frog leg

Newsletter of the Declining Amphibian Populations Task Force - South Asia

No. 11, August 2004

Molecular phylogeny of Indian Anura (Amphibia: Lissamphibia): a preliminary report

M.S. Dharne[1], H.V. Ghate [2]*, A.D. Padhye [3], Y.S. Shouche [1]

[1] *National Centre for Cell Science, Pune Maharashtra 411007, India.*

[2] *Zoology Department, Modern College, Pune, Maharashtra 411005, India.*

[3] *Zoology Department, Garware College, Pune, Maharashtra 411004, India.*

* *Corresponding author email: hemantghate@hotmail.com*

Anuran amphibians are a problematic group. Most frogs and toads look very similar, in general, as the overall body form is highly conserved. Families such as Ranidae are cosmopolitan and are distributed over most of the suitable habitats of the world but many are species of very restricted distribution. The systematics of this group (especially higher level phylogenetic relationship of Anura) is still incompletely resolved (Emerson *et al.*, 2000) and, until recently, many morphologically similar forms were lumped in to one or few genera. Genus *Rana* being one such speciose genus that had to be divided into several subgenera and several subgenera had to be elevated to generic rank in recent years. Even today the situation is far from clear as many Asiatic genera and species need to be studied in detail. For example genus *Philautus* is still problematic and genus *Polypedates* is included as subgenus of *Rhacophorus* by some but treated as valid genus by others. Earlier only morphological characters were available to base our phylogenetic interpretation but now the situation has changed. With the advent of PCR amplification technique and automated DNA sequencing facilities, it has become possible to construct "phylogenetic trees" on the basis of similarities/dissimilarities of gene sequences. The use of DNA sequences in anuran systematics is increasingly becoming popular and useful as a phylogenetic tool, as pointed out by Dawood, Channing and Bogart (2002). This analysis provides abundant data (or character states) for inferring phylogeny accurately as morphological data may be affected by adaptive traits or convergent evolution. These kinds of "molecular data" are rapidly being accumulated on the amphibians of different parts the world, as is evident from the sequences of various genes deposited in GenBank. Very few species of the Indian subcontinent have been studied at molecular level so far and hence we have initiated these studies at Pune (Modern College and National Centre for Cell Science, Pune, Maharashtra, India). The purpose of this project is:

1) to test some of the phylogenetic relationships proposed recently among the species of genus *Rana* sensu Boulenger;
2) to find out if there are any cryptic species, among local amphibians, that are morphologically indistinguishable; 3) to see how many of the recently suggested genera / families form monophyletic groups; and
4) to acquire molecular data for all the available species.

It must be emphasized that there are still surprises in store for us as far as Indian frogs are considered, as shown by recent discovery of an interesting frog by Biju and Bossuyt (2003). This frog turned out to be belonging to a new family, and, of course, to a new genus and species! This discovery has further thrown light on our ancient relationship with Seychelles.

It is our considered opinion that every effort must be made now to collect molecular, ethological and ecological data for our amphibians, whenever possible. This is not to suggest that morphological data are obsolete, because both, morphological and molecular data have been shown to contain significant phylogenetic information (Emerson *et al.*, 2000) to settle the issues in systematics and that no new classification is proposed without such data. This will improve knowledge of the systematics of our anurans and reduce the burden of names that become synonyms. We agree with Vences and Glaw (2001), who have pointed out problems with respect to systematics of superfamily Ranoidea and suggested that polyphyletic taxa be excluded and at the most a few paraphyletic taxa may be accepted.

Our analysis, at present, is based on sequence comparison of a small fragment of mitochondrial 12S rDNA (small subunit of mitochondrial ribosomal RNA gene) among common frogs and toads found in and around Pune. This is only a preliminary report, much analysis remains to be done, and we have tissue samples of at least 25 more species (from different parts of India) at hand that are being sequenced and analysed. We are presenting only a couple of "phylogenetic trees" here and rest of the analysis will be published in a detailed paper.

Salient findings of the project

The genus *Fejervarya* with species like *F. limnocharis*, *F. rufescens*, *F. greenii* and *F. syhadrensis*, is a complex group and
perhaps many different species are today recognized as *Fejervarya limnocharis. Hoplobatrachus* is closer to *Spheroteca* and the various species of *Spherotheca,* with *Hoplobatrachus,* form a clade with *Euphlyctis cyanophlyctis* as a sister species.

Limnonectes brevipalmata is closer to *Fejervarya sp.* from the northeast. In short, *Hoplobatrachus, Limnonectes, Fejervarya and Spherotheca* as well as *Euphlyctis* (all formerly under genus *Rana* and now placed in different genera) are grouped in a large clade. Relationship between these genera needs additional studies. These findings are more or less similar to those of Marmayou *et al*. (2000) who also found *Hoplobatrachus chinensis* (= *rugulosus*) and *Spherotheca pluvialis* forming a clade with *Fejervarya limnocharis* as a sister species. Richards, Nussbaum and Raxworthy (2000) found African *Hoplobatrachus occipitalis* and *Fejervarya limnocharis* to be in one clade while Vences *et al*. (2000) found *Fejervarya limnocharis* to be sister species to a clade of *Sphereotheca dobsoni* and *S. breviceps*. With respect to *F. greenii*, it is believed to be present in Sri Lanka only. It is necessary now to see whether morphologically similar species found in India is close to true *F. greenii* or it is a different species or perhaps a variant of *F. limnocharis,* as suggested by some workers (see Dutta, 1997).

Bootstrap support for all the clade/subclades in the group of species presented here, as shown in figure, is mostly above 50%. Further, it is clear that *Euphlyctis* is also closer to Ceratobatrachinae (sensu Dubois, 1992) members, as also found by Kosuch *et al*. (2001) and its earlier inclusion in subfamily Dicroglossinae (Marmayou *et al*., 2000) along with the genera *Phrynoglossus* and *Occidozyga* needs reconsideration.

All *Nyctibatrachus* species that we analysed are in one clade supported by high bootstrap score. Inclusion of available GenBank sequences of *N. major* and *N. aliciae* indicates that the latter two are different species as compared to our group. *Nyctibatrachus major* appears to be a basal species. *Nyctibatrachus* from Raigad and Tamhini-Mulshi are similar and are morphologically closer to *N. major* but it appears to be different (at molecular level) from the available *N. major* sequence. There are perhaps one or two cryptic species in this genus. It is also apparent that the two species *of Indirana* are forming separate clade that is basal to all other Ranidae members. These are not close to *Nyctibatrachus* as previously suggested. Thus it would be better to separate these two genera under Indiraninae and Nyctibatrachinae (rather than in one subfamily Ranixalinae sensu Dubois, 1992), as has been adopted by Bossuyt and Milinkovitch (2000). Our data are similar to that of Bossuyt and Milinkovitch (2000).

Limnonectes kuhli (sequence from GenBank) and *L. brevipalmata* included in this study are widely separated in this analysis, as shown earlier by Vences *et al*. (2000) and *L. kuhli* is sister to the large group containing *Hoplobatrachus*, *Fejervarya*, *Spherotheca*, *Euphlyctis* and *Limnonectes brevipalmata* (although the bootstrap support to this node is below 50%).

Microhylidae are grouped together, however *Rana malabarica* is present as a sister to this microhylid group in Figure 1. When other *Rana* sequences available from GenBank are included in the analysis and Microhylidae members are removed, *Rana malabarica* groups with other *Rana* species. *Nyctibatrachus* species are then grouped with *Rana* members in a clade (Figure 2).

Bufo scaber, *B. stomaticus* and *B. melanostictus* form one clade, similarly *Polypedates leucomystax* and *P. maculatus* form one clade, as expected. We are using the name *Polypedates* instead of *Rhacophorus* as used by Wilkinson *et al*. (2002). *Indotyphlus* or *Bufo* species were used as an outgroup. Traditional Rhacophoridae has been now considered as subfamily Rhacophorinae of family Ranidae (see Dubois, 1992), though there is no unanimity about this placement. More species are necessary to resolve this problem.

When African species of the genus *Tomopterna* sensu stricto (as suggested by Marmayou *et al*., 2000) are included (using GenBank sequences) in the analysis (data not shown here), these species form a separate monophyletic clade supporting the contention of other workers (Vences *et al*., 2000) that genus *Tomopterna* (as previously recognized) should be split to different genera. It is also certain that the Asian genus *Spherotheca* (also formerly treated as *Tomopterna*) is a totally different group with only similar morphological features and habits.

We feel that phylogeny work involving "*Fejervarya -Limnonectes complex*" of species will be a challenging task and much more material from different parts of the Indian subcontinent and Asia will have to be studied. It is also apparent that species *Fejervarya limnocharis*, as presently recognized, is perhaps a complex of many species / subspecies spread over entire Asia. Sumida et al. (2002), who have very recently analysed *limnocharis* populations from different islands of Japan and Taiwan, are of the opinion that- "...hereafter it will be necessary to bring together all aspects of the systematics of *R. limnocharis* complex widely distributed in Southeast Asia, and to comprehensively elucidate the phylogenetic relationships and evolutionary processes among them using various techniques such as morphological and ecological observations, karyotype and allozyme analysis, molecular DNA sequence analysis and crossing experiments.."

We thank our Institutions for facilities and encouragement. We also thank Dr. S.K. Dutta (Utkal University, Bhubaneshwar) and Dr. M.S. Ravichandran (ZSI, Chennai) who helped sorting some out taxonomical problems. Thanks are also due to Mr. Vivek Gour-Broome for sharing his experience and relevant data with respect to the local amphibians. We also thank Dr. Franky Bossuyt for encouragement and for pointing some errors that could now be corrected at proofs stage.

Materials and Methods

High Molecular weight genomic DNA was extracted from the crushed and powdered muscle and liver tissues or even toe-clips according to standard methods (Sambrook et al., 1989). Mitochondrial 12 S rDNA was partially amplified (380 bp) using the vertebrate universal primers **UniL1091** and **UniH1478** (Kocher et al., 1989). Amplification reactions were performed in PTC-200 MJR. For 25 ìl of reaction, conditions followed were: 94^{0}C denaturation for 1 min, 55^{0}C annealing for 1 min and 72^{0}C extension for 1 min, for 35 cycles. Positive PCR products were checked by agarose gel electrophoresis. PCR products were purified by PEG-NaCl protocol (20%PEG and 2.5mM NaCl).

