# FROGLOG

IUCN/SSC Declining Amphibian Populations Task Force

February 1996, Number 16

This issue of FROGLOG is an overview of some of the work which Canadians have been conducting on amphibian populations, including investigation of stressors which affect amphibian survivorship, monitoring by volunteers and the rehabilitation of amphibian habitats. Stan Orchard, the National Coordinator of the Working Group on Amphibian and Reptile Conservation in Canada, provides a summary of the Canadian efforts to address the issue of declining amphibian populations; following authors summarize specific amphibian projects. We hope that other countries will find this information interesting and we look forward to them using FROGLOG to summarise their ongoing projects and findings.

Christine Bishop (Guest Editor)

## Declining Amphibian Populations in Canada: Then and Now

In the fall of 1991 a workshop was convened in Burlington, Ontario by Christine Bishop (Canadian Wildlife Service) and Bob Johnson (Metro Toronto Zoo) to discuss declining amphibian populations in Canada. By the meeting's conclusion we were a *bona fide* organization with coordinators in each province and territory. Also, we had chaired subgroups covering intensive monitoring, diseases, historical databases, and environmental contaminants. The acronym 'DAPCAN', coined by David Green (McGill University), was immediately adopted by the group to simplify the more cumbersome title, 'Task Force on Declining Amphibian Populations in Canada'. David Green, was elected the first National Coordinator and DAPCAN became a force that organized and mobilized herpetologists in Canada to an unprecedented degree.

Since our initial meeting in 1991 we have held four successful conferences in different cities across Canada, at which 150 scientific papers have been presented and abstracts printed. We have also published the proceedings of our first conference and have a book in production that summarizes our findings during the first three years. We have spawned numerous volunteer programs to monitor and document amphibian population trends across Canada and have been instrumental in focusing researchers on declining amphibian population studies.

After considerable debate at DAPCAN V, in 1995, general agreement was reached that the mission statement of our organization should be broadened to include reptiles. This was a logical step because it consolidates the herpetological conservation research community in Canada. Our greatest concern was that this move should not dissolve or in any way detract from the ongoing work of DAPCAN. DAPCAN and its IUCN/SSC mandate will continue but as a subdivision of the 'Working Group on Amphibian and Reptile Conservation in Canada'. The first meeting of this group, and the sixth meeting of DAPCAN, will be held on the campus of the University of Calgary, in Calgary, Alberta from October 5-7th, 1996. For further details, contact the National Co-ordinator (address page 4).

In the past we have relied on government grants to launch and sustain our projects, but governments are cutting back and we felt that we must become more self–reliant. Thus, we have resolved to make our organization officially non–profit/charitable so that we can issue tax–deductible receipts and thereafter actively solicit donations from public and private sources. We will also build on the cooperative links that we have forged between scientists of many specialities, the concerned general public, the media, and myriad environmental agencies. Our organization will continue to benefit science, education, and environmental awareness in Canada and will redouble its efforts to find answers to where and why amphibian populations are declining.

Stan A. Orchard, DAPCAN, National Coordinator (address page 4).

## Amphibian Monitoring in Canada

In 1992 volunteer observers began monitoring the calls of male anurans in Canada. By 1995 there were large scale call count monitoring programs in Nova Scotia, Québec, Ontario, Manitoba, and Saskatchewan involving over 400 observers. Observers are asked to report on the species calling and the intensity of the calling for three minutes per night during the peak amphibian breeding season (April–July). Observers could choose their monitoring site which can be either a rural roadside, their backyard or a favourite pond or marsh. The program has proven to be very popular with the general public, partly because we send volunteers tapes of the frog calls, and people are excited about learning the calls of animals other than birds! In Ontario, the Long Point Bird Observatory and Environment Canada Marsh Monitoring Program combines surveys of marsh birds and amphibians in Great Lakes wetlands to assess the need for rehabilitation of a marsh or to determine whether a rehabilitation project has been successful. In Nova Scotia, the program focuses on a single species, the spring peeper (*Pseudacris crucifer*) and volunteers call the provincial museum on the first date of the spring that spring peepers are heard. As well as providing presence/absence data, this also promotes awareness of amphibians because the locations of the first dates of calling are reported on television weather maps.

