CHAPTER 5

BIOLOGY OF *BUFO STOMATICUS* AND *MICROHYLA ORNATA* IN ITS NATURAL HABITAT IN VADODARA DISTRICT

5.1. INTRODUCTION

Amphibians with a few exceptions have a typical biphasic life history pattern. Adults migrate to breeding sites, deposit eggs and return to terrestrial habitats (Duellman and Trueb, 1986). They fall into three categories with respect to their breeding habitats. The first category is composed of those that breed in permanent water, the second breed in temporary and seasonal pools and the third groups breed out of water. The latter group still requires a moist location to deposit its eggs (Porter, 1972). Thus, all the amphibians rely upon seasonal and geographical patterns of precipitation which govern their distributions (Porter, 1972; Duellman and Trueb, 1986).

Anurans prefer the safest habitat for reproduction wherein the survival of the tadpoles is maximum. For this reason, they breed in diverse water bodies ranging from highly ephemeral to permanent ponds (Wellborn *et al.*, 1996). Different permanent habitats like streams, stream side ponds, lakes and wetlands are used by the anurans for reproduction. Whereas, temporary water bodies like small phytotelmata, pools of water formed in various plant structures such as bromeliad tanks, modified leaves, open fruits, nut capsules and small puddles are frequently used for egg and tadpole deposition by tropical frogs (Caldwell and de Araujo, 1998). Those species breeding in temporary water bodies are explosive breeders, compared to the amphibians which breed in permanent water bodies, as they have to complete their development and metamorphosis within short hydro period (Richter and Seigel, 2002).

Amphibian tadpoles have to depend on many factors which bring changes in their aquatic environment. Interaction among the biotic and the abiotic characteristics of the water body influence the tadpole ecology (Morin, 1983; Smith, 1983; Wilbur, 1987; Dunson and Travis, 1991). Study of impact of biotic and abiotic factors on the growth and survival of the tadpole is ecologically relevant, because larval growth rates are important in determining individual fitness and in shaping amphibian communities. Biotic factors that influence amphibian development include, but are not limited to, food availability (Kamat, 1962; Travis 1984; Alford and Harris, 1988; Berven and Chadra, 1988; Crump, 1989), predation (Skelly and

Werner, 1990; Wilbur and Fauth, 1990; McCollum and VanBuskirk, 1996), population density (Richards, 1958; Licht, 1967; Brockelman, 1969; Wilbur, 1972, 1976, 1977*a*, *b*; Gromko *et al.*, 1973; Wilbur and Collins, 1973; John and Fenster, 1975; Smith- Gill and Gill, 1978; Smith-Gill and Berven, 1979; Semlitsch and Caldwell, 1982; Berven and Chadra, 1988; Newman, 1989; Scott, 1990), and inter- and intraspecific chemical signaling (Smith-Gill and Berven, 1979; Werner, 1986). Abiotic factors can directly affect the efficiency of variety of organismal functions and are capable of determining how successful a population can be in their environment (Dunham *et al.*, 1989). The local distribution of the tadpole species may also be affected by the abiotic characteristics of the water bodies. Some abiotic factors that influence metamorphosis are water temperature (Smith-Gill and Berven, 1979; Newman, 1989; Hayes *et al.*, 1993), dissolved oxygen (Crowder *et al.*, 1998), water, pH (Clark and Hall, 1985; Freda and Dunson, 1986), photoperiod (Wright *et al.*, 1990), pond drying (Wilbur, 1987; Petranka and Sih, 1986), and pond size (Heyer *et al.*, 1975; Pearman, 1995; Skelly, 1996). Though all these factors makes the survival of the tadpole difficult still they are able to cope up as they are highly plastic in their adaptations and the ability to camouflage against the enemies makes their survival possible to some extent (Wassersug, 1975; Heyer *et al.*, 1975).

Tadpoles are transient consumers often reaching high population densities in periodic pond ecosystems without fish (Flecker *et al.*, 1999) where they can influence the primary production, nutrient flux and competitive interactions (Seale, 1980; Bronmark *et al.*, 1991; Kupferberg, 1997a). Gut analyses of tadpoles from natural pond ecosystems carried out by various workers demonstrates a broad spectrum of food including various species of algae, protozoa, macrophytes, rotifers and crustaceans, pollen, detritus and live or dead animals including conspecific or heterospecific tadpoles (Li and Lin, 1935; Savage, 1952; Gosner, 1959; Kamat, 1962; Heyer, 1973; Sabnis and Kolhatkar, 1977; Sabnis and Kuthe, 1980; Sekar, 1990 a,b; 1992a, b; Kupferberg, 1997b). Moreover, because of their complex life cycles, amphibians are important links between freshwater and their terrestrial surroundings. When metamorphosed they leave the water habitat and the assimilated nutrients are transferred to land ecosystem (Seale, 1980). Data on tadpole nourishment are therefore essential for a better understanding of aquatic-terrestrial nutrient balances.

Tadpoles exhibit structural diversities that are associated with their habitat, foraging behaviour and predator avoidance (Duellman and Trueb, 1986). The Morphological differences in body shape, tail and oral disc have extensively been used by morphologists to establish relationships among genera and families of anuran amphibians (Boulenger and Annandale, 1918; Noble, 1927; Orton, 1953). The difference in the oral morphology of

anuran tadpoles specifically reflects adaptive radiations of each species to exploit different parts of the available food base in the pond ecosystem (Lynch, 1973). Studies on tadpole morphology are thus crucial to comprehend their ecology and therefore likely is the foremost factor interpreting most of the aspects of their biology (Altig and McDiarmid, 1999).

In addition to the identification of each tadpole species it is also of primary importance to observe and document the life history of each amphibian species (Wilbur, 1980). Of the total number of species of amphibians in the world, India has 262 species which is less than 5% of the world amphibian species diversity (Frost *et al.*, 2006).The development of amphibian tadpoles in the Indian region has been scantily investigated. Perusal of the literature showed that life history of the species belonging to the family Dicroglossidae *viz. Hoplobatrachus tigerinus* (McCann, 1932; Dutta and Mohanty-Hejmadi, 1976), *Rana cyanophylctis* (Mohanty-Hejmadi and Dutta, 1979), *Indirana beddomii* (Kuramoto and Joshy, 2002), *Indirana leithii* (Chari and Daniel, 1953), *Rana curtipes* (Sekar, 1990b), *Rana breviceps* (Mohanty-Hejmadi *et al.*, 1979a) and *Rana temporalis* (Hiragond and Saidapur, 1999) has been studied. Whereas species studied from other families like Rhacophoridae include *Rhacophorous malabricus* (Sekar, 1990a) and *Polypedates maculatus*, (McCann, 1932; Mohanty- Hejmadi and Dutta, 1988; Girish and Saidapur, 1999). Studies have also been done on rare and secretive Microhylids like *Ramanella Montana* (Krishna *et al.*, 2004) and *Ramanella variegata* (Dutta *et al.*, 1990-91) and *Uperodon systoma* (Bhaduri and Daniel, 1956; Mohanty-Hejmadi *et al.*, 1979b). Previous studies on anuran larvae have primarily concentrated on their systematics and natural history, however the distribution, habitat use and ecological interactions have received petite attention.

Compared to other states of India, Gujarat is relatively poor in terms of amphibian diversity (Naik and Vinod, 1993). Among the 262 species of amphibians known in India, only 18 species of anuran and one species of caecilian are reported from Gujarat (Naik and Vinod, 1996). Nine species of amphibians belonging to the order anura is reported from Vadodara district, Central Gujarat (Chapter-4). There are nine genera and four families representing the order anura in this district. However, there is only limited information available on the description of larval morphology of these anurans from the state as well as from the district (Suresh *et al.*, 2005). *Bufo stomaticus* was recorded as a common species of toad in the disturbed areas, while *Microhyla ornata* is common Microhylid found in undisturbed areas of Vadodara district (Chapter-1). Very little information about the bioecology of these two species is available. Workers like Mohanty-Hejmadii *et al.*, (1980), Padhye and Ghate (1989), Dey and Gupta (2002) described in brief the early development of *M. ornata*. Khan (2002) from Pakistan has described the development of *B. stomaticus*. In this chapter the

morphology, morphometry and ecology of the tadpoles of *B. stomaticus* and *M. ornata* from Vadodara district are described. Pertinent biotic and abiotic factors were also determined to understand their influence on the survival of tadpoles in the natural environment. The present study is relevant to amphibian conservation particularly in the light of reports of world wide amphibian decline (Wake, 1990). Additonally, these informations will vastly improve our basic understanding of the oral morphology and feeding ecology of the tadpoles in question.

Moreover, being sensitive to waterborne pollutants, structural anomalies in tadpoles are widely reported (Rowe *et al.*, 1996; Sparling and Lowe, 1996). Therefore, a deviation from the normal morphology of the tadpole can be used as a biomarker to the aquatic pollution.

5.2. INTENSIVE STUDY SITES

Two study areas were selected to understand the breeding biology of the selected species of anurans. These areas were appraised after a reconnaissance survey. Many permanent and temporary water bodies were surveyed extensively during the years 2004 and 2005. However the breeding sites of both the species were selected at random within each of the study sites. The study sites included:

Site – I Timbi (Position: $22^0$21'08" N and $73^0$18'02" E)

This study area included many permanent and temporary water bodies. The permanent water bodies had water throughout the year; however the temporary ones were filled with water only during monsoon after which they get dried.

Site-II Sindhrot (Position: $22^0$19'38" N and $73^0$04'13" E)

Many temporary as well as permanent water bodies were encountered in this study area. After monsoon slowly the stagnation sets in and the water level begins to reduce in many of the permanent water bodies whereas the temporary water bodies generally dries up after postmonsoon.

5.3. METHODOLOGY

5.3.1. Sampling

During the inventory studies (Chapter 4) it was found that tadpoles of *Bufo stomaticus* belonging to Bufonidae family and *Microhyla ornata* of Microhylidae family were common in the study sites. Identification of the amphibians, their eggs and larvae, is an important pre-requisite to an ecological study. Henceforth, the larval biology of these two species were

scrutinised during monsoon, from June 2004 to September 2004 and June 2005 to September 2005 (For details refer Chapter 3)

5.3.2. Morphometric Studies

For the morphometric studies, tadpoles of both the species were caught in the field with the help of dipnet. They were then, transported to laboratory and examined under stereo microscope (Leica MZ 16 A) for identification. Further confirmation was done by referring to standard publications (Daniel, 2001; Chanda, 2002). However, the developmental stages were identified as described by Gosner (1960). Finer morphological features were observed under the stereomicroscope and photographed with a CCD camera. Morphometric measurements were recorded using calibrated digital caliper (Mitutoyo, Japan). Drawings of general morphology land marks were made with a camera lucida (Figure 5.5).

5.3.3. Scanning Electron Microscopic Analysis

Oral disc of tadpoles at Gosner's stage-37 were examined under Scanning Electron Microscope. The oral anatomy was examined in JEOL-5610LV Scanning Electron Microscope.

5.3.4. Gut Content Analysis

Both the species of tadpoles were groped into prehindlimb and hindlimb stage and fifteen tadpoles per stage and per species were examined for the gut content analysis. The gut content were then analyzed under a binocular Research Microscope (Leica DMRB).

5.3.5. Physicochemical Analysis

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 ($PO_4^{3-}-P$), nitrate-nitrogen (NO_3^--N), and total solids (Chapter-3). Water samples of one litre each were taken from one location per pond. Sampling was done once a week in each month from June to September in the year 2004 and 2005. Samples were analysed on the same day and these information were then amalgamated to represent the yearly data.