The purified DNA samples were sequenced for 430 bp region, using ABI 310 DNA sequencer by capillary electrophoresis as per manufacturer's

Figure 1: A strict consensus neighbor-joining tree drawn by MEGA VERSION 2.1, showing the relationship between Rhacophoridae, Ranidae, Microhylidae and Bufonidae, with *Indotyphlus* sp. as outgroup

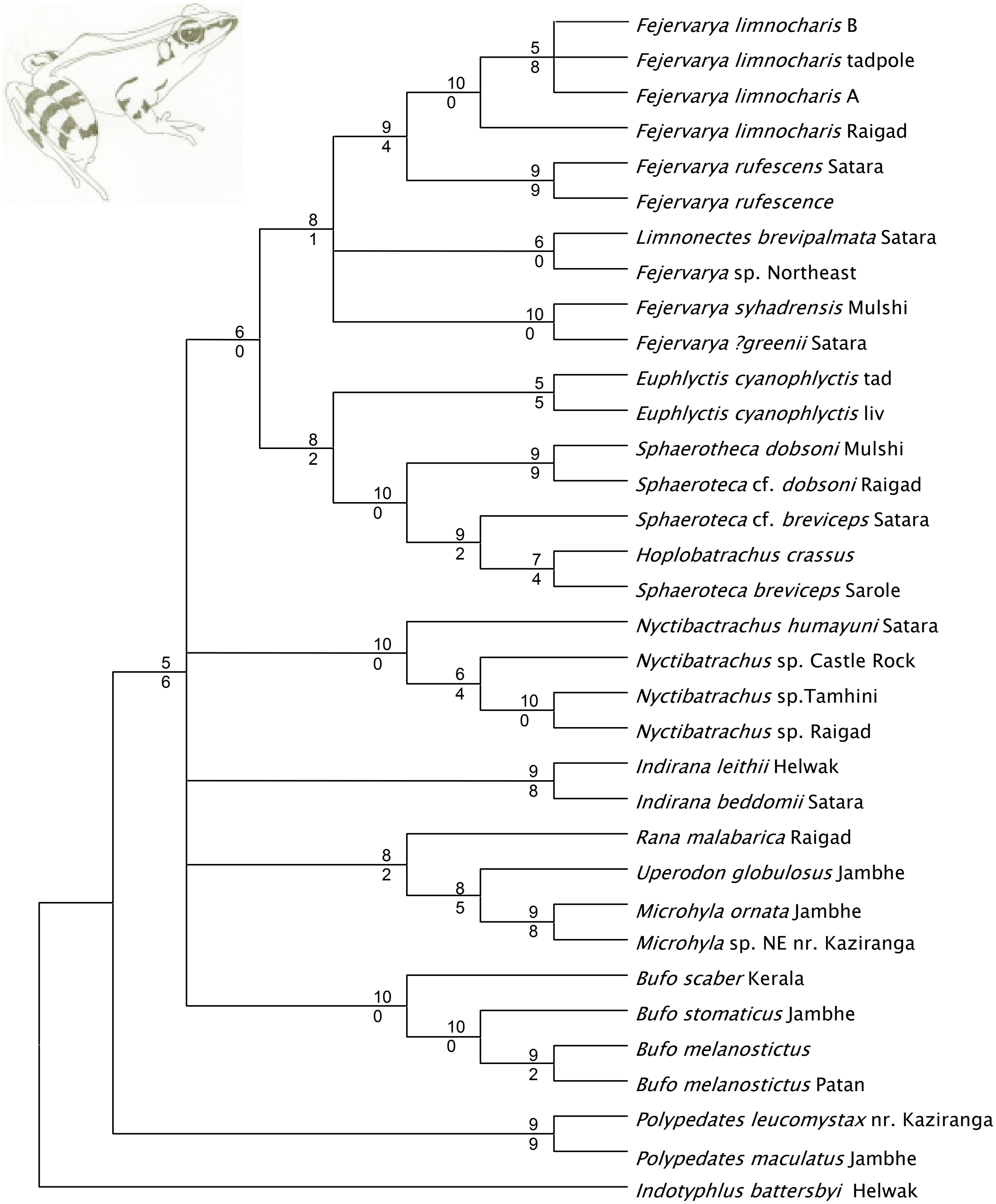
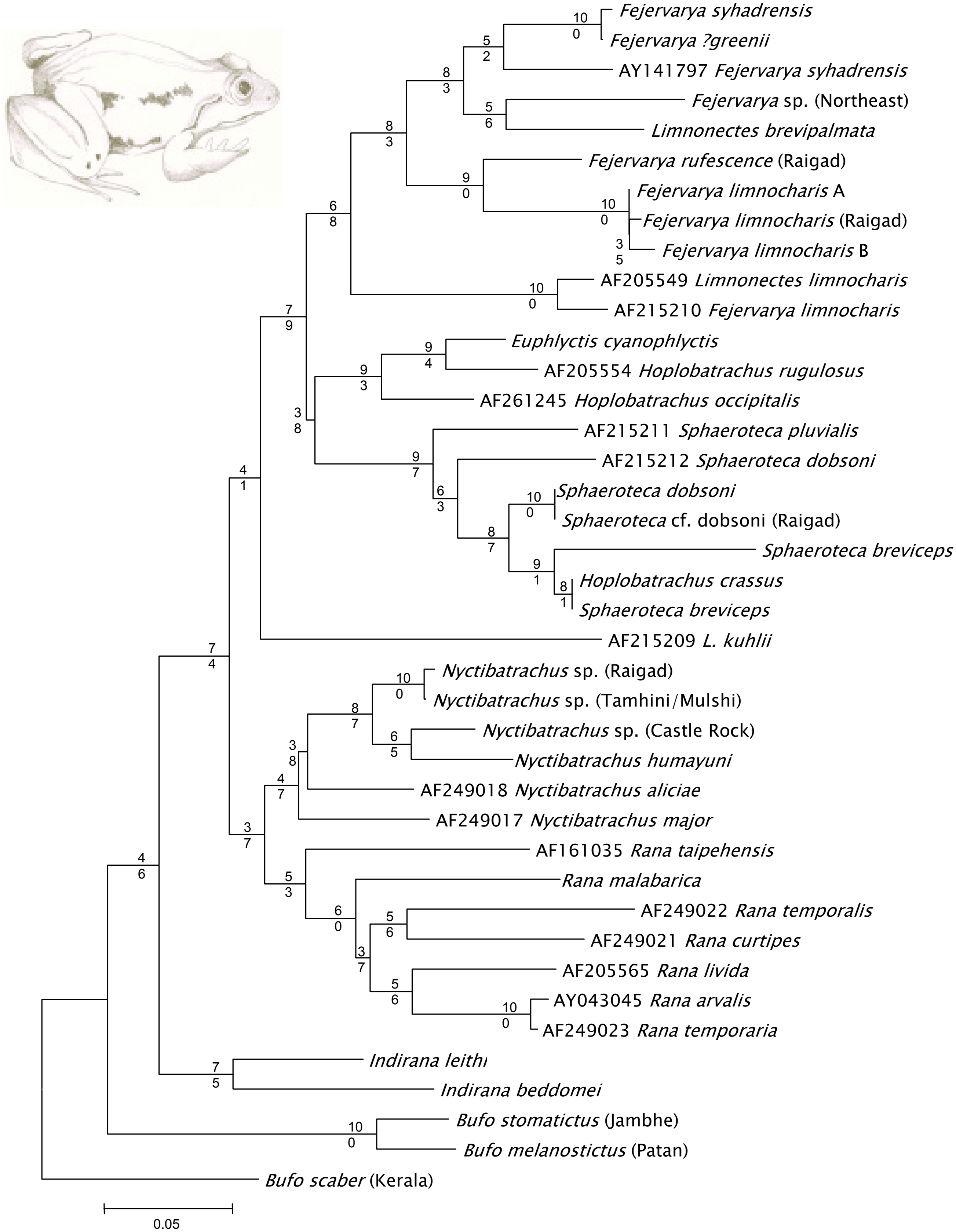


Figure 2: Phylogenetic relationship drawn using Jukes-Cantor parameter, indicating the relationship among Indian Anura using 12 S rRNA gene as a molecular marker, supported with bootstrap values ranging from 35 to 100, outgroup *Bufo*.



instructions. The sequencing reactions were performed using UniL 1091 primer on PE Gene Amp 9700 PCR machine. The conditions were 96^{0}C denaturation for 10sec, 50^{0}C annealing for 5 sec and 60^{0}C extension for 4 sec using fluorescent dye labeled nucleotides.

Sequences obtained were analyzed for closest homology database search by standard BLAST search provided by NCBI (http:/www.ncbi.nlm.nih.gov/blast). The sequences are being deposited in GenBank. The sequences were primarily aligned with default parameters of CLUSTAL W online software (http:/www.ebi.ac.uk). All taxa (Transition and Transversion) were weighted uniformly in the analysis.

Phylogenetic relationship among investigated taxa were analyzed by using Neighbor-Joining (NJ) method as described by Kumar *et al*., (2001), using the MEGA Version 2.1 (Molecular Evolutionary Genetic Analysis), in which genetic distance was measured by Jukes-Cantor (JC) model.

References

Biju, S.D. and Bossuyt, F. (2003). New frog family from India reveals an ancient biogeographical link with the Seychelles. *Nature* 425: 711-714

Bossuyt, F. & M.C. Milinkovitch (2000). Convergent adaptive radiations in Madagascan and Asian Ranid frogs reveal covariation between larval and adult traits. *Proceeding of National Academy of Science, USA*. 97: 6585-6590

Dawood, A., A. Channing & J.P. Bogart (2001). A molecular phylogeny of the frog genus Tomopterna in southern Africa: Examining species boundaries with mitochondrial 12S rRNA sequence data. *Molecular Phylogenetics and Evolution* 22: 407-413.

Dutta, S.K. (1997). *Amphibians of India and Sri Lanka*. Odyssey Publishing House, Bhubaneswar, Orissa.

Dubois, A. (1992). Notes sur la classification des Ranidae (Amphibiens Anoures). *Extrait du Bulletin Mensuel de la Societe Linneenne de Lyon* 61: 305-352.

Emerson, S., C. Richards, R.C. Drewes & K.M. Kjer (2000). On the relationship among Ranoid frogs: A review of the evidence. *Herpetologica* 56: 209-230.

Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S.F. Paabo, S.F. Villablanca & A. Wilson (1989). Dynamics of Mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceeding of National Academy of Science, USA*. 86: 6196-6200

Kosuch, J., M. Vences, A. Dubois, A. Ohler & W. Bohmer (2001). Out of Asia: Mitochondrial DNA evidence for an Oriental origin of tiger frogs, genus *Hoplobatrachus. Molecular Phylogenetics and Evolution* 21: 398-407

Kumar, S., K. Tamura, I.B. Jakobsen & M. Nei (2001). MEGA2: Molecular Evolutionary Genetics Analysis Software, Bioinformatics

Marmayou, J., A. Dubois, A. Ohler, E. Pasquet & A. Tillier (2000). *Compte Rendu Academie de Sciences, Paris., Sciences de la vie*, 323: 287-297

Richards, C., R.A. Nussbaum & C.J. Raxworthy (2000). Phylogenetic relationships within the Madagascan boophids and mantellids as elucidated by mitochondrial ribosomal genes. *African Journal of Herpetology* 49: 23-32.

Sambrook, J., E.F. Fritsch & T. Maniatis (1989). *Molecular Cloning: A laboratory manual.* Cold Spring Harbor, New York.

Vences, M., F. Glaw, J. Kosuch, I. Das & M. Veith (2000). Polyphyly of *Tomopterna* (Amphibia:Ranidae)based on sequences of mitochondrial 16S and 12S rRNA genes, and ecological biogeography of Malagasy relict amphibian groups. Diversite et Endemisme a Madagaskar, pp. 229-242. In: Lourenco, W.R. & S.M. Goodman (Eds.). *Memoires de la Societe de Biogeographie*. Paris.

Vences, M. & F. Glaw (2001). When molecules claim for taxonomic changes: New proposals on the classification of Old World treefrogs. *Spixiana* 24: 85-92

Wilkinson, J.A., R.C. Drewes & O.L. Tatum (2002). A molecular phylogenetic analysis of the family Rhacophoridae with an emphasis on the Asian and African genera. *Molecular Phylogenetics and Evolution* 24: 265-273.

2002 CAMP-GAA Report

The much-awaited Report on the status of amphibians of South Asia as worked out in the Conservation Assessment and Management Plan Workshop and Global Amphibian Assessment in July 2002 is to be launched soon in September 2004. The delay in the assessments have been due to the continuing confusion in amphibian taxonomy. This is hopefully resolved to some extent, but no surprises if it adds to more confusion, given the various sources and styles of species identification in the region and the overwhelming influence of foreign experts with specimens for comparison.

The Report will be in two forms -- the global perspective from GAA, which will have an analysis of the status of all amphibians of the World. The CAMP Report will have a regional focus with global assessments of endemic amphibians of the region and regional and national assessments for all the South Asian countries of amphibians with wider distribution.

Also to expect in these reports are a plethora of new species assessments, some of them yet formally undescribed.

Some salient features of the South Asian Amphibian CAMP Report will include:

1. Species assessments of all the described amphibians of the region according to the 2001 IUCN Red List Criteria and Categories.
2. Distribution information
3. Threats
4. Approximate range and area of occupancy
5. Synonyms
6. Distribution maps
7. Conservation measures / recommendations
8. Global status of endemic species
9. Regional status in South Asia
10. National status within each South Asian country.

Some Field Techniques and Sampling Methods for Amphibians

Anslem de Silva
Chair, DAPTF, Working group, Sri Lanka
Convener, CBSG Sri Lanka Net Work
kalds@sltnet.lk

This handout was prepared for the "Amphibian Hands-on Training" workshop at Thrissur, Kerala from 24 to 28 June, 2002. All techniques and sampling methods are collated from various books and papers on field techniques and sampling methods (see references), except the canopy technique, which the author carried out for one year at Horton Plains Ecosystem (a 3000-odd-hectare cloud forest at 2100m asl in Sri Lanka). It was felt that access to some papers listed may be difficult for Asian students to find. However, interested students can consult a few books, especially *Measuring and monitoring biological diversity: standard methods for amphibians* by Ronald Heyer *et al.* (1994), *Sampling amphibians in lentic habitats* by Deanna Olson *et al.* (1997) and *Herpetofauna workers' manual* by Tony Gent and S. Gibson.

Few important tips

Before commencing a field study first attend to the following:

1. Check for the ownership of the land you intend studying. If it belongs to the Department of Wildlife, Forestry, Company plantation or private sector, obtain approvals to carry out your project. State the names of the participants and their address, dates and times you will conduct the study.
2. Inquire from watchers and people around the study area you wish to conduct the study for possible trap guns, unprotected wells and wild animals (wild elephants, leopards etc).
3. Obtain relevant maps of the study area (e.g. contour, vegetation, soil etc).
4. If it is a short term study, check for the best season for the amphibians in the area you wish to study.
5. Laminate keys that you will use in the field (e.g. Gosner's scale of staging anuran larvae).
6. Print or duplicate the survey forms according to the number of plots, transects you wish to conduct. Duplicate a few extra copies which could be attached along with the final report.

Basic items required

Some basic items required by a field researcher:

1. Wellingtons or high boots for the entire team.
2. Powerful head lamps or torches for the entire team.
3. A good camera with a flash and close up lenses, tripod and other accessories.
4. Snake sticks and probing sticks - These could be home made or imported.
5. Dip-net.
6. Preservatives with relevant containers.

The following items could be carried in the multiple pocket field kits worn by professional cameramen. I find this jacket very useful and convenient which could carry all the following items.

1. A GPS with an altimeter. If a GPS is not available include a compass and an altimeter
2. Hygrometer to measure the Rh.
3. Thermometer to record the air temperature
4. Gastric canula for stomach flushing
5. Litmus paper for quick assessment of pH
6. Measuring tapes (in millimeters)
7. Collecting bags
8. A pocket knife
9. Pen and pencil
10. Rubber bands
11. Field note book
12. Plastic rain cape
13. Empty film cans for fragile specimens (gastrapod shells)
14. Strong nylon rope (5 to 10 meters).
15. Field water bottle.
16. First aid items such as a bandage, medicated plaster, antiseptic with cotton wool and a few paracetamol tablets.

Steps in designing a study

1. Define the goals of the study*
2. Select the study area
3. Select a suitable protocol for meeting the study goals
4. Select specific survey sites within the study area
5. Determine what data will be collected at each site
6. Determine what data analyses will be used to be sure the study design and survey protocol will provide the necessary data
7. Assemble and train field workers
8. Conduct the field work
9. Analyze the data
10. Prepare and distribute the final report
11. Publish results in a peer-reviewed journal

*(Is the goal to determine what amphibians occur in an area to measure their relative abundances, or to estimate the population size?)

Species presence is the most fundamental information that can be gained from a biological survey.

Relative abundance is the most commonly used measure of amphibian abundance. Abundance can be calculated in reference to either time, area, or effort (e.g. number of traps per night) for example, visual encounter surveys for amphibian larvae results in an index of individuals per hour for each species found [e.g 30 adult Common Hour-glass Tree-frogs (*Polypedates cruciger*) per hour]. Area-constrained surveys result in an index of individuals per area searched (e.g. 23 larval *Limnonectes* per $10m^2$). Sometimes the time spent searching is predominated and the technique is referred to as a time constrained survey.

Determination of population size is difficult and is normally done only as part of a detailed research involving a single species or a few ecologically similar species occupying a specific site (e.g. frogs and newts in a signal pond). To estimate population size, individual amphibians are usually marked, released, and recaptured. Several statistical models can be used to estimate population size based on the population of marked and unmarked individuals in subsequent samples (Donnelly & Guyer, 1994).

The study area

The study area must be clearly delineated. There are three basic ways to define the study area: by watershed, by management area, and by the habitat or range of a species.

Species habitat or range

This type of survey focuses on an individual species, typically one which is listed or believed to be rare. A single species survey would include all suitable habitats for that species within either the entire geographic range or an area of interest such as a National Forest or a State Park.

Complete survey

Complete surveys conducted in a naturally defined area (e.g. a watershed) provide the best information on the population status of amphibians, especially if the study area has boundaries which are barriers to dispersal. A complete survey includes all aquatic habitats within the study area, e.g., the entire length of all streams and rivers, and all ponds, lakes, and meadows.

Some survey methods

1. Visual encounter surveys (VES):

The field researchers walk through the study site for a prescribed time period, systematically searching for amphibians.

2. Audio strip transects (AST):

All calling frogs along a line transect is counted.

3. Transect sampling:

Transect sampling is ideal for studying elevational gradients from lowlands to uplands depending on the area of the study site. Mark the transects on the map (of the study area) so that it will cover most of the important vegetation and aquatic habitats. Five plots, a 100m x 6m each, leaving a gap of 50 to 100m should be used as a transect.

4. Quadrate sampling of forest floor frogs:

This simple technique by Allmon (1991), Jaeger & Inger (1994) is a widely used and accepted technique conducted in many parts of the world in assessing the forest litter frogs. A barrier is made of plastic netting measuring 5mx5m and 45cm high, and it is erected using metal rods (50cm long). Care should be taken not to walk inside the quadrate while erecting the netting.

Two or three persons, usually on their hands and knees, remove leaf-litter, logs, and boulders; approximately 3-4cm of the soil is checked for fosserial amphibians and their eggs, using standard gardening tools. One observer checks for animals attempting to escape.

The altitude in meters and other environmental data within the quadrate is recorded; relative humidity 1.5m above the ground inside the quadrate, air temperature (1.5m above the ground), and soil temperature in centigrade 1cm inside the soil is recorded as well.

The moisture content of the litter in the centre of the quadrate is measured by collecting all the leaf-litter within an area of $652\ cm^{2}$. The leaf-litter has to be collected from the centre of the plot into a polythene bag, which should be immediately tied securely with a rubber band to prevent moisture escaping and weighed. A label with the plot number is pasted on the bag. The litter emptied into a metal container should be placed inside an oven. The temperature should be raised to $150\ ^{o}C$. The oven is then switched off to allow gradual cooling. The dried litter is transferred into the same bag and re-weighed. The litter moisture content is calculated by subtracting the moisture content of the dried mass from the wet mass.

5. A Modified Drift Fence For Capturing Tree frogs:

Drift Fences have become standard equipment for catching amphibians, and their use has become central to studies on demography, population dynamics, and behavior of amphibians.

The fence consists of a support structure made of PVC pipes and nylon strings and a flexible barrier made of clear, roll plastic.

