GENERAL CONSIDERATIONS

THEY'RE SLIPPERY, SLIMY CRITTERS. SOME SWIM, OTHERS HOP OR CRAWL. SOMETIMES YOU NEVER NOTICE THEY ARE AROUND. OTHER TIMES YOU HEAR THEM ALL OVER THE PLACE.

We're talking about frogs, toads, caecilians and salamanders. They might look different from one another, but they all share one thing in common. They're amphibians!

The word "amphibian" comes from a combination of two Greek words, "Amphi," which means "both," and "bios," which means life. It means "living in two ways" and refers to the distinct two-stage life-history pattern, characteristic of most members of this group. Most amphibian eggs hatch into free-swimming, gill-breathing larvae, these aquatic larvae undergo a radical alteration of their body structure (metamorphosis), including the development of lungs, in order to live on land, but there are many interesting exceptions to this general way of reproduction (Duellman and Trueb, 1986).

They are believed to be evolved from a common ancestry of Sarcopterygian bony fish during the Devonian period. The body structure of amphibians both past and present, shows the advances made on their fish ancestors (Feller and Hedges, 1998).

Amphibians have a naturally complex life cycle that involves both aquatic and terrestrial environments, and therefore directly exposing individuals to changes in their environment. Their skin and eggs are highly permeable, which makes them sensitive to small changes in the environment (Blaustein and Wake, 1990). Furthermore, amphibians are ectothermic, enabling differences in temperature to have direct effects on their body temperature and metabolic processes (Blaustein *et al.*, 2001). Hence they are often considered sensitive indicators of environmental conditions (Blaustein and Wake, 1990; Wake, 1991; Alford and Richards, 1999; Carey, 1999). Some scientists believe that amphibians can be viewed collectively as a kind of 'global warning system', so that their decline is a serious cause of concern for the planet. (Wake, 1991)

Carolus Linnaeus (1758) first used the term amphibians for all animals other than fish, birds and mammals. He considered both reptiles and amphibians as "foul and loathsome" animals and grouped them together. The name "AMPHIBIA" was restricted to frogs, toads, salamanders and caecilians by Latreille (1825). Since, this group of vertebrates has got adapted to many different aquatic and terrestrial habitats; it has developed a remarkable diversity in life histories. By the carboniferous period the amphibians had diversified greatly. Though, many of these forms have become extinct now. The present living species of Amphibia are but, a fraction of what existed in the past. Today, they are present on every continent except Antarctica, and can be found in almost all habitat types from dry, inhospitable deserts to lush tropical rainforests. All the living amphibians throughout the world have been grouped in 3 orders, namely Caudata (Salamanders and Newts), Gymnophiona (Caecilians) and Anura (Frogs and Toads). According to Frost, (2007) the number of amphibian species till date is 6091 throughout the world, a number that often changes to reflect new and redefined species. Out of these, there are about 556 species recognized as caudate and are grouped into 9 families, whereas the caecilians are grouped into 3 families comprising of 173 species. Forty five families are currently recognized as anurans, representing more than 5,362 species and thus making the order Anura the largest of the three.

India apparently has the most diverse fauna in the Oriental region with more than 262 species (Chanda and Ghosh, 1988; Inger and Dutta, 1996; Frost *et al.*, 2007), and more being discovered regularly (Biju and Bossuyt, 2003, 2005a, 2005b, 2005c and 2006). The earliest study of amphibians in India however, dates back to fauna volumes published by Boulenger (1890, 1920). Later, Fergusson (1904), Annandale (1907-1919), Rao (1915-1937), McCann (1932-1946) and Smith (1935 and 1943) contributed to the major works on Indian amphibians. In the later years Daniel (1953-1975) worked on taxonomy, ecology and distribution of Indian amphibians. However, discovery of quite a few frogs and caecilians (Dubois *et al.*, 2001; Gower *et al.*, 2004; Bhatta *et al.*, 2007) in the recent years exemplifies that our knowledge on the amphibian diversity of this region is still far from complete. Several studies have been carried out on amphibian communities in different ecosystems world over (Inger and Voris, 1993; Hecnar and Closkey, 1996). However, there exists only limited information on the amphibian community structure in India (Dash and Mahanta, 1993; Andrews *et al.*, 2005a, b).