5.3.6. Controlled Experiments

Controlled experiments were performed to understand the pH tolerance of both the species of tadpoles. Moreover, experiments were also performed in the lab to know the predatory behaviour of the selected species of fishes.

5.3.7. Statistical Analysis

All the parameters characterized by continuous data were analyzed to give group means and standard deviation. 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).

The physicochemical parameters and the density of tadpoles at different study sites were compared using the Bray-Curtis index of similarity and based on the similarity a dendrogram for different study sites was plotted (Programme PRIMER, Version 5.2.4.). The various physicochemical variables were subjected to Principal component analysis (PCA) by using the programme PRIMER (Version 5.2.4). Variable with factor loading larger than 0.40 were considered to have significant contributions to principal components (Manly, 1990).

5.4. RESULTS AND DISCUSSION

5.4.1. Breeding Period

Factors which affect oviposition have an important bearing on amphibian breeding biology. Packer (1960) found that breeding migration of *Terich rivularis* was provoked by rainfall. Alcalaa (1962) pointed out the necessity of rain for breeding to occur in *Rhacophorous leucomystax*. Mahapatro and Dash (1990) reported that oviposition in *B. stomaticus* is initiated by onset of monsoon rains. In the present study, breeding of *B. stomaticus* and *M. ornata* coincided with monsoon, as reported by other workers (Ferguson, 1904; McCann, 1932; Daniel, 1975). Rain is necessary for breeding this is supported by the fact that more clutches were collected in July and August the months of heavy rains (Figure 5.1). However, with the onset of rain the ambient temperature decreased from 40^0C in May to 33^0C in July. This decrease in air temperature was coupled with high humidity. Dimmit and Ruibal (1980) have listed sound and vibration as the primary factors while temperature, time of the day, amount of rainfall on preceding day and change in soil moisture as secondary factors that may affect emergence of *Scophiopus couchi* from their winter burrows. Therefore, it could be logical to surmise that factors like rainfall, decrease in temperature

and increase in humidity together might be influencing the breeding response in the species studied.

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. Breeding period started from mid June and lasted till the end of August or early September. Unlike the toads, *M. ornata* began their breeding activity only after the area started receiving few consecutive monsoon showers, they breed during the mid monsoon (Mid July-late August or early September) (Figure 5.1). Breeding during the late or mid monsoon is also reported for other Microhylids species (Dutta *et al.*, 1990-91). This could be because *M. ornata* is a burrowing animal and breeds in temporary rainwater pools, which form usually by mid monsoon after repeated rains.

5.4.2. Breeding Characteristics

Many male and female adults of *B. stomaticus* were found to horde the same site for breeding. A single breeding aggregation with 45 males and 30 females were sighted from one of the study area (Figure 5.9). While in case of the Microhylid *M. ornata*, 4-7 adults were found congregating at the breeding site, which is a smaller congregation as compared to that seen in the toads. Mature anurans exhibit distinct sexual dimorphism. In both the species the breeding male size was found to be less than that of the females (Table 5.1). This is in agreement with other reports that say in anurans adult females are typically larger than their male counter parts (Crump, 1974). Howard (1978) and Hemelaar (1983) concluded that adult female toads are bigger than adult male toads. In *B. stomaticus*, the males developed secondary sexual characters like yellowish vocal sac and black cornified patches on the inner side of the first and second fingers. However, not all males present at the breeding site showed this character. In the Microhylid, the males often inflated the vocal sac to its full capacity, which is pale in colour (Figure 5.6).

5.4.3. Call

Males of *B. stomaticus* were observed calling from the land as well as from the aquatic medium. On land they were sighted positioned on the ground, raised slightly on their front legs while calling and kept changing their calling direction. This has the obvious reason to attract females from different point. The males were then found moving towards the water bodies. As the female approached, the male stops calling and the pair forms amplexus. Each call was of 20–30s duration with a gap of 20-25s. Voice could be syllablised as kraw-kraw-kraw. Males called throughout the night beginning at 1830 hrs and lasting until 0700

hrs. In case of *M. ornata* the calling began much later, calling started at 2130 hrs and continued throughout the night. The males were always seen calling from near by water bodies and once the females approached they formed a pair.

5.4.4. Reproductive Mode

A great variety of reproductive modes are utilized by the tropical anurans (Crump, 1972, 1974; Duellman, 1978). Crump (1974) has classified the reproductive mode into three categories 1) aquatic development, when eggs are deposited in water and tadpoles develop in water; 2) semiterrestrial development, when eggs are deposited out of water and tadpoles develop in water and 3) terrestrial development, when eggs and larvae are independent of standing water. *B. stomaticus* is a terrestrial animal and were found throughout the year except in very extreme climates while *M. ornata* is a burrowing form, predominantly seen during the monsoon (personal observation). 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 tadpole's takes place in water.

5.4.5. Courtship and Amplexus

During the breeding season male produced a characteristic sound to attract female conspecifics. Males of *B. stomaticus* were trying to mount a single female and were kicking against each other with their hindlegs. In between one managed to clasp the female, still the other toads tried to dislodge the successful one and were moving randomly in water. The competing males were found trying to dislodge the amplexed male. Such type of competition was not observed in *M. ornata*. Amplexus in *B. stomaticus* and *M. ornata* is axillary, wherein the male clung to the female by holding it below the armpit (Figure 5.7). Axillary mode of amplexus is seen in so-called "advanced" frogs (Lynch, 1973; Duellman and Trueb, 1986).

5.4.6. Spawn

Eggs of *B. stomaticus* were laid in the form of long single strings (Figure 5.10, 5.11), with eggs embedded in the string. Eggs strings were pale translucent yellowish green in colour and were found loosely wound around vegetation, broken branches and objects like broom. The clutch size varied from 6000-8000. 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. The clutch size in *M. ornata* ranged from 150-400 eggs (Table 5.2, Figure 5.12). Padhye and Ghate (1988 a,b) recorded the number of eggs per spawn in *M. ornata* to be as small as 62 eggs or large as 1327 eggs. In the current study the egg diameter of this Microhylid species ranged from 1.8-2.0mm which is much less than that reported by Padhye and Ghate

(1988b). Moreover the colour of the egg reported by these workers is brownish while in the present study colour of the eggs was pale yellowish. Each fertilized egg is surrounded by layer of jelly which in turn is loosely attached to the jelly of another egg and this forms a single layer of spawn (Figure 5.13). In the current study the egg masses of *M. ornata* were found floating on the water or were found adhered to vegetation in the water bodies. The egg laying habits of *M. ornata* is similar to that of other Microhylids (Dutta *et al.*, 1990-91). Number of egg masses deposited ranged from 1 to 3 per site. The clutch size in Microhylid *Ramenella variegata* is reported to be 575–1417, while that of *Ramenella obscura* from Sri Lanka is 557(Saidapur, 1989).

5.4.7. Habitat utilization by tadpoles

The distribution of tadpoles among habitats suggests that species select specific site for reproduction. Anuran tadpoles are reported from a broad range of habitats like stream, stream side pond, forest ponds, lakes, wetland, arboreal water bodies, leaf litter, etc. (Hero, 1991). Both the species of tadpoles in the present study were encountered only in lentic ecosystem. They were found in permanent ponds and temporary water bodies (small ditches and puddles) formed due to rain water. There is no report of both the species breeding in lotic system. Though both of them co-occurred in 25-30% of the water bodies, no competition was observed suggesting that both the species of tadpoles could partition the habitats within water bodies. Heyer (1973) observed spatial segregation of living space among the inhabiting tadpoles at a seasonal tropical location in Thailand. Spatial segregation was also seen in the present study among the species of tadpoles found in the same water bodies. 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.

B. stomaticus tadpoles are active swimmers and feeders, and normally keep to the lower levels of the natural water bodies like ponds and small ditches. They were also seen attaching themselves to the sides of small tanks. According to Altig and Johnston (1989), *B. stomaticus* larvae belong to lentic benthic habitat guild. Tadpoles of this group rasp food from submerged surfaces with the help of their keratinized mouth parts and their eyes are dorsal. However, *M. ornata* tadpoles belong to lentic suspension feeder guild, they inhabit the water column by sitting quiescently in the water column and pumping water through buccopharyngeal structure to entrap suspended particles, as they lack keratinized mouth. Partioning of resource by the tadpoles avoid competition among them and there is a peaceful coexistence among the tadpoles in the same habitat. Thus the tadpole species showed habitat specificity within the water bodies. 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).

5.4.8. Tadpole Aggregation and Density

The term 'aggregation' is generally applied to tadpoles as meaning any distinct grouping or massing of individuals regardless of the cause (Bragg, 1968). Several species of tadpoles form conspicuous aggregation. Three major hypothesis have been proposed to explain aggregation in tadpoles 1) Thermotaxy (Brattstrom and Waren, 1955) 2) Enhanced feeding (Bragg, 1940, 1954; Richmond, 1947; Duellman and Lescura, 1973; Beiswenger, 1975; Katz *et al.*, 1981) and 3) Reduced Predation (Black, 1970)

One of the characteristics of Bufonid larvae is to form schools and feed along the marginal water (Beiswenger and Test, 1967; Beiswenger, 1975, 1978; Breden and Kelly, 1982). In the present study, formation of schools in case of *B. stomaticus* was not seen, on the contrary they were more solitary, while *M. ornata* formed feeding schools in the watercolumn. They are not conspicuously coloured to human observer but were seen to form aggregations that were clearly visible. When disturbed they moved together. However the reason for the tadpoles of *M. ornata* forming aggregation is unclear.

Densities of Bufo tadpoles were comparatively higher than the Microhylid in all the study areas (Table 5.8). The difference in the clutchsize (ibid, p.9) of these two anurans might be one of the reason for the disparity in the densities.

5.4.9. Morphometry and Description of Tadpoles of *Bufo stomaticus*

Morphometric details of tadpoles belonging to different developmental stages are given in Table 5.1. In dorsal view the body is mostly globular to ovoid; it is depressed as it is wider than high. The external nares are prominent and closer to the eyes than to the snout (Figure 5.22, 5.23, 5.24). 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. They are positioned beneath the longitudinal axis and directed posteriorly. Spiracular opening is situated slightly closer to the end of the body than to the snout and is at a level between the apex of the caudal myotomes and the hindlimbs. Its opening is rounded to oval. Snout spiracular distance (SSD) is 71% of snout vent length (SVL) and 29% of total body length (TBL) length. 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. The dorsal fins show pigmentation, however, the pigmentation is absent or minimum in the ventral fins. The height of the tail is maximum at the midpoint of the tail length. Height of the dorsal fin is greater than that of the ventral fin. The height of the dorsal fin increases from the base of the tail to just past the midpoint of the tail from where the margin of the fin runs almost parallel to the tail musculature. Over the entire length, the margin of the ventral fin runs parallel to the muscular tail. Degeneration of the tail takes place from Gosner stage 42. Live tadpoles are usually black in colour.