6. Canopy sampling:

Very few canopy studies have been conducted in Asia. The present method I used in my study at Herton Plains National Park (HPNP) was devised to check canopy and tree hole amphibian species.

i) A 10x10 meter quadrate was demarcated using a yellow nylon cord. A cluster of ten to fifty plots should be assessed 100m. apart.

ii) Two people climbed and investigated trees over 50cm dbh inside this quadrate. They checked the: a) crevices, b) tree holes, c)moss and ferns that grow on the trunk and branches and d) the canopy. The second person on the ground checked the loose bark of the trunk up to eye level and stays alert to catch any animal falling/jumping from the canopy.

iii) Specially made wire probes were used to check the tree holes. If a frog was present in a tree hole the dimensions of the tree hole was taken using a Holtain steel spring tape, and the height from the ground. If water was present the Ph was taken.

7. Patch sampling:

Amphibians are often found in specific microhabitats or patches such as underneath logs, tree holes and bromeliads. Patch sampling can be used to determine the number, relative abundances and densities in such patches.

8. Sampling at breeding sites:

Some species are common at breeding ponds and other water bodies. Data on the sex ratio, sizes and patterns could be collected at such sites.

9. Sampling of amphibian larvae:

There are many techniques of sampling anuran larvae: live seining, dip-net and the use of a bucket. I found seining smaller ponds to be effective and easy.

Additional tips for field workers

1. Method for measuring caecilians and salamanders in the field:

Caecilians and salamanders are difficult to measure in the field as they are slimy and because of their sinuous movements. Hence the following simple home made apparatus will help a field worker to measure these amphibians accurately. A transparent 2mm thick Plexiglas (Perspex) (30 x 15cm), a base plate 32 x 15cm on which a sponge (sheet) 1.5cm thick and 29 x 13cm pasted or fixed. The Plexiglas is attached with hinges. When measuring a caecilian or a salamander, the animal is kept on the inner surface of the Plexiglas. Moisten the Plexiglas before keeping the animal). Immediately cover the specimen with the sponge surfaced plate. The stop bolts prevent the full weight of the cover pressing on the animal. Measurements, photographing and other observations could be conducted without handling and harming the animal.

2. Staging anuran embryos and larvae:

The widely used key for staging anuran tadpoles is the Gosner's (1960) scale. I laminate this for use in the field.

3. Single hand restraining a frog or toad:

Hold the knee joint firmly between thumb and forefinger.

4. Marking techniques:
i. Toe clipping
ii. Tattooing
iii. Waistbands
iv. Photographic records of dorsal patterns

5. Stomach flushing:

The flushing apparatus consists of a syringe to which flexible tubing or a gastric canula is fixed. The frogs could be anesthetized using aqueous solution of tricaine methanesulfonate (1 g/l). However, I prefer not to anesthetize the animal.

Voucher specimen

Fixation and preservation:

Voucher specimen should be initially kept in proper positions so that it could be studied easily, fingers and toes extended so that webbing is distinct. Most specimens in major museums are initially preserved in 10% formalin and later transferred to ethanol. However, to prevent postmortem decomposition of soft amphibian tissues; and for rapid fixation the SSAR recommends formalin-alcohol-acetic acid (FAA) solution. Mix 10 parts of commercial (40%) formalin, 50 parts 95% alcohol (ethyl or isopropyl), 40 parts water and 2 parts glacial acetic acid (the acetic acid should be added just before use as it evaporates).

Tissue samples:

One or two toe tips are sufficient for mitochondrial DNA analysis.

Photographic vouchers:

Good colour photographs of the dorsal, ventral and lateral aspect including the fingers and toes with webbing, could be used as a voucher if the authorities do not allow preservation of the specimens.

Some Recommended References

Allmon, W.D. (1991). A plot study of forest floor frogs, Central Amazon, Brazil. *Journal of Tropical Ecology* 7: 503-522.

Burke, R.L. & S.R. Humphrey (1987). Rarity as a criterion for endangerment in Florida's fauna. *Oryx* 21(2): 97-102.

Burke, R.L. (1991). Relocations, repatriations and translocations of amphibians and reptiles: taking a broader view. *Herpetologica* 47(3): 350-357.

Bury, R.B. & P.S. Corn (1991). Sampling methods for amphibians in streams in the Pacific Northwest. *United States Department of Agriculture,* 29pp.

Donnelly M.A. & C. Guyer (1994). Mark-recapture, pp.183-200. In: Heyer *et al* *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians.* Smithsonian Institution Press.

Fellers, G.M. & K.L. Freel (1995). A standard protocol for surveying aquatic amphibians. *United States Department of the Interior,* 117pp.

French, D.D. (1994). Hierarchical richness index (HRI): a simple procedure for scoring 'richness', for use with grouped data. *Biological Conservation* 69: 207-212.

Gent, T. & S. Gibson (1998). Herpetofauna workers' manual. Peterborough, Joint Nature Conservation Committee 152pp.

Gosner, K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16: 183-190.

Jaeger, R.G. & R.F. Inger (1994). Quadrate sampling, pp. 97-102. In: Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians.

Heyer, W.A. *et al.* (Eds.) Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Smithsonian Institute, USA.

Leclerc, J. & D. Courtois (1993). A simple stomach flushing method for ranid frogs. *Herpetological review.* 24(4): 142-143.

Magurran, A.E. (1998) Ecological diversity and its measurement. Croom Helm Limited, London, 179pp.

Murphy, C.G. (1993). A modified drift fence for capturing tree frogs. *Herpetological review.* 24(4): 143-145

Olson D.H., W.P. Leonard & R.B. Bury (1997). Sampling amphibians in lentic habitats: methods and approaches for the Pacific Northwest. Society for Northwest vertebrate Biology, Olympia, Washington, 134pp.

Pisani G.R. (1973). A guide to preservation techniques for amphibians and reptiles. *Society for the Study of Amphibians and Reptiles. USA* 22pp.

Rice, T.M. & D.H. Taylor (1993). A new method for marking waistbands to mark anurans. *Herpetological Review* 24(4): 141-142.

Rodgem, W.A. (1991). Techniques for wildlife census in India, a field manual. Wildlife Institute of India, Dehra Dun, 82pp.

Designing amphibian population monitoring protocol

Karthikeyan Vasudevan

The importance of a "reliable" amphibian population monitoring protocol cannot be emphasized better in the wake of clearly documented amphibian declines globally (Houlahan *et al.*, 2000). In the early 1990s there were intense debates on what 'amphibian declines' were and how one should differentiate them from human impacts and natural population fluctuations (Peclunan & Wilbur, 1994). Today, there is no doubt that 'amphibian declines' are unique and can be identified as distinct, from other forms of biological diversity loss. The progress of our understanding of the phenomenon has led to identifying causal mechanisms (Kiesecker & Blaustein, 1995; Daszak *et al.*, 1999). The reports of amphibian declines have used variety of methods, those range from opportunistic records to rigorous experimental work or use of remote sensed data. Irrespective of the tool used to arrive at a judgement on 'amphibian declines' the primary objective of unambiguously documenting the change in amphibian population remains unchanged. This has thrown a challenge to herpeto-logists round the world to carry out intensive surveys using appropriate monitoring designs. The appropriateness of any monitoring design depends on the objective(s) of the study, the method(s) to be employed, and the amphibian(s) in question. However, there are some overriding factors that need to taken into consideration during the design of a protocol, which we will discuss here.

More the merrier

Every study will aim to sight as many target groups as possible. The primary consideration for the choice of a method should be 'Increase Detection'. Increased observations on the species will provide a better insight to the researcher about the biology of the species. The biologist should view any method that provides poor detection seriously and consultation with other biologists and a statistician is recommended. Too many zero detections in the data set will increase the variance and result in an inaccurate estimate. Stratification of sampling can be a choice that one might want to explore.

Improvisation is key to solving this problem, statisticians have a great deal of theoretical knowledge on addressing issues related to low detection. The use of adaptive cluster sampling is an example of such improvisation (Vasudevan *et al*., 1999).

Blind man's bluff

Recently, some statisticians opened the Pandora's box by highlighting the significance of spatial autocorrelation and its implications at arriving at reliable estimates. Why is that the poor biologist is always pinned against the wall? Biologists should tread carefully during the early stages of any study. One should ensure that the sampling is randomized so that each data point is providing a measure that reflects the 'true estimate' and the variation associated with it. Randomization is a painful process if one were roughing it out on the mountains looking for frogs, but it should be better to go through it than get nailed by the number pundits at the end of the study.

Doing the same thing again and again

A researcher might be arduously keeping counts of frogs in a pitfall trap, but these counts are within a sample. A 'large sample' will have several counts of animals repeated in a way that the mean of the sample will reflect a reliable estimate of the abundance of the frog in that area. If the same biologists asks what is a 'large sample'? The answer to this question is not easy. The size of a sample is inversely related to the amount of variability it captures. A statistician might go ahead and suggest that data based on counts in samples would tend to be normally distributed when samples are greater than 40. However, this is not thumb rule, one needs to examine the data as it is being collected. It varies with the target population and how heterogeneous it is.

The data dart board example

'Precision and accuracy' are two important attributes of a sample. How best the sample describes the 'universe' rests on these two attributes. Both precision and accuracy are desirable and should be aimed during any sampling design. However, in most studies in field biology accuracy is desired over precision. Monitoring protocols should build in these attributes to the sample in order to achieve successful results.

When everything else remains constant

Not all relationships in the data can be meaningful and can be attributed as causal mechanisms to the change documented in the population of amphibians. In order to tease out 'real causal relationships' one has to sieve the data through filters called 'controls'. In effect, these controls refine the analysis and remove extraneous influence on the data other than those that one wants to explore a causal relationship. Again one's wisdom during designing comes into fore, prior knowledge of the system plays a very important role. In the case of amphibian declines there are some good examples of this (Broomhall *et al*., 2000). Statistical tests cannot take care of the problem of interfering variables if proper controls are not built in the sampling design in the first place.

Ten most often committed mistakes

1. Sample only in places where the animal is found.
2. Continue the same sampling strategy when one has poor detections.
3. Stratify sampling without prior knowledge of the distribution of the target species.
4. Sample at constant intervals or proportions.
5. Few replicates and numerous pseudoreplicates.
6. Checking variance in data, when the period for data collection is over.
7. Statistical tests and software's are godly things; they will rectify all the above mistakes.
8. Avoid complications by not providing controls.
9. Null models are too much maths just discard them.
10. The results should always comply with the findings by renowned or reputed biologists. If not the data is faulty.