Gujarat is the westernmost state of India situated between 20^{0}6' and 24^{0}42' north latitude and 68^{0}10' and 74^{0}28' east longitude. This region has various types of habitat, ranging from dry desert to moist deciduous forests and seashore along coastal islands with mangrove forests and mud flats. It is one of the most diversified states with respect to its biodiversity (State of the Environment (SoE), Gujarat 2001). However, the amphibians are one of the poorly studied groups of fauna in Gujarat. This group of fauna is neglected even in the fundamental works of Gazetteer of India. Nonetheless, first detailed and systemic study on amphibian in this region was initiated by Sarkar (1984) which reported the presence of 9 species. Later, Naik and Vinod (1992, 1993) documented the amphibians, which added the description of new species. Previous studies (Naik and Vinod, 1992, 1993, 1996; Vyas, 1996) on amphibians of Gujarat have mainly focused on systematics and preparation of checklists. However, the comprehension on amphibian communities is also important for formulating important conservation measures. Hitherto no quantitative ecological studies have been done on the amphibian community for any ecosystem within Gujarat. Thus during the present study an attempt was made to determine the species richness, abundance and community structure of amphibians through spatial and temporal variation in Vadodara, central Gujarat (Chapter 1). The study was conducted in three non-urban areas *viz.* Timbi, Sindhrot and Fofalia and one urban area *viz.* MSU campus from Vadodara district.

The amphibians found from Vadodara belonged to nine genera, four families and one order. All these species belonged to the order Anura, however no caecilians were encountered during the present study. Therefore, it can be construed that caecilians might be restricted only to south Gujarat as reported by other workers (Siliwal *et al.*, 2003, Pilo *et al.*, 2004). Family Dicroglossidae and Microhylidae had the maximum number of species accounting for 33.3% each of the entire amphibian fauna of Vadodara, while Bufonidae and Rhacophoridae showed less percentage of species. The species encountered during the present study were *Euphlyctis cyanophlyctis, Hoplobatrachus tigerinus, Fejervarya limnocharis, Bufo stomaticus, Duttaphrynus melanostictus, Kaloula pulchra, Microhyla ornata, Uperodon systoma and Polypedates maculatus.* Amongst the sites selected to study the amphibian community structure, Fofalia showed the maximum species richness and diversity, followed by Timbi and Sindhrot. However, the least diversity as well as species richness was observed in MSU campus. The high diversity at Fofalia can be attributed to its diverse vegetation and comparatively low urbanization as compared to MSU campus. Krishnamurthy (2003) while studying the amphibians at Western Ghats gave credence to the present notion of greater amphibian species abundance and diversity at non-urban sites than at urban sites. The data on population density reveals a high average density at Timbi and low density at Fofalia. On the contrary, Fofalia registered high evenness value, compared to other sites, suggesting that the species here are more evenly distributed.

Further, comparison between two communities at the study sites was made with the help of similarity coefficient and the coefficients of community. These values were high between Timbi and Sindhrot and therefore, it can be inferred that these two sites are similar in its amphibian composition. However, the low similarity coefficient value between Fofalia and MSU Campus revealed that the community structure of these two areas were less similar.

Moreover, an attempt was also made to further divide the habitats as described by Dash and Mahanta (1993) into vertical and horizontal components such as arboreal, terrestrial, terrestrial burrowing, aquatic margin and aquatic. Subsequent analysis revealed that *E. cyanophlyctis* was the only aquatic species, whereas *H. tigerinus* and *F. limnocharis* were semi aquatic frequently encountered at the margin of the water bodies. Both the Bufonids *viz. B. stomaticus* and *D. melanostictus* were terrestrial and commonly seen in open grounds, less vegetational areas and near the human habitations, whereas the microhylids like *M. ornata, K. pulchra* and *U. systoma* were categorized as terrestrial burrowing due to their burrowing habit. However, *P. maculatus* was the lone arboreal species.