Part of the amphibian monitoring program has involved evaluating inter–observer variation, the length of the survey period, and the relationships between call counts, chorus size and population densities. We have found that three minute point counts provide an adequate balance between stop duration and number of stops in areas with species diversity and climate similar to those in Ontario. There is a bias associated with observer experience; inexperienced observers tend to underestimate the number of calling individuals. It is therefore important to know the level of experience of observers. Because of the nature of the bias, apparently stable or increasing populations may actually be declining, whereas apparently declining populations probably indicate actual declines. The results of monitoring must be interpreted with consideration of the specific habitat requirements and breeding behaviour of the species involved.

For more information on validation of amphibian call counts contact: Leonard Shirose, email: leonard.shirose@cciw.ca. or at the address given for Christine Bishop, below.

For more information on amphibian monitoring in Canada, contact: Christine Bishop, Canadian Wildlife Service, Environment Canada, Box 5050, Burlington, Ontario L7R 4A6; 905–336–6434 (fax); 905–336–4843 (phone), email: cab.bishop@cciw.ca

For information on critical elements of the extensive cooperative monitoring programme, between Canada and the United States (NAAMP) see the Web site: http://www.im.nbs.gov

## Habitat Rehabilitation for Reptiles and Amphibians on the Great Lakes

Environment Canada has produced a new publication entitled *Habitat rehabilitation in the Great Lakes – Techniques for Enhancing Biodiversity* which reports the habitat requirements and methods for creating habitat in the Great Lakes for animals including six species of amphibians. It includes detailed examples, methods and case studies of habitats created.

This publication is available on the Internet Web site at the URL: http://www.cciw.ca/glimr/metadata/habitat–rehabilitation/intro.html

Paper copies can be requested through Christine Bishop (address above).

## Adopt-A-Pond Wetland Conservation Programme

The Metro Toronto Zoo encourages habitat preservation and restoration. The Metro Toronto Zoo's *Adopt–A–Pond* programme was created to encourage schools to undertake pond 'adoptions' by visiting a wetland, restoring a wetland, building a pond or conducting classroom activities associated with wetland and frog conservation. All participating students receive wetland 'adoption' certificates.

A recent success story comes from St. Anne elementary school in Richmond Hill, Ontario. The school contacted us in the fall of 1994 when a drainage ditch behind their school yard dried out too quickly killing all the toad tadpoles. A restoration plan was developed with the help of the Richmond Hill parks department. Three small ponds were dug into the drainage ditch. We were happy to hear that the toads were able to metamorphose before the ditch dried up in spring 1995!

Over 1200 schools already participate in the programme, We are committed to involving all 5,000 Ontario primary and secondary schools and then linking them through homeowners' backyards, industrial lands, hydro rights-of-way and railway lines.

Heather Gosselin, Metro Toronto Zoo, P.O. Box 280, West Hill, Ontario M1E 4R5.

## Environmental Contaminants and Amphibians in Canada

### Pesticides and Behaviour in Tadpoles

Research into the effects of pesticides on amphibians has been conducted at Trent University in Peterborough, Ontario since 1989. Short–term exposures have been used to assess the sensitivity to pesticides of native amphibians such as wood frogs (*Rana sylvatica*), bullfrogs (*R. catesbeiana*), green frogs (*R. clamitans*), leopard frogs (*R. pipiens*), American toads (*Bufo americanus*) and spotted salamanders (*Ambystoma maculatum*). Low concentrations of pesticides were used in order to mimic concentrations that might occur in water following typical field applications. The low concentrations employed often caused only sublethal effects such as paralysis and an inability to move away from a gentle prod (used to test escape and predator avoidance behaviour). Pesticides that have been studied include the insecticides permethrin, fenvalerate, fenitrothion, tebufenozide and endosulfan and the herbicides triclopyr, glyphosate, hexazinone, triallate, trifluralin and bromoxynil.