REFERENCES

Houlahan J,E., C.S. Findlay, B.R. Scmidt, A.H. Meyer and S.L. Kuzmin (2000). Quantitative evidence for global amphibian population declines. Nature 404: 752-755.

Peclunan, J.H.K., H.M. Wilbur (1994). Putting declining amphibian populations in perspective: natural fluctuations and human impacts. *Herpetological* 50: 65-84.

Kiesecker, J.M. and A.R. Blaustein (1995). Synergy between UV-B. Radiation and a pathogen magnifies amphibian embryo mortality in nature. *PNAS* 92:11049,11052.

Daszak, P., L. Berger, A.A. Cunningham, A.D. Hyatt, D.E. Green, and R. Spear (1999). Emerging infectious diseases and amphibian population declines. Emerging Infectious Diseases 5: 735-748.

Vasudevan K., R. Chellam and A. Kumar (1999). Structure and composition of forest floor amphibians in Kalakkad-Mundanthurai Tiger Reserve. *Current Science*

Broomhall, S.D., W.S. Osborne, and R.B. Cunningham (2000). Comparative effects of ambient Ultraviolet-B radiation on two sympatric species of Australian frogs. *Conservation Biology* 14: 420-427.

Decline of *Rana taipehensis* population in southern Assam

Mithra Dey
Senior Lecturer, Department of Zoology, G.C. College, Silchar 788004, India.
Email: mdey_19@ indiatimes.com

The northeastern region of India has been identified as one of the global biodiversity hot spots and is the home of about 72 species of amphibians (Chanda, 2002). The Barak Valley of southern Assam comprises three districts, *viz*., Cachar, Hailakandi and Karimganj (24°82' & 25°82'N and 92°15' & 93°15'E) and abounds in wetlands of various sizes, formed in the flood plains of River Barak and its tributaries, with low hills strewn inbetween. The myriad freshwater ecosystems along with thick tropical vegetation offer excellent habitats for anurans. During a survey conducted between November 1996 and December 2002, in this region ten species of anurans belonging to eight genera and four families where recorded (Table 1). These include *Microhyla ornata*, *Kaloula pulchra*, *Euphlyctis cyanophylictis*, *Fejervarya limnocharis*, *Hoplobatrachus tigerinus*, *Rana taipehensis*, *Polypedates leucomystax*, *Bufo melanostictus*, *Rana* sp. 1 and *Rana* sp. 2.

Rana taipehensis belonging to Family Ranidae was caught from a low lying marshy area in Silchar (24°50'N, 92° 51'E) in Cachar District. The area is thickly vegetated with *Enhydra fluctuans*, *Commelina benghalensis*, *Eclipta prostrata* and *Eicchornia* sp. This was the only habitat from which the frog was collected out of the total 41 sites surveyed. The number of specimens caught was only three (one in March 1997 and two in March 1998) and has not been sighted since then. (One of the specimens was deposited at ZSI Kolkata ZSI-A9090, one retained by the author and one was released in its habitat).

The limited number of specimen caught and the restricted distribution observed indicates that this species has a habitat preference and is becoming rare in this area. The sight from which this specimen was recorded is being drastically altered due to construction of a residential complex. Silchar is rapidly undergoing urbanization and low lying areas are being reclaimed for development. Ahmed and Goswamy(1999) also observed *Rana taipehensis* to be a rare species in and around Guwahati, Assam. Subsequently, Dahmer *et al.*, (2001) assigned this species threatened status and recommended statutory protection in Hong Kong while Li Yi Cheng and Lin Hua Chin (2002) reported it as Vulnerable with restricted distributed from Taiwan. However, Choudhury *et al.* (2001) have observed it to be abundant in Kamrup District of Assam.

The primary reason for decline is habitat destruction due to urbanization and increasing human settlement. In addition, improper waste disposal like polythene bags plastics etc, into the unused ponds as well as the marshy habitat is resulting in loss of breeding grounds. It is therefore essential to develop awareness among the local residents regarding the conservation of this species and also give protection to the habitat in which it was found. It is also suggested that artificial rearing of this species can be taken up to prevent its total loss from this area.

ACKNOWLEDGEMENT

I am grateful to Dr. S.K. Chanda, Zoological Survey of India, Kolkatta for identifying the *Rana taipenhensis* specimens and I also gratefully acknowledge UGC for financial assistance.

Table 1: Anuran species of Barak Valley recorded during November 1996-December 2002

Species	Registration No.	Status
Family: Bufonidae		
1. *Bufo melanostictus* Schneider 1799	ZSI A 9092, 9093	Abundant
Family: Ranidae		
2. *Euphlyctis cyanophlyctis* Schneider 1799	ZSI A 9076, 9081-86	Abundant
3. *Fejervarya limnocharis* Gravenhorst, 1829	ZSI A 9087, 88	Abundant
4. *Hoplobatrachus tigerinus* Daudin, 1803	ZSI A 9089	Common
5. *Rana taipehensis* Van Denburgh, 1909	ZSI A 9090	Very rare
Family: Microhylidae		
6. *Kaloula pulchra* Gray, 1831	ZSI A 9094	Rare
7. *Microhyla ornata* Dumeril & Bibron, 1841	ZSI A 9080, 9079	Rare
Family: Rhacophoridae		
8. *Polypedates leucomystax* Gravenhorst, 1829	ZSI A 9091	Abundant
9. *Rana* sp. 1		Rare
10. *Rana* sp 2		Rare

REFERENCES

Ahmed, M. and U.C. Goswami (1999) Amphibians in and around Gauhati University, Guwahati, Assam. *Frogleg* 3(2): 3

Chanda, S.K. (2002). Northeast India is one of the biodiversity hotspots conservation sites of the world. *Frogleg* 10: 10.

Choudhury, N.K., B. Hussain, M. Baruah, S. Saikia and S. Sengupta (2001). Fauna of Kamrup District, Assam, with notes on their natural history. *Hamadryad* 26(2): 276-282.

Dahmer, T.D, K.H. Kai and M.L. Felley (2001). Porcupine, Newsletter of the Deptt. of Ecology and Biodiversity, Univ. of Hong Kong.

Cheng, L.Y. and L.H. Chin (2002). Program of Taipei Grass Frog (*Rana taipehensis*) *in situ*. Taipei Zoo, Taiwan in *Zoo's Print* XVII(11): 15.

Hoplobatrachus crassus swallowing a *Polypedates maculatus* in Sri Lanka

Roshan K. Rodrigo, V.A.M.P.K Samarawickrama, Naalin Perera, M. Sandun, J. Perera, T.N. Peries and C.N.B. Bambaradeniy

IUCN-The World Conservation Union, 53, Horton Place, Colombo 07, Sri Lanka.

Email: rodrigo_roshan@hotmail.com

The observation was made on 2 November 2002 in Kalametiya Wildlife Sanctuary around 2030hr. The location was adjacent to the road leading to Kiula -Kalametiya fishing harbour and the habitat was a seasonal pond. The shallow pond had accumulated water from the mid inter-monsoon showers. Grasslands and scrublands were the surrounding vegetations. Species identification was done by their colour patterns and other morphological character based on Dutta and Manamendra-Arachchi (1996). Prey (*P. maculatus*) was identified before it was swallowed; its colour pattern and well developed disk on tips of digits.

An adult *H. crassus* (Jerdon's Bull Frog) was swallowing another adult *P. maculatus* (Chunam Tree-frog) at the edge of the pond. *P. maculatus* was being swallowed slowly from its head end. Swallowing took place for about 8 minutes. *Microhyla ornata* (Ornate narrow mouth frog), *Fejervarya limnocharis* (Common paddy field frog) and *Uperodon systoma* (Balloon frog) were also recorded from the same pond during the time of observation. Most amphibian larvae are cannibalistic (Pough *et al.*, 2001). Bambaradeniya (1997) had recored a *H. crassus* swallowing a microhylid (*Microhyla rubrum*) in a rice field at Bathalagoda, Sri Lanka.

ACKNOWLEDGEMENT

I would like to thank Dr. Deepani Jayantha for her kind assistance to prepare this short communication.

REFERENCES

Bambaradeniya, C.N.B. (1997). Observation of a Ranid (*Hoplobatrachus crassus*) swallowing a Microhylid (*Microhyla rubrum*). *Lyriocephalus* 3(2): 32.

De Silva, A. (1996). The herpetofauna of Sri Lanka: a brief review. Published by the author, pp. 38-43.

Dutta, S.K. and K. Manamendra-Arachchi (1996). *The Amphibian Fauna of Sri Lanka*. The Wildlife Heritage Trust, Sri Lanka.

Pough, F.H. *et al.* (2001). *Herpetology*, pp. 436-437. 2nd Edition. Prentice-Hall, Inc. New Jersey.

ABSTRACTS FROM VARIOUS JOURNALS

Conservation Biology, 2001, 15(4): 903–913

Amphibian declines and environmental change: Use of remote-sensing data to identify environmental correlates

Cynthia Carey, W. Ronald Heyer, John Wilkinson, Ross A. Alford, J.W. Arntzen, Tim Halliday, Laura Hungerford, Karen R. Lips, Elizabeth M. Middleton, Stan A. Orchard & A. Stanley Rand

Populations of many amphibian species are declining worldwide, and a few species appear to have become extinct. In an attempt to evaluate the potential usefulness of remote-sensing techniques as a tool for identifying the causes of these declines, we compiled a database that contains descriptions of 120 localities, both at which declines have been documented and at which no declines are yet known. The number of species involved, dates and degree of declines, habitat characteristics, and other factors are provided for each locality. Four relatively undisturbed areas in northeastern Australia, Costa Rica-Panama, central Colorado, and Puerto Rico were chosen for examination of environmental correlates coincident with mass mortalities at these localities. We used data predicted by models or collected by satellites, airplanes, or direct sampling on the ground to evaluate variations over time in temperature, precipitation, wind direction, UV-B radiation, and concentrations of certain contaminants at these sites. We asked whether unusual changes in these environmental variables occurred either just in advance of or concurrent with dates of amphibian mass mortalities. The variation in certain environmental variables documented by others (Alexander & Eischeid 2001; Middleton et al. 2001; Stallard 2001 [all this issue] appears unlikely to have directly caused amphibian deaths. But correlations between these environmental changes and the occurrence of amphibian die-offs invite further investigation into synergistic interactions among environmental variables and possible indirect causal relationships.