Oral morphology of anuran tadpoles differs specifically reflecting adaptive radiations of each species to exploit different parts of the available food base in the pond ecosystem (Boulenger and Annandale, 1918; Noble, 1927; Orton, 1953; Lynch, 1973). 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 tadpoles is positioned anteroventral and is emarginated laterally (Table 5.4, Figure 5.14, 5.15). It has a single row of rounded, delicate membranous marginal papillae, which are broadly interrupted anteriorly and posteriorly. 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 upper and the lower jaw sheaths (beak) are edged with sharp, pigmented and keratinized serrations (Figure 5.16). Moreover, the serrations of the beak are not uniform (Figure 5.17); some of the serrated ends are pointed while few of them are blunt. 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 (Figure 5.18) however, its inner corners are overlapped by upper beak. The sharp edges of beak might be helping in scraping the food material. The relative amount of dark keratinization of the jaw sheaths could be used as a useful distinguishing character among different species of tadpoles (Altig *et al.*, 1998). The *B. stomaticus* tadpoles, it was observed, have jaw sheaths that are dark and extend nearly the entire width. Labial Tooth Row Formula (LTRF) of a tadpole is used in depicting, number and arrangement of tooth rows on its oral disc (Altig and Johnston, 1989). The LTRF of *B. stomaticus* is 2(2)/3 wherein the length of the individual tooth row varies. 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 and measures 0.75 mm. These teeth are laterally cuspidate with 11-12 cusps (Figure 5.19, 5.20). The jaw sheaths and teeth are used to remove material from substrate. 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 (Table 5.4). By the end of stage 42 the beak and the teeth rows slowly starts disappearing.

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. The relatively short period of development is characteristic of tropical species which have to take advantage of transitional aquatic habitats during the monsoon (Heyer, 1973). Upon metamorphosis these froglets developed red spots on the dorsal side (Figure 5.25), 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, 1988). An initial period of maximum growth (with minimal development) is preceded and followed by periods of significant development and little growth. ANOVA test indicates that the rate of growth differs significantly between the three groups of tadpoles (Table 5.3). Strauss and Altig (1992) stated that most of the growth of tadpole follows the exponential phase of sigmoid curve. Most anuran larvae are considerably flexible in their growth rates and development (Wilbur and Collins, 1973).

5.4.10. Morphometry and Description of Tadpoles of *Microhyla ornata*

Morphometric parameters of ornata tadpoles belonging to different development stages are given in Table 5.5. In dorsal view the body is mostly ovoid. The external nares are very small compared to that of the tadpoles of *B. stomaticus*. Narial opening is not visible clearly and is oval in shape with a well defined rim. Pigmentation on the rim of nares differentiates it from rest of the body. Nares are closer to the eyes than to the snout. The eyes are positioned laterally and are protruding. The interorbital space is very large compared to the internarial distance. Interorbital space is more than 80% of bodywidth. Spiracle is single and median. It is not clearly visible as seen 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, opening medial, tubular, directed posteriorly and not linked to the ventral fin. Tail is long and very fragile. Tail length is almost 65% of the body length. The tail musculature is poorly developed with thickness less than 1 mm and it touches the fin at the posterior end. The tail tip is sub acuminate. The height of the tail is maximum at the midpoint of the tail

length. The ventral fin is larger in size compared to the dorsal fin. Pigmentation is more in the ventral fin and this reduces near the tail tip (Figure 5.26, 5.27, 5.28).

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 whereas the oral disc is without any teeth or beak (Figure 5.12). an moreover, the marginal papillae are absent. Absence of typical oral disc and its anterodorsal mouth, reflects its filter feeding microphagus habit. Position of oral disc on the body of tadpole reflects its feeding ecology, food preference and methodology employed by it for food acquisition (Altig and Johnston, 1989). Dey and Gupta (2002) have recorded the total length of tadpoles at four limb stage, less than that at two limb stage, while in the present study the total length of tadpoles at four limbed stage is more than compared to the tadpoles at two limbed stage. 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 (Figure 5.29). The pattern of the growth curve in *M. ornata* is similar to that seen in the tadpoles of *B. stomaticus* (Figure 5.2).

5.4.11. Ecological Considerations

Heyer (1973) and Torres-Orozco *et al.*, (2002) are of the opinion that the occurrence of tadpole species in the aquatic system depends both on the species as well as on biotic and abiotic environmental factors. Amphibian egg and larval development are directly related to water temperature (Herreid and Kinney, 1967), so the temperature regime of breeding sites influences successful hatching, tadpole growth (Herreid and Kinney, 1967) and metamorphosis (Wilbur, 1987). Temperature has been found to be an important factor, in the growth and development of larvae. Water temperature of the water bodies in the present study, where the tadpoles were found, ranged between 26°C and 30°C. Both the tadpole species were observed more on the periphery of the water bodies i.e. the shallow zone, wherein the temperature of the water is more. This behaviour probably reflects a preference for warm water. Preference for higher temperature has been documented for several anuran species and is usually explained through its benefits in terms of growth rate (Huey and Kingsolver, 1993; Barandun and Reyer, 1997). 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($r=0.997$, $p \leq 0.05$) (Table 5.9). According to Putnam and Bennett (1981) increase in body temperature results in increase in performance capacity. *Bufo boreas* (Brattstrom, 1962), *Bufo canorus* (Mullally, 1953) and *Bufo terrestris* (Noland and Ultsch, 1981) larvae were observed to select the warmest areas of the pond.

Much research has been done on the effect of acidic environment on amphibian (Pierce, 1985; Freda, 1986). According to Strijbosch (1979) pH is one of the most important habitat characteristics for the inhabiting amphibians. 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. Controlled experiment in lab showed that both the tadpole species did not survive below pH 5.5. At pH 6.5 the tadpoles of *B. stomaticus* showed delayed growth whereas more 50% mortality was observed at pH 6.0. One of the major effects of acidic environment on these animals is the depression of the larval growth rates (Cummins, 1986; Freda and Dunson, 1985, 1986; Ling *et al.*, 1986). Both acid tolerant and acid sensitive species show inhibition in growth of tadpoles even with a short term exposure to acidity (Freda and Dunson, 1986; Pierce and Montgomery, 1989). Andren *et al.*, (1989) suggested that amphibian population breeding in acidic ponds over several generations undergo adaptation to acidic condition. There is a range in species sensitivities to acidity, some species such as *R. sylvatica* and *R. clamitans* which are more tolerant are found in more acidic habitats (Gosner and Black, 1957; Saber and Dunson, 1978). *M. ornata* tadpoles were found to be more sensitive to acidic pH, as more than 50% mortality was observed below pH 6.5. The effect of alkaline medium on different species of tadpoles has been less explored. However, during the present study, the controlled experiment performed revealed that tadpoles of *M. ornata* are found sensitive to pH above 9 while the tadpoles of *B. stomaticus* were much 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.

Aquatic habitats vary widely in many chemical characteristics, but for air-breathing animals dissolved oxygen (DO) concentration is one among the most important limiting parameter. Ponds are subject to large DO fluctuations on a daily and seasonal basis (Noland and Ultsch 1981; Nie *et al.*, 1999). Variation in DO can have important effects on community organization and interspecific interactions (Dunson and Travis, 1991; Wellborn *et al.*, 1996). Upon hatching, tadpoles begin to breathe via internal gills, pumping water through their mouths and out a small, tube like structure located on the left side of the body (Souder, 2000). However, 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 ($r=0.997$, $p \leq 0.05$)(Table 5.10), however no such correlation was apparent with the density of *B. stomaticus* tadpoles. It appears that abundant oxygen may be needed for the pond to support the tadpoles of *M. ornata*. In the current study the tadpoles of Bufonids were observed in water bodies where the dissolved oxygen value was as low as 0.5mg/l. Under low oxygen conditions tadpoles are able to meet their respiratory requirements through the

process of bobbing (swimming to the surface for air). To breathe air in, tadpoles fill their buccal cavity with air at the water surfaces (Altig and Mcdiarmid, 1999). Bobbing was noted in *B. stomaticus* tadpoles towards the end of metamorphosis suggesting that under low oxygen condition they meet their requirement through bobbing. One advantage of precocious airbreathing may be to facilitate metamorphic plasticity. Metamorphosis requires the possession of well developed lungs, and the process of air-breathing enhances pulmonary development (Bruce *et al.*, 1994) and maintenance (Crowder *et al.*, 1998). Low DO imposes severe demand on respiratory system and generally constrains activity, it may create a refuge for hypoxia-tolerant prey species if their predators are unable to tolerate hypoxia (Poulin *et al.*, 1987). However, air breathing is their most rapid and vital response; though non-air-breathing tadpoles can survive low DO conditions for short periods (Noland and Ultsch, 1981) atmospheric oxygen is probably necessary for long-term survival under sustained hypoxia. Breeding sites of both these tadpoles also differed in the depth of the water bodies. The ponds of *B. stomaticus* were comparatively shallower than those of the Microhylids. The depth of *M. ornata* tadpoles positively correlated with the density

One of the effects of high total solids is increased turbidity in an aquatic habitat. Increased turbidity influences biota, affects characteristics such as ecological conditions, resource availability and species interaction (Hart, 1990). In the present study it was observed that water bodies with the tadpoles of *M. ornata* had low total solid level while *B. stomaticus* tadpoles preferred water bodies with high total solids (Figure 5.30). The probable reason for differential preference for water bodies with total solid level however needs to be further evaluated.

In a lentic ecosystem nitrates-nitrogen are produced by the bacterial decomposition of ammonia and organic materials. High levels of nitrites will prevent fish from carrying on normal respiration and at the same time may be detrimental to tadpoles (Marco and Blaustein, 1999). Studies have shown that the toxicity of nitrate compounds to amphibians increases with increasing nitrate concentrations and exposure times (Baker and Waights, 1994; Hecnar, 1995; Xu and Oldham, 1997; Marco and Blaustein, 1999; Schuytema and Nebeker, 1999). Nitrate has been shown to affect body length, larval period length, and feeding activity in other species as well (Hecnar 1995; Oldham *et al.*, 1997; Rouse *et al.*, 1999). In the present study the nitrate-nitrogen ($NO_3^- - N$) in the water bodies where both the species of tadpoles were found, averaged from to 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 (Rouse *et al.*, 1999). However, in the present study no stastically significant correlation was observed

between the nitrate-nitrogen and density of tadpoles. The mean phosphate-phosphorous value of the water bodies ranged from 0.08 to 0.26 mg/L.

The various measures of water chemistry were subjected to principal component analysis so as to evaluate the microhabitat requirement for the presence of tadpoles. Principal component analysis showed that the first three components accounted for 87.5% of the variation in the data (Table 5.15, 5.16). Subsequent component contributed 9.7% of variation. The first component axis (PC 1) was characterized by tadpole density, total solids, phosphate-phosphorous and the second principal component axis (PC2) was characterized by watertemperature and pH. The third principal component had high component loading with water temperature, total oxidised nitrogen and dissolved oxygen. The factors highly correlated with these three component were those that directly related to the water variables and indicate the characteristics of the water bodies.

5.4.12. Vegetation

Habitat structural complexity of any ecosystem can strongly affect biotic interactions. In particular, the intensity of predator-prey interactions can be mediated by habitat complexity because the effectiveness of predators often decreases in structurally complex habitats (Werner *et al.*, 1983; Babbit and Jordan, 1996). Typically, increased habitat complexity reduces predation rates by providing refuges for prey or by decreasing predator efficiency (Savino and Stein, 1982; Werner *et al.*, 1983). Water bodies with tadpoles of *B. stomaticus* had more vegetation compared to that seen near the water bodies of *M. ornate.* Ephemeral ponds with tadpoles of *M. ornata* were more open with only few species of plants around it (Figure 5.10). 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*).