It is well known that animals are not randomly distributed in space and are expected to select microhabitats to increase their survival rate and reproductive success (Orians and Wittenberger, 1991; Pulliam and Danielson, 1991). This microhabitat selection would be particularly important for animals inhabiting heterogeneous environments that are easily affected by abiotic factors (Nie *et al.*, 1999; Eterovick and Barros, 2003). In order to comprehend how the amphibians utilize their environment, niche breadth and niche overlap was studied (Chapter 1). It was measured by observing the distribution of individual organism with a set of resources (Andrews *et al.*, 2005a). Among the anuran species, *B. stomaticus*, had the highest standardized niche breadth score of 0.3391 followed by *H. tigerinus* (0.2900), thus indicating that these species utilize a broad spectrum of microhabitat. Quite the reverse, the Microhylid *viz. M. ornata, K. pulchra and U. systoma* had very low niche breath scores indicating that they use narrow range of microhabitat.

Further, microhabitat association between two species was measured with Horn's index of niche overlap. In the present study *B. stomaticus/D. melanostictus, E. cyanophylictis/F. limnocharis* and *E. cyanophylictis/H .tigerinus* showed maximum value of niche overlap at different sites, symptomatic of high association between these species. Nevertheless, the Microhylids *M. ornata/U. systoma* were also more associated in terms of the microhabitat resource utilization, while the Rhacophorid *P. maculatus* being arboreal showed 0% overlap with any other species.

Thus, the analysis of amphibian community structure of Vadodara district reveals that species diversity and species richness is more in non-urban area as compared to the urban area and therefore, shows a characteristic pattern of disturbance. The difference in the species richness and diversity between the urban and non-urban areas could be attributed to the habitat loss/modification associated with rampant urbanization. However, the data attained from this study can be used as a baseline data for comparison in the future and also to initiate similar studies elsewhere.

Further, it is well documented that amphibian assemblages in a particular area may respond to the success in their breeding. Therefore, studies on the breeding biology of amphibian species are crucial to comprehend relevant amphibian conservation particularly in the light of reports of world wide amphibian decline (Wake, 1990). Hence, the breeding biology of *B. stomaticus* and *M. ornata,* two most common species from Vadodara district were studied (Chapter 2). Pertinent biotic and abiotic factors were also determined to understand their influence on the survival of tadpoles in the natural environment.

In the present study, breeding of *B. stomaticus* and *M. ornata* was found to coincide with monsoon, as reported by other workers (Ferguson, 1904; McCann, 1932; Daniel, 1975). Rain is necessary for breeding which is supported by the fact that more clutches were collected in July and August the months of heavy rains. According to Khan and Malik (1987) *B. stomaticus* is the first amphibian to arrive at the flooded areas and quickly form very noisy choruses. The same observation was made during the current study wherein it was observed that the marbled toad, *B. stomaticus* breeds soon after the onset of the monsoon whereas unlike the toads, *M. ornata* began their breeding activity only after the area started receiving few consecutive monsoon showers. Mature anurans exhibit distinct sexual dimorphism. In both the species the breeding male size was found to be less than that of the females. This is in agreement with other reports that state that, in anurans adult females are typically larger than their male counter parts (Crump, 1974). Both the species of anuran come to the water body only during breeding period and have aquatic mode of development *i.e* from amplexus to the complete development of tadpoles, takes place in water. Amplexus in *B. stomaticus* and *M. ornata* is axillary, wherein the male clung to the female by holding it below the armpit. The clutch size in bufonid varied from 6000-8000. Eggs strings were single, pale yellowish green in colour and were found loosely wound around vegetation, broken branches and objects like broom. The egg diameter ranged from 1.45 mm to 1.6mm. Mahapatro and Dash (1990) found the clutch size in *B. stomaticus* to be 9000 to 11000. However, the clutch size in *M. ornata* ranged from 150-400 eggs and were found to be floating on the surface of the water or attached to some vegetation. Densities of Bufo tadpoles were comparatively higher than the Microhylid in all the study areas. The difference in the clutch size of these two anurans might be one of the reason for the disparity in the densities

