AMPHIBIANS WORKSHOP
Taylor Ranch, Idaho
15-16 July 1994

Charles R. Peterson
Department of Biological Sciences, Idaho State University
Idaho Museum of Natural History
Campus Box 8007
Pocatello, Idaho 83209

I. Introduction

A. Sources of Information
B. Characteristics of Amphibians
C. Importance of Amphibians
D. Amphibian Declines
E. Inventory, Monitoring, and Research Needs

II. Sampling Techniques

A. Incidental observations
B. Seize and capture (manual, nets, rakes, etc.)
C. Trapping
(pit and funnel traps with drift fences; minnow traps)
D. Artificial Cover
E. Electroshocking
F. Calling
G. Road driving

III. Checklist of Possible Amphibians of the Frank

Long-toed Salamander (Ambystoma macrodactylum)	confirmed
Idaho Giant Salamander (Dicamptodon aterrimus)	probable
Western Toad (Bufo boreas)	confirmed
Tailed Frog (Ascaphus truei)	confirmed
Pacific Treefrog (Pseudacris regilla)	probable
Western Chorus Frog (Pseudacris triseriata)	possible
Spotted Frog (Rana pretiosa)	confirmed

IV. Species Accounts
A. Range
B. Descriptions - adults, eggs, larvae, and juveniles
F. Habitat
G. Activity patterns
H. Calling
I. Reproduction
I. Sampling techniques

References

Blaustein A.R., and D.B. Wake. 1990. Declining amphibians: a global trend? Trends in ecology and evolution. 5:203-204.

Blaustein, A.R., P.D. Hoffman, D.G. Hokit, J.M. Kiesecker, S.C. Wells, and J.B. Hays. 1994. UV repair and resistance to solar UV-B in amphibian eggs: A link to population declines?

Campbell, H.W. and S.P. Christman. 1982. Field techniques for herpetofaunal community analysis. Pp. 193-200 in Scott, N.J. (ed.), Herpetological communities: A symposium for the Society for the Study of AMphibians and Reptiles and the Herpetologists League. Wildlife Research Report 13, U.S. Fish and Wildlife Seice, U.S. Dept. of the Interior, Washington, D.C.

Cooperider, A.Y., R.J. Boyd, and H.R. Stuart. Inventory and monitoring of wildlife habitat. U.S. Dept. of the Interior, Bureau of Land Management Service Center, Denver, CO. 858 pp.

Corn, P.S. and R.B. Bury. 1989. Logging in Western Oregon: responses of headwater habitats and stream amphibians. Forest Ecology and Management, 29(1989):39-57.

Corn, P.S. and R.B. Bury. 1990. Sampling methods for terrestrial amphibians and reptiles. USDA Forest Service, Pacific Northwest Research Station, Portland, Oregon. General technical Report PNW-GTR-256.

Corn, P.S. and J.C. Fogleman. 1984. Extinction of montane populations of the northern leopard frog (Rana pipiens) in Colorado. Journal of Herpetology, 18(2):147-152.

Grant, B.W. A.T. Tucker, J.E. Lovich, A.M. Mills, P.M. Dixon, and J.W. Gibbons. 1992. The use of coverboards in estimating patterns of reptile and amphibian diversity. Pp. 379-403 in Wildlife 2001:Populations. D.R. McCullough and R.H. Barrett (eds.). Elsevier Science Publishers, Essex, England.

Halliday, T. and K. Adler. 1987. The encyclopedia of amphibians and reptiles. Facts on File Publications, New York. 143 pp.

Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.C. Hayek, and M.S. Foster (editots). 1994. Measuring and monitoring biological diversity: standard methods for amphibians. Smithsonian Institution Press. 364 pp.

Karns, D.R. 1986. Field herpetology: methods for the study of amphibians and reptiles in Minnesota. Occasional Paper No. 18, Division of Comparative Biology, James Ford Bell Museum of Natural History, University of Minnesota, Minneapolis. (Contains good descriptions of techniques for finding and monitoring populations).

Leonard, W.P., H.A. Brown, L.C. Jones, K.R. McAllister, and R.M. Storm. 1993. Amphibians of Washington and Oregon. Seattle Audubon Society, Seattle, Washington. [excellent color photographs]

Linder, A.D. and E. Fichter. 1977. The amphibians and reptiles of Idaho. Idaho State University Press, Pocatello, ID. 78 pp.

Mosely, R. and C. Groves. 1992. Rare, threatened, and endangered plants and animals of Idaho. Conservation Data Center, Nongame and Endangered Wildlife Program, Idaho Department of Fish and Game. 38 pp.

References (continued)

Nussbaum, R.A. E.D. Brodie, and R.M. Storm. 1983. Amphibians and reptiles of the Pacific Northwest. University Press of Idaho, Moscow. 332 p. The best general source of information on the amphibians and reptiles of Idaho).

Peterson, C.R. and M.E. Dorcas. 1992. The use of automated data acquisition techniques in monitoring amphibian and reptile populations. Pp 369-378 In: Wildlife 2001: Populations. D.R. McCullough and R.H. Barrett, (eds.). Elsevier Applied Science, London.

Peterson, C.R. and M.E. Dorcas. 1994. Automated data acquisition. Pp. 47-57In: Measuring and monitoring biological diversity - Standard methods for amphibians. Heyer, W.R., M. Donnelly, R.W. McDiarmid, L.C. Hayek, and M.S. Foster (editors). Smithsonian Institution Press, Washington, D.C.

Pough, F.H., J.B. Heiser, and W.N. McFarland. 1989. Vertebrate Life. Third edition. Macmillan Publishing Co., New York. (An excellent textbook on vertebrate zoology; especially information on amphibians and reptiles).

Pisani, G.R. 1973. A guide to preservation techniques for amphibians and reptiles. Herp. Circular. No. 1. 22 p. Society for the Study of Amphibians and reptiles.

Scott, N.S. and R.A. Seigel. 1992. The management of amphibian and reptile populations: species priorities and mtehodological and theoretical constraints. Pp. 343-368 In: Wildlife 2001: Populations. D.R. McCullough and R.H. Barrett, (eds.). Elsevier Applied Science, London.

Stebbins, R.C. 1985. A field guide to western reptiles and amphibians. Houghton Mifflin Co., Boston. 336 pp. (The best field guide to the amphibians and reptiles of the western United States).

Turner, F.B. 1958. Life history of the western spotted frog in Yellowstone National Park. Herpetologica, 14:96-100.

Turner, F.B. 1960. Population structure and dynamics of the western spotted frog, Rana p. pretiosa Baird & Girard, in Yellowstone Park, Wyoming. Ecological Monographs, 30:251-278.

Vial, J.L. (editor) 1992. Froglog - IUCN/SSC Declining Amphibians Task Force Newsletter. March, 1992, 1.

Welsh, H.H. 1987. Monitoring herpetofauna in woodland habitats of northwestern california and southwestern Oregon: A comprehensive strategy. Gen. Tech. Rep. PSW-100. Berkeley, CA. Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture.

Welsh, H.H., A.J. Lind, and D. L. Waters. 1991. Monitoring frogs and toads on Region 5 National Forests. R-5 Fish Habitat Relationship Technical Bulletin. Number 4, May 1991. 12 p.

Welsh, H.H. and A.J. Lind. In press. Structure of herpetofauanal assemblage of the Douglas-fir/hardwood forests of northern California and southwestern Oregon. Pages XX-XX in L.F. Ruggiero, K.B. Aubry, A.B. Carey, and M.H. Huff, tech. coords. Wildlife and vegetation of unmanaged Douglas-fir forests. USDA For. Serv. Gen Tech. Rep. PNW-GTR-XXX. [Contains a discussion of management strategies to maintain a viable herpetofauana]

DAPTF Directors and Chairs

BOARD OF DIRECTORS

*William R. Branch
Port Elizabeth Museum
P.O. Box 13147
Humewood, 6013
SOUTH AFRICA (Code 27)
Phone: (041) 561051 Fax: (041) 562175

Alain Dubois
Laboratoire des Reptiles et Amphibians
Musée National d'Histoire Naturelle
25, Rue Cuvier
75005 Paris
FRANCE (Code 33)
Phone: (1) 40 79 34 87 Fax: (1) 40 79 34 84

*Timothy R. Halliday
Biology Department
The Open University
Walton Hall
Milton Keynes, MK7 6AA
UNITED KINGDOM (Code 44)
Phone: 0908-653831 Fax: 0908-654167

Sergius L. Kuzmin
Institute of Evolutionary Morphology
and Ecology of Animals
Academy of Sciences
Leninsky Prospekt, 33,
Moscow 117071
RUSSIA

Robert Johnson
Metropolitan Toronto Zoo
P.O. Box 280
West Hill, Ontario, M1E 4R5
CANADA
Phone: (416) 392-5968 Fax: (416) 392-4979

James B. Murphy
Dallas Zoo
621 E. Clarendon Dr.
Dallas, TX 75203, USA
Phone: (214) 670-7573 Fax: (214) 670-7450

*Jaime F. Péfauer
Departamento de Biología
Facultad de Ciencias
Universidad de los Andes
Merida, VENEZUELA 5101 (Code 58)
Fax: 74 40 1286

George B. Rabb, IUCN/SSC
Chicago Zoological Society
Brookfield, IL 60513, USA
Phone: (708) 485-0263 Fax: (708) 485-3532

*Michael J. Tyler
Department of Zoology
University of Adelaide
GPO Box 498
Adelaide, South Australia 5001
AUSTRALIA (Code 61)
Fax: 8 223 5817

David B. Wake (Chair)
Museum of Vertebrate Zoology
University of California
Berkeley, CA 94720, USA
Phone: (510) 642-3567 Fax: (510) 643-8238

William Winner (Ex officio)
Center for Analysis of Environmental Change
Oregon State University
Corvallis, OR 97333, USA
Phone: (503) 737-1744 Fax: (503) 737-3399

John W. Wright
Museum of Natural History
Los Angeles, CA 90007, USA
Phone: (213) 744-3371

* Also serves as Organizational Chairperson for Regional Working Group.