- -

Conservation Biology, 2001, 15(4): 914–929

Evaluating ultraviolet radiation exposure with satellite data at sites of amphibian declines in Central and South America

Elizabeth M. Middleton, Jay R. Herman, Edward A. Celarier, John W. Wilkinson, Cynthia Carey & Robert J. Rusin

Many amphibian species have experienced substantial population declines or have disappeared altogether during the last several decades at a number of amphibian survey sites in Central and South America. Our study addresses the use of trends in solar UV-B radiation exposure (280–320 nm) at these sites over the last two decades, derived from the Total Ozone Mapping Spectrometer satellite data. It is intended to demonstrate a role for satellite observations in determining whether UV-B radiation is a contributing factor in amphibian declines. We used these data to calculate the daily erythemal (sunburning)) UV-B, or UV-B$_{ery}$ exposure at the latitude, longitude and elevation of each of 20 survey sites. The annually averaged UV-B$_{ery}$ dose,as well as the maximum values, have been increasing in both Central and South America, with higher levels reached at the Central American sites. The annually averaged UV-B$_{ery}$ exposure increased significantly from 1979–1998 at all 11 Central American sites we examined (r^2 =0.60–0.79; p d” 0.015) with smaller but significant increases at five of the nine South American sites (r^2=0.24–0.42;p d” 0.05). The number of days having the highest UV-B exposure (e”6.75 kJ/m^2/day) increased in both regions from ,< 40 days per year to approximately 58 days per year in 1998 (r^2= 0.24–0.42; p d” 0.001). In Central America, the contribution of these very high UV-B$_{ery}$ total increased from approximately 5 to approximately 15% over the 19-year period, but actual daily exposures for each species are unknown. A UV-B ratio, the highest monthly UV-B exposure relative to the annual average for the highest UV-B category (e” 6.75 kJ/m2), increased in both regions over this time period (r^2 = 00.73;p d” 0.001). This UV index was consistently higher for Central America, where species declines have been the most severe. These results should justify further research into whether UV-B $_{ery}$ radiation plays a role in amphibian population declines and extinctions. We discuss synergy among UV-B radiation and other factors, especially those associated with alterations of water chemistry (e.g., acidification) in aqueous habitats.

- -

Conservation Biology, 2001, 15(4): 943–953

Possible environmental factors underlying amphibian decline in eastern Puerto Rico: analysis of U.S. government data archives

Rober F. Stallard

The past three decades have seen major declines in populations of several species of amphibians at high elevations in eastern Puerto Rico, a region unique in the humid tropics because of the degree of environmental monitoring that has taken place through the efforts of U.S. government agencies. I examined changes in environmental conditions by examining time-series data sets that extend back at least into the 1980s, a period when frog populations were declining. The data include forest cover; annual mean, minimum, and maximum daily temperature; annual rainfall; rain and stream chemistry; and atmospheric-dust transport. I examined satellite imagery and air-chemistry samples from a single National Aeronautics and Space Administration aircraft flight across the Caribbean showing patches of pollutants, described as thin sheets or lenses, in the lower troposphere. The main source of these pollutants appeared to be fires from land clearing and deforestation, primarily in Africa. Some pollutant concentrations were high and, in the case of ozone, approached health limits set for urban air. Urban pollution impinging on Puerto Rico, dust generation from Africa (potential soil pathogens) and tropical forest burning (gaseous pollutants) have all increased during the last three decades, overlapping the timing of amphibian declines in eastern Puerto Rico. None of the data sets pointed directly to changes so extreme that they might be considered a direct lethal cause of amphibian declines in Puerto Rico. More experimental research is required to link any of these environmental factors to this problem.

- -

Conservation Biology, 2001, 15(4): 930–942

Climate variability in regions of amphibian declines

Michael A. Alexander & Jon K. Eischeid

We explored the relationship between amphibian declines and climate variations in Colorado (U.S.A), Puerto Rico, Costa Rica-Panama and Queensland (Australia) through two sources of data: output from the National Center for Environmental Prediction "reanalysis system" and area-averaged station data. The reanalysis system merges observations from airplanes, land stations, satellites, ships, and weather balloons with output from a weather-forecast model to create global fields of atmospheric variables. Station data consisted of temperature and precipitation measured with thermometers and rain gauges at fixed locations. Temperatures were near normal in Colorado when the amphibian declines occurred in the 1970s, whereas in Central America temperatures were warmer than normal, especially during the dry season. The station data from Puerto Rico and Australia indicated that temperatures were above normal during the period of amphibian declines, but reanalysis did not show such a clear temperature signal. Although declines occurred while the temperature and precipitation anomalies in some of the regions were large and of extended duration, the anomalies were not beyond the range of normal variability. Thus, unusual climate, as measured by regional estimates of temperature and precipitation, is unlikely to be the direct cause of amphibian declines, but it may have indirectly contributed to them. Previous researchers have noted that the declines appear to have propagated from northwest to southeast from Costa Rica to Panama and from southeast to northwest in Queensland, Australia. Wind has the potential to transport pathogens that cause amphibian mortality. The mean direction of the near-surface winds tended to parrallel the path of amphibian declines from July-October in Central America and from May-July in Australia. The wind direction was highly variable, however, and the propagation rate of amphibian declines was much slower than the mean wind speed. In addition, the most likely pathogen is a chytrid fungus that does not produce desiccation-resistant spores. Thus, if wind is involved in the progpagation of amphibian declines, it is through a complex set of processes.

Conservation Biology, 2001, 15(4): 1175-1178

Climate warming and calling phenology of frogs near Ithaca, New York, 1900-1999

James P. Gibbs & Alvin R. Breisch

Because ambient temperature strongly influences reproduction in frogs, the seasonal timing of frog calling provides a sensitive index of biotic response to climate change. Over the last century, daily temperatures increased during 5 of the 8 months key to gametogenesis in frogs and toads near Ithaca, New York (U.S.A). Earliest dates of calling frogs recorded by Albert Hazen Wright between 1900 and 1912 near Ithaca were compared to those from the New York State Amphibian and Reptile Atlas Project for 1990-1999 for the three countries surrounding Ithaca. Four species are now calling 10-13 days earlier, two are unchanged, and none is calling later. The data suggest that climate has warmed in central New York State during this century and has resulted in earlier breeding in some amphibians - a possible first indication of biotic response to climate change in eastern North America.

Conservation Biology, 2001, 15(4): 1064-1070

Transfer of a pathogen from fish to amphibians

Joseph M. Kiesecker, Andrew R. Blaustein & Cheri L. Miller

Ecological studies of exotic species focus primarily on how invaders directly affect particular resident species. In contrast, little is known about the indirect effects of introduced species on native communities, including how pathogens may be spread by introduced species. We provide evidence suggesting that introduced fish may serve as a vector for a pathogenic oomycete, *Saprolegnia ferax,* that has been associated with embryonic mortality of amphibians in the Cascade Mountains of Oregon, U.S.A. In laboratory experiments, mortality induced by *S. ferax* was greater in western toad (*Bufo boreas*) embryos exposed directly to hatchery-reared rainbow trout (*Oncorhynchus mukiss*) experimentally infected with *S. ferax* and hatchery reared trout not experimentally infected than in control embryos. Embryos also developed significant *S. ferax* infections when raised on soil that was exposed to trout experimentally infected with *S. ferax* Furthermore, toad embryos exposed to *S. ferax* isolated from sites where *Saprolegnia* outbreaks are common experienced higher mortality than embryos exposed to *S. ferax* isolated from sites where *Saprolegnia* outbreaks have not occurred. Given the widespread practice of introducing hatchery-reared fishes, we suggest that fish used in stocking programs could be an important vector for diseases responsible for amphibian losses.

Conservation Biology, 2001, 15(4): 1071-1078

Effects of road traffic on two amphibian species of differing vagility

Laurie W. Carr & lenore Fahrig

Vehicular traffic can be a major source of mortality for some species. Highly vagile organisms may be at a disadvantage in landscapes with roads because they are more likely to encounter roads and incur traffic mortality. To test this prediction, we assessed the population abundance of two anuran species of differing vagility, the leopard frog (*Rana pipiens*, more vagile) and the green frog (*Rana clamitans*, less vagile), at 30 breeding ponds. Traffic density, an index of the amount of potential traffic mortality, was measured in concentric circles radiating from the ponds out to 5 km. We conducted multiple linear regressions relating population abundance to traffic density, pond variables, and landscape habitat variables and found that leopard frog population density was negatively affected by traffic density within a radius of 1.5 km. There was no evidence that the presence of vehicular traffic affected green frog populations. These results suggest that traffic mortality can cause population declines and that more vagile species may be more vulnerable to road mortality than less vagile species.

Conservation Biology, 2001, 15(4): 1079-1090

Edge effects on lizards and frogs in tropical forest fragments

Martin A. Schlaepfer & Thomas A. Gavin

We investigated whether forest-pasture edges affect the distribution of an assemblage of smaller vertebrate ectotherms in a consistent and predictable manner. We describe the abundance and distribution of two species of anoline lizards (*Norops*) and five species of leaf-litter frogs (*Eleutherodactylus*) along the edges and in the interiors of nine forest fragments near Las Cruces, Costa Rica. Over 4 months, we surveyed 44 pairs of plots by visual encounter. In each pair of plots, one was immediately adjacent to the pasture and the second was within the forest "interior". Both plots of a pair were searched simultaneously. This block design controlled for the effects of weather, topography, and searcher ability. The distribution of all species was highly variable with respect to edges. Only two species of frogs, *Eleutherodactylus podiciferus* and *E. cruentus*, were significantly more abundant in interior plots than in edge plots, although not consistently so. Both species of *Norops* lizards were more abundant in interior plots than in edge plots, although not consistently so. Both species of *Norops* lizards were more abundant along forest edges during the dry season. Both *Norops* species and several *Eleutherodactylus* species, however, appeared to become more abundant in the forest interior after the onset of the wet season, suggesting a seasonal edge effect. In *Norops polylepis*, the most abundant anole, rates of ectoparasitism were lower along edges than in forest interiors. The magnitude of the edge effect on any one species was not influenced by the size of fragments or by the distance of the interior plot from the nearest edge. We believe that edge effects should not be defined by the distance to which they are detected. Rather, they should be viewed as highly dynamic in space and time; taxa appear to respond to different components of edge effects according to their particular biological requirements.