Furthermore, spatial segregation among the tadpoles of Bufonid and Microhylids were observed during the present study. Tadpoles of *B. stomaticus* are benthic whereas *M. ornata* tadpoles are suspension feeder and were always encountered on the surface suggesting resource partitioning within water bodies. According to Altig and Johnston (1989) *B. stomaticus* larvae belong to lentic benthic habitat guild and *M. ornata* tadpoles belong to lentic suspension feeder guild. The ecological implication of spatial segregation rather than selecting different food categories are unclear, though feeding disturbance by other species may be an important factor in explaining spatial segregation of tadpole species (Richmond, 1947; Heyer,1973; Steinwascher, 1978; Odendaal *et al.*, 1984).

It was also observed that *M. ornata* tadpoles form conspicuous aggregation in the water column. Several hypothesis have been proposed to explain aggregation in tadpoles (Brattstrom and Waren, 1955; Duellman and Lescura, 1973; Beiswenger, 1975; Katz *et al.*, 1981), however the reason for formation of aggregation in Microhylid tadpoles, in the present study, is unclear.

Additionally, when the morphology and morphometry of the tadpoles were studied (Chapter 2) it was observed that *B. stomaticus* tadpole is mostly globular to ovoid, nonetheless it is wider than high. The external nares are prominent and closer to the eyes than to the snout. Narial opening is very prominent and is oval in shape with a well defined rim. The eyes are positioned dorsally and are slightly protruding. The interorbital space is almost the double of the internarial distance. Spiracle is sinistral, conical, very short and attached to the body wall except for its tip which is free. Vent tube is of moderate size, opening medial, tubular, directed posteriorly and not linked to the ventral fin. Tail length is almost 58-60% of the TBL. The tail musculature is moderately developed and it does not touch the fin at the posterior end. Height of the dorsal fin is greater than that of the ventral fin. Live tadpoles are usually black in colour.

It is also known that oral disc are widely modified according to the ecological and dietary specialization of the tadpoles (Altig and Johnston, 1966), reflecting wide arrays of food preference and different methodologies employed in feeding. The oral disc of the *B. stomaticus* tadpoles is positioned anteroventral and is emarginated laterally. It has a single row of rounded, delicate membranous marginal papillae, which are broadly interrupted anteriorly and posteriorly. Although, the sub marginal papillae are absent. The major part of the oral disc is the anterior and the posterior labia on which rows of keratinized, spiny teeth are arranged. The beaks are edged with sharp, pigmented and keratinized serrations which are not uniform. The serrated, keratinized jaw sheaths and labial teeth, it is reported, allow the tadpoles to graze periphyton effectively (Kupferberg *et al.*, 1994; Kupferberg, 1997a; Altig and McDiarmid, 1999). The lower beak is 'V'shaped however, its inner corners are overlapped by upper beak. The labial tooth row formula of *B. stomaticus* is 2(2)/3. The first anterior tooth row (A-1) is complete while the second tooth row (A-2) in the anterior labium is incomplete and is interrupted with a large median gap. All the posterior tooth rows are complete. In the posterior labium the relative length of the teeth rows are P-1>P-2>P-3; P-3 being the shortest. These teeth are laterally cuspidate with 11-12 cusps. The jaw sheaths are also used in chopping larger pieces of material into sizes that fit into the mouth (Altig and Mcdiarmid, 1999). However, oral disc attains its maximum size (45% of the Body Width) in the tadpoles of stage 36-41. By the end of stage 42 the beak and the teeth rows slowly starts disappearing.

Further, the tadpoles of *B. stomaticus* analyzed in present study fall in Orton's type-IV category (Orton, 1953). The tadpoles in the present study took 20-25 days to complete metamorphosis in the natural environment. Upon metamorphosis these froglets developed red spots on the dorsal side, which disappeared once they were adult. The growth curves in these tadpoles (Figure 5.2) were found to be similar to that of other anurans (Mohanty-Hejmadi and Dutta, 1986). Strauss and Altig (1992) stated that most of the growth of tadpole follows the exponential phase of sigmoid curve.