In one study, for instance, we exposed neural stage embryos and newly hatched tadpoles of green frogs to low levels of the herbicide glyphosate. Following 96 hours of exposure to the herbicide, surviving animals were moved to fresh water. Nominal glyphosate concentrations of 1.2 to 4.0 ppm initially caused tadpoles paralysis from which they eventually recovered. During the first 24 hours of exposure to 8.0 ppm, all tadpoles either died or were completely paralysed. Furthermore, almost all of the survivors from the first 24 hours of exposure died before the completion of the 96-hour exposure period. Follow–up tests indicated that much of the toxicity could be attributed to the surfactant used in the RoundUp® formulation of glyphosate.

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### Measuring the Health of Frogs in Agricultural Habitats Subjected to Pesticides in Southern Québec

A number of clinical approaches were used to determine the health of anurans living in agricultural habitats with the aim of identifying the most useful methods for detecting sublethal effects of agricultural pollutants. In the summers of 1992 and 1993, green frog (*Rana clamitans*), leopard frog (*R. pipiens*, bullfrog (*R. catesbeiana*) and American toad (*Bufo americanus*) were collected from ponds and ditches in potato fields, sweet corn fields, and uncultivated areas of similar habitat. Die–offs, outbreaks of disease and hind limb deformities were seen in habitats subjected to pesticide applications as well as in natural sites. In total 12.4% of metamorphosing anurans (0–68.5% animals per site; n = 853 individuals) were malformed in farmland habitats compared to 0.7% (0–7.7% animals per site; n = 271 individuals) in control sites. However, this difference in the proportion of deformities between control sites and croplands was not statistically significant ($p = 0.15$). Results of hematologic and blood biochemical analyses of adults appeared consistent with a normal health status in all locations. Flow cytometry revealed genomic disruption in adult and metamorphosing individuals from both potato and sweet corn fields. Deformed individuals from the potato fields had increased genome size variability. Similar genomic effects were found in pesticide–exposed individuals showing no apparent physical or physiological problems. High genotoxicity values for water samples taken from the cultivated sites were also found. Clinical examination and flow cytometry are therefore practical health assessment techniques for the examination of sublethal effects of environmental contaminants on frogs. This investigation indicates a general mutagenic effect of agricultural pollutants on anurans, in addition to potential teratogenic and pathogenic effects in some cases.

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### Apple Orchard Insecticide and Fungicide Effects on Ranid Populations in Ontario

The health of northern leopard frogs *Rana pipiens* and green frogs *Rana clamitans* was evaluated at eight breeding sites (permanent ponds or ditches), four of which were situated in apple orchards. Mature males were weighed, measured and aged. Tissues were taken to determine genetic variation, gonadosomatic, hepatosomatic and fat body indices, detoxification enzyme (EROD) activity, and levels of circulating steroid hormones. Embryos and larvae were subjected to *in situ* (caging) and ambient pond-water (laboratory) assays, and to toxicity tests of pesticides used in orchards. Significant differences in adult male size, age and condition, and in tadpole growth were detected between study site populations, but differences did not always distinguish reference site individuals from orchard site individuals. Male green frogs at one orchard site exhibited hepatic EROD induction, indicating that they had been exposed to pollutants. Caged embryos and larvae suffered high mortality at some orchard sites during *in situ* assays. The embryos and larvae caged in reference sites exhibited high hatching success (over 90%) and high survival rates (over 85%) during the two week assays, indicating that mortality in orchard ponds was probably due to stressful environmental conditions. Toxicity tests revealed that the pesticide diazinon, and the formulations Dithane® DG, Guthion® 50WP, and Thiodan® 50WP caused mortality, deformities, and/or growth inhibition in embryos and tadpoles. Residues of three of these compounds were detected in some orchard ponds, and, therefore, could have detrimentally affected wild egg and tadpole stages. High average heterozygosity values in sampled adult populations suggested that extensive immigration and emigration were occurring. This supports suspicions that breeding populations were subpopulations of larger metapopulations.

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### Impact of Reproductive and Developmental Toxicants on Populations of Mudpuppies

We examined the impact of persistent organochlorines on a long–lived, aquatic salamander, the mudpuppy (*Necturus maculosus*) through the comparison of populations along pollution gradients in the St. Lawrence and Ottawa River system (Québec and Ontario).