Zoos' Print Journal, 2002, 17(3): 723-728

A redescription of *Gegeneophis carnosus* (Beddome, 1870) (Amphibia: Gymnophiona)

G.M. Malathesh, K.R. Gundappa, S. Ravichandra Reddy & Katre Shakuntala

Eight specimens of the lesser known *Gegeneophis carnosus* (Beddome) sighted and collected during June-September 1996 from the General Cariappa Biodiversity Park at Madikeri, Karnataka State, India are redescribed with details of their morphology, morphometry and comparative taxonomic analysis. The species appears to be an altitudinal one, restricted in its distribution to the wet semi-evergreen forest range of the Western Ghat belt of Kerala and Karnataka and inhabits a unique cryptic subterranean habitat.

Zoos' Print Journal, 2002, 17(3): 735-740

An overview of amphibian fauna of Maharashtra State

A.D. Padhye & H.V. Ghate

There are 43 species of amphibia in Maharashtra State of the 224 species found in India, distributed among six different families as: Icthyophiidae, Caecilidae, Bufonidae, Microhylidae, Ranidae and Rhacophoridae. Out of seven genera endemic to the Western Ghats, three are represented in Maharashtra State.

Zoos' Print Journal, 2002, 17(4): 757-763

An overview of amphibian fauna of Pune District with special reference to their status in and around Pune City

A.D. Padhye, Mukul Mahabaleshwarkar & H.V. Ghate

For the last 30 years, Pune has been a center of industrialization and concomitant urbanization. As a result the city is rapidly growing, in size as well as in population, at the cost of its environment. This has severely impacted the flora and fauna of the city and its outskirts. We have been studying the impact of urbanization on amphibians for the last few years. Our studies reveal that there has been a loss of one-third of amphibian species from the city area in the last 25 years, mainly due to habitat destruction. The situation is similar in other townships throughout Pune District.

Zoos' Print Journal, 2003, 18(6): 1117-1125

Amphibian fauna of Nagaland with nineteen new records from the state including five new records for India

J. Meren Ao , Sabitry Bordoloi & Annemarie Ohler

The amphibian fauna of Nagaland is updated to 32 species, 19 of which are new to the state and five species, *Megophrys wuliangshanensis*, *Megophrys glandulosa*, *Amolops viridimaculatus*, *Rana humeralis* and *Rhacophorus gongshanensis*, are new to India. The elements that compose this fauna are complex and include species from Himalayan region from north-eastern montane region and also from the Indian subcontinent.

Zoos' Print Journal, 2002, 17(12): 951-953

A note on morphometry of tadpole and adult of Microhyla Ornata (Duméril & Bibron) (Anura: Microhylidae)

Mithra Dey and Abhik Gupta

The morphometry of the tadpole of *Microhyla ornata* (Duméril & Bibron) including morphometric measurements of adult, in addition to those made by earlier studies, were carried out in and around Silchar, Assam. These measurements were made in four developmental stages of tadpoles and in froglet and adult, with notes on their ecology. The morphological and morphometric changes associated with growth and metamorphosis and their possible adaptational significance are discussed.

Zoos' Print Journal, 2003, 18(2): 1007-1010

Impact of some pesticides on the growth of tadpoles of common Indian toad *Bufo Melanostictus* Schneider

Mercy Mathew and M.I. Andrews

Impact of Organophosphate fungicide (Hinosan™) and Organochlorine (Endosulfan™) on the growth of tadpoles of *Bufo melanostictus* was studied. The weight and length of tadpoles exposed to both Hinosan and Endosulfan decreased in all pesticide concentrations, (0.001 to 0.04ppm Endosulfan and 0.1 to 0.45ppm Hinosan) with maximum reduction on thirtieth day. Endosulfan was found to be more toxic than Hinosan to tadpoles of this toad.

Zootaxa 2003, 351: 1-10
www.mapress.com/zootaxa/

A new species of *Gegeneophis* Peters (Amphibia: Gymnophiona: Caeciliidae) from southern Maharashtra, India, with a key to the species of the genus

Varad Giri , Mark Wilkinson & David J. Gower

A new species of Indian caeciliid caecilian, *Gegeneophis danieli* (Amphibia: Gymnophiona), is described from a single specimen from the Western Ghats of southern Maharashtra. This distinctive species differs from all other Indian caeciliids in having more numerous secondary annuli that are not restricted to the posterior half of the body. A new key to the species of *Gegeneophis* is presented, and the diversity of the genus is briefly discussed.

Nature, 16 October 2003, 425: 711-714
www.nature.com/nature

New frog family from India reveals an ancient biogeographical link with the Seychelles

S.D. Biju & Franky Bossuyt

About 96% of the more than 4,800 living anuran species1 belong to the Neobatrachia or advanced frogs2-4. Because of the extremely poor representation of these animals in the Mesozoic fossil record, hypotheses on their early evolution have to rely largely on extant taxa. Here we report the discovery of a burrowing frog from India that is noticeably distinct from known taxa in all anuran families. Phylogenetic analyses of 2.8 kilobases of mitochondrial and nuclear DNA unambiguously designate this frog as the sister taxon of Sooglossidae, a family exclusively occurring on two granitic islands of the Seychelles archipelago8. Furthermore, molecular clock analyses9 uncover the branch leading to both taxa as an ancient split in the crowngroup Neobatrachia. Our discovery discloses a lineage that may have been more diverse on Indo-Madagascar in the Cretaceous period, but now only comprises four species on the Seychelles and a sole survivor in India. Because of its very distinct morphology and an inferred origin that is earlier than several neobatrachian families10, we recognize this frog as a new family.

Current Science, 10 January 2004, 86(1): 211-216

Jurassic frogs and the evolution of amphibian endemism in the Western Ghats

Sushil K. Dutta, Karthikeyan Vasudevan, M. S. Chaitra, Kartik Shanker & Ramesh K. Aggarwal

The diversity of frogs and toads (Anurans) in tropical evergreen forests has recently gained importance with reports of several new species1. We describe here a fossorial frog taxon related to the African Heleophrynidae and Seychellian Sooglossidae from the Western Ghats of India. This frog possesses a suite of unique ancient characters indicating that it is a transitional form between Archaeobatrachians and Neobatrachians. Molecular clock analysis based on the nucleotide diversity in mitochondrial 12S and 16S genes dates this frog as a Gondwana relic, which evolved 150-195 Mya during the mid-Jurassic period. With this taxon, the evolution of endemism in the Western Ghats and other Gondwana break up landmasses is now dated much before the Cretaceous-Tertiary boundary. We propose that sea level surges in the late Jurassic2 isolated tablelands creating insular amphibian fauna. Reduction in area may have promoted stochastic extinctions and resulted in amphibian endemism. Our study reinforces the conservation significance of the Western Ghats as major global hotspot of biodiversity. The habitat of this endemic amphibian lineage is currently endangered due to various upcoming dam projects, which is a cause of serious conservation concern.

Hamadryad, 2001, 26(2): 235-246

Description of advertisement calls of five *Bufo* species (Bufonidae) from South and South-east Asia.

Stephane Grosjean & Alain Dubois

The advertisement calls of five Asian species of the genus *Bufo* are described, three of them (*Bufo asper*, *Bufo parvus* and *Bufo stomaticus*) for the first time. For each species, numerous temporal parameters are given, as well as the mean values of the frequency bands. Each call is illustrated by an oscillogram and a spectrogram, and is compared with available data from the literature for the calls of the same as well as closely-related species.

Hamadryad 2001, 26(2): 253-260

Herpetofaunal mortality on roads in the Anamalai hills, southern Western Ghats.

S.P. Vijayakumar, Karthikeyan Vasudevan & N.M. Ishwar

We sampled road-killed amphibians and reptiles from the highway segments passing through rainforest fragments and tea gardens in the Anamalai hills in order to evaluate the effects of vehicular traffic on the herpetofauna. There was greater mortality among amphibians than reptiles due to vehicular movement. A total of 73 reptiles belonging to 24 species, 13 genera and seven families and 311 amphibians belonging to eight genera and five families killed on the highway were recorded. These figures included several endemic species of amphibians and reptiles. Rainfall resulted in increased activity of amphibians and uropeltids, thereby making them vulnerable to road traffic. Amphibian roadkills were associated with coffee and forest habitats while reptiles were found most often in forested habitats. Road segments passing through tea plantations had the lowest number of amphibian and reptile roadkills compared with other vegetation categories. Possible explanations for differences in species richness and relative counts in the forest in relation to roadkill are discussed. The long-term effects of mortality due to roads is of conservation importance considering the low abundance of herpetofauna in the rainforests.

Hamadryad, 2001, 26(2): 276-282

Amphibian fauna of Kamrup District, Assam, with notes on their natural history.

N.K. Choudhury, B. Hussain, M. Baruah, S. Saikia & S. Sengupta

The amphibian fauna of Assam state, north-eastern India has been poorly evaluated and so far, 30 species have been reported. Kamrup District of Assam with an area of 4,345 km^2 shows a great diversity of habitats. In the present study, we report 20 species of amphibian from the District. This includes two species (*Philautus garo* and *Ichthyophis garoensis*) that are being reported for the first time the state.

Hamadryad, 2002, 27(1):62-70

***Rana* (*Hylarana*) *sensu* Dubois (1992) in India and the identity of *Hylorana tytleri* Theobald, 1868.**

A. Ohler & P.K. Mallick

Morphometric comparison of frogs of the subgenus *Rana (Hylorana)* (s. str., Dubois, 1992) showed siginificant differences between the populations from West Bengal, India and those of *Rana erythraea* and *Rana taipehensis* from more eastern regions. Study of the holotype of *Hylorana tytleri* shows that for the Indian and Nepalese populations the name Rana *(Hylarana)* tytleri is available. A key to the species of *Rana (Hylarana)* and the known distributions of *Rana tytleri, Rana erythraea* and *Rana taipehensis* are provided.

Hamadryad, 2002, 27(1): 71-77

Tadpoles of *Indirana beddomii* (Anura: Ranidae).

Mitsuru Kuramoto & S.H. Joshy

External and internal morphology of the semiterrestrial tadpoles of *Indirana beddomii* (stages 37 and 39) from south-western India was examined. Advanced tadpoles had well-developed hind limbs with nearly the same ratio to head-body length as in adults. However, the fore limbs were still in the early stages of development and most skeletal components were cartilaginous. These indicate that the hind limbs develop precociously. In spite of their semiterrestrial life, the tadpoles did not have lungs. With strongly serrated beaks and branched, curved labial teeth, they feed on green algae and diatoms. Comparisons with previously described tadpoles of the genus *Indirana* and with semiterrestrial tadpoles in other anuran taxa are discussed.