5.4.13. Food

The data on the food items of *B. stomaticus* and *M. ornata* tadpoles is shown in the Table 5.12 and 5.13. Tadpoles of both the species were largely herbivorous and a variety of algal component constituted the major food items. 14 species of phytoplankton and 8 species of zooplankton, while 7 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 (Table 5.12 and 5.13). Tadpoles collected from Sindhrot had maximum number of food items in their gut. Nevertheless, food ingestion does not necessarily mean food digestion (Hegner, 1923; Hendricks, 1973). According to Savage (1952) algae which are important as food are those with thin cell walls (eg. *Scendesmus*) and that the cell contents are made available to the tadpoles after rupturing of the wall by the jaws and labial teeth during ingestion and later by peristaltic action in the gut. In the current study, tadpoles of *B. stomaticus* collected from Timbi had high percentage of *Scendesmus* in their gut. Presumably these were abundant in that particular period and sites and therefore consumed by the tadpoles. *Merismopedia* was prominent in the gut of the tadpoles collected from Sindhrot. In addition, they also showed a good percentage of *Anabaena*. The filamentous blue-green algae *Anabaena* promotes growth in tadpoles and is digested more thoroughly than the other algae (Pryor, 2003). It should be noted that ability of tadpoles to digest nuisance, bloom-forming algae such as *Anabaena* has important implications in freshwater resource management (Dickman, 1968; Pryor, 2003).

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). Costa and Balasubramanium (1965) showed from stomach content analysis that *Rhacophorus cruciger* larvae are qualitatively non-discriminant in the food that they ingest. Similar analyses for *Rana clamitans* tadpoles showed that these larvae are qualitatively and quantitatively non discriminant in their suspension feeding (Farlowe, 1928).

The percentage occurrence of rotifers and diatoms (*Keratella, Lecane, Scendesmus, Coscinodiscus* etc.) which are suspended in the water, were high in the tadpoles of the Microhylid *M. ornata*. This might be due to the nature of water body (fresh and muddy water with meagre algal component). This could also be attributed to the oral features of these tadpoles. Position of oral disc on the body of tadpole reflects its feeding ecology, food preference and methodology employed by it for food acquisition (Altig and Johnston, 1989).

There was notably no difference in terms of food composition for both the species of tadpoles, between the prehindlimb and hindlimb stages; food items were almost similar 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 its weaker siblings. Crump (1983) suggested that cannibalism might be particularly common in species that reproduce in ephemeral ponds. 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). A few genera have even reversed the main ecophysiological adaptation of tadpoles (filter-feeding) and have become secondary carnivores and/or cannibals (Polis and Myers, 1985; Altig and McDiarmid, 1999). In the current study, it was observed that the tadpoles of *B. stomaticus* were able to feed both on the planktonic community by means of filtration, and on a large variety of substrates (including algaes and carrion) by rasping, scraping and chopping with their jaw sheaths and labial teeth. Thus they can be categorized as opportunistic feeders, feeding on the available food sources.

5.4.14. Predators

There can be direct and indirect effects of predators on the population and community dynamics of a prey. Indirect effects can be due to predators' direct interaction with resource and this in turn can alter the behaviour as well as the abundance of species which are dependent on these resources (Dethier and Duggins, 1984; Carpenter *et al.*, 1985; Strauss, 1991; Werner, 1992). Direct effects of Predators may be due to their consumption of prey and also due to their influence of prey performances and behaviour (Schoener, 1993; Harris, 1995; Menge, 1995). Some of the potential tadpole predators in the present study were insects like beetles, water bugs, and vertebrate predators like fishes and birds (5.14). Eggs of the amphibians were more vulnerable to predators than tadpoles due to mobility factor. Howard (1978) reported high mortality among egg masses of bullfrogs. The current study reports birds like *Ardeola grayii, Bubulcus ibis, Egretta garzetta and Halcyon smyrnensis* were the major vertebrate predators preying mainly on the eggs of both the amphibian species than the tadpoles.

In addition to other predators, fishes are also known to eliminate amphibian larvae from some of the habitat (Petranka, 1983). Studies have demonstrated direct and negative effects of fish on amphibian population through predation on eggs or tadpoles (Semlitsch, 1988; Sih, 1992; Bronmark and Edenhamn, 1994; Gamradt and Kats, 1996; Skelly, 1996; Smith *et al.*, 1999). In the present study it was observed that the water bodies which were already occupied by fishes were not selected by both the species for laying eggs. These vertebrate predators are commonly present in permanent water bodies than in temporary water bodies. This could be one of the factors for the amphibians to select temporary water body for breeding. *Poecilia sp.* and *Gambusia affinis* were the two key fish predators of the tadpoles. Controlled experiments in the lab showed that these predators readily consumed

the tadpoles as well as the eggs of both the species of anurans studied. Temporary water bodies occupied by these two species were devoid of any tadpoles. Thus, it can be said that the distribution of the tadpoles were strongly related to fish predation pressure. There are reports that some tadpoles species avoid predators because of their larger body size (Heyer *et al.*, 1975; Semlitsch and Gibbons, 1988; Gascon, 1992). 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. Such secretions, if present, do not seem to repel these two species of fishes in the present study. Almost all of the released *M. ornata* tadpoles were consumed by *Poecilia.* Nevertheless, unpalatibility of some tadpole species is well documented as an important deterrent to some of the predatory fishes (Kruse and stone, 1984; Kats *et al.*, 1988; Cecil and Just, 1979). Some tadpoles of the family Bufonide are known to be toxic to their predators (Licht, 1968; Brodie and Formanowijcrz, 1987). In the present study, beetles like *Hydrophilus acuminatus* and water bugs belonging to the genus *Sphaerodema* were the most common invertebrate predators encountered. *Sphaerodema* were encountered at both the sites. They were observed in both temporary as well as permanent water bodies with tadpole eggs. According to Aditya and Raut (2001) *Sphaerodema* is a potential predator in freshwater ecosystem.

5.4.15. Water Bodies

Permanent ponds contain large predator which is not encountered in temporary ponds (Skelly, 1996). The numerous predators in the permanent ponds are thought to be the most important source of mortality to the tadpoles (Skelly, 1996; Wellborn *et al.*, 1996; Williamson and Bull, 1999). Survival of *Hyla regilla* tadpoles and *Rana aurora* tadpoles was lower in the permanent ponds as compared to the temporary ponds (Adams, 2000). 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. Heyer *et al.*, (1975) suggested that frogs breeding in small, temporary ponds are less likely to be affected by predation. Roth and Jackson, (1987) in the experimental studies found that pools of smaller size had lower densities of predators and the disappearance of *Hyla cinerea* larvae was lower in small ponds compared to larger water body. According to Crump (1974) animals using the temporary and small water bodies to breed are regarded as most advanced as they utilize the available nearby resources for the survival. In the current study also tadpoles of both the species were found predominantly in temporary water bodies than the permanent ones (Figure 5.3). Cluster analysis of both the study sites based on various physicochemical

parameter of water bodies and density of the tadpoles reveals that water bodies with tadpoles of *M. ornata* at Timbi and Sindhrot during both the year are closely related with a similarity of about 98.23%, and 97.64% while similarity of 95.84% and 89.84% exists between these variables at Timbi and Sindhrot, with tadpoles of *B. stomaticus*. The association between the water bodies of *B. stomaticus* and *M. ornata* was comparatively less similar (Figure 5.17). Therefore, it is logical to surmise that both these anuran larvae use water bodies that differ in their physicochemical variables (Figure 5.4, 5.5).

TABLE 5.1 Snout-vent length of male and female *Bufo stomaticus* and *Microhyla ornata* during the breeding season

Species	Snout-Vent length (mm)	
	Male	Female
B. stomaticus	45.75 ± 6.95	54.75 ± 6.70
M. ornata	19.5 ± 2.02	23.6

TABLE 5.2 Certain reproductive and developmental parameters of *B. stomaticus* and *M. ornata*

Species	Breeding month	Clutch size (Volume count)	Days to metamorphosis
B. stomaticus	Mid-June, July, August, September	6000-8000	25-30 days
M. ornata	Mid-July, August, September	150-400	35-40 days

TABLE 5.3 Morphometric measurements (in mm) of the tadpoles of *B. stomaticus* (X ± SD)

PARAMETERS	TADPOLE STAGE		
	25-30(n=6)	31-36(n=6)	37-41(n=6)
Total body length	12.40 ± 2.48^{a}	17.32 ± 0.91^{b}	18.06 ± 2.9^{c}
Snout-Vent length	5.55 ± 0.79^{a}	7.0 ± 0.88^{b}	7.2 ± 1.23^{bc}
Body depth	2.45 ± 0.83^{a}	2.71 ± 0.58^{b}	2.66 ± 0.56^{bc}
Body width	3.31 ± 0.48^{a}	4.01 ± 0.30^{b}	4.58 ± 0.91^{c}
Inter Orbital Distance	0.94 ± 0.14^{a}	1.32 ± 0.07^{b}	1.42 ± 0.22^{bc}
Inter Narial Distance	0.59 ± 0.18^{a}	0.66 ± 0.07^{b}	0.71 ± 0.11^{bc}
Snout to Narial Distance	0.86 ± 0.11^{a}	1.01 ± 0.13^{b}	1.24 ± 0.31^{bc}
Snout to Eye Distance	1.57 ± 0.46^{a}	1.96 ± 0.20^{b}	1.90 ± 0.17^{bc}
Spiracle to Snout	3.6 ± 0.43^{a}	5.11 ± 0.57^{b}	5.33 ± 0.30^{c}
Tail Length	6.91 ± 0.57^{a}	10.63 ± 0.78^{b}	10.86 ± 1.84^{c}
Tail Height	2.68 ± 0.96^{a}	3.82 ± 0.57^{b}	4.26 ± 0.57^{c}
Dorsal fin Height	0.86 ± 0.40^{a}	1.29 ± 0.19^{b}	1.46 ± 0.10^{bc}
Ventral fin Height	0.66 ± 0.28^{a}	0.68 ± 0.07^{b}	0.74 ± 0.10^{bc}
Tail Muscle Height	1.22 ± 0.33^{a}	1.59 ± 0.14^{b}	$1.56 \pm .06^{bc}$
Tail Muscle thickness	0.7 ± 0.11^{a}	0.98 ± 0.20^{b}	0.92 ± 0.11^{bc}

Mean difference is significant at the 0.05 level ($p \leq 0.05$)

Values with same superscript are not statistically significant for each parameter

TABLE 5.4 Features of oral disc in *B. stomaticus* tadpoles

FEATURES	TADPOLE STAGE		
	25-30	31-36	37-41
Orientation of oral disc	Antero-ventral	Antero-ventral	Antero-ventral
Labial Papillae	Present	present	present
Row of Labial papillae	One	One	One
Submarginal papillae	Absent	absent	absent
Oral disc width	1.56 ± 0.16 mm	1.92 ± 1.9 mm	2.0 ± .07 mm
Beak Upper/Lower	Serrated	Serrated	Serrated
Tooth	Laterally cuspidate	Laterally cuspidate	Laterally cuspidate
Cusp	Present	present	present
LTRF	2(2)/3	2(2)/3	2(2)/3

TABLE 5.5 Morphometric measurements (in mm) of the tadpoles of *M. ornata* (X ± SD)

PARAMETERS	TADPOLE STAGE		
	25-30(n=6)	31-36(n=6)	37-41(n=6)
Total body length	16.93 ± 0.65^{a}	21.13 ± 0.76^{b}	22.12 ± 1.29^{bc}
Snout-Vent length	6.15 ± 0.42^{a}	7.11 ± 0.26^{b}	7.82 ± 0.28^{c}
Body depth	3.09 ± 0.13^{a}	4.11 ± 0.20^{b}	4.24 ± 0.22^{bc}
Body width	3.51 ± 0.24^{a}	5.40 ± 0.50^{b}	5.62 ± 0.18^{bc}
Inter Orbital Distance	3.1 ± 0.07^{a}	4.43 ± 0.29^{b}	4.54 ± 0.27^{bc}
Inter Narial Distance	0.70 ± 0.07^{a}	0.79 ± 0.04^{b}	0.80 ± 0.09^{bc}
Snout to Narial Distance	1.13 ± 0.09^{a}	1.32 ± 0.11^{b}	1.45 ± 0.14^{bc}
Snout to Eye Distance	1.60 ± 0.06^{a}	2.16 ± 0.14^{b}	2.23 ± 0.18^{bc}
Tail Length	10.78 ± 0.37^{a}	14.02 ± 0.65^{b}	14.11 ± 1.12^{bc}
Tail Height	3.79 ± 0.26^{a}	4.39 ± 0.11^{b}	4.39 ± 0.19^{bc}
Dorsal fin Height	1.03 ± 0.07^{a}	1.19 ± 0.05^{ab}	1.22 ± 0.16^{b}
Ventral fin Height	1.53 ± 0.15^{a}	1.92 ± 0.13^{b}	1.83 ± 0.11^{bc}
Tail Muscle Height	1.03 ± 0.08^{a}	1.22 ± 0.07^{b}	1.20 ± 0.12^{bc}
Tail Muscle thickness	0.78 ± 0.07^{a}	0.98 ± 0.20^{b}	0.95 ± 0.03^{bc}

Mean difference is significant at the 0.05 level ($p \leq 0.05$)

Values with same superscript are not statistically significant for each parameter.

