Project Anuran is a research expedition founded as a joint initiative between students from the University of Edinburgh and the University of Belize (formerly University College Belize). The project’s main research aim is to establish an intensive monitoring program of frogs and toads in the region around the research station of the Natural History Museum, Las Cuevas, in the Chiquibul Forest Reserve, Belize. During the summer of 2000, the initial field phase of the project collected baseline data on the diversity and relative abundance of anurans, alongside basic ecological information such as habitat associations, community patterns and calling behavior. The output of the project is able to contribute directly to the monitoring efforts of the DAPTF in Belize (formerly University College Belize). The project’s main research aim is to establish an intensive monitoring program of frogs and toads in the region around the research station of the Natural History Museum, Las Cuevas, in the Chiquibul Forest Reserve, Belize. During the summer of 2000, the initial field phase of the project collected baseline data on the diversity and relative abundance of anurans, alongside basic ecological information such as habitat associations, community patterns and calling behavior. The output of the project is able to contribute directly to the monitoring efforts of the DAPTF in the Yucatan region, under the Mayan Forest Anuran Monitoring Project (MAYAMON), coordinated by Dr. Jack Meyer.

Fieldwork is conducted in the immediate vicinity of the research station, in an area of broadleaf subtropical wet forest interspersed by evergreen forest. Standing at 500 m elevation, Las Cuevas is within the northern foothills of the Maya Mountains of Belize, which form part of one of the largest expanses of continuous forest in Central America. Although the area was extensively cleared during the peak of the Maya civilization (ca. 800 AD), apart from selective logging for mahogany and cedar, and harvesting of latex from the sapodilla tree, it has seen little further human disturbance. In this regard it represents an excellent location from which to monitor the dynamics of anuran populations in relatively pristine tropical environments. Such monitoring will hopefully provide an opportunity for future reflection on potential indirect human perturbations as possible culprits for apparently mysterious amphibian declines in such areas (sensu the golden toad of Costa Rica).

The focus of our efforts was on vocalizing species, predominantly of the family Hylidae. Eight breeding sites, representing a range of sub-habitat types from large, open permanent ponds to small ephemeral forest pools, were assessed over a total of 25 surveillance nights. Surveillance of each site ran from 1900 to 0300 (or earlier if calling ceased), and data were collected at hourly intervals. Recordings were made on a nominal scale of the audible abundance of each species present following MAYAMON protocol, and an additional measure of calling intensity (calls per minute) was taken to allow a more detailed consideration of calling behavior and reproductive effort. In addition to these biotic data, measurements were made to record the abiotic environment on each survey night, and each site was mapped in detail to describe its floral community. This intensive methodology allowed a relatively comprehensive assessment of vocalizing anurans around Las Cuevas, with 12 species being recorded in particular detail. As well as a photographic record, digital recordings were made of each species which will form a valuable contribution to the work of future monitoring groups (see www.projectanuran.org). A number of pleasant surprises were uncovered, including evidence of a high local abundance of the regionally rare Morelet’s tree frog, Agalychnis moreletti. Aside from an improved understanding of diversity and relative population abundance, an insight was gained into the habitat preferences of each species, their calling patterns and the extent of temporal separation of each species in an assemblage. Such information is highly valuable in establishing optimal future monitoring programs of these populations. Collection of data on habitat requirements and community structure, over and above simple measures of diversity and abundance, allows a more multidimensional approach to monitoring to be taken. It is hoped that a more detailed picture will provide an enhanced ability to identify changes in assemblage structure and the potential onset of any future population declines.

A second direction of our work during Phase I was to conduct a preliminary assessment of ground dwelling species, predominantly of the genus Eleutherodactylus. Over one hundred man-hours of visual encounter surveys, in addition to five permanent drift-fence arrays, produced an initially disappointing result with four species, and less than 30 individuals recorded in total. This work serves to highlight both the low local abundance of these species and the notorious difficulty of fairly representing such a cryptic group. Interestingly, during a week’s surveillance of a region of Caribbean pine (Pinus caribaea), a much higher abundance was found, including an as yet unidentified ranid specimen. Eight specimens collected were deposited in the Natural History Museum (London), where they will form an important part of the ongoing revision of this little known but highly diverse genus.