Hamadryad, 2002, 27(1): 90-98

Observations on geographic variation in the Asian Frog, *Hoplobatrachus rugulosus* (Anura: Ranidae).

Timothy D. Schmalz & George R. Zug

This study examines a series of morphometric, meristic, and

colouration traits in the wide-ranging *Hoplobatrachus rugulosus*. Comparison of four samples (Myanmar, Thailand, Hong Kong, Taiwan) reveals minor differences within and among the sample localities, but the differences do not display concordant patterns of geographic variation of the twenty-three traits examined. All populations display sexual dimorphism in morphometric traits, no dimorphism in hindfoot webbing or dorsal rugosity, and dimorphism of colouration in Taiwan and Myanmar populations. Discriminant analysis of the morphometric traits provides evidence of modest segregation of the sample localities, but the small sample sizes make these results equivocal. Eastern *H. rugulosus* averages larger and more rugose than western populations. Taiwan frogs usually have spotted bellies, and spotting decreases westward to immaculate bellies in Myanmar frogs. Hong Kong frogs have less hindfoot webbing than the three other populations. Other traits differ, but their geographic patterns do not match that of the preceding ones or display another single pattern.

Hamadryad, 2003, 27(2): 248-253
Chromosomal sex determination in anuran amphibians.
Samarendra Nath Banerjee & Samar Chakrabarti

The sex chromosome constitution in amphibians has been a conflicting issue, with occurrence of heteromorphic sex chromosome being demonstrated in 55 species of the over 5,000 extant species. Among anurans, cytogenetically recognizable heteromorphic sex chromosomes have been confirmed in four species.

Hamadryad, 2004, 28(1&2): 7-18
A synopsis of the amphibian fauna of Goa
A.K. Sarkar & Sukumar Ray

This report deals with 27 species in 12 genera, five familes and two orders representing the amphibian fauna of the State of Goa State, western India, based on collections in the Zoological Survey of India that were made between March, 1966 and July, 1979. A total of 397 specimens of amphibians are being reported. Four additional species (including *Microhyla rubra, Indirana beddomii, Limnonectes brevipalmatus* and *Gegeneophis ramaswami*) are recorded for the first time from Goa. A microhylid, *Ramanella mormorata* Rao, which was recorded by Das and Whitaker (1997) from southern Goa after over half a centuary, is here being reported from both the northern and southern parts of Goa. Diagnoses have been added for determination of species.

Hamadryad, 2004, 28(1&2): 98-104
An overview of the biodiversity of Indian caecilians (Amphibia: Gymnophiona)
M.S. Ravichandran

A brief overview of the caecilian diversity of India is presented. The fauna consists of 21 species in three families, concentrated primarily in the Western Ghats, but also in the Eastern Ghats and in north-eastern India. The taxonomy, distribution, ecology, and life history of caecilians in India, as elsewhere, are inadequately studied, but some recent progress is reviewed. Studies are hampered by the small sample size available for most species. Improved taxonomy is urgently required, and this can form the basis for other studies, including conservation assessments.

Field study, collection, identification, legal & ethical issues, conservation and distribution status of South Asian amphibians

S.K. Dutta
Co-Chair, DAPTF-South Asia
Department of Zoology, Utkal University, Bhubaneswar 751004, Orissa, India.
E-mail: sk dutta@vahoo.com

The present report includes some of the parameters, which relate to amphibian studies in South Asia. The first issue relates to field study, which can not be compared with studies being conducted in other countries. This is due to several reasons and the most noteworthy is the inaccessible geographic localities, specifically in the western and the Eastern Himalayan region, the northeast India, the Western and Eastern Ghat Ranges of India and the northern Sri Lankan region. Ironically, some of these areas are rich with amphibian species. As field study involves money, expertise, man power, coordination between the field researcher and the policy makers and also time frame, it is a mandatory task to maintain balance between and within the above parameters. For example, some researchers might complain about financial constraints and others about proper facility. However, these are not the major problems involved in field study. In order to accomplish a successful field study, one needs to do a variety of home works (establish contact, standardize field methodologies, select locality and species or species groups, review the past and the present status of species known from the locality and lastly arrangement of field trips at a suitable time of the year). Selection of locality and time period of field study are the most important factors involved in a successful field survey. However. without proper expertise one can not expect to gain substantially from a field study and this seems to be a major hurdle in our region. I do not mean that all the field researchers are without proper expertise, but there is not enough coordination between the researchers and also not enough exchange of ideas. There are several examples of solitary field studies and the findings are not published with highly rated standards. Under the circumstances, we should try to evolve a working methodology for a coordinated field survey and this will enhance our knowledge in the field.

The second parameter is quite important from research and conservation point of view, because the term “collection” is the most critical word so far as the biodiversity conservation is concerned. Currently, the policy makers of most of the South Asian countries are quite sensitive when one talks about collection of fauna and flora for conservation studies. In fact. majority of us does not understand the value of collection and subsequent study. Collection does not mean killing and killing does not mean destroying the biodiversity. However, we should understand the reason of collection, what to collect, when to collect and where to collect. Once, these facts are clear, the next step is to make people understand the importance of collection. We should understand that biodiversity conservation needs collection and if necessary killing or sacrificing biotic components. Let us look into an important chapter in taxonomy, which is type concept. Infact, “type” or the “types” are the specimens of a species, which are the results of “sacrifice”. Does this mean that the specimens have been killed for nothing. Of course not, but a lot of us does not understand this and on the other hand we create several problems to workers involved in assessing the biodiversity. The above comments need to be discussed in detail.

Identification is a perennial problem in our subcontinent and this is associated with several factors. The most noteworthy is the lack of expertise, which does not mean non-availability of experienced researchers. What I mean is the proper knowledge in the field of identification and this revolves around literature, long term research involvement, knowledge of the types and character selection etc. However, some of us are in a hurry for identifying the species by themselves and also many of us search for "identification key" to settle down with putting a name to a specimen. To me this is a gross mistake, because it needs time to learn the identification techniques. A printed "key for identification" can not be useful enough to identify your specimen. It is necessary that you work in the field, study the ecology, behavior and breeding of the species, analyze the characters and do plenty of homework with existing literature. Lastly, it might be possible to finalize the identification task. The whole exercise might involve several years, but not several days or months, which is a general practice with some of us. To overcome the above problem, we again need cooperation and coordination among and between individuals.

Legal and ethical issues are many and I will stress upon a few. Most of the South Asian countries have their own laws and regulations for conservation of biotic community and species. For example, India has Wildlife Protection Act (1972), which is being updated time to time. However, there are some peculiar restrictions, which never permit a researcher to conduct a proper field survey and collect samples. This is a major hazard in India and I am sure several of us face this problem. Another recent issue relates to ethics and currently animal ethics committee is everywhere. The main task of animal ethics committee is either to approve or disapprove animal sacrifice for research. Perhaps the worst sufferers in the hands of animal ethical committee could be taxonomists and systematists, who certainly need to sacrifice animals as and when required. However, I am still confused whether we taxonomists or systematists still need the approval of the animal ethics committee for sacrificing organisms as and when required. Is it possible to seek the approval of the committee when we are conducting field survey when specimens need to be sacrificed? I also do not understand the deal between the authority involved in issuing collection permits (for example the Forest Department of Govt. of India or of a state) and the animal ethics committee.

Conservation issues are many and currently we are quite aware with the conservation status of maga species of vertebrates. So far as the amphibians are concerned, the conservation status is practically nil (except few species protected by the Wildlife Protection Act). However, we are desperately trying to link our research involvement with conservation, because the fascinating word "conservation" is being utilized in our research proposals to pull money or more money from funding agencies. This I feel is a desperate attempt to murder "conservation" of amphibians in our country, because without a substantial contribution, we use the term and continue our goal. As 1 understand, without a proper understanding of the biology of a species, we cannot say anything about conservation of that species. We do not have proper and detailed biological data of our species.

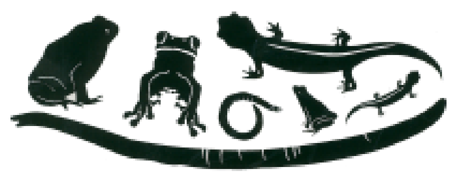
So far as the amphibians are concerned, evaluation of their distribution status is an old story, because there are plenty of literature on distribution records. However, we need to quantify, the amount of data available on distribution of a species. Of course, most of us will agree to the fact that, "not enough" is known about the distribution record of more than 50% of our amphibian species. This seems to be an alarming number. How can we overcome this problem? Certainly, more fieldwork is needed, but again in appropriate place and time of the year. Another problem associated with amphibians is their discontinuous distribution record over a large geographic range and this is certainly an artifact of inadequate field survey, coupled with gross identification errors.

The last comment deals with the ethics surrounding publication and referred specimens. Infact, the voucher specimens and the types of new species should be available to the herpetological communities for further study and scrutiny. Hence, until unless the researchers deposit their specimen in a recognized national or international repository, it makes no sense to provide new locality records or describe new species. There are several instances where new species have been described without designating the types or deposition in proper organizations, which are not accessible to herpetologists.

Special thanks to the following organisations for the technical and monetary support provided to run the Declining Amphibian Populations Task Force-South Asia network and in the publication of this Newsletter.

DAPTF-SA CBSG-I FRAWG CBSG-SA

ZOO OUTREACH ORGANISATION DAPTF SSC IUCN WILD WILDLIFE INFORMATION LIAISON DEVELOPMENT



frog leg
August 2004 No. 11

Editor: Sanjay Molur
Edtorial Advisor: Sally Walker
DAPTF-SA Co-chairs: Sushil Dutta & Sanjay Molur

frog leg is the Newsletter of the Declining Amphibian Populations Task Force - South Asia, the regonal network of the Declining Amphibian Populations Task Force, SSC, IUCN.

frog leg is published by Zoo Outreach Organisation and Conservation Breeding Specialist Group India as a service to the amphibian conservation community as well as conservation actioners and enthusiasts of South Asia.

For communication:
DAPTF-SA, c/o Zoo Outreach Organisation
PB 1683, 29-1 Bharathi Colony
Peelamedu, Coimbatore
Tamil Nadu 641004, India.
E-mail: herpinvert@vsnl.com

frog leg is available online at www.zoosprint.org