In dorsal view the tadpoles of *M. oranta* is mostly ovoid. The external nares are very small and the narial opening is not visible clearly. The nares are closer to the eyes than to the snout. The eyes are positioned laterally and are protruding with a large interorbital space. Interorbital distance is more than 80% of body width. These tadpoles have a single median spiracle. Though present, it is not clearly visible in the tadpoles of *B. stomaticus.* Snout spiracular distance is 62% of snout vent length and 23% of total body length. Vent tube is of moderate size, tubular and has a median opening. Tail is long and very fragile with a subacuminate tip. Tail length is almost 65% of the body length. The tail musculature is poorly developed and it touches the fin at the posterior end. The ventral fin is larger in size compared to the dorsal fin. Tadpoles of this species belong to the Type-II of Orton's category (Orton, 1953). Living tadpoles are usually transparent. The mouth of the tadpoles is positioned on the dorsal surface of the body. Oral disc is without any teeth or beak. Absence of typical oral disc and its anterodorsal mouth reflects its filter feeding microphagus habit. The tadpoles took 35-40 days to complete metamorphosis in the natural environment. The froglet show the characteristic pattern on the back as seen in the adult.

The pattern of the growth curve in *M. ornata* is similar to that seen in the tadpoles of *B. stomaticus.*

In the current study the density of microhylid tadpoles were not significantly correlated with the temperature, but the density of the tadpoles of bufonids significantly correlated with temperature. *Bufo* tadpoles are known to select higher temperatures in the field (Noland and Ultsch, 1981) and according to Putnam and Bennett (1981) increase in body temperature results in increase in performance capacity. However, from the present study also it can be deduced that the selection of warmer water bodies by the Bufonids might favor a higher metabolic rate and faster development.

Another abiotic factor considered to be the most important habitat characteristics for the inhabiting amphibians is pH (Strijbosch 1979). Inhibition of growth at low pH has been observed in several different species of tadpoles (Freda and Dunson, 1985, 1986; Cummins, 1986) however, growth rate of *Pseudacris triseriata* tadpoles were not significantly affected by high pH as reported by Kaufman and Gerlanc (2005). In the present study it was seen that the tadpoles in the natural habitat survived successfully at a pH ranging between 7.0 and 7.8. *M. ornata* tadpoles were found to be more sensitive to acidic pH, as more than 50% mortality was observed below pH 6.5, whereas the Bufonid tadpoles are tolerant to this pH. However, in natural environment, no correlation could be established between the density of the tadpoles and the pH of the water bodies.

Ponds are subject to large DO fluctuations on a daily and seasonal basis (Noland and Ultsch 1981; Nie *et al.*, 1999) Tadpoles of *M. ornata* were found in water bodies with high DO concentration as compared to the water bodies having tadpoles of *B. stomaticus.* Dissolved oxygen was significantly correlated with the density of *M. ornata* tadpoles, however no such correlation was apparent with the density of *B. stomaticus* tadpoles. Bufonids tadpoles were found in the water bodies with DO values less than 0.5 mg/L. Under low oxygen conditions tadpoles are able to meet their respiratory requirements through the process of bobbing (swimming to the surface for air) and to breathe air in; tadpoles fill their buccal cavity with air at the water surfaces (Mcdiarmid and Altig, 1999). Bobbing was noted in *B. stomaticus* tadpoles towards the end of metamorphosis suggesting that under low oxygen condition they meet their requirement through this phenomenon.

In the present study the total oxidized nitrogen in the water bodies where both the species of tadpoles were found, averaged from 0.19 mg/L to 0.48 mg/L. There is a great deal of variation in the nitrate tolerance in different species of tadpoles (Smith *et al.*, 2005).

However, in the present study no statistically significant correlation was observed between the total oxidized nitrogen and density of tadpoles.