WORKING GROUP CHAIRS

K.D. Arudpragasam, *Co-Chair, Sri Lanka*
University of Colombo
SRI LANKA

W.R. Breckenridge, *Co-Chair, Sri Lanka*
Department of Zoology
University of Peradeniya
Peradeniya
SRI LANKA

David M. Green, *National Coordinator, Canada*
Redpath Museum
McGill University
Montreal, PQ H3A 2K6
CANADA
Phone: (514) 398-4088

*Kuang-Yang Lue, *Taiwan*
Department of Biology
88 Roosevelt Rd., Sec. 5
11718, Taipei, Taiwan
REPUBLIC OF CHINA (Code 86)
Phone: (02) 932-5234 Fax: (02) 931-3904

Jay M. Savage, *Lower Central America-Costa Rica/Panama*
Department of Biology
University of Miami
Coral Gables, FL 33142, USA
Phone: (305) 284-2130 Fax: (305) 284-3039

Annie Zuiderwijk, *Western Europe*
Institute of Taxonomic Zoology
University of Amsterdam
P.O. Box 4766 - 1009 AT Amsterdam
THE NETHERLANDS (Code 31)
Phone: (20) 5256624 Fax: (20) 5257238

*(Acceptance pending)

U.S. Regions

Ronn Altig, *Mississippi Delta (AR,MS,LA)*
P.O. Drawer GY
Department of Biological Science
Mississippi State University
Mississippi State, MS 39762
Phone: (601) 325-7561 Fax: (601) 325-7939

Andrew R. Blaustein, *Co-Chair, Pacific Northwest* (WA,OR,AL)
Department of Zoology
Oregon State University
Corvallis, OR 97331-2914
Phone: (503) 737-5356

Ronald A. Brandon, *Central (IA,MO,IL,IN,OH)*
Department of Zoology
Southern Illinois University
Carbondale, IL 62901
Phone: (618) 536-2314

Richard C. Bruce, *Appalachian (KY,TN,NC,VA,WV)*
Highlands Biological Station
P.O. Box 580
Highlands, NC 28741
Phone: (704) 526-2602

R. Bruce Bury, *Co-Chair, Rocky Mountains (ID,MT,WY,CO,UT)*
National Ecology Research Center
4512 McMurray Ave.
Fort Collins, CO 80525-3400
Phone: (303) 226-9270

Janalee P. Caldwell, *Southern Plains (OK,TX)*
Oklahoma Museum of Natural History
1335 Asp Ave.
Norman, OK 73019-0606
Phone: (405) 325-4712 Fax: (405) 325-7771

P. Stephen Corn, *Co-Chair, Rocky Mountains (ID,MT,WY,CO,UT)*
National Ecology Research Center
4512 McMurray Ave.
Fort Collins, CO 80525-3400

C. Kenneth Dodd, Jr., *Southeast (AL,GA,FL,SC)*
National Ecology Research Center
412 NE 16th Avenue (Room 250)
Gainesville, FL 32601
Phone: (904) 372-2571 Fax: (904) 374-8080

Gary M. Fellers, *Cal/Neva (CA,NV)*
Point Reyes National Seashore
Point Reyes, CA 94956
Phone: (415) 663-8522

A. Ross Kiester, *Co-Chair, Pacific Northwest (WA,OR,AL)*
USDA Forest Service
3200 SW Jefferson Way
Corvallis, OR 97331
Phone: (503) 750-7269

James E. Platz, *Northern Plains (ND,SD,NE,KS)*
Department of Biology
Creighton University
2500 California St.
Omaha, NE 68178
Fax: (910) 622-0287

Norman J. Scott, Jr., *Southwest (AZ,NM)*
Department of Biology
University of New Mexico
Albuquerque, NM 87131
Phone: (505) 766-3903

Richard L. Wyman, *Northeast (ME,NH,VT,NY,PA,MD,DE,MA,RI,CT,NJ,DC)*
Edmund Niles Huyck Preserve, Inc.
P.O. Box 188
Rensselaerville, NY 12147
Phone: (518) 797-3440

Issue Specific

W. Ronald Heyer, *Monitoring Protocols*
Division of Amphibians and Reptiles
US National Museum of Natural History
(NHB Mail Stop 162)
Washington, DC 20560, USA
Phone: (202) 357-2195

Roy McDiarmid (Will succeed WRH)
National Fish and Wildlife Laboratory
U.S. National Museum of Natural History
(NHB Mail Stop 162)
Washington, D.C. 20560, USA
Phone: (202) 357-2780

James L. Vial, *Documentary Resources*
(Address given below)

FROGLOG

Newsletter of the IUCN/SSC Task Force on Declining Amphibians

Dr. James L. Vial
Task Force Coordinator, *Editor*
Center for Analysis of Environmental Change
EPA Environmental Research Laboratory
200 South West 35th Street
Corvallis, Oregon 97333, USA
(503) 754-4798
Telefax (503) 754-4799

Funding for FROGLOG is provided in part by a special donation from Frog's Leap Vineyards, St. Helena, California 95474, USA

Amphibian and Reptile Observation Form

Please provide whatever information you can, even if you are unsure of the species. Thank you.

Species_____ Number of Animals_____

Observation Date _____/_____/_____ (*month:day:year*) Time_____am pm (*circle one*)

Observer Name(s) _____ Phone No:_____

Address_____

Affiliation/Relevant Experience_____

Have you seen this species before?_____

Description of Animal_____

_____

_____ Did you photograph the animal? _____

Description of Animal's Behavior_____

_____

_____

Animal's Location_____

_____

Drainage _____ County _____ State_____

Elevation_____ UTM_____E_____N

Habitat_____

_____

Weather_____

_____

General Comments_____

_____

Please return to:

Dr. Chuck Peterson
Idaho Museum of Natural History
Box 8007, Idaho State University
Pocatello, Idaho 83209
(208) 236-3922 [office] (208) 236-4570 [FAX]

Date	Time	Observer(s)	Species	Location	Remarks (#'s, weather, habitat, behavior, etc.)

AMPHIBIAN SURVEY DATA SHEET – US FISH & WILDLIFE SERVICE, 4512 McMURRY AVE. FT. COLLINS, CO 80525-3400

(circle choice for shaded variables; supply value for others) (ver. 2/7/92)

DATE	BEGIN TIME	END TIME	OBSERVERS

LOCALITY

STATE	COUNTY	MAP NAME	OWNER	ELEVATION (circle scale) M FT

T	R	S	SECTION DESCRIPTION	UTM ZONE	NORTHING (or LAT)	EASTING (or LON)

AMPHIBIAN AND/OR GARTER SNAKE SPECIES PRESENT (INDICATE NUMBERS IN CATEGORIES IF POSSIBLE) — CIRCLE METHOD AND INDICATE IF VOUCHER SPECIMEN WAS COLLECTED

SPECIES	ADULTS/JUVENILES	CALLING?	TADPOLES/LARVAE	EGG MASSES	METHOD
		Y N			VISUAL/AURAL ID DIP NET/SEINE HAND COLLECTED TRAPPED VOUCHER COLLECTED? YES NO
		Y N			VISUAL/AURAL ID DIP NET/SEINE HAND COLLECTED TRAPPED VOUCHER COLLECTED? YES NO
		Y N			VISUAL/AURAL ID DIP NET/SEINE HAND COLLECTED TRAPPED VOUCHER COLLECTED? YES NO
		Y N			VISUAL/AURAL ID DIP NET/SEINE HAND COLLECTED TRAPPED VOUCHER COLLECTED? YES NO
		Y N			VISUAL/AURAL ID DIP NET/SEINE HAND COLLECTED TRAPPED VOUCHER COLLECTED? YES NO

FISH PRESENT?	YES ??? NO	FISH SPECIES:			
ENTIRE SITE SEARCHED?	YES NO	IF NO, INDICATE AREA			METERS OF SHORELINE M² OF HABITAT

PHYSICAL AND CHEMICAL ENVIRONMENT (CHEMISTRY VARIABLES OPTIONAL – USE EXTRA SPACES FOR ADDITIONAL MEASUREMENTS)

WEATHER:	CLEAR	OVERCAST	RAIN	SNOW	WIND:	CALM	LIGHT	STRONG

AIR TEMP (circle scale) °C °F	WATER TEMP (circle scale) °C °F	COLOR:	CLEAR	STAINED	TURBIDITY:	CLEAR	CLOUDY

pH	ANC			

SITE DESCRIPTIONS – (SKETCH SITE AND PUT ADDITIONAL COMMENTS ON BACK OF SHEET)
OMIT THIS SECTION IF DATA HAVE BEEN COLLECTED ON A PREVIOUS VISIT