TABLE 5.6 Physicochemical parameters of the water bodies at the study site

Sites		Timbi	Sindhrot	Timbi	Sindhrot
Parameters	Year	*B. stomaticus*		*M. ornata*	
Water temp.	2004	28.9 ± 1.65	29.6 ± 0.75	27.8 ± 0.9	30.1± 0.9
	2005	29.1 ± 0.86	27.6 ± 1.11	28.8 ± 1.6	28.6 ± 0.8
pH	2004	7.48 ± 0.06	7.78±.02	7.76 ± 0.32	7.64 ± 0.05
	2005	7.71±0.06	7.81±0.43	7.63 ± 0.31	7.75 ± 0.09
$NO_3^- - N$	2004	0.19 ± 0.18	0.48± 0.10	0.19 ± 0.02	0.24 ± 0.17
	2005	0.21± 0.16	0.39 ± 0.18	0.20 ± 0.04	0.28 ± 0.28
$PO_4^{3-} - P$	2004	0.13 ± 0.05	0.26 ± 0.20	0.09 ± 0.01	0.10 ± 0.03
	2005	0.16 ± 0.07	0.22 ± 0.21	0.08 ± 0.02	0.11 ± 0.04
DO	2004	4.15 ± 0.11	2.74 ± 0.71	3.64 ± 0.15	5.35 ± 0.48
	2005	1.15 ± 0.70	3.59 ± 1.32	4.29 ± 0.94	4.16 ± 0.94
COD	2004	11.2 ± 1.53	11.73 ± 0.54	13.33 ± 0.54	7.2 ± 0.92
	2005	10.66 ± 0.90	8.53 ± 1.53	8.53 ± 0.50	11.2 ± 0.50
TS	2004	65.3 ± 28.9	257.6 ± 85.7	76.4 ± 26.0	60.4 ± 10.1
	2005	125.73 ± 61.2	315.7 ± 49.1	61.3 ± 15.2	62.37 ±17.8

Table 5.7 Summary of pH tolerance level by *B. stomaticus* and *M. ornata* tadpoles

Tadpole species	pH	No. of tadpoles used	No. of tadpoles survived
B. stomaticus	5.5	30	0
	5.8	30	2
	6.0	30	12
	6.5	30	27
	7.0	30	30
	7.5	30	30
	8.0	30	30
	8.5	30	21
	9.0	30	13
	9.5	30	7
	10.0	30	2
M. ornata	6.0	30	0
	6.5	30	9
	7.0	30	30
	7.5	30	30
	8.0	30	28
	8.5	30	22
	9.0	30	4
	9.5	30	0
	10.0	30	0

TABLE 5.8 Density=number of tadpoles/m^2

Species	Year	Timbi	Sindhrot
M. ornata	2004	6.9 ± 6.8	16.9 ± 9.3
	2005	10.5 ± 2.4	9.2 ± 1.9
B. stomaticus	2004	340.2 ± 68.7	406.25 ± 35.44
	2005	230 ± 51.8	346.7 ± 61.4

TABLE 5.9 Linear relationships between density of the *B. stomaticus* tadpoles and physicochemical variables of the water bodies

	Correlation coefficient(r)	r^2
TS	-0.818	0.6684
DO	0.997*	0.9936
PO_4^{3-} – P	0.172	0.02961
NO_3^- – N	0.278	0.07709
pH	-.736	0.5416
Water temperature	0.991*	0.9820

* Correlation is significant at p≤0.05

TABLE 5.10 Linear relationships between density of the *M. ornata* tadpoles and physicochemical variables of the water bodies

	Correlation coefficient(r)	r^2
TS	0.484	0.2343
DO	0.971*	0.9436
PO_4^{3-} – P	0.636	0.4046
NO_3^- – N	0.742	0.550
pH	0.141	0.01980
Water temperature	0.327	0.1068

* Correlation is significant at p≤0.05

TABLE 5.11 Vegetation around the water bodies of tadpoles of *B. stomaticus* and *M. ornata*

Sr. No.	*B. stomaticus*	*M. Ornata*
	Plants species	
1.	*Asteracanthus longifolia*	*Parthenium Sp*
2.	*Parthenium sp.*	Grass
3.	*Ammannia baccifera*	*Cyprus sp.*
4.	*Amaranthus spinosus*	
5.	*Urena lobata*	
6.	*Cassia occidentalis*	

7.	*Cassia tora*	
8.	*Pongania tora*	
9.	*Phyllanthus amara*	
10.	*Commelina sp.*	
11.	*Cyprus sp.*	

TABLE 5.12 Percentage of food items in the gut of tadpoles of *B. stomaticus*

Intestinal contents	Timbi		Sindhrot	
	Pre-hind limb stage	Hindlimb stage	Pre-hind limb stage	Hindlimb stage
Merismopedia sp.	17.80%	11%	25.52%	20.6 %
Phacus sp.	6.75%	5.80%	13.11%	9.69%
Scendesmus sp.	18.32%	20.47%	8.45%	8.57%
Coscinodiscus sp.	14.20%	9.95%	4.72%	3.53%
Oedogonium sp.	15.95%	17.42%	1.00%	8.52%
Anaebaena sp.	3.25%	5.22%	10.22%	9.05%
Ankistrodesmus	------	------	3.47%	3.76%
Navicula sp.	2.75%	1.79%	6.20%	3.71%
Nitzachia sp.	------	-----	5.96%	3.16%
Synedra sp.	1.35%	2.76%	6.70%	5.27%
Fragellaria sp.	6.86%	2.48%	------	----
Eudorina sp.	2.34%	4.84%	7.20%	8.50%
Volvox sp.	-----	2.48%	4.96%	5.64%
Cosmarium sp.	6.77%	7.46%	------	-----
Spirulina sp.	------	------	1.36%	2.25%
Paramoecium sp.	2.98%	1.38%	-----	1.12%
Keratella sp.	------	6.77%	1.25%	0.75%
Brachionus sp.	2.95%	2.07%	0.25%	0.90%
Asplancha sp.	----	1.38%	0.50%	0.90%
Lecane sp.	0.57%	1.53%	0.25%	0.97%
Daphnia sp.	-----	-----	-----	3.76%
Nematode worm	-----	----	-----	0.62%
Unsegmented worm	-----	----	-----	.60%

TABLE 5.13 Percentage of food items in the gut of tadpoles of *M. ornata*

Intestinal contents	Timbi		Sindhrot	
	Pre-hind limb stage	Hindlimb stage	Pre-hind limb stage	Hindlimb stage
Syndra sp.	----	3.07%	18.26%	23.95%
Coscinodiscus sp.	17.64%	7.69%	10.54%	17.24%
Nitzachia sp.	----	-----	8.36%	10.34%
Cosmarium sp.	8.82%	10.76%	-----	-----
Volvox sp.	----	2.05%	12.92%	8.62%
Euglena sp.	----	2.56%	----	-----
Phacus sp.	4.41%	5.64%	----	5.17%
Eggs	8.82%	15.38%	8.15%	4.25%
Filinia sp.	13.23%	7.69%	15%	12.06%
Lecane sp.	10.29%	13.33%	8.26%	6.66%
Keratella sp.	14.70%	9.23%	2.35%	1.95%
Lepadella sp.	13%	6.15%	2.50%	1.50%
Brachionus sp.	1.47%	11.28%	12.98%	10.5%
Daphnia sp.	7.35%	5.12%	-----	0.75%

TABLE 5.14 Distribution of potential predators in both the study sites

Sr. No.	Predator species	Timbi	Sindhrot
	BIRDS		
1.	*Ardeola grayii*	+	--
2.	*Bubulcus ibis*	+	--
3.	*Egretta garzetta*	+	--
4.	*Halcyon smyrnensis*	+	+
5.	FISHES		
6.	*Poecilia sp.*	+	+
7.	*Gambusia affinis*	+	+
8.	INSECTS		
9.	*Hydrophilus acuminatus* (beetle)	--	+
10.	*Sphaerodema acuminatus* (bug)	+	+
11.	*Sphaerodema rusticum*	---	+

+ Present; -- absent

TABLE 5.15 Summary of principal component analysis (PCA) of microhabitat variables

PC	Eigenvalues	%Variation	Cum.%Variation
1	4.55	56.8	56.8
2	1.49	18.6	75.4
3	1.13	14.1	89.5
4	0.71	8.9	98.4
5	0.06	0.8	99.1

TABLE 5.16 Values of the physicochemical parameters from principal component analysis (PCA) of the study sites

Variable	PC1	PC2	PC3
Density	-0.400	-0.386	0.042
Temperature	0.380	0.393	-0.290
TS	-0.441	0.177	-0.080
DO	0.278	0.130	-0.485
$PO_4^{3-} - P$	-0.450	-0.004	-0.205
$NO_3^- - N$	-0.390	0.175	-0.447
pH	-0.256	0.640	-0.022

TABLE 5.17 Cluster analysis similarity matrix of the study sites during the study period 2004 and 2005

	04 T.M.O	04 S. M.O	05 T.M.O	05 S. M.O	04 T.B.S	04 S.B.S	05 T.B.S	05 S.B.S
04 T. M.O								
04 S. M.O	93.358							
05 T M.O	95.401	97.64						
05 S. M.O	98.226	94.92	96.935					
04 T. B.S	68.173	69.875	68.76	68.239				
04 S. B.S	61.788	61.808	60.427	62.106	87.895			
05 T. B.S	69.999	69.578	68.364	70.104	89.841	87.81		
05 S. B.S	62.95	62.54	61.359	63.075	87.639	95.84	87.524	