Water bodies with tadpoles of *B. stomaticus* had more vegetation compared to that seen near the water bodies of *M. ornata*. Ephemeral ponds with tadpoles of *M. ornata* were more open with only few species of plants around it. Plant cover may play a role in the survival of these species. Babbitt and Jordan (1996) found that increased plant density resulted in decreased predation by aquatic insects on southern toads (*Bufo terrestris*).

Tadpoles of both the species were largely herbivorous having major food items in the form of a variety of algal components. Fourteen species of phytoplankton and eight species of zooplankton, while seven species of phytoplankton and zooplankton each were recorded from the stomach content of *B. stomaticus* and *M. ornata* tadpoles respectively. Majority of the tadpoles belonging to different taxa are largely herbivores, though their diets may vary widely across the environments (Savage, 1952). Percentage of food items in the gut of the tadpoles differed from both the sites. In the current study, tadpoles of both the species seemed to feed randomly without any discrimination, on whatever was available in the particular water body where they grew up. Tadpoles are relatively indiscriminate feeders (Farlowe, 1928; Jenssen, 1967; Dickman, 1968; Wassersug, 1972; Seale and Beckvar, 1980) though they may forage selectively under certain circumstances (Kamat, 1962; Kupferberg, 1997a). There was notably no difference in terms of food composition for both the species of tadpoles, between the prehindlimb and hindlimb stages having almost similar food items in both the stages. Tadpoles of *B. stomaticus* were seen scavenging on dead bodies of drowned animals (Figure 5.31). In the absence of food resources, they also showed cannibalism by devouring their weaker siblings. According to some reports, many tadpoles may supplement their diets with animal protein through predation or scavenging on conspecific and heterospecific eggs and tadpoles (Crump, 1983, 1986, 1990; Tejedo, 1991).

Some of the potential tadpole predators in the present study were insects like beetles, water bugs, and vertebrate predators like fishes and birds. Eggs of the amphibians were more vulnerable to predators than tadpoles due to mobility factor. Some tadpoles of the family Bufonidae are known to be toxic to their predators (Licht, 1968; Brodie *et al.*, 1978). Rao (1917) reported that *Microhyla* tadpoles escape predation due to offensive, acidic secretions of the cephalic gland. Rao (1917) further mentioned that fishes reject these tadpoles even if forced. Nevertheless, in the present study such secretions, if present, do not seem to repel fishes like *Poecilia sp.* and *Gambusia sp.*

In the present study *B. stomaticus* and *M. ornata* were found selecting temporary water bodies to deposit their eggs. It could be hypothesized that presence of predators like fish in the permanent water bodies might have forced the animal to select a place like seasonal water bodies which can form a safe abode for the developing young ones.

In recent years, conservationists have become concerned about the decline in amphibian population. These results will help direct conservation efforts by identifying characteristics of water bodies that indicate a high potential for providing suitable breeding habitats for each of these species.

The current wave of interest in amphibian population biology as well as in the possibility that there is a global pattern of decline and loss was initiated in 1989 at the First World Congress of Herpetology (Barinaga, 1990). By 1993 more than 500 populations of frogs and salamanders on five continents were listed as declining or of conservation concern. At least now there is a consensus that alarming declines of amphibians have occurred (Kuzmin, 1994; Blaustein *et al.*, 1994). As most amphibians are exposed to terrestrial and aquatic habitats at different stages of their lifecycles, and because they have highly permeable skins, they may be more sensitive to environmental toxins or to changes in patterns of temperature or rainfall than any other terrestrial vertebrate. 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 Introduced Species (Bradford, 1989; Bradford *et al.*, 1994; Bronmark and Edenhamn, 1994; Knapp 1996; Kats and Ferrer, 2003); Climate Change (Pounds *et al.*, 1999; Kiesecker *et al.*, 2001 and Carey and Alexander 2003); UV-B Radiation (Kerr and McElroy, 1993; Cummins, 2002; Kats *et al.*, 2002; Blaustein *et al.*, 2003; Heyer *et al.*, 2003); Habitat Modification and Pollution (Johnson, 1992; Elmberg, 1993; Carey and Bryant, 1995; Hecnar and M'Closkey, 1996; Hecnar, 1997; Semlitsch and Bodie, 1998)and lastly but not the least Diseases (Berger *et al.*, 1998; Lips *et al.*, 1999; Daszak *et al.*, 2003).