ORIGIN:	NATURAL	MAN-MADE	DRAINAGE:	PERMANENT	OCCASIONAL	NONE

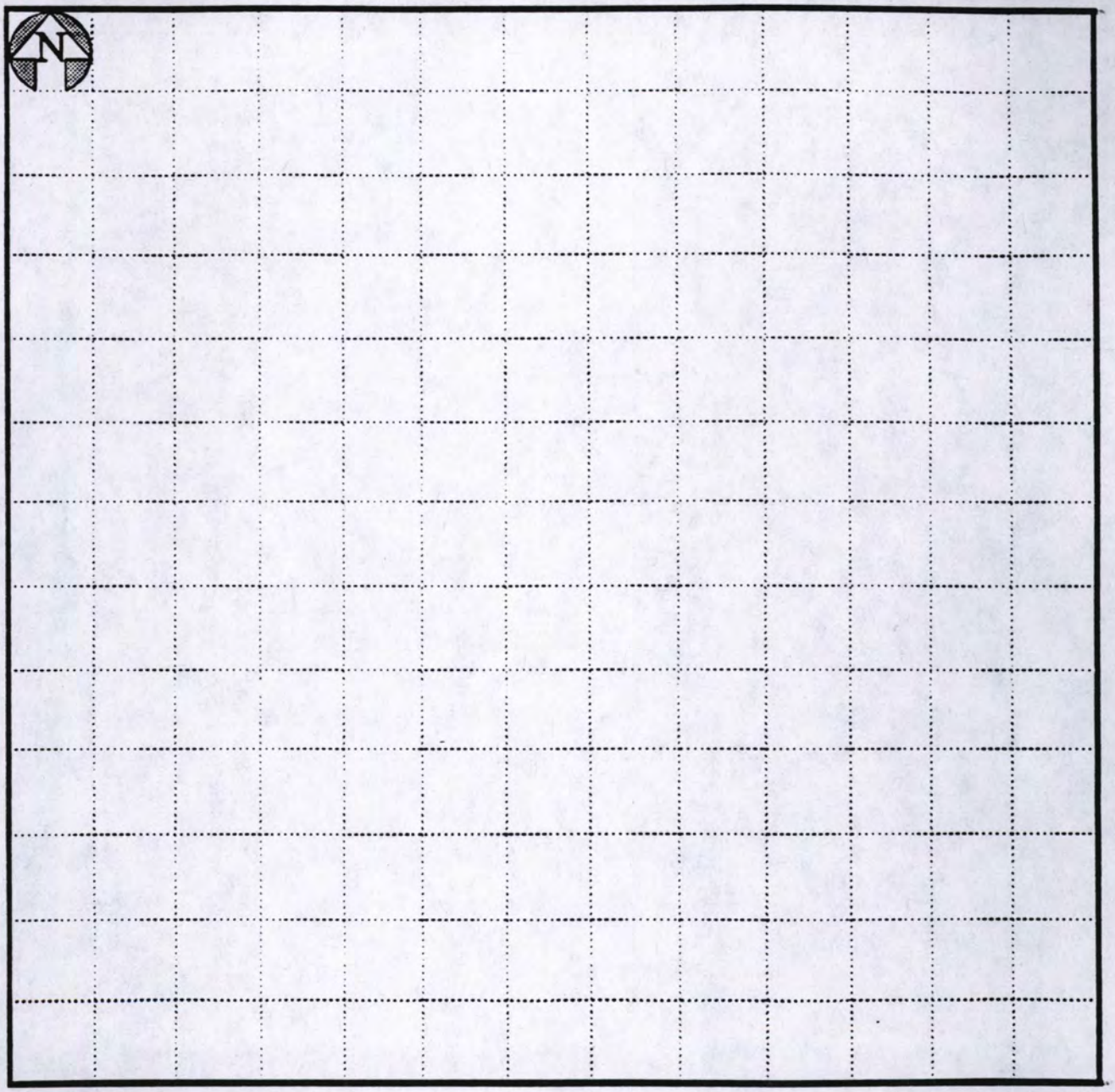
DESCRIPTION:	PERMANENT LAKE/POND	TEMPORARY LAKE/POND	MARSH/BOG	STREAM	SPRING/SEEP	ACTIVE BEAVER POND	INACTIVE BEAVER POND

SITE LENGTH (M)	SITE WIDTH (M)	MAXIMUM DEPTH:	< 1 M	1 – 2 M	> 2 M

STREAM ORDER	1	2	3	4	5 +
PRIMARY SUBSTRATE:	SILT/MUD	SAND/GRAVEL	COBBLE	BOULDER/BEDROCK	OTHER

% OF POND LAKE MARGIN WITH EMERGENT VEGETATION:	0	1 – 25	25 – 50	> 50
EMERGENT VEGETATION SPECIES (LIST IN ORDER OF ABUNDANCE)				
NORTH SHORELINE CHARACTERS:	SHALLOWS PRESENT	SHALLOWS ABSENT	EMERGENT VEG PRESENT	EMERGENT VEG ABSENT
DISTANCE (M) TO FOREST EDGE:	FOREST TREE SPECIES:			

ROUGH SKETCH OF SITE GRID SPACING IS ___ METERS BETWEEN LINES



ADDITIONAL NOTES:

It appears complex and intimidating, but actually can be completed in a short amount of time after [illegible] to be circled, and the remaining variables are numerical and easy to determine. The data sheet is divided into four sections, divided by double lines. Each section describes a cohesive set of variables. In addition the back of the sheet includes a grid for a rough sketch of the site and space for additional comments. The map is optional, but the future value of the data is enhanced if it is supplied.

SECTION 1 – LOCALITY *These data are essential. Many amphibian surveys have been hampered by the inability to relocate exact locations in the historical record. Some of this information can be completed in the office after the survey.*

DATE: Use the format DD-MMM-YY (e.g., 05-APR-92).

BEGIN TIME: List the time survey of habitat for amphibians began in 24 hour format.

END TIME: List the time the survey ended in 24 hour format. (The total time (END TIME - BEGIN TIME) should reflect only the amount of time spent searching for amphibians. Total time plus number of observers may be used to assess relative abundance.)

OBSERVERS: List names or initials of all persons involved in searching.

LOCALITY: Describe the *specific* geographic location of the site. Use air distance in two directions (e.g., 5km N and 7.5 km W) of a map landmark that likely will not change (distance from a large town or city is not all that helpful).

STATE: Use the 2-letter abbreviation.

COUNTY:

MAP NAME: List the name of the U.S.G.S. quadrangle or other map used to locate the site.

OWNER: List the public land manager (e.g., Roosevelt Nat. Forest or Rocky Mtn NP), or name of the owner if the site is on private land (listing the owner's name will make it clear that you did not trespass to survey the site).

ELEVATION: Circle the scale used; meters are preferred.

T: township **R:** range **S:** section

SECTION DESCRIPTION: Describe the location of the site within the section (e.g., SE ¼ or NE ¼ of SE ¼)

UTM ZONE, NORTHING, EASTING: Universal Transverse Mercator coordinates are preferred over longitude and latitude. The UTM zone is listed on newer topographic maps. If you are using a map without the UTM grid, substitute latitude for Northing and longitude for Easting.

SECTION 2 – SPECIES DATA *List all amphibian species observed. If garter snakes are seen, list them here also.*

SPECIES: Use the scientific name. Convenient shorthand is to use a 4-letter code made up of the first 2 letters of the genus and species (e.g., *Rana sylvatica* would be RASY).

ADULTS/JUVENILES: Indicate presence with a check, but numbers seen are more valuable data

CALLING?: Circle Y if frogs are vocalizing in a breeding chorus, of if a breeding aggregation of species that don't call (e.g., *Bufo boreas*) is observed.

TADPOLES/LARVAE: Same as for adults/juveniles

EGG MASSES: Same as above. Numbers of egg masses are especially valuable data. If possible, describe the developmental stage of eggs in the space for additional notes on the back of the form.

METHOD: Circle how observations were made: **VISUAL/AURAL ID** – species identified without picking it up, either by sight or by recognition of the breeding call; **HAND COLLECTED** – animal was picked up and identified in the field (higher confidence than visual id); **DIP NET/SEINE** – the usual method of collection for larvae; **TRAPPED** – minnow-type traps are also used for larvae; **VOUCHER COLLECTED?** – circle yes or no (voucher specimens are recommended for every site, *especially if identification is uncertain and for larvae*). Indicate voucher status in addition to method used.

FISH PRESENT?: If yes, list species if you can. Circle the question marks if you are not certain, but suspect that fish are present.

ENTIRE SITE SEARCHED?: If no, list either the meters of shoreline or the area (m^2) of habitat (e.g., amount of wet meadow) searched.

SECTION 3 – PHYSICAL AND CHEMICAL DATA *Water chemistry data are difficult to collect accurately without thorough planning and quality equipment; these data are optional. Weather data are important for determining the quality of the observations (e.g., was an absence of amphibians due to observations made during a blizzard?)*

WEATHER, WIND: Indicate atmospheric conditions

AIR TEMPERATURE: Take at chest height in shade. The Celsius scale is preferred.

WATER TEMPERATURE: Take 1 meter from margin and at 2 cm depth, or where egg masses are observed.

COLOR: This is a qualitative assessment of whether the water clear or tea-colored from organic (humic) acids.

TURBIDITY: This is a qualitative assessment of whether the water clear or clouded from suspended particulate matter.

SECTION 4 – HABITAT DESCRIPTION *These data are important for developing hypotheses to explain changes in abundance of amphibians. This section needs to be filled out only once for each site (a reasonable amphibian survey should include at least 2 – 3 visits to each site in one season).*

ORIGIN: Decide whether the lake is a natural geologic formation or man-made. Bodies of water enlarged by a dam are problematic. List them as man-made, but add an explanation in the space for additional notes on the back of the form.

DRAINAGE: Circle whether the site has permanent drainage, no drainage, or occasional drainage. Determining the potential for occasional drainage requires judgement. Look for clues in the topography and vegetation.

DESCRIPTION: Decide how best to describe the site. If there is evidence of past or present beaver activity, circle one of these choices in addition to your choice.