The standard survey methods used were effective in identifying and describing the activity of the majority of anuran species found. However, it is important to recognize, as noted above, that some species are consistently misrepresented using traditional techniques. One such group is what can perhaps be termed the ‘explosive’ breeders, such as the burrowing toad (Rhinophrynus dorsalis), and Mexican tree frog (Smilisca baudinii), whose true numbers are revealed only after intense rains and in suitable clearings. It is crucial that such considerations...
are incorporated into future monitoring programs in order to obtain a more representative picture of amphibian communities (even if it means impromptu surveys in the middle of a storm at night).

Following the success of Phase I of Project Anuran, a second field phase is due to be conducted in the summer of 2001, with the intention of developing a long-term monitoring program. The dearth of long-term, and adequately quantitative, studies of amphibian populations has all too frequently been identified as the major shackle to improving our understanding of global declines and their potential causes. Such information is critical in order to distinguish between natural inter-annual variation in populations and the progression of true, long-term trends. Ecosystems are dauntingly complex phenomena, and few more so than tropical rainforests, where a seemingly infinite number of dimensions of variability, both spatial and temporal, serve to confound the naive ecologist. Survey schemes of sufficient duration and breadth, so as to allow a high degree of confidence in the predictions of declines and assessment of decline rates, are a pivotal step towards the vital unraveling of this complexity. While little can be done to rectify the lack of historical records, it is in our hands to ensure the continued provision of new, accurate data sets. It is here that we consider undergraduate contributions, such as Project Anuran, are able to offer a significant contribution. Our preliminary survey during 2000 showed that the gathering of detailed information on amphibian populations does not require highly trained field ecologists. Rather, it is our experience that with support from appropriate experts and institutions, undergraduate biologists can offer significant manpower, resources and boundless enthusiasm to monitoring work. It is necessary here to highlight both the importance and invaluable contribution that can be made from close liaison between workers in visiting and host countries. The continued involvement of both Edinburgh and Belize students in Project Anuran is a crucial necessity in maintaining its long-term operation.

In light of these comments, we would like to take this opportunity to express our sincere thanks for the continued support of a number of scientists in our work, namely, Dr. Peter Stafford of the Natural History Museum (London).

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**Disinfection of Ambystoma tigrinum Virus (ATV)**

By Jesse Brunner & Tim Sesterhenn

Ambystoma tigrinum Virus (ATV) and related ranaviruses from North America, Europe and Australia (Daszak et al. 1999) are highly lethal to amphibians, and are frequently associated with epizootics of amphibian populations. Researchers have recognized that they may inadvertently play a role in spreading these and other pathogens and have been urged to implement common-sense disinfection protocols to avoid spreading disease. Common methods include washing waders, boots, and nets with a 10% bleach solution or with Quat-128. Our lab uses 70% ethanol or isopropyl alcohol to disinfect surgical equipment when PIT tagging or taking tail clips in the lab and field. Ethanol is a standard disinfectant in most laboratories. Given that ranaviruses can survive up to several months when dried (Langdon 1989; J. Brunner, unpublished data) and that the effectiveness of chemical disinfectants can be variable (Springthorpe & Sattar 1990) we tested the effectiveness of chemical disinfectants and that the virus samples were still highly active. Soaking samples in 70% isopropyl alcohol and 10% bleach, however, completely inactivated the virus. One virion remained viable after 45 minutes of exposure to 70% ethanol, not complete inactivation, but over a million-fold reduction in active virus.

Ethanol, isopropyl alcohol, and bleach are all adequate disinfectants for viral contamination, but must be used methodically. It is not enough to simply spray surfaces. Instead, liberally pour the disinfectant on spills and soak or scrub waders and other equipment. Also, it is essential to keep disinfectants fresh: in an initial trial we found that the 70% ethanol we had been using in the lab was ineffective, presumably because it had degraded. Heat, ultraviolet light and organic matter degrade chlorine compounds quickly (Springthorpe and Sattar 1990). Each of these disinfectants is readily available and, especially cheap. Disinfection, then, is simply a matter of following adequate protocols.

