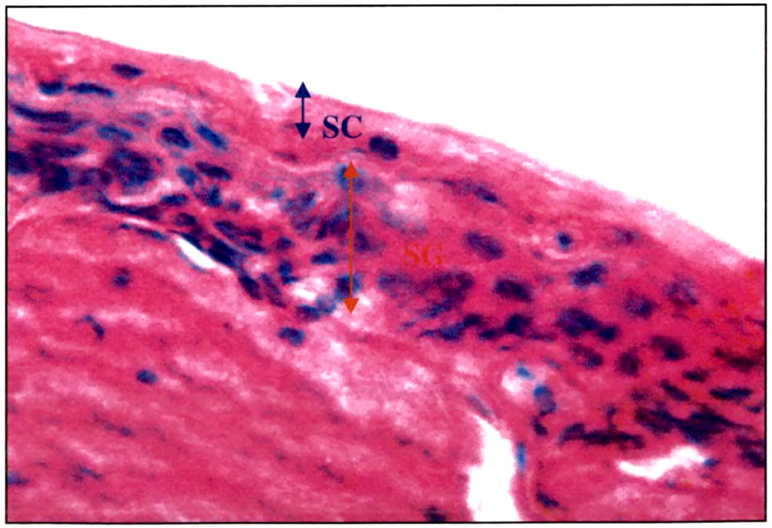

FIGURE 6.24 Section SC (↔) region of skin from a *Mixophyes fasciolatus* with mostly empty sporangia present. Note empty collapsing sporangium (arrow) and one containing bacteria (B). One sporangium is divided by an internal septum (S), (Berger *et al.*, 1999).