LENGTH, WIDTH: Record the maximum length and width of lakes and ponds. For streams, record the length and average width of the reach searched.

MAXIMUM DEPTH: Most times, you will not have access to a boat, so estimate depth (deep lakes are usually not important to amphibians).

STREAM ORDER: This is an index of stream size, and you will need a topographic map to determine it. First-order streams have no tributaries, second-order streams are formed by the confluence of two 1st-order streams, third-order streams are formed by the confluence of two 2nd-order streams, and so on.

PRIMARY SUBSTRATE: Circle the type that covers the majority of the bottom of the site.

EMERGENT VEGETATION: Circle the percentage of the margin of the site with emergent vegetation present, and list the dominant species. If you are botanically-disadvantaged, list the categories of the dominant species (e.g., cattail, sedges, etc.).

NORTH SHORELINE CHARACTERS: Describe the north shore of a lake or pond in terms of shallow water and emergent vegetation. This is important in evaluating quality of breeding habitat in some mountain locations.

FOREST CHARACTERS: List the closest distance between the water and the surrounding forest, and list the most common tree species. Leave these fields blank if there is no forest. Describe other surrounding habitat types in the notes section on the back of the form.

AMPHIBIAN POPULATION MONITORING DATA SHEET

Study Site _____

Observers/Teams[1] _____

Date _____ Begin Time _____ End Time _____ Total Search Time[2] _____

Air Temperatures at 1 m[3] _____ Water Temperatures at 2 cm[4] _____

Wind[5] _____ Cloud Cover _____% Radiation[6] _____

Remarks about Environmental Conditions:[7]

_____

_____

SPECIES[8]	ADULTS/JUVENILES[9]	TADPOLES/LARVAE[10]	EGG MASSES (#)	CALLING? (Y/N,#)
Tiger Salamander				
Western Toad				
Chorus Frog				
Spotted Frog				

Remarks:[11]

_____

_____

_____

Notes:

[1] Indicate if observers searched together or independently.
[2] Total search time = the elapsed time X the number of "teams" searching.
[3] Shade thermometer when measuring air temperature.
[4] Measure water temperatures in sunlit and shaded water 1 m from shore or where egg masses are observed.
[5] Estimate wind speed (still, light, moderate, heavy).
[6] Estimate solar radiation conditions (sun blocked completely or partially by clouds, clear, etc.)
[7] Indicate any significant changes in environmental conditions since the last visit (e.g., changes in water level).
[8] In the unlikely event that you see some species not in this table (e.g., a leopard frog), describe it under remarks.
[9] Indicate numbers of individuals. If you can distinguish adults, juveniles, and newly metamorphosed individuals, please do so.
[10] Indicate the appropriate category for tadpole numbers observed (e.g., 1-10, 10-100, > 100, > 1000).
[11] Under remarks, indicate any other significant observations (e.g., the presence of predators such as fish, garter snakes, and sandhill cranes). Please include suggestions for improving this data form.

If you have questions, please contact:

Charles R. Peterson
Dept. of Biology
Idaho State University
Campus Box 8007
Pocatello, ID 83209
208-236-3922

Steve Corn
U.S. Fish and Wildlife Service
National Ecology Research Center
4512 McMurry Ave.
Fort Collins, CO 80525
303-226-9464 FTS-323-5464

A GUIDE TO PRESERVATION TECHNIQUES FOR AMPHIBIANS AND REPTILES

George R. Pisani
Museum of Natural History
University of Kansas
Lawrence, Kansas 66044

Illustrations
by

Patricia May and Tom Swearingen
Museum of Natural History
University of Kansas

Table of Contents

INTRODUCTION

Over the years, several informative works describing the preservation of amphibians and reptiles have been published. Most of these have been intended for relatively limited distribution by the institutions or individuals publishing them. This fact, coupled with new laws pertaining to syringes and certain drugs used for killing specimens, warrants an additional treatment of the subject. This article is an attempt to combine a complete survey of current techniques with a page size that the individual collector can conveniently carry in the field.

In an age when so many wild species and areas of suitable habitat are at the threshold of extermination, it seems advisable at the outset to include a plea for conservation in this booklet. Current museum collections contain excellent samples of various North American species of reptiles and amphibians from certain areas within their ranges. In these instances, it is a needless waste to collect and preserve additional material when this will not add appreciably to our knowledge of these creatures. I am not referring to such collecting as may be associated with the compiling of a synoptic teaching collection by a school or to collection of specimens needed for a particular aspect of research, but rather to the capture and preservation of animals simply to amass a collection which may never be used for scientific or educational purposes. There are numerous geographic areas, including several in North America, in which the amphibians and reptiles are poorly known. Collections from these areas can add measurably to our herpetological knowledge. Persons wishing to learn of the desirability of specimens from particular areas should consult with herpetologists at nearby universities, museums, zoos, etc.

I would like to express my thanks to Woodrow W. Barber, Hobart M. Smith, William E. Duellman, Joseph T. Collins, Clarence J. McCoy, and George Iannarone for furnishing helpful material and/or advice and to Phyllis Shaffer, Judy Hamilton, Leanne Johnson, and Ginger Stiggins for typing assistance. Thanks are also expressed to Jaime Villa for preparing much of the "International Shipment" section.

FIELD NOTES

Specimens not accompanied by data identifying the collection locality are virtually useless to scientific investigators. The more data available for a specimen, the greater its value in research. Hence, keeping accurate, complete field notes is necessary. Many times, data felt to be trivial at the time of collection may prove to be quite useful when many observations are pooled. Field notes should be written in waterproof ink ("Pelikan" brand is preferred by many) using only one side of each page. Several brands of waterproof ink will "run" if alcohol is accidentally spilled on the page, hence care should be used in selecting ink. A worthwhile technique is to carry a small notebook for on-the-spot data taking. Then transfer these data into the permanent field notes as soon afterward as possible.

The following is a representative outline of data included in field notes (numbers refer to those in figure 1a):

1. Locality- Do not record locality with reference to business establishments. Use towns or mapped roadways; determine distance from an automobile odometer (if available), or estimate distance carefully from maps. It is not unusual for roads to be rerouted, renumbered or both. It is therefore advisable to refer to roads indicated in a good atlas to which future reference can be made. In the U. S., the American Highway Atlas (Gousha Co., Chicago) is suitable. If collecting in areas remote from roadways, locate the collecting site as accurately as possible from U. S. Geological Survey topographic maps. Collectors in foreign countries should try to obtain accurate, detailed maps of the areas in which they are working. Elevation of the locality should also be recorded whenever possible.

2. Date- Always write out the name of the month, or indicate month by a Roman numeral; 6-10-71 could refer either to June 10th or October 6th.

3. Name(s) of all collector(s) present.

4. Time of collecting.

fig.1a

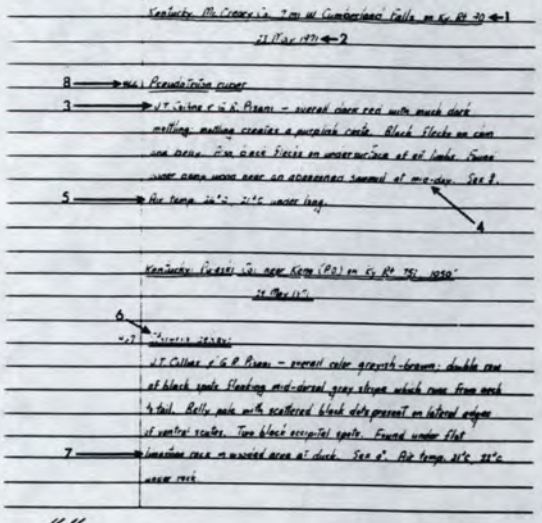
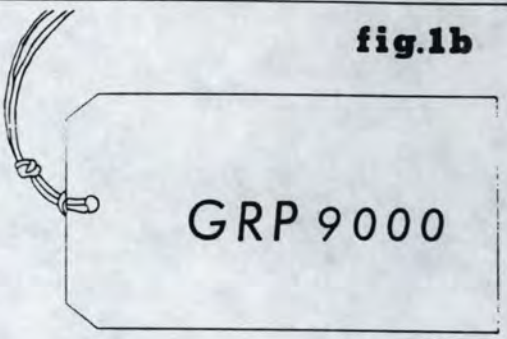


fig.1b



5. Air temperature and other appropriate weather notes- It is often useful to note existing cloud cover and moisture conditions, as well as general weather conditions preceding the collection.

6. Species- List all species collected plus the number collected of each, followed by species which may be observed but not collected. Accurate color notes are a worthwhile inclusion, especially when collecting in regions having a poorly known herpetofauna. It is also worthwhile to take accurate color notes when an atypically colored individual of a well-known species is encountered.

7. Microhabitat of species collected and any significant behavior (courtship, defensive display, etc.) observed.

8. Field number- It is useful to carry a series of numbered field tags on collecting trips. These should be printed on heavy paper in permanent ink. Satisfactory tags can also be made with one of the commercially available label makers that imprint plastic tape. Thread can be sewn through the numbered tags, but the backing on the tape should not be removed. Avoid using colored thread, or thread made from synthetics such as nylon, which may be destroyed by preservatives. White, cotton carpet thread is suitable for tagging. After threading the tag, tie a small knot in the string as shown in figure 1b. Each specimen should be assigned its own number, which greatly simplifies the task of keeping specimens and localities associated. Testes, stomach contents, photos, tape recordings, etc. are assigned the same number to increase efficiency in future analyses. Field tags should be securely tied (with a square knot) to specimens as illustrated in Plates 1 and 2. Lizards possessing femoral pores should be tagged by knotting the string below the knee; this avoids covering the pores with string. Tags in the field series should be numbered independently from the catalog series discussed in a later section.