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). In many ways, it negatively affects natural ecosystems and aquatic systems in particular. Urbanization had significantly altered stream habitat in southern California leading to negative effects on the diversity and abundance of native amphibians (Riley *et al.*, 2005). Therefore, in order to know whether urbanization has any major impact on the amphibians, water bodies within the Vadodara city were evaluated for their physicochemical characteristics and these variables were correlated with species abundance of the inhabitant amphibians (Chapter 3).

The investigated water bodies are permanent and though they were filled with water in all the seasons, periodical fluctuation in the water levels throughout the study period was observed. Minimum temperature recorded was 18°C during winters whereas maximum temperature went up to 32.4°C during the summers. However, the temperature during the monsoon was moderate and ranged between the summer and the winter values. There was no significant correlation observed between the species abundance and water temperature.

The mean pH value in all the studied water bodies ranged from 7.43 to10.29 during the study period. Majority of the water bodies were alkaline with higher pH value and more than 75% of the water bodies were having pH value higher 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 whereas the monsoon values were low.

The chemical oxygen demand values (COD) ranged from 12.8 to 135.2 mg/L, while the dissolved oxygen (DO) ranged from 0.43 to 7.5 mg/L. Low values for COD was documented during the monsoon, whereas the same were high during the summers. COD values higher than 120 mg/L was recorded from Pond A, C and Pond H. 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. The ponds with high COD showed total absence of any amphibian and thus may be a cause of concern. However, no significant statistical correlation was observed between COD values and the species abundance

The present study reveals phosphate-phosphorous($PO_4^{3-}-P$) level between 0.1 and 1.8 mg/L. Higher $PO_4^{3-}-P$ value was recorded during the summer and during this period, out of nine water bodies, eight showed the $PO_4^{3-}-P$ concentration more than 0.2mg/L. Mean values of nitrate-nitrogen ($NO_3^{-}-N$) at most sampling sites were higher than 0.1 mg/L due to eutrophication. Species abundance showed statistically significant bivariate negative correlation with $PO_4^{3-}-P$ in one of the pond, the correlation between amphibian abundance and $NO_3^{-}-N$ was weak and statistically non significant. Johnson and Chase

(2004) have observed high incidences of parasite infestation in the frogs that reside in water having elevated levels of phosphorous.

Breeding of Amphibians *viz. E. cyanophlyctis* and *H. tigerinus* was observed only in 33% (Pond D, F and I) of the water bodies, which had comparatively lower concentration of phosphate-phosphorous, nitrate-nitrogen and low level of COD. Moreover, they were relatively less eutrophicated than other water bodies. 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). All the studied water bodies in Vadodara were found eutrophicated with varying degree of eutrophication. The high nutrient values possibly contribute towards eutrophication. Results of other studies also support the notion of eutrophic condition induced frog embryo mortality and malformations (Boyer and Grue, 1995).

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 get adapted taking advantage of man- made or degraded sites that are unsuitable for many other frog species. Thus, they are versatile and adaptable animals, able to inhabit small and degraded sites. Species such as *Limnodynastes peronii* and *Litoria aurea* have also got adapted to such changes in their environment (Hengl and Burgin, 2002; Pyke, 1999). Additionally, Rubbo and Kiesecker (2005) have hypothesized that certain species of amphibians are more sensitive to the adverse effects of urban development. Thus it can be said that urbanisation resulting into eutrophicated water bodies with higher level of organic, inorganic pollutants and higher levels of nutrients, together might be a major contributor towards disappearance and low abundance of amphibians.