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Check out the DAPTF Fieldwork Code of Practice at:
- http://www.mpm.edu/collect/vertzo/herp/Dapft/ftcode_e.html (English)

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Alytes facilitate amplexus. The best known of what Littlejohn (1977) calls 1991). There are not many examples defending a retreat (Stewart and Rand 1960), scream when threatened by a predator (Yerkes 1903), or call in 1992, Bush 1993), scream when threatened by a predator (Yerkes 1903), or call in defending a retreat (Stewart and Rand 1991). There are not many examples of what Littlejohn (1977) calls reciprocal calling. In these species the sexes alternate calls that seem to facilitate amplexus. The best known include Alytes (Heinzmann 1970, Marquez 1992, Bush 1993), Xenopus (Picker 1980) and Tomodactylus (Dixon 1957).

A number of other reports of reciprocal calling have been published, most of them quite recently. In some of these cases other studies of the same species in other places, or of closely related species have not reported female calling. Female calling has been reported in Pelobates cultripes in Spain (Salvador 1986), Hyperolius marmoratus in Malawi (Stewart 1967), Rana blythi in Borneo (Emerson 1992), Rana ridibunda in England (Frazer 1983) Rana virgatipes (Given 1993), and Rana palustris in eastern North America (Roble 1986). Rana, and Polyedates in northwest India (Roy 1995 and 1997), Leptodactylus fallax in Dominica (Davis et al. 2000), Eleutherodactylus guanahacabibes in Cuba (Dias & Estrada 2000), Eleutherodactylus podiciferus in Costa Rica (Schlaepfer et al. 1998) and Phyllomedusa trinitatis in Trinidad (Kenny 1966). Schlaepfer et al. (1998) suggest that reciprocal calling occurs mostly in species with a long breeding season, diffuse breeding choruses, simple male calls, and in some cases, male parental care.

The behaviour of most frogs, particularly tropical frogs, is poorly known and in some of these cases it is quite possible that female calling had been overlooked. In most cases female calls are reported as being softer than those of the males and often infrequent. Reciprocal calling would be easy to overlook by someone who was biased to assume automatically that any frog heard calling is a male.

Some of the other reports are not so easy to explain. For example, Polyedates leucomyostax females were reported to call by Roy in NW India; this species was studied by Narins et al. (1998) in Malaysia who described the repertoire but reported no female calling. Stewart watched a female Hyperolius marmoratus call in Malawi but intensive studies of breeding and communication in South Africa (Passmore et al. 1992) report no female calling. Perhaps these are examples of geographical variation in calling behavior but another explanation is also possible.

Perhaps the hormone systems of these calling females had been distorted by pollutant chemicals? When Hannigan & Kelley (1986) treated female Xenopus with androgens their calls became more male-like. Penna & Somers (1992) showed that if they manipulated the hormone system of Hyla cinerea with injections of AVT and/or implants of the steroid hormone testosterone they could induce females to call. It is known that DDT and related chemicals can act as a steroid mimic in several classes of vertebrates (Colborn & Clement 1992, fide Hayes 1997) and Hayes (1997) has shown that “…estrogen (E2) treatment [of larvae] can result in ovarian differentiation or testicular differentiation, depending on the species. Paradoxically, E2-treatment may also result in either testicular differentiation or ovarian differentiation in a single species depending on the dose of the treatment…” (p146). Hayes argues that steroid-mimicking environmental contaminants may be involved in amphibian population declines.

If environmental contaminants can be involved in an amphibian population decline by producing deformed tadpoles as Hayes suggests, then it seems possible that these steroid mimics might affect the hormone balance of amphibians less severely impacted by the chemicals. Perhaps chemicals introduced by man into the environment might act on some females to induce them to call. Certainly there are many chemicals used in agriculture as pest control and fertilizer around the world. These may be distributed far beyond the immediate area of application by water and as dust or aerosols in the air (Cohen and Pinkerton 1966). These pollutants have been detected in what appear to be undisturbed environments. If Saharan dust can be blown to Florida (Stallard pers. com.) there is probably no frog on the planet that is not potentially in contact with pollution.

The case is certainly not proven but it seems possible that at least some of the recent reports of female frog calling are the results of chemical pollutants introduced by humans into the environment. To mix a metaphor, if frogs are “a canary in the coal mine” whose disappearance is warning us of environmental degradation, it may be important to notice when the canary sings.