Of the above data, numbers 1 (locality), 2 (date), 3 (name(s) of collector(s), and 8 (field number) represent the minimum data which should be recorded. If specimens are donated to an institution, the field notes should be donated with them. Do not include field notes in the same container used to hold specimens.

KILLING OF SPECIMENS

It is essential that live herpetological specimens be killed in such a manner as to leave the muscles in a relaxed state. Following this, they can be fixed, or hardened, in standardized positions which enables researchers to examine them conveniently and most accurately (refer to Plates 1 and 2). Many books recommend that reptiles be killed by hypodermic injection of aqueous sodium pentobarbital (Nembutal) into the heart. This technique is indeed excellent, but the reader should be aware that Nembutal is not a generally available drug, its possession being closely regulated by the Federal Bureau of Narcotics and Dangerous Drugs. It is possible for qualified persons to obtain a permit to purchase Nembutal, but the application procedure is best begun several months in advance of anticipated need. For additional details concerning the permit, the reader is urged to consult representatives of the above-mentioned Bureau at the Federal centers in most large cities. State and local regulations should also be checked. Commercial Nembutal is sold at a concentration of 50 mg/cc. The form of Nembutal sold as a syrupy elixer should be avoided. Commercjal Nembutal may be used directly for larger specimens (over 5 pounds body weight), and diluted 1:5 with water for smaller reptiles; for very small specimens such as Typhlops or small Scincella it is possible to dilute to 1:10 and retain effectiveness. Nembutal diluted 1:10 can also be used on larger specimens, but death will be delayed. One cc (used commercial strength) injected into the heart is generally sufficient to quickly kill an animal of the bulk (volume) of a 3 foot timber rattlesnake (Crotalus horridus). Position of the heart in snakes can often be judged by closely watching the ventral plates on the anterior 1/3 of the body to detect heartbeat. Injection anywhere into the anterior 1/3 of the body cavity is also effective, but death is not as rapid as from heart injection. Other reptiles can be killed by injection into the heart region. Do not attempt to inject specimens which are so small or thin as to be heavily damaged by the needles at hand.

A number of other effective killing means are available. Turtles may be chloroformed if care is taken not to allow them to stiffen. Confining the turtle with a chloroform moistened rag or cotton wad in a closed container for 15-30 minutes (Cook, 1965) should suffice. The use of chloroform on other reptiles is definitely not recommended, as severe contortion usually results. Trichloroethylene or ether may be substituted for chloroform with good results, and can be used on most reptiles. Most specimens can be killed by confinement with either trichloroethylene or ether for 5 minutes beyond the time the animal loses the ability to right itself when turned over. These liquids are available to the public from either biological supply houses or certain drugstores. Their use may be superior to Nembutal when working with small, fragile animals like some tropical geckos. Caution should be observed with ether, as it is highly flammable and can, under certain storage conditions, explode. Read labels carefully.

All amphibians and a number of smaller reptiles (e.g. small, tropical geckos) are easily killed by immersing them in a solution of Chloretone (hydrous chlorobutanol). A stock supply is commonly prepared as a saturated solution of Chloretone in 95% ethanol. This stock solution may be conveniently carried in a small vial; 2 cc of it added to a pint of water is effective. The solution should be kept tightly covered when not in use, and can be used over and over; its strength will diminish with use.

Various other means are suitable for killing reptiles and amphibians. Securing the animal(s) in a cloth sack and immersing the sack in warm (110°-120°F; 43-47°C) water is effective, but specimens should be removed shortly after death. Specimens may also be immersed in alcohol (15-25% for amphibians; 50-60% for reptiles). Though the method is not recommended, bags containing reptiles may also be left exposed to direct sunlight until death from overheating occurs. Great care must be used however, as dehydration and accompanying contortion can happen quickly; amphibians should never be killed in this way. Both procaine hydrochloride (Livezey, 1958) and succinylcholine chloride (Anectine) (Lambert, 1967) have been used effectively as killing agents; however, their availability is usually restricted like that of Nembutal.

Recent drug laws have greatly increased the difficulty of obtaining syringes for preserving purposes. State laws may also vary in the regulation of the above-mentioned chemicals. It is often possible to obtain necessary supplies through institutions, particularly in return for depositing desired specimens.

FIXING

The purpose of fixation is to preserve the actual morphological state and color of the specimen, and to prepare the tissues for microscopic examination. Hence, the fixative should kill tissue quickly; penetrate it uniformly and rapidly; prevent postmortem decomposition; not distort the tissue; and should prepare the tissue for staining. No single fixative will do all of these things, so various compromises must be made.

The most widely accepted and suitable general fixatives for field use are:

1) Formalin ("Formol" or "Formalina" in Spanish; "das Formaldehyd" in German)- Sold commercially as a solution of approximately 40 percent formaldehyde gas in water, formalin is the most widely used field fixative. For purposes of dilution, commercial formalin is usually considered as 100%, and can be used in 10% strength (1 part formalin: 9 parts water) for fixation. Formalin may be buffered (which helps to reduce discoloration of specimens) by mixing 1 tablespoon of baking soda or borax with each pint of 10% formalin. Generally sold as a liquid (often in drugstores), it is also available as a solid polymer (paraformaldehyde), which is convenient for saving weight and space in transport. Huheey (1963) recommends sealing 16 grams of paraformaldehyde and 4 grams of anhydrous sodium carbonate in packets for field transport; 1 packet added to 400 ml (about 1/2 quart) of water makes a 10% solution of buffered formalin. Premixed, buffered paraformaldehyde powder is available from Carolina Biological Supply House. Paraformaldehyde alone can be obtained from Eastman Organic Chemicals, Rochester, New York. Formalin, while an excellent general fixative, is highly irritating to the user's skin and (as a vapor) to mucous membranes. It is not uncommon for users to develop strong allergies

to formalin. Also, formalin has a tendency to cause swelling of several types of tissue, rendering them unsuitable for some histological purposes.

2) FAA (formalin-alcohol-acetic acid)- Prepared by mixing 10 parts commercial formalin, 50 parts of 95% alcohol (ethyl or isopropyl), 40 parts water and 2 parts glacial acetic acid. FAA penetrates tissue far better than formalin alone, and has less tendency to cause cell distortion. The rapid tissue penetration can also be an aid to preserving valuable specimens found dead and, perhaps, partially decomposed. The primary disadvantages of FAA are: the need to mix several components; and, the necessary alcohol and acetic acid may not be available in certain localities. FAA is not available in powder form, but can be premixed without the water to reduce volume in transport; water may be added later. If FAA is to be used extensively in hot regions, it is recommended that the acetic acid be added just prior to actual use, as it quickly evaporates from the solution; containers may be cooled by wrapping them in wet rags and shading them to retard evaporation of acetic acid.

3) Alcohol- If neither formalin nor FAA are available, alcohol may be used as a fixative. Cook (1965) recommends ethanol (95% for reptiles; 70% for amphibians) or isopropanol to fix in the absence of other solutions, but the latter is not desirable.

4) Special- A large number of other fixatives exist, each being useful for different types of tissues, and studies. Bouin's solution (75 parts saturated aqueous picric acid, 25 parts commercial formalin, 5 parts glacial acetic acid) is especially useful for field preservation of testes to be used in spermatogenesis studies. Testes may be placed in vials of Bouin's and safely kept there for long periods of time without distortion of cells; the remainder of the specimen may be fixed with FAA or formalin. For a complete discussion of special fixatives, the reader is referred to Guyer (1961) and similar texts.

5) Miscellaneous- If a valuable specimen must be saved and no other solutions are available, a number of emergency measures are possible. The specimen may be frozen or packed in strong brine until preservative can be obtained. Liquor is generally not a suitable source of alcohol, as 110 proof liquor is only 55% ethanol. However, strong tequila (about 160 proof) may be useful; rubbing alcohol can also be used. These, however, are only desperation measures and it is usually more beneficial to get the specimen into a proper fixative (hospitals, local schools, etc. are suggested as possible sources).

It is always preferable to introduce fixative into the body cavity, as specimens (particularly reptiles and larger amphibians) can decompose internally if simply placed in fixative. Enough fixative should be injected to fill, but not distend, the animal. Care should also be taken not to damage the femoral pores of many lizards by puncturing them with the needle. The neck of turtles should be completely extended and the mouth held open with wood, cork, or tightly wadded paper prior to fixation. Excellent neck extension can be obtained by hooking the dead turtle's upper jaw over a nail or broken branch and letting the animal's hanging weight pull the neck out straight prior to injecting it. The upper jaw can also be hooked over a paper clip placed over the edge of the fixing tray, and the neck then drawn out. One hemipenis of male lizards and snakes should be partially everted with thumb pressure on the base of the tail, followed by injection to completely evert it as indicated on the front cover. The hemipenis should not be permitted to remain incompletely everted; thread may be tied around the base of the fully everted hemipenis to help retain fluid within it. It is also an acceptable practice to evert the hemipenis by injection of fixative alone. Typical sites for injection of preservative are starred in Plate 2. Tails of lizards and snakes should be slit lengthwise, being very careful not to break the tail off; sharp instruments are a "must." Large amounts of fixative can be conveniently handled in the injection apparatus designed by Jackson (1971), although the author has never felt at a disadvantage using larger syringes. If no injection apparatus is available, the specimen should be deeply slit in several places ventrally and placed belly-up in fixative. Spread the sides of the slits to admit fixative more easily. Avoid cutting the anal plates of snakes and lizards and femoral pores of lizards.