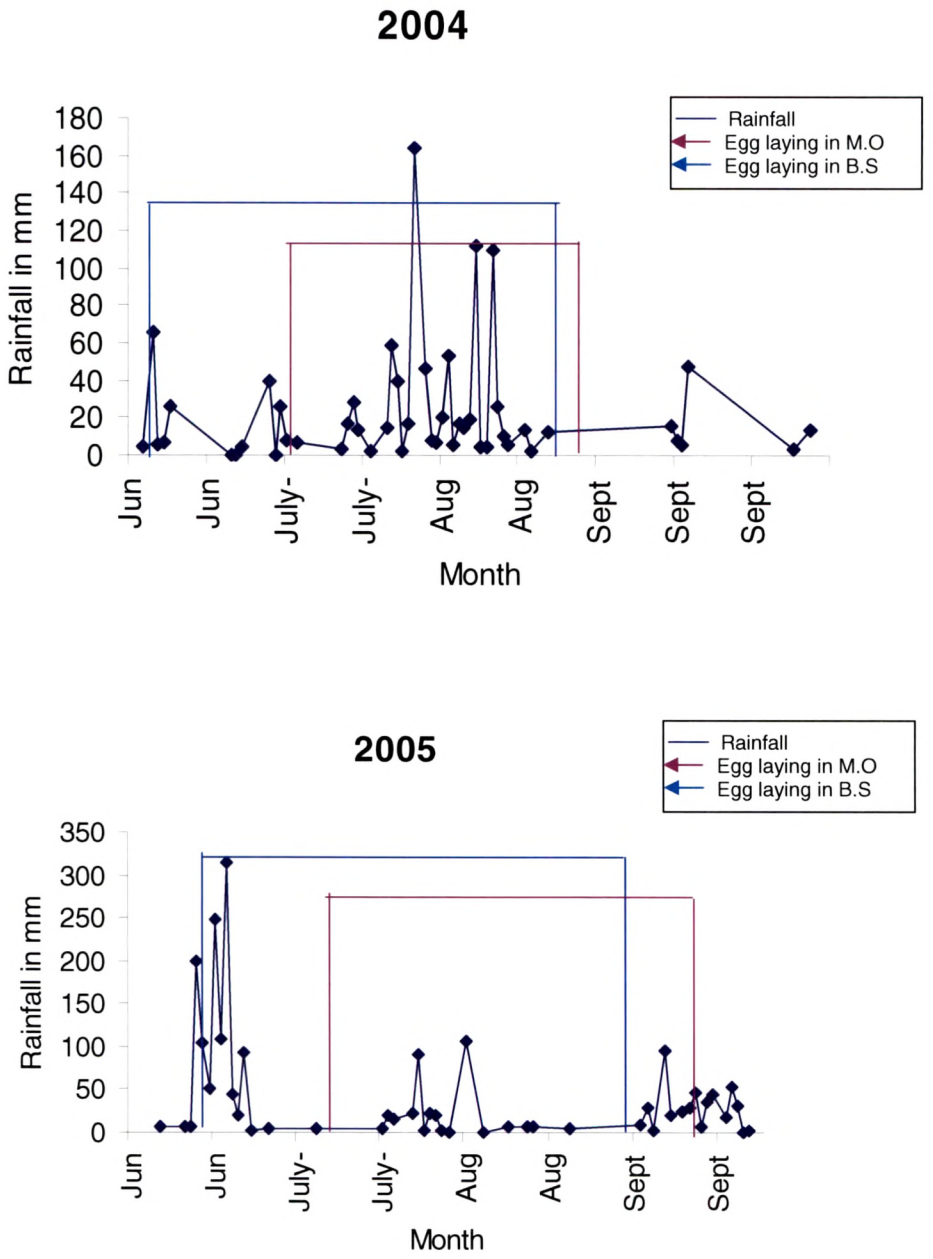


FIGURE 5.1 Rainfall and breeding seasonality of *B. stomaticus* and *M. ornata* in the study sites during the year 2004 and 2005.

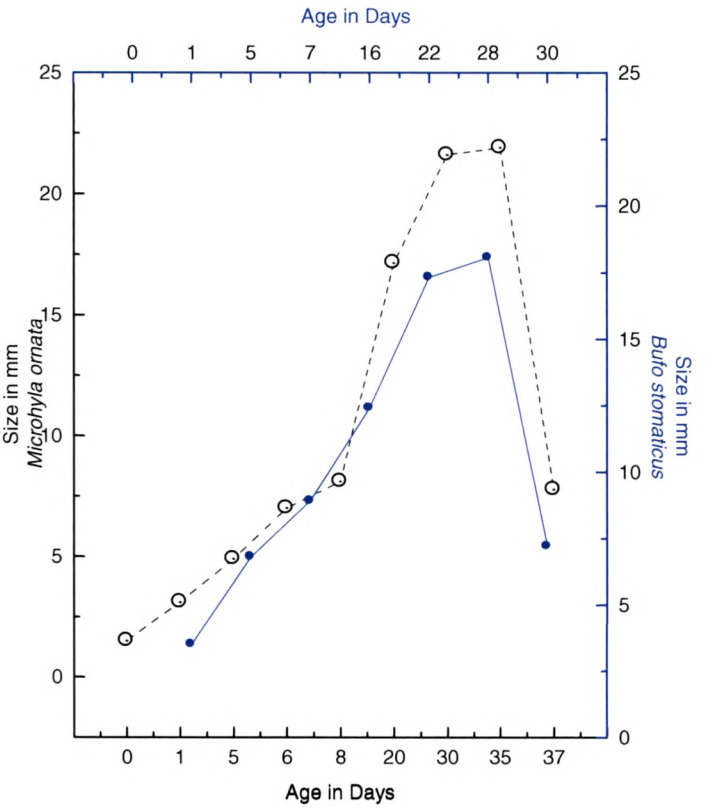


FIGURE 5.2 Growth curves of tadpoles of *B. stomaticus* and *M. ornata*

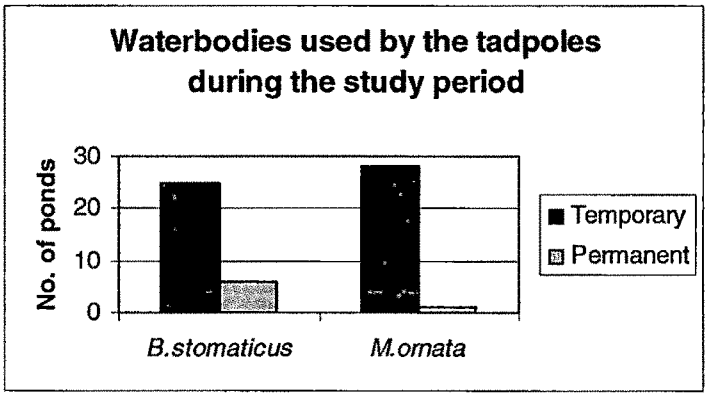


FIGURE 5.3 Water bodies used by the tadpoles during the study period

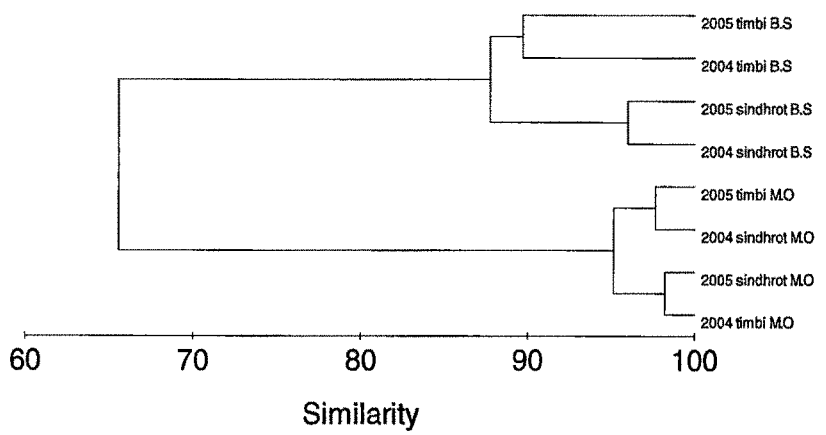
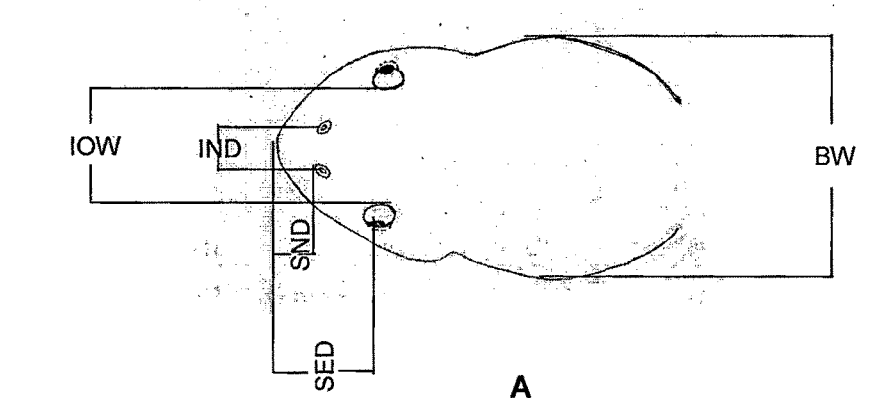
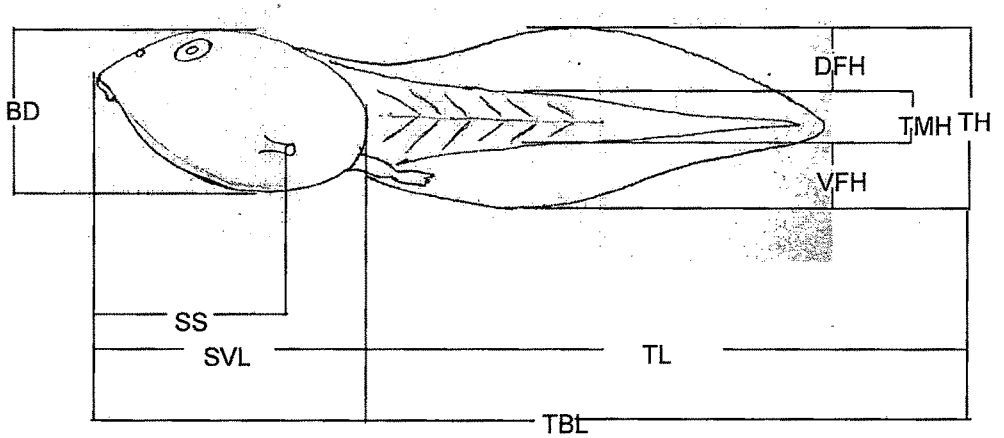


FIGURE 5.4 Cluster analysis of water bodies at both the site during the study period examining the similarity of their tadpole density and habitat variable



A



B

FIGURE 5.5 Schematization of the landmarks used for morphometric measurements
A-General profile, **B** - Lateral view;

BD-Body depth; BW-Body width, DFH- Dorsal fin height; IND- Internarial distance; IOB-Inter orbital distance;SED- Snout eye distance; SND- Snout narial distance; SS- Snout spiracle distance; SVL- Snout vent length; TBL Total body length; TH- Tail height; TL- Total length; TMH- Tail muscle height; VFH- Ventral fin height