Concordant with habitat destruction, environmental contamination due to organic and inorganic compounds derived from agricultural discharge and the effluents containing high levels of heavy metals from industries also have deleterious consequences on the amphibian population (Lee and Stuebing, 1990; Hsu *et al.*, 2006; Loumbourdis *et al.*, 2007). Studies by earlier workers have shown that among the aquatic organisms, amphibians are the most sensitive to aquatic contaminants (Nebeker *et al.*, 1994; Loumbourdis and Wary, 1998). *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 (personal observation). They were commonly encountered in the sites which were polluted by the effluent discharge from the industries like electroplating unit, plastic industries, chemical producing factories, automobile manufacturing industries and metal fabricating unit as well as tyre manufacturing companies. Therefore, in the present study the concentrations of heavy metals in tissues of *E. cyanophlyctis* were analyzed (Chapter-3). Thus, accumulation study on the heavy metal content of these animals will thus enable a more extensive evaluation of the degree of contamination by heavy metals.

The result demonstrated a high nickel (Ni) concentration in both the tissues of frogs collected from polluted sites when compared to the non-polluted area. The elevated level of Ni in the tissue indicates heavy metal pollution in these areas. The relatively higher level of Ni in the liver and kidney of *Euphlyctis cyanophlyctis* 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 has indicated unusually high amount of Ni. The World Health Organization (WHO) classifies nickel compounds as carcinogen. 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 (Cd) in the kidney and liver of frogs from the polluted sites as compared to the non-polluted sites. This may adversely affect the amphibians in the long run due to the extremely long biological half-life (about 30 years) of cadmium in both human and animal bodies and its ability to accumulate in tissues, and in particular in the kidneys. *Rana ridibunda*, when exposed to 200 ppm aqueous solutions of cadmium showed major histological and histochemical alterations in the liver and kidney (Loumbourdis, 2005). However, no significant difference was observed between the contents of chromium in the liver and kidney of *E. cyanophlyctis* 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.

A significant bioaccumulation of heavy metal on the frog population studied, suggest that they can be used as bioindicators of pollution. Further it is also suggested that precaution is needed to be taken in order to prevent further heavy metal pollution, which can otherwise be dangerous to the aquatic organisms as well as to the humans.

Apart from habitat destruction and pollution, another major cause for the decline of amphibian population worldwide is the disease "Chytridiomycosis". The effects of chytridiomycosis on amphibian populations, in America, Europe, Australia and New

Zealand, have been devastating with at least one population driven to extinction, and worsened status for other threatened species (Berger *et al.*, 1998; Daszak *et al.*, 1999; Weldon *et al.*, 2004). Surveying the extant and archived specimens, *Batrachochytrium* has been found in every continent that has amphibians, except Asia (Bosh *et al.*, 2000; Speare and Berger, 2000; Weldon, 2002). 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. Due to lack of any such studies in this region the present study also focused 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 (Chapter 3).

The 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). The normal thickness of stratum corneum(SC) as observed in the present study is 2-5µm, while the infected SC measures about 60µm (Berger *et al.,* 1998). 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. Therefore, it can be inferred that the much hyped chytridiomycosis is not contributing to the decline of amphibian population if any, in Gujarat.

It can be therefore, summarised from the present study that the amphibian fauna of Vadodara district is scanty, accounting for only 3% of the total Indian amphibians. However, not surprisingly, the sites away from the city harbour greater species richness. Subsequent analysis of habitat utilization and niche preference revealed the existence of definite resource partitioning for greater coexistence. Moreover, from the study it is apparent that the change in land use pattern together with domestic and industrial discharges act as the major contributors towards low species abundance in this region as against the popular belief of Chytridiomycosis induced population loss reported from many parts of the world. Nevertheless, comprehensive screenings extending several parts of the region need to be conducted before one conclusively pronounce the area free from the threats of dreaded Chytridiomycosis.

Lastly, after analyzing the various factors that would possibly contribute to amphibian decline one can't help but agree with **Robert Fisher,** a research biologist with the US

Geological Survey (USGS), who remarked "We continue to discover that these declines are not driven by single 'smoking gun' causes but instead are the result of synergistic effects of multiple stressors in the environments inhabited by amphibians".