Once the animal is injected or slit, it is most conveniently fixed by placing it (after proper positioning) between pieces of white paper toweling moistened liberally with fixative. This can be done in shallow, covered plastic or rustproof metal pans. Surgical instrument pans with sliding metal covers are handy for this. Avoid colored towels, as the colors dissolve in the fixative and stain the specimen.

Preferred positions for fixing and sites for field tag attachment are illustrated in Plates 1 and 2. Amphisbaenids and caecilians should be fixed in the same position as snakes; it is useful to fix these with the mouth open, as this greatly facilitates examination of oral characters later on. Lizards with long tails should be fixed with the tails bent as shown. Frogs and toads may be positioned with the sole of the foot down (Duellman, 1962). However, because this position obscures many hind limb and anal characters, others feel that anurans are best fixed with the hind limbs in the position shown in Plate 1c. Toes and fingers should always be straight and spread apart. Small amphibians need not be injected or slit prior to positioning, as the fixative will penetrate to the body cavity quite easily. Small amphibians and lizards may have the field tag tied around the body just anterior to the pelvic region.

Amphibian eggs and larvae are best fixed and stored by dropping them directly into jars of 10% formalin; preserve entire egg clutches whenever possible. Many amphibians attach their eggs to leaves, twigs, etc. Whenever it is practical, these items should be preserved with the eggs in situ, as the latter are often severely damaged by attempts to disengage them. Change the formalin on eggs and larvae after about 12 hours. Reptile eggs should be measured (length and width, in millimeters), then injected.

All specimens should be allowed to remain in fixative for 24 hours.

Very Large Specimens (too large to be conveniently stored entire in liquid)

1. Snakes- Obtain the snout to vent and tail lengths (in mm). Then skin by making a long ventral incision to the side of the mid-line; leave the head and tail attached to the skin, severing these from the carcass (avoid cutting the anal plate), and then inject head and tail (evert hemipenis if male) with fixative. With boids, sever the hind part just

Plate 1. Amphibians

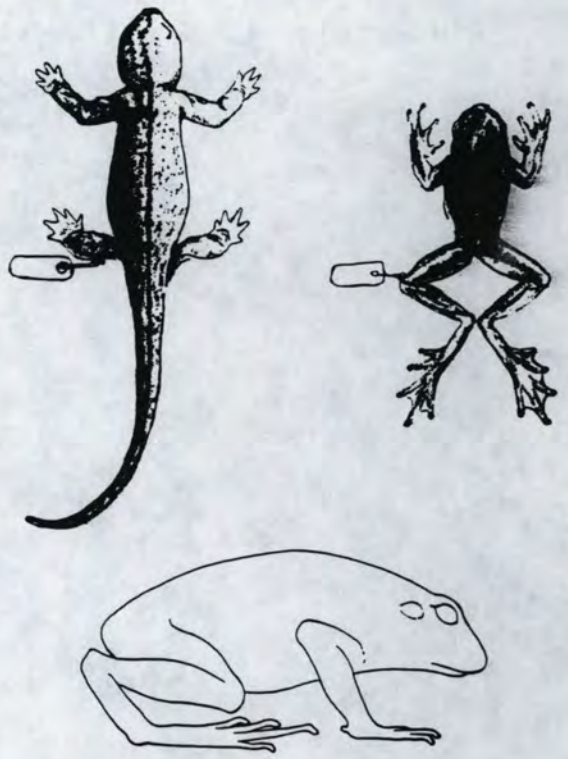
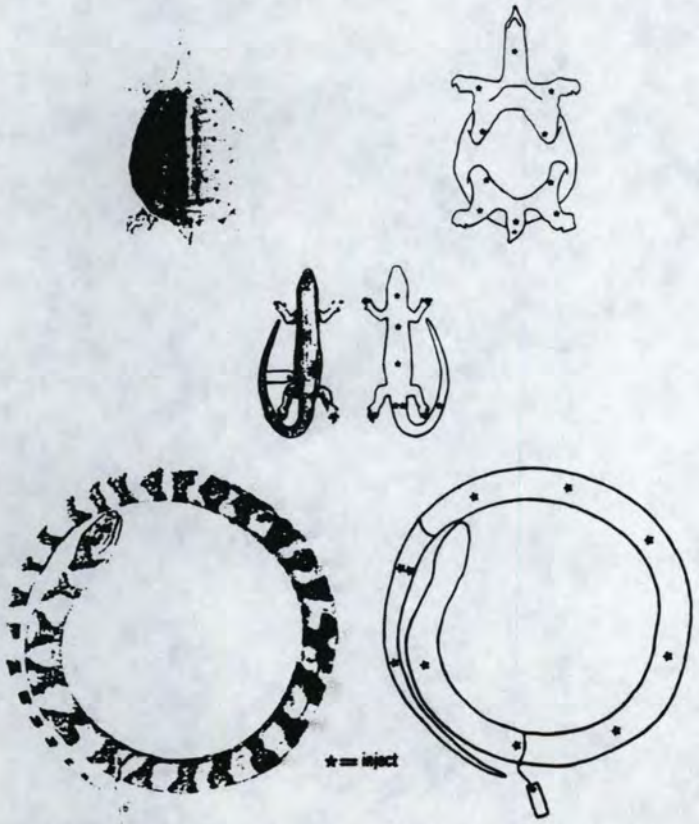


Plate 2. Reptiles



ahead of the bony, vestigial pelvic elements. The skin may now be preserved by covering the flesh side with cloth or absorbent paper, rolling loosely and immersing in fixative, or by rubbing with borax or arsenical soap, rolling and drying. In this latter instance, it is best to preserve the head and tail separately in liquid. If the specimen is a male, a testis should also be preserved. Reproductive condition of females should be noted (i.e.- number of ova present, size of the largest ovum, etc.). Embryos, especially those of poorly known species should be preserved in liquid; it is preferrable to do this by preserving the entire oviduct rather than by removing embryos.

2. Turtles- Avoid cutting the shell. It is preferable to cut the head, neck and forelegs out as one unit, the hind legs and tail as a second unit and preserve these in liquid. The stomach and reproductive organs should also be preserved in liquid. Carefully clean out and dry the shell.

3. Crocodilians- Measure and skin the specimen as for snakes, except that the tails should be skinned as well. Feet may be left attached (inject with fixative), instead of skinned out. Rub the skin with borax or arsenical soap and dry.

SHIPPING

Once they have been properly fixed, most herpetological specimens may conveniently be transported by wrapping them loosely in cheesecloth or white paper towel which has been liberally moistened with alcohol (70% ethanol or 40% isopropanol), or the fixative, then sealing them in two plastic bags (one within the other, individually closed by twisting the end and knotting or securing with a rubber band). Several small specimens may be wrapped in a single length of cheesecloth by laying the cloth out flat, spacing the specimens down the length of it, folding the sides over the animals and rolling the cloth loosely, like a rug.

Thus packed, the specimens occupy minimum space and weight, important factors when they must be transported any distance or mailed. Specimens will remain in

ondition for several weeks, so long as the bags are well sealed to retard e and evaporation. Cotton in sheet form may be substituted for cheesecloth, bulkier and may adhere to the scales of some rough-scaled species of lizards, nen it is moist.

s being shipped (parcel post is a convenient means) should be carefully ed and clearly marked "PRESERVED SCIENTIFIC SPECIMENS." Packages often are t to much "wear and tear," so, effort in preparation pays off! Paint cans ying sizes make leakproof, sturdy mailing containers. Plastic bags containing ens may be simply placed in the can and extra space filled with wadded rags ir. Bags with heavy specimens should never be placed on top of lighter ones. address labels should be typed or written with permanent ink. Place one se in the can with the specimens, tape a second to the side of the can. the third label to the paper used to wrap the parcel.

aint cans are not too costly, and a source of supply can generally be found sulting local paint stores. Watching auction notices sometimes turns up a store that is going out of business and may have cans. Remove handles from efore use. In lieu of cans, specimens may be packed in any durable container. postal regulations for size and weight restrictions before packing extremely or unwieldy parcels. Specimens such as large turtle shells or the skins of crocodilians may have to be sent via freight, and again, secure packing is . Shipment of all crocodilian specimens is subject to stringent regulation, y of these species are endangered animals. Collectors planning to take should carefully check customs regulations for import restrictions as well cking capture laws in countries where the animals occur. Proper arrangements ten be made through the institution where one proposes to deposit the specimens.

enerally, specimens should never be sent in glass containers. Obvious tions to this are amphibian eggs (and sometimes, larvae) and very fragile ens. These should be placed in the smallest containers needed to hold the ens plus fluid to maintain them; fluid should fill the containers, which e heavily padded with cardboard or cotton. If rigid plastic tubing of tient diameter is available, break resistant containers may be fashioned t by cutting an appropriate length, stoppering one end, enclosing specimens luid, then sealing the other end. The tube may be wrapped lengthwise with wire to secure the stoppers. Plastic vials are available from some biological supply houses; larger drugstores may also furnish the names of suppliers of these. Be sure to only use vials which can be securely closed (screw-on or snap-on lid). Again, pay special attention to wrapping such containers.

INTERNATIONAL SHIPMENTS

Collectors should be aware of proper methods for shipping specimens internationally. Donation of all or part of a collection to institutions outside one's own country serves to:

1. make synoptic herpetofaunal collections of different areas available to as many researchers as possible,

2. prevent the loss (through war, neglect, earthquakes and other damage) of valuable collections deposited entirely in a single institution, and

3. place the herpetologist in contact with colleagues in foreign institutions; this frequently leads to a most beneficial exchange of ideas and data, thus advancing herpetology as a field of study.