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**A Hierarchical Approach in Studying the Effects of an Insecticide on Amphibian Communities**

By Michelle D. Boone, Christine M. Bridges & Nathan E. Mills

Widespread pesticide use makes it increasingly likely that non-target species will be exposed to chemical contamination. Amphibians that breed opportunistically in temporary wetlands in roadside ditches or agricultural floodplains may be especially vulnerable to pesticide exposure, chronically or occasionally, depending on the length of the species’ larval period and time of breeding. Toxicological research, however, emphasizes the lethal effects of short-term exposure on a few model organisms. This research, therefore, has limited application to understanding the role of contaminants in communities.

At the University of Missouri, we have conducted research on the effects of an insecticide in the laboratory, field and in semi-natural ponds, research funded in part by seed grants from DAPTIF in 1997 (CMB), 1998 (MDB), and 2000 (NEM). We have used the chemical carbaryl (the active ingredient in Sevin), a short-lived carbamate that acts through acetylcholinesterase inhibition, which may serve as a model chemical for neurotoxins (i.e., carbamates and organophosphates).

We have completed experiments that clarify the effect of carbaryl and allow us to make predictions about the effects of other neurotoxins.

**Laboratory Studies**

Laboratory studies are a necessary starting point to understand how contaminant effects, manifested at the community level, begin. Basic toxicological data like LC50s (lethal concentration to 50% of the population) indicate that concentrations that induce mortality in larval amphibians are greater than found in the environment (Bridges 1999a). Additionally, vulnerability to lethal levels of carbaryl varies widely among species and populations (Bridges & Semlitsch 2000), and tadpoles most sensitive to high levels of carbaryl are less fit under natural field conditions (Semlitsch et al. 2000). Expected environmental concentrations (EECs), however, are typically much lower. The effects of sublethal concentrations, therefore, may be more relevant to amphibian communities because they may directly affect time and size to metamorphosis, or indirectly affect survival. For example, tadpoles demonstrate a reduction in swimming performance and activity levels with a sublethal exposure to carbaryl (Bridges 1997), which may negatively impact time and mass at metamorphosis. Exposed tadpoles also exhibit non-adaptive predator avoidance responses (Bridges 1999b), which can alter predator-prey dynamics (Bridges 1999c). When tadpoles are chronically exposed to concentrations of carbaryl that are an order of magnitude lower than EECs, there is still a dramatic increase in mortality and a high incidence of deformities (Bridges 2000). Carbaryl’s potency can also be increased by temperature (Boone & Bridges 1999) and ultraviolet levels (Zaga et al. 1998), factors that are often not accounted for. The results from laboratory studies suggest that short-term chemical exposure could lead to effects that outlive the chemical and negatively impact responses beyond the larval stage or metamorphosis.

**Field Studies: Cattle Tank Ponds and Experimental Wetlands**

Because amphibians in more complex systems may be affected by...
contaminants differently than individuals reared in controlled laboratory conditions, it is necessary to determine if predicted effects in the laboratory are applicable in the field. Outdoor cattle tank studies demonstrate that short-lived contaminants at EECs can impact mass, time, and survival to metamorphosis, although sometimes in unexpected ways. For instance, the biotic environment can influence the potency of carbaryl; carbaryl’s effect changes with the predator environment and initial larval density (Boone & Semlitsch 2001a, 2001b). In one case, more Woodhouse’s toads (Bufo woodhousii) survived to metamorphosis at high density when exposed to carbaryl than in low density or control environments (Boone & Semlitsch 2001b). In a study considering the effects of multiple doses of carbaryl, exposure to carbaryl three times enhanced survival and size at metamorphosis under high density conditions (Boone et al. 2001); these results suggested that carbaryl may affect metamorphosis by stimulating stress hormones, as well as acting through the food chain. Even in large experimental wetlands, which include a range of factors typically excluded in cattle tank studies, a short-lived contaminant can alter amphibian abundance and mass at metamorphosis (Boone 2000); effects in the field were similar to those in cattle tank studies. While laboratory studies predicted reduced mass or survival from EECs of carbaryl, field studies often indicate that carbaryl has stimulatory effects on these responses. Therefore, laboratory work was not necessarily predictive of the response of amphibians in the field.