FIGURE 5.6 Adult *M. ornata*

FIGURE 5.7 Amplecting pair of *B. stomaticus*

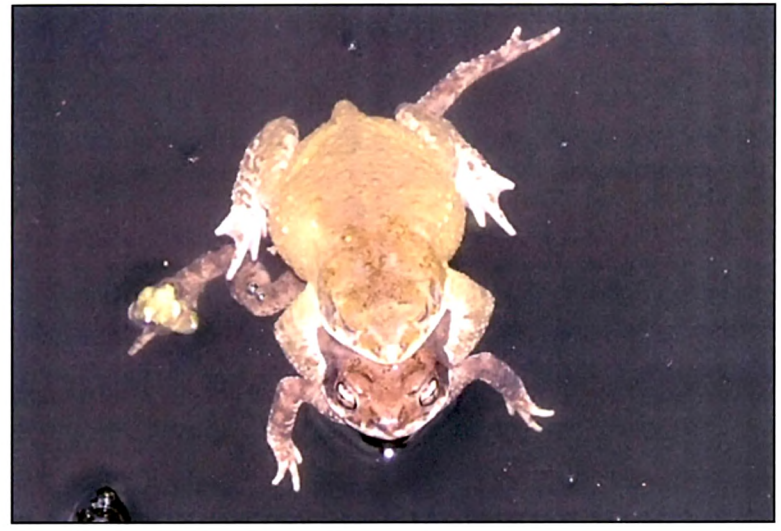


FIGURE 5.8 Breeding site (→)of *M. ornata*

FIGURE 5.9 Breeding site of *B.stomaticus*

FIGURE 5.10 Egg mass of *B. stomaticus*

FIGURE 5.11 Single string of eggs of *B. stomaticus*

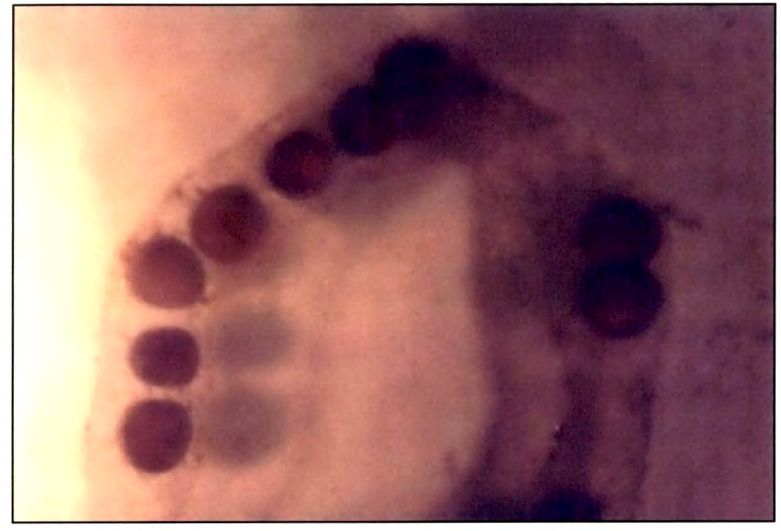


FIGURE 5.12 Egg mass of *M. ornata*

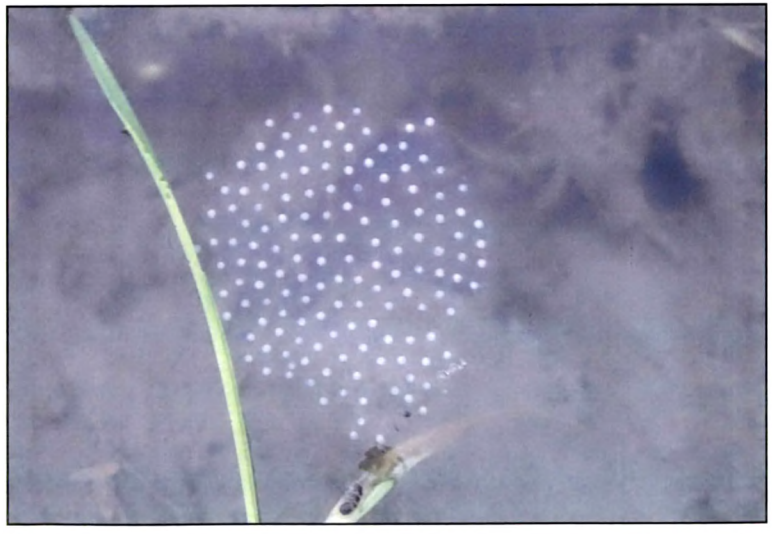


FIGURE 5.13 Single egg of *M. ornata*

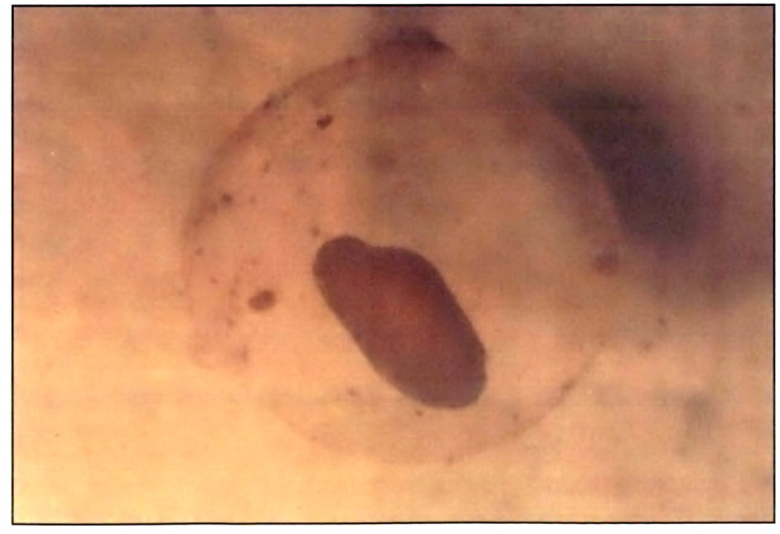


FIGURE 5.14 Oral disc of *B. stomaticus* tadpole at Gosner stage-37

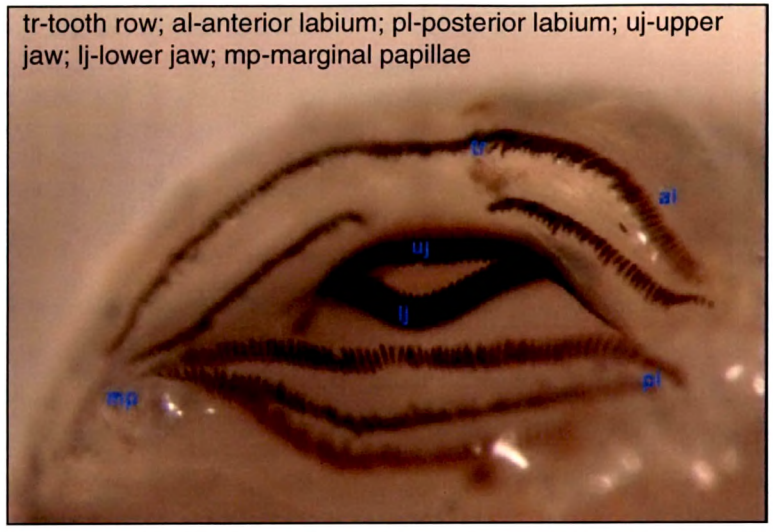


FIGURE 5.15 Scanning electron micrograph of oral disc of *B.stomaticus* tadpole

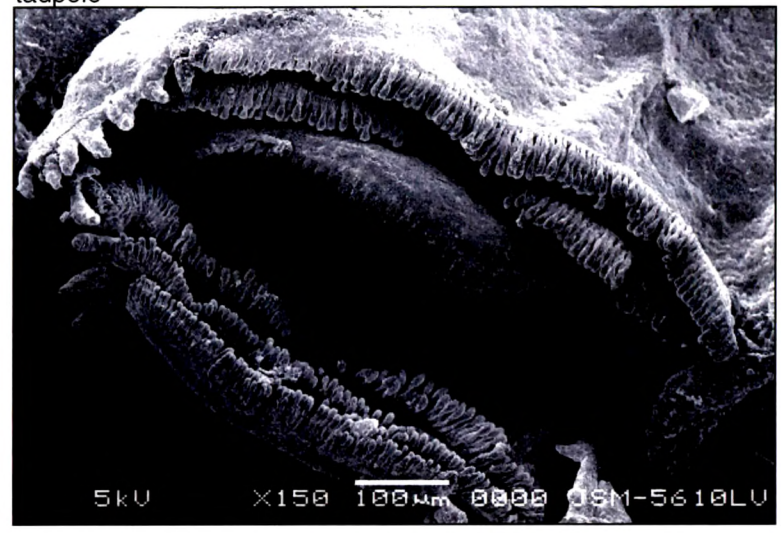


FIGURE 5.16 Scanning electron micrograph showing the beak of *B.stomaticus* tadpole

FIGURE 5.17 Scanning electron micrograph showing the serration in the beak of *B. stomaticus* tadpole

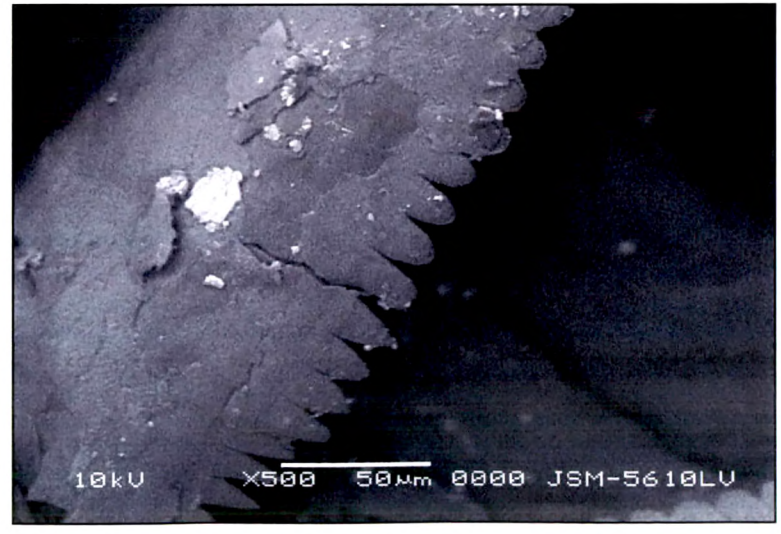


FIGURE 5.18 Scanning electron micrograph showing lower beak (↑) of *B. stomaticus* tadpole

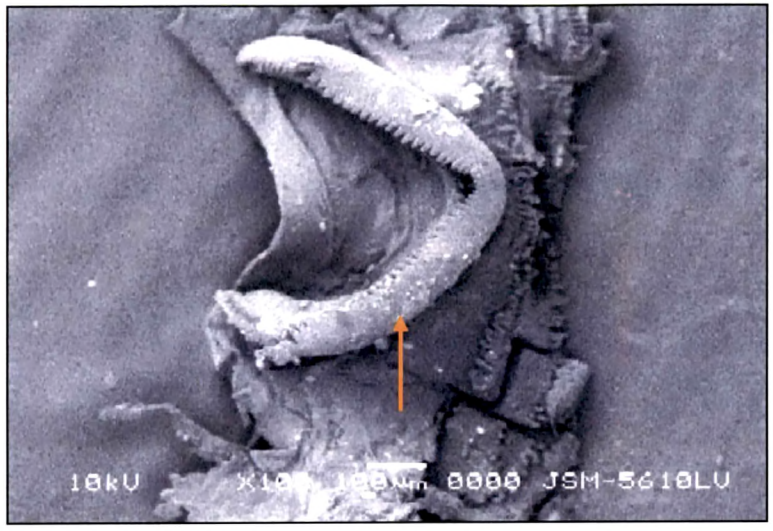


FIGURE 5.19 Scanning electron micrograph of larval *B. stomaticus* teeth showing cusps (←) in the upper labium.