The private hoarding of specimens by any person is a waste of valuable biological data, and can lead to overcollecting (i.e.--researchers may gather specimens from areas already represented, though inaccessible, in private collections). It is with the above points in mind, and the hope that more collectors will decide to enter into donation, exchange or loan relationships with foreign institutions, that the following guidelines are presented.

The methods of packing described in the preceding section are adequate for international shipment. Generally, mail is the most convenient means of sending packages which are not too heavy or bulky. Parcels sent via surface ("ordinary") mail should have extra preservative added to the specimen bags, as they may take as long as 4 months to reach their destination. Persons

ing specimens internationally should check local mail regulations on el size, weight and any special packing provisions. The shipper may also equired to affix various postal and customs "declaration tags" to parcels. e tags vary with parcel destination and are generally provided by the tal service.

Very large or heavy packages will have to be sent via freight (air, n, ship). The sender will be required to complete a "waybill" (avail- e from the carrier) listing, among other things, the nature and value of contents. To avoid excess charges, package and waybill should be marked Commercial Value." Postal services in all countries have the legal right nspect all packages. Intensive efforts to curtail the traffic of narco- and other restricted drugs has led to the extensive exercising of this nt, and the fact that several persons have attempted to smuggle drugs with- specimen containers has not aided the situation. Inspectors often open stic bags of specimens, and may be unaware of the need to reseal them. causes loss of fluid and dehydration and probable loss of the specimens. is therefore advisable to include two copies of the following statement with parcel (one pasted on the outside and one sealed within):

PECTION OFFICER: This package contains dead, preserved amphibians and/or tiles packed in plastic bags. As the specimens have great scientific ue and will be ruined if not kept moist in their preservative, it is erative that the bags be tightly resealed after inspection to avoid poration or leakage of preservative. Thank you.

SPECTOR POSTAL: Este paquete contiene ejemplares de anfibios y/o reptiles ertos, preservados y empacados en bolsas plasticas. Puesto que los emplares son de valor cientifico y se arruinan si no permanecen en su qui servativo, se suplica que, despues de abrir las bolsas para sp rlas, las cierre hermeticamente para evitar que el liquido se apore o se derrame. Gracias.

AUTORIDADES ALSANDEGARIAS: Este volume contem anfibios e repteis mortos, nservados em sacos plasticos. Como o conteudo tem valor cientifico e se tragara se nao for mantido humido no preservativo, pedese que apos abrir sacos para inspecao os mesmos sejam firmemente fechados para evitar aporacao ou derramamento do liquido. Obrigado.

Biologists should also be aware that the international shipment of specimens (alive or preserved) is being ever more closely regulated for conservation reasons. Shipments of preserved animals sent to the USA must be accompanied by a list bearing the number and scientific name of all specimens included. The importer (in the USA) must obtain a special permit from the Bureau of Sport Fisheries and Wildlife (Dept. of the Interior) in order to receive foreign shipments of preserved or live specimens.

Live shipments are additionally regulated by the Dept. of Agriculture and the Public Health Service. In all cases, endangered species are covered by regulations separate from species not currently considered endangered. You are urged to carefully investigate all legal aspects of international shipment before preparing to send animals.

STORAGE AND LABELLING

This section is not intended to be a complete guide to curatorial technique. Rather, it is meant to serve as a set of capsule directions for those wishing to start a preserved herpetological collection. A detailed discussion of curatorial technique may be found in Slevin (1927).

Preserved collections are best maintained in alcohol. Suitable alcohol generally costs about the same (per gallon) as formaldehyde, and alcohol-stored specimens are far easier to work with. Formaldehyde also tends to corrode metal lids and containers. Most collectors will be deterred from using ethanol by the high tax imposed upon its sales. Isopropanol is far cheaper, and is entirely satisfactory for storage of specimens. Methanol should never be used. Concentrati of 50% is suitable for reptiles, while 40% is better for amphibians. Both ethanol and isopropanol are generally sold at 95% concentration; 526 ml of this plus 474 ml of water make one liter of 50% concentration (421 ml alcohol + 479 ml water for 40%). Specimens being transferred from formalin or FAA fixation to alcohol must first be soaked in water for 48 hours. Failure to soak the specimen often results in its being severely dehydrated by the alcohol. Properly fixed specimens will not be harmed by this method. If material is desired for use in histological work, selected pieces of tissue should remain in 30% alcohol

for 24 hours, then 24 hours of 50% alcohol before going to final storage (omit the water soak). Do not pack specimens tightly in the jar. Snakes fixed in the position illustrated earlier will readily coil in jars for storage.

Each specimen retained in the collection should be assigned a catalog (in addition to the aforementioned "field number"). Amphibian eggs and larvae and reptile eggs may be cataloged with a single tag designating one clutch or lot. This number should be entered in a permanent catalog (using waterproof ink), along with the species name, date of capture/preservation, sex, locality, ecological notes and name of collector. Tags may be tied in the same region as the field tag. Collector's field number should also be entered. As a cross-reference, it is useful to maintain a card file (by taxonomic family) in which a single card is used for each species. On this card may be entered numbers from the catalog that apply to these species.

It is convenient to place a label bearing species name, catalog numbers and locality data with each container. These should be written in permanent ink on heavy, durable paper. That produced by Byron-Weston Mills under the name "Linen Record Ledger", 100% cotton and linen fiber, 36 lb. and Dennison Paper Company's product "Resistall Index Bristol", 100% rag, 110 lb. wt. are both excellent and are available through printing shops. The label may either be placed within transparent containers, or attached to the outside of opaque ones with masking tape. If moderate cost can be withstood, external labels can be placed within tie-on, plastic label-holders. A typical museum label is shown in Figure 2.

Specimen jars should be stored in cool places to help retard evaporation of preservative, and should never be exposed to sunlight, as specimen colors are rapidly faded by such exposure. Placing a piece of Parafilm sheet (available from Carolina Biological Supply House, Burlington, North Carolina) over jar mouths before screwing on the cap will also reduce evaporation. Containers should be checked periodically and fluid level maintained. Well preserved and cared for collections make valuable teaching and research tools.

COLOR PRESERVATION

While preserving the morphological state of herpetological specimens has never presented any severe hurdles to collectors, preservation of color is quite another matter. All currently used, popular preserving fluids are alcoholic and/or acidic to some degree. Therefore, it usually is not too long before most pigments are dissolved by such fluids and extracted from the specimens. Amphibians seem particularly vulnerable in this regard, though the effect on reptiles is noticeable.

Previously, the only acceptable method of retaining amphibian skin color was that described in Cook (1965). Basically, this consists of skinning the specimen, confining all cuts to the ventral surfaces of body and limbs. The skin is then floated flesh-side up in a pan of water and remaining particles of tissue are removed. The skin next is floated flesh-side down and spread out in a second pan. A wet piece of cardboard may then be brought up beneath the skin, which is rubbed lightly to flatten it and remove trapped air. The cardboard-skin preparation may be dried on blotting paper until moist, then placed between layers of blotting paper and thoroughly dried with heavy weights (such as books) on top of it; it may also be placed in a plant press. Reptiles may be similarily prepared. In all cases, the carcass should be preserved in fluid and tagged with the same number as the skin. Skins thus prepared should be stored in the dark and not exposed to prolonged light.

The above technique, while useful, is tedious. Windsor (1971) has described a technique for using 50% saturated, aqueous ammonium sulfate solution as a preservative of frogs. As the compound is an aqueous, neutral salt, no pigment was dissolved and natural color was still evident in the specimens 6 months after preparation. Total fixing time should be at least 36 hours.

Specimens may be stored in buffered formalin (10%) or isopropanol (40%) to which liquid Ionol-40 R has been added (White and Peters, 1969). Storage should be in dark places which are not subjected to heat much above 70°F. The formalin/Ionol method has been successfully used with herpetological material by Mr. Woodrow Barber, Biology Department, University of Kentucky at Morehead, and by Dr. George Iannarone, Chicago Academy of Sciences (personal communication).

Powdered Ionol should not be used, as it is difficult to prepare a stable solution of it in preservative. Ionol is sold by the Shell Oil Company (Chemicals Division).

While the two chemical methods discussed above have not been widely used with herpetological material, their success on a limited scale coupled with the value of accurate color preservation suggests that they should be more thoroughly investigated.

LITERATURE CITED

Cook, Francis R. 1965. Collecting and preserving amphibians and reptiles, p. 128-151, In R. M. Anderson (ed)., Methods of collecting and preserving vertebrate animals. Nat. Mus. Canada, Bull. No. 69, Biol. Ser.: 1-199.

Duellman, William E. 1962. Directions for preserving amphibians and reptiles, p. 37-40.In E. Raymond Hall (ed)., Collecting and preparing study specimens of vertebrates. Misc. Publ. Univ. Kansas Mus. Nat. Hist., (30): 1-46.

Guyer, Michael F. 1961. Animal micrology, 5th impression. Univ. Chicago Press, Ill.; xviii + 327 pp.

Huheey, James E. 1963. Concerning the use of paraformaldehyde as a field preservative. Copeia, 1963(1):192-193.

Jackson, Morris K. 1971. A new "syringe" for injecting herptiles. Herpetol. Rev., 3(4):75.

Lambert, Lewis H., Jr. 1967. 'Anectine': a herpetological killing agent. Herpetologica, 23(2):135-136.

Livezey, Robert L. 1958. Procaine hydrochloride as a killing agent for reptiles and amphibians. Herpetologica, 13(4):280.

Slevin, Joseph R. 1927. The making of a scientific collection of reptiles and amphibians. Proc. Calif. Acad. Sci., 16(9):231-259.