Although the mudpuppies were found to accumulate polychlorinated biphenyls (PCBs) and organochlorine pesticides in ovarian tissues at concentrations apt to cause reproductive and developmental dysfunction (maximum levels: total PCBs=58.2 mg/kg wwt, DDE=1.66 mg/kg wwt) no differences were found between sites in the circulating levels of sexual steroids, fecundity, egg diameters and the gonadosomatic index of vitellogenic females. However, X–ray analyses revealed high prevalences of limb deformities in adults from contaminated sites (maximum level: 58.3%), where individuals were significantly more likely to develop limb defects relative to reference sites (8.7–8.9%). The frequencies of terata, including oligodactyly and polydactyly, were positively correlated with the concentrations of embryotoxic and teratogenic chemicals, such as certain congeners of PCBs, in gonads.

Although other causal agents, such as pathogens, cannot be disregarded, contaminants are potentially involved. Observed defects could originate from the effects of pollutants either on the regeneration of injured limbs or during critical stages of larval development. High frequency of minor defects in adults might signal that severe malformations, compromising survival, are prevalent at earlier stages. We found an ageing of the population at the most polluted site, suggestive of low recruitment, which supports the hypothesis of toxic stress during early development. Investigation of the effect of toxicity on the corticosterone producing axis, which is closely involved in the stress response, suggests that the ability to promptly elevate corticosterone levels, in response to stimulation by corticotropin (ACTH) or acute stress, is reduced by persistent exposure to organochlorines.

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### New Database on Effects of Pollutants on Amphibians and Reptiles

The Canadian Wildlife Service is compiling an electronic bibliography and database on amphibian and reptile toxicology. We are collecting data on the toxicity of environmental contaminants, and information on the experimental methods that have been used in amphibian toxicology. The database will summarize toxicity and environmental contaminant residue data for amphibians which have been published after 1988 and will contain all available data on the effects of environmental contaminants on reptiles. Data on effects of pollutants can be accessed by the type of contaminant, or species of interest.

For information on the reptile and amphibian toxicology database, or to obtain a paper copy of a Canadian Wildlife Service summary of the amphibian toxicology literature up to 1988, please contact: Bruce Pauli, Environment Canada, Canadian Wildlife Service, National Wildlife Research Centre, Hull, Québec, CANADA K1A 0H3, phone: (819) 953–2634, fax: (819) 953–6612.
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## Ultra–Violet Radiation Work in Canada

### The Impact of Ultra–Violet Radiation on Developing Amphibian Eggs and Tadpoles

We augmented ultra–violet–b (UV–B) radiation in otherwise natural conditions by suspending UV–B lamps over the water at three pond or marsh sites in south central Ontario. We tested the sensitivity of embryos and newly hatched tadpoles of four species of frogs (wood frog *Rana sylvatica,* American toad *Bufo americanus*, green frog *Rana clamitans*, and bullfrog *Rana catesbeiana*) to both incident and augmented UV–B. Experiments were conducted, from April to August 1995, in relatively open areas of the marshes where differential shading could be avoided. Experimental flow–through containers under UV-B lamps were covered with cellulose acetate, which blocked all wavelengths below 290nm. Half of the containers exposed only to incident radiation also had cellulose acetate lids, while the other half were covered with Mylar D, which blocks all ultra–violet radiation. We recorded hatching success of embryos and mortality or abnormality of embryos or tadpoles. UV–B radiation was measured with an Optronics full spectrum Spectroradiometer. Of the total incident radiation, 290-800nm, that reached the water surface in the middle of a sunny day in June, UV–B constituted 0.38%, and at five cm below the surface, the level of eggs or tadpoles lying on the bottom of a submerged container, this was reduced to only 0.1%. Under the lamps, at the bottom of the submerged containers covered with cellulose acetate, this increased to 0.5%. Embryos hatched successfully under the UV–B, in excess of 80%, a rate not significantly different from that under the other two conditions. However, mortality of tadpoles of all four species was significantly greater under UV–B.