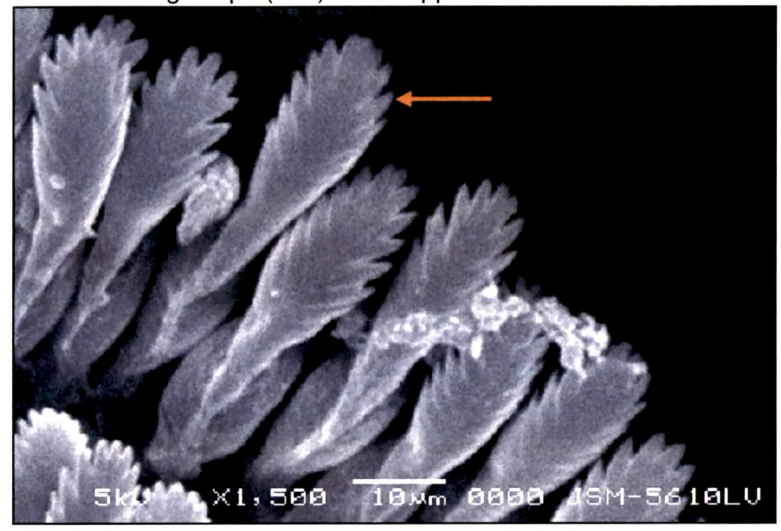


FIGURE 5.20 Scanning electron micrograph of larval *B. stomaticus* teeth in the lower labium

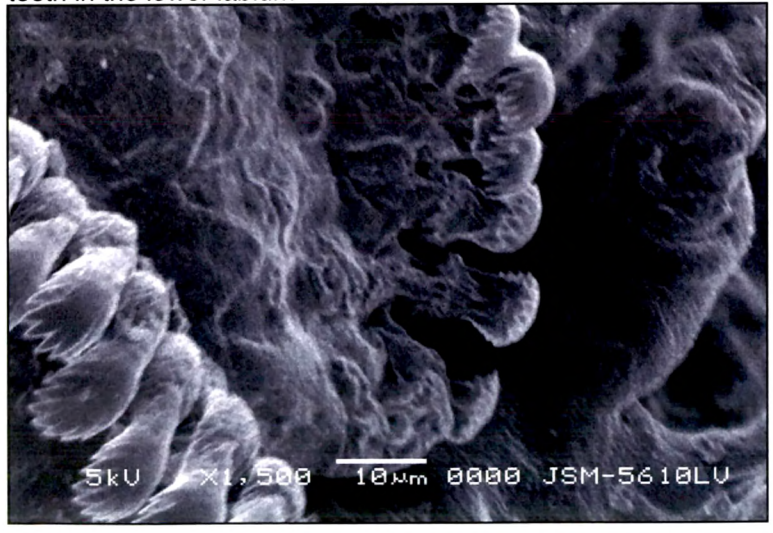


FIGURE 5.21 Scanning electron micrograph showing the oral disc of *M. ornata* tadpole at gosner stage 37

FIGURE 5.22 Tadpole of *B. stomaticus* at Gosner stage-21

FIGURE 5.23 Tadpole of B. stomaticus at Gosner stage-37

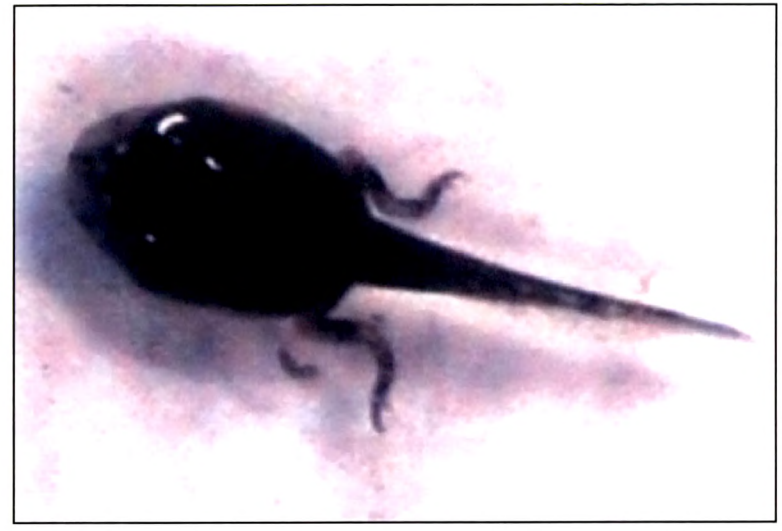


FIGURE 5.24 *B. stomaticus* tadpole at Gosner stage-44

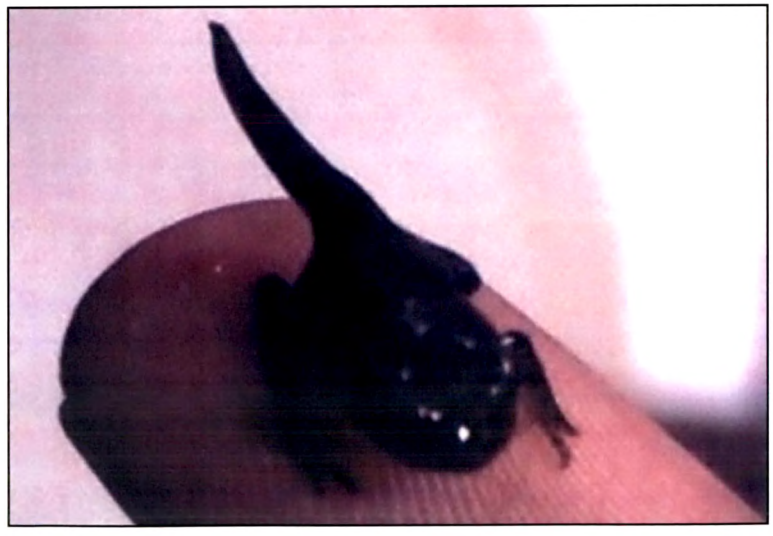


FIGURE 5.25 Froglet of *B. stomaticus* showing red spots

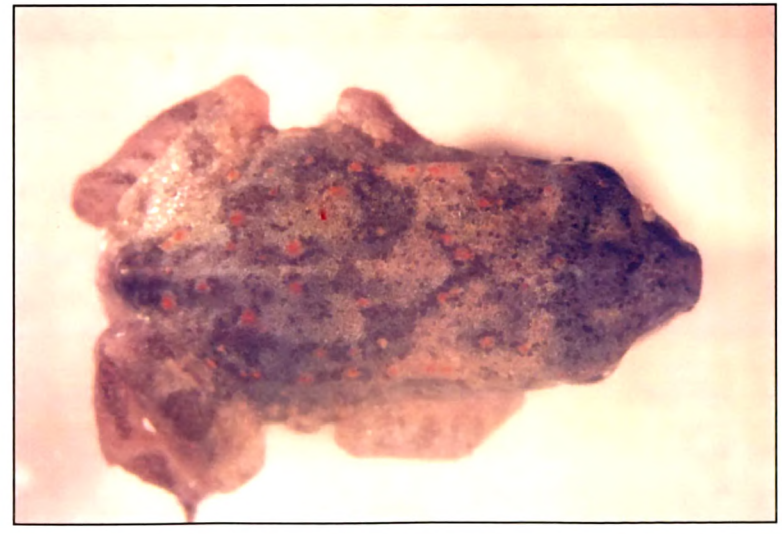


FIGURE 5.26 Developing eggs of *M. ornata*

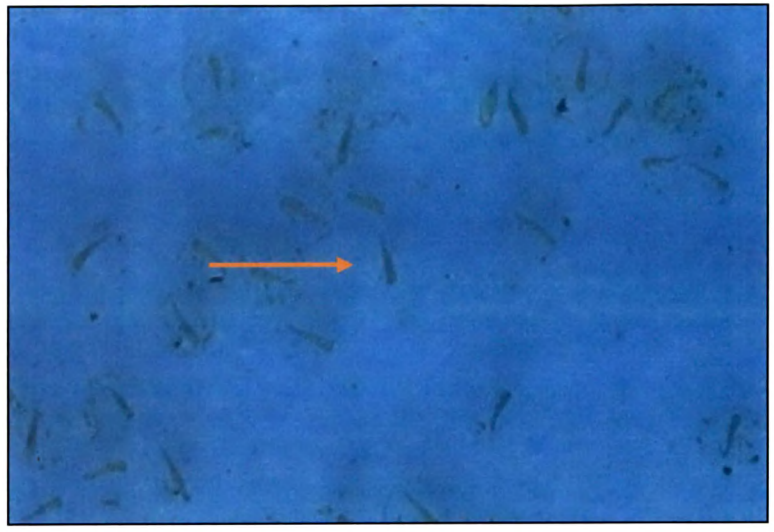


FIGURE 5.27 Tadpoles of *M. ornata* at gosner stage-30

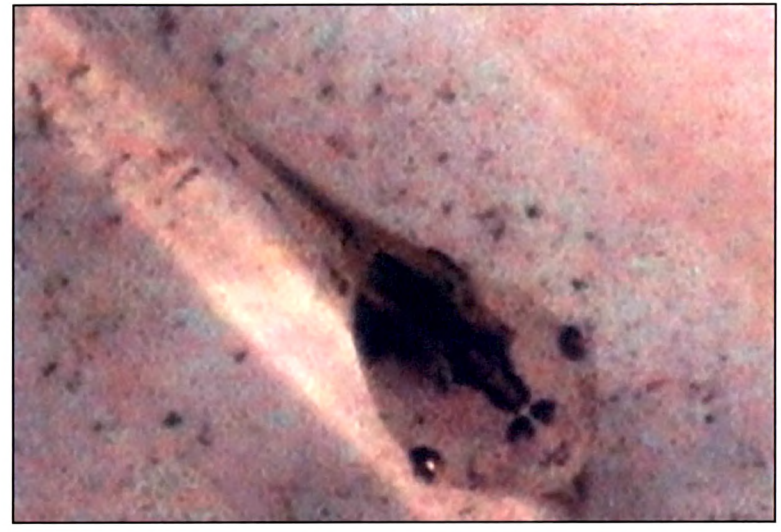


FIGURE 5.28 *M. ornata* tadpoles at Gosner stage-42

FIGURE 5.29 Froglet of *M. ornata*

FIGURE 5.30 High density of *B. stomaticus* tadpoles (→) in the water body

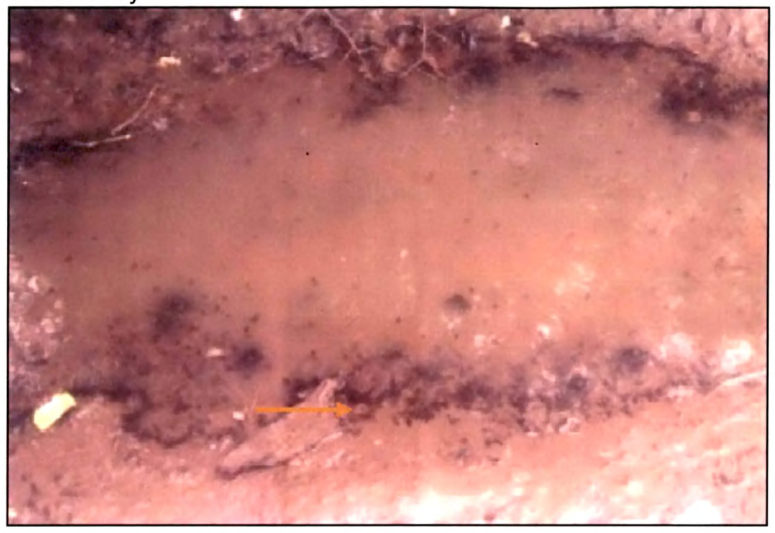


FIGURE 5.31 Tadpoles of *B. stomaticus* (→)feeding on carrion