In a community, carbaryl can directly alter a species’ behavior/physiology or indirectly alter the biotic community (which could account for “positive” chemical effects). Studies designed to distinguish indirect and direct effects of carbaryl indicate that, in the field, the direct effects of carbaryl on metamorphosis were small to nonexistent. Effects on metamorphosis in response to carbaryl-induced changes in the aquatic community (i.e. indirect effects) were much greater. For example, even when tadpoles were not exposed to carbaryl, spring peeper (Pseudacris crucifer) and southern leopard frog (Rana sphenopechala) tadpoles grew faster and were larger at metamorphosis when raised in communities previously exposed to carbaryl, even though survival did not vary (Mills & Semlitsch, unpublished data). Indirect effects (via effects on phytoplankton and zooplankton) were far more important in influencing responses of anurans at metamorphosis, and this outcome emphasizes the significance of understanding the effects of a contaminant in more realistic and complex conditions.

In conclusion, by studying the effects of carbaryl on amphibians in the laboratory, field and semi-natural ponds, we are developing a good understanding of how this broad-spectrum insecticide could affect amphibian communities in nature. Our results suggest that, despite its short half-life (hours to days in our studies), carbaryl can directly affect behavior at EECs, and carbaryl can alter the food web of the community resulting in changes in species abundance and size and time to metamorphosis. Our studies demonstrate the importance of incorporating genetic variation, biological realism, and realistic exposures in discerning how contaminants affect community processes. Additionally, our work illustrates how even a short-lived contaminant may alter the structure of amphibian communities by direct or indirect effects on individual species. These results suggest that environmentally relevant levels of contaminants could alter abundance of species, both positively and negatively, and that contaminants believed benign in the environment may alter communities and could, potentially, contribute to reductions in biodiversity and population size over time.

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Report on the Roundtable
Organized by the DAPTF Monitoring Protocols Working Group, 15 July 2001

The aim of the roundtable was to provide a forum for all those involved with monitoring programmes within Europe, and to exchange ideas and information, with a view to formulating a programme of priority activities for the DAPTF Working Group within Europe. A panel, which included representatives from the SEH Conservation and Mapping Committees, was convened to provide a focus for the discussion and to answer specific questions regarding monitoring issues within Europe. A total of 21 participants, representing 12 countries, ensured a wide-ranging and comprehensive exploration of activities and priorities. At the end of the session a five-point action plan for Europe was agreed as follows:

1) The DAPTF Monitoring Protocols Working Group will develop a website that will act as a co-ordination and information centre for monitoring schemes across Europe. Organizations charged with running national monitoring programmes would be encouraged to provide information about the protocols being used in their own country, with appropriate links to more detailed information as necessary.

2) As it would not be logistically possible to address monitoring issues in all species in all countries, the working group would seek to prioritize its work at both the species and regional levels. At the species level, the initial focus would be on widespread rather than rare or locally distributed species (e.g. Rana temporaria, Triturus cristatus, Bombina bombina, B. variegata, Bufo calamita and Hyla arborea). Regional priorities would be identified as the information gained through the website described in (1) was consolidated and analyzed.

3) Rather than moving towards single, standardized protocols for individual species or regions, the DAPTF Working Group would seek to embrace the diversity of methods that are currently in use across Europe, but attempt to provide a single framework within which they can be executed.

4) Recognizing that the degree of professional involvement in monitoring protocols varies considerably across Europe, the DAPTF Working Group would seek to embrace protocols that can be used by both professional and voluntary workers.

5) The DAPTF Working Group would maintain links with the SEH Conservation and Mapping Committees, as well as other organizations involved with amphibian monitoring, to ensure that effort was coordinated and complementary.

This plan is by no means a definitive list of actions, and will no doubt evolve as new information comes to light. It is hoped that work will shortly begin on the development of the website, and any organization that is running a national or regional monitoring scheme and who was not represented at the meeting is invited to contact me. I am indebted to Kurt Grossenbacher, Helmut Faber and Patrick Haffner for agreeing to sit on the panel, and to Milan Vogrin and the organisers of the SEH meeting in Zalec for providing facilities for the roundtable.