In *Rana clamitans* stage-sensitivity to UV-B was examined. Mortality of 1–2 week old tadpoles under the lamps was rapid and almost total; much higher than mortality of newly hatched tadpoles. We consider these results to be preliminary, and will refine the experiments in 1996. We anticipate that the greatest challenge will be to understand the impact of UV-B on the chemistry of pond water.

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### Effects of UV–B on amphibian development: embryonic and larval survival of *Hyla regilla* and *Rana pretiosa*

In April and May 1995, we exposed newly–laid eggs of *Hyla regilla* and *Rana pretiosa* to three experimental treatments: (1) control: UV–B blocked by means of a filter (Acrylite® OP3, Cyro Canada), (2) ambient UV–B (with or without an OP4 filter that transmitted all wavelengths) and (3) enhanced UV–B (with or without an OP4 filter). In Treatment 3, we enhanced ambient UV–B by 15% and 30% above levels at mid–day with fluorescent lamps that had a peak output in the range 280–320 nm. After hatching, we continued to expose larvae to the three UV–B regimes. There were eight replicates of each treatment for *R. pretiosa* eggs and four replicates of all other treatments.

The hatching success of *R. pretiosa* was lower in the enhanced UV–B treatment (56.0%) than in the ambient UV–B (89.8%) and control (81.0%) treatments. In contrast, the hatching success of *H. regilla* did not differ among the treatments. The survival of larvae of both species was significantly reduced in the enhanced UV–B treatment after two months but was similar in the control and ambient UV–B treatments. Very few *R. pretiosa* larvae survived under the enhanced UV–B regime.

We conclude that ambient UV–B levels at our study site on Vancouver

Island, B.C., Canada, did not affect the embryonic or early larval survival of either of the species. However, both species are potentially vulnerable to increases in solar UV–B predicted for the next decades. We are presently examining histological changes in embryos and larvae exposed to the different UV–B regimes and plan to carry out follow–up studies next spring, pending appropriate funding.

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### Effects of Ultraviolet Radiation on Amphibian Eggs

Ultraviolet light has been postulated as a casual factor in the apparent decline of amphibian populations. We tested the effects of ultraviolet–a (320–400nm) and ultraviolet–b (290–320nm) on eggs of *Rana sylvatica.* We also examined the transmission of ultraviolet–b through egg jelly of several amphibian species. No effect of ultraviolet–a was found at exposures twice the intensity of normal outdoor levels. Embryos were exposed to artificially high intensity UV–B, generated by unshielded UV–B lamps (302nm) or to reduced irradiance levels achieved by acetate filters. All embryos exposed to 30 minutes or more of artificially high intensity UV–B died. After exposure to high intensity UV–B of 15 minutes or less or at ecologically relevant levels, there was no effect on hatching success. There was a higher proportion of abnormal embryos after 10 or 15 minute exposure to high intensity UV–B at 12$^{o}$C compared to 20$^{o}$C. Jelly surrounding ova in amphibian eggs can effectively reduce UV–B transmission through the egg mass. Our results suggest that incident UV–B in Ontario is not likely to be detrimental to embryos of *R. sylvatica*.

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## Long-Term Population Studies

### Ranid Population Monitoring in Algonquin Provincial Park

Since 1985, the population dynamics of three ranids (*R. catesbeiana, R. clamitans, and R. septentrionalis*) have been investigated using mark-recapture techniques. This ongoing, long-term study is establishing basic life history and population parameters as well as determining population status in the relatively pristine environment of Algonquin Provincial Park, Ontario. To date, research indicates that, although all three populations fluctuate annually, there is no apparent long-term decline in abundances.

To further our understanding of population dynamics, we have recently begun to age individual frogs using skeletochronology. Initial results are encouraging in that several bullfrogs (*R. catesbeiana*) of known age, from the mark-recapture study, have been aged accurately. Bullfrogs are of particular concern because they have been reported to be declining in many parts of their range. This decline has been attributed, in part, to extensive commercial harvest. In 1996, we hope to compare the age-structure in our population with that of harvested populations. Other future research will examine longevity, sex ratio dynamics, distribution of reproductive success, and growth rates.

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### Variation in Abundance and Age Structure in Fowler's Toads, *Bufo fowleri*, at Long Point, Ontario

Fowler's toad (*Bufo fowleri*) is considered a vulnerable species in Canada because of its restricted range in the country and evidence that some populations have disappeared. I have been monitoring *Bufo fowleri* over eight consecutive breeding seasons (1988-1995) at Long Point, Ontario by tracking and marking all calling males within a study area stretching along 10 km of Lake Erie shoreline. Reliable estimates of total numbers of toads were based upon continuous mark/recapture data throughout each breeding season. The numbers of males rose markedly from 12 in 1988 to 487 (+/-49) in 1991 and then declined to 115 (+/-12) in 1994 and 49 (+/-7) in 1995. Fluctuations is mean size and in age structure also occured. Males in 1989, 1994, and 1995 were significantly larger than in other years. In 1992, when toads were abundant, 24% of adult males were one year old but in 1994 only 5% of males were one year old. In each of those years, only one four-year-old was found but in 1995, at least a fifth of the breeding chorus was four years old. This evidence of fewer but larger and older toads in 1994 and 1995, especially, implies that poor summer growth rates in 1993 and 1994 may have determined the decline in calling male numbers simply by diminishing the early recruitment of yearling males into the adult chorus without correlation to the total abundance of postmetamorphic toads. Because age structure among adults is evidently variable and chorus behaviour is weather-dependant, chorus size is highly stochastic. Since there is no stable age structure, simple population growth models cannot be applied. Furthermore, neither the spring field studies nor reference to weather records could identify consistent factors correlated with either the increase in adult males beginning in 1990-91 or the declines of 1994 and 1995. All of this underscores the extreme difficulty of predicting population numbers, much less trends, in randomly fluctuating, short-lived species.

David M. Green (address below)

## New Book on Declines in Canadian Amphibian Populations

*Amphibians in Decline*, edited by David M. Green, contains contributions from 52 authors, in 29 chapters. While concentrating on Canada it represents a benchmark for investigations of amphibian population abundance. Several demographic studies focus on amphibian population trends in Ontario, Québec, and Alberta. New data on amphibian distributions in various other parts of the country are also presented and genetic aspects of amphibian population biology are examined. Several chapters consider extensive monitoring of anuran populations, sampling methods for terrestrial salamanders, and surveying of amphibian larvae. The known and probable effects of forestry, acid precipitation, pesticides, disease, global warming, and ultra–violet radiation on amphibian populations are examined.

The volume concludes with a general discussion of amphibian population declines, identifying the problems and seeking answers. Current information on the status of each of the 43 species of amphibians found in Canada is presented. All chapters are peer–reviewed and have abstracts in French and English. The manuscript copy of the book has been submitted for review and, if accepted, will be a joint publication of the Society for the Study of Amphibians and Reptiles and the Canadian Association of Herpetologists in the new series *Herpetological Conservation*.

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## DAPTF Working Group Chairs

***Canada***

Please note a new contact address for Stan A. Orchard, DAPCAN, National Coordinator, 1745 Bank Street, Victoria, British Columbia, V8R 4V7, Phone/fax: 604-595-7556,
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***Chemical Contaminants***

The DAPTF gratefully acknowledges the work of Mike Tyler in setting up this working group. The new Chair is:

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Christine Bishop is a wildlife toxicologist with the Canadian Wildlife Service, of the federal department of environment (Environment Canada). The Working Group will attempt to keep herpetologists and the public up to date with information on the effects of pollutants on amphibians, through FROGLOG. The Canadian Wildlife Service, through Bruce Pauli (see above), will be maintaining a computerized database on toxicological references on amphibians and reptiles which will be completed in mid–1996. However, ongoing contributions to the database, especially reports unavailable in journals, are needed to maintain up to date listings. If you would like to be involved with this Working Group or become a contact for information on a specific type of contaminant (eg. trace metals, acid deposition, chlorinated hydrocarbons, herbicides, insecticides, molluscicides) please contact Christine Bishop.

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