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Assessing the Conservation Status of Australian Frogs

IUCN - ASH Workshop
6-9 February 2001
Funded by IUCN & Environment Australia, Hosted by Australian Society of Herpetologists in the Department of Zoology, University of Tasmania

As part of the new IUCN Global Amphibian Assessment process, Craig Hilton-Taylor (IUCN) and Dr. Jean-Marc Hero (ASH/Griffith University) organised and completed a national workshop to assess the conservation status of Australian Frogs in February 2001. ASH President, Professor Roy Swain, hosted the workshop at the University of Tasmania. Over twenty amphibian biologists attended with expertise covering all regions of Australia. The primary objective was to facilitate an urgently required update of the current IUCN listings for the 213 currently described frog species in Australia. The findings from this workshop were open to discussion for 3 months following the workshop before the final listing was posted on behalf of the group (see below). This information will be used to update the IUCN listing and will be submitted to Environment Australia later in 2001 for consideration in updating the Australian Commonwealth listing under the EPBC Act (2000).

Following was a focus on the Australian amphibian species; Critically Endangered 14 species; Endangered 8 species; Vulnerable 15 species; Near Threatened 4 species; Data Deficient 22 species; Least Concern 147 species. The listing for each species is available at the following www site:

Considering the first 2 categories, 17 species (8%) of Australian frog species are listed as extinct or critically endangered. Combining the first 3 categories, 25 (11.7%) of Australian frog species are currently threatened with extinction. A total of 40 Australian frog species (18.8 %) are currently threatened or vulnerable to extinction.

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Recent studies of European frogs reveal complexities of the link with UV-B

From Tim Halliday, DAPTF International Director

Elevated levels of UV-B radiation, resulting from thinning of the ozone layer, have been shown to have an adverse effect on the survival of eggs and larvae of some anuran species, but not of others. The European common frog (Rana temporaria) appears to be one of those species that is unaffected by elevated UV-B. For example, Cummins et al. (1999) found that artificially elevated UV-B did not increase mortality and Håkkinen et al. (2001) have found that, in Finland, R. temporaria is not affected by ambient UV-B, whereas both the moor frog (R. arvailis) and the common toad (Bufo bufo) are.

A study by Hofer and Mokri (2000) revealed that the skin of R. temporaria tadpoles contains a UV-B absorbing substance, in effect a sunscreen. This may be an alternative, or an additional adaptation to counter the effects of UV-B to the DNA-repair enzyme photolyase that has been found in some North American UV-B resistant frogs, such as Hyla regilla (Blaustein et al. 1994).

Rana temporaria may not be as well-provided against UV-B as it seems, however. Pahkala et al. (2000)
found that embryos protected from ambient UV-B in the field showed no difference from exposed embryos, in terms of hatching rate or frequency of abnormalities; they were, however, significantly larger as hatchlings. In a subsequent lab study (Pahkala et al. 2001), this group has identified a ‘carry-over’ effect from the embryo to the larval stage. Individuals exposed to elevated UV-B as early embryos developed into larvae that metamorphosed later, at a smaller size, and with a higher frequency of developmental abnormalities than individuals shielded from UV-B or exposed to normal levels.

These studies suggest that the effects of UV-B on amphibians can be very subtle and, therefore, hard to detect, and that species that appear to be unaffected by elevated UV-B may not in fact be so.

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A major effort, using volunteers, is being made to find gastric brooding frogs (*Rheobatrachus vitellinus*), which disappeared 15 years ago, in the Eungela National Park, Queensland, Australia.

Froglog welcomes short contributions (500-1000 words) on any research, discoveries or conservation news relating to the amphibian decline phenomenon. Success stories as well as tales of impending disaster are sought! We encourage authors describing original research to first make submissions to a fully-refereed journal and then, if appropriate, to publish a precis or synopsis in Froglog.

Please submit potential contributions to John Wilkinson at the main office address below. E-mail submissions are encouraged (DAPTF@open.ac.uk). In order to speed your article into print, please, if possible, make your submissions SINGLE spaced and use the font Helvetica 9-point. Refer to this or any recent issue of Froglog for format, and please note the preferred format of any references cited!

FROGLOG is the bi-monthly newsletter of the Declining Amphibian Populations Task Force. John W. Wilkinson, Editor, Department of Biological Sciences, The Open University, Walton Hall, Milton Keynes, MK7 6AA, U.K.

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