White, David A. and Edward J. Peters. 1969. A method of preserving color in aquatic vertebrates and invertebrates. Turtox News, 47(9):296-297.

Windsor, Donald A. 1971. Ammonium sulfate as a preservative which does not remove color from frogs. Copeia, 1971(2):356-357.

INDEX

AMPHIBIAN WORKSHOP

Taylor Ranch
18 July 1992

Charles R. Peterson
Department of Biological Sciences, Idaho State University
Idaho Museum of Natural History
Campus Box 8007
Pocatello, Idaho 83209
(208) 236-3922

I. Introduction

II. Checklist of Possible Amphibians

III. Species Accounts (slides)
- A. Range map
- B. Adults
- C. Eggs
- D. Larvae
- E. Juveniles
- F. Habitat
- G. Activity patterns
- H. Calls

IV. Sampling Techniques
- A. Incidental observations (forms)
- B. Seize and capture
- C. Time constrained Searches
- D. Area constrained searches
- E. Trapping (pit and funnel traps with drift fences)
- F. Seining
- G. Calling surveys
- H. Road driving

V. Surveys - USFWS Sample Site Data Form

VI. Monitoring Programs

VII. Preservation Techniques

VIII. Examination of Preserved and Living Specimens

IX. References

CHECKLIST OF IDAHO AMPHIBIANS*

Order Urodela

Family Ambystomatidae

Ambystoma tigrinum	Tiger Salamander
× *Ambystoma macrodactylum*	Long-Toed Salamander
✗ *Dicamptodon aterrimus*	Idaho Giant Salamander

Family Plethodontidae

Plethodon idahoensis	Couer d'Alene Salamander

Family Salamandridae

Taricha granulosa	Roughskin Newt

Order Anura

Family Bufonidae

✗ *Bufo boreas*	Western Toad
Bufo woodhousii	Woodhouse's Toad

Family Hylidae

✗ *Pseudacris* (= *Hyla*) *regilla*	Pacific Chorus Frog
Pseudacris triseriata	Western Chorus Frog

Family Leiopelmatidae

✗ *Ascaphus truei*	Tailed Frog

Family Pelobatidae

Spea (= *Scaphiopus*) *intermontana*	Great Basin Spadefoot

Family Ranidae

Rana catesbeiana	Bullfrog
Rana pipiens	Northern Leopard Frog
✗ *Rana pretiosa*	Spotted Frog
Rana sylvatica	Wood Frog

*Names are consistent with Collins, J.T. (1990). Standard Common and Current Scientific Names for North American Amphibians and Reptiles. Third Edition. SSAR Herpetological Circular No. 19: 1-41.

CHECKLIST OF IDAHO REPTILES*

Order Testudines - Turtles

Family Emydidae

Chrysemys picta	Painted Turtle
Clemmys marmorata	Western Pond Turtle

Order Squamata
Suborder Lacertilia - Lizards

Family Anguidae

Elgaria coerulea (= *Gerrhonotus*)	Northern Alligator Lizard

Family Iguanidae

Crotaphytus bicinctores	Mojave Black-collared Lizard
Gambelia wislizeni	Longnose Leopard Lizard
Phrynosoma douglassi	Short-horned Lizard
Phrynosoma platyrhinos	Desert Horned Lizard
Sceloporus graciosus	Sagebrush Lizard
Sceloporus occidentalis	Western Fence Lizard
Uta stansburiana	Side-blotched Lizard

Family Scincidae

Eumeces skiltonianus	Western Skink

Family Teiidae

Cnemidophorus tigris	Western Whiptail

Suborder Ophidia - Snakes
Family Boidae

Charina bottae	Rubber Boa

Family Colubridae

Coluber constrictor	Racer
Diadophis punctatus	Ringneck Snake
Hypsiglena torquata	Night Snake
Masticophis taeniatus	Striped Whipsnake
Pituophis catenifer	Gopher Snake
Rhinocheilus lecontei	Longnose Snake
Sonora semiannulata	Ground Snake
Thamnophis elegans	Western Terrestrial Garter Snake
Thamnophis sirtalis	Common Garter Snake

Family Viperidae

Crotalus viridis	Western Rattlesnake

*Names are consistent with Collins, J.T. (1990). Standard Common and Current Scientific Names for North American Amphibians and Reptiles. Third Edition. SSAR Herpetological Circular No. 19: 1-41.

FROGLOG

IUCN/SSC Declining Amphibian Populations Task Force

March, 1992, No. 1

Coordinator's Column

The DAPTF has been established by the International Union for the Conservation of Nature (IUCN), Species Survival Commission (SSC) to organize a global monitoring program for (1) determining the status of amphibian populations (2) assessing the implications of any declines (3) studying potential causative factors and (4) making appropriate policy recommendations based upon these findings. The Coordinating Council, administered by the Coordinator, includes researchers, liaison officers of societies and agencies as well as other interested parties, all of whom serve as communicators.

As of the last week in January, the Task Force became equipped and manned at the projected level when it occupied its present facilities at the Environmental Research Laboratory in Corvallis, Oregon. In addition to the recent acquisition of computer hardware, we now have a full time information systems manager in the person of Tony Clem. Once our system is interfaced we shall initiate an electronic database and other activities designed to serve as a viable communications network.

We are still in need of Regional Working Group Chairperson for the U.S. Great Lakes area (WI, MIN, MI).

Priority has been given to organization of a Working Group to assist in compilation of a comprehensive bibliography of reports relating to amphibian populations that will be generated and maintained at the Coordinator's office. We wish to include titles of primary and secondary literature, thesis and dissertations, as well as names of earlier investigators who have archived their field notes at a repository. This resource will be freely available to those wishing to make comparisons with contemporary studies.

Anyone interested in these or related studies are invited to join the DAPTF. Please send your name, address and telecommunications number(s), indicating your interest or participation, to the Coordinator's address.

Canada Launches Major Initiative

(The following is edited and condensed from a report by Hinrich Kaiser, *Redpath Museum, McGill University, on the workshop "Declines in Canadian amphibian populations: designing a national monitoring strategy" held at the Canada Centre for Inland Waters in Burlington, Ontario, on October 5 and 6, 1991.* Bull. CAH/ACH 5(2):1-4.)

The workshop in Burlington, organized by Christine Bishop (Canadian Wildlife Service) and Bob Johnson (Metro Toronto Zoo), constituted the first comprehensive attempt to address the declining amphibian phenomenon from a Canadian viewpoint. The problem of amphibian declines has become an urgent concern among Canadian herpetologists. Participation of researchers in universities, governments and private organizations was truly exceptional. This meeting was the largest gathering of Canadian herpetologists in memory.

In her opening remarks, Bishop stated that the aim of the meeting was to create a framework to monitor Canadian amphibian declines and the factors causing them. Johnson, a DAPTF Board of Directors member, highlighted the problem. Speakers representing the various provinces gave depressing status reports on amphibian populations throughout Canada. In many cases, these were anecdotal accounts, although causal relationships between declines and anthropogenic events can be confirmed in all too many cases. Invariably, each speaker referred to the basic lack of knowledge about the amphibians in question: distributions are insufficiently known, causes behind disappearances are uncertain, and habitat surveys are insufficiently detailed.

The introduction of non-native amphibian species and sports fish, mismanagement of wetlands, human intrusion, and logging, have all been identified as damaging to amphibian populations in more than one province. All were cited as being at least partly responsible for population declines in British Columbia. In Nova Scotia fragmented habitats and the resulting inbreeding within many species have produced increased frequencies of albinism and extra-limbed individuals. A well-documented problem is shown by *Rana pipiens*, stemming from the sale of over a million frogs to biological supply companies in the U.S. until die-offs began in 1975. In the middle 1970s, the famous Manitoba frog holes were empty, and despite an eight-year ban on picking frogs their numbers have not much increased.

Natural events, such as droughts, may be in part responsible for declines observed in populations in Saskatchewan. An outbreak of red leg disease in 1976 resulted in many deaths of *Rana pipiens* in Alberta. Recent observations on *Rana catesbeiana* in the Algonquin area showed that the average weight of calling bullfrogs at two separate sites differed significantly. It is unknown whether life history, social structure or harvesting contributed to this phenomenon.

In Ontario and Quebec, amphibian monitoring has been going on for some time. Since 1984, Ontario has received a total of 52,000 records from 2,700 volunteers and has also compiled a bibliography of herpetology including ca. 1,400 references. In Quebec, 5,400 records are reported.

However, it is puzzling that some species seem entirely unaffected. It has been suggested that certain ones may be rebounding from natural, cyclical events and that there may be positive changes observed in many areas within the next few years.

The afternoon talks centered on the monitoring of amphibian populations, including reports of projects that have produced quantitative data. Data show the best estimate is gained by intensive study. This method has actually been employed in a four-year study of Fowler's toads at Long Point. These toads have dramatically increased in numbers since the study began, likely an effect of the water level rise in Lake Erie.

Among other concerns presented was the importance of: experimental design, timing and length of study; preservations of natural conditions of the habitat; measuring both natural and anthropogenic environmental factors; generating a genetic database during monitoring; larval stages in relation to reproductive success and gene flow; pathological conditions present in the populations; and determining the effects of contaminants upon entire populations.

Open discussions began on the second day. It was first determined that the Working Group will be a research coordinating body for investigating the hypothesis that amphibian populations are in decline. If this hypothesis is supported, the group should then seek ways to reverse the declines. It was agreed that

goal is best served by separately sidering historical data, intensive monig studies, and extensive monitoring ects.

The intensive monitoring group dised how to approach the monitoring cess. Life history research must be current with the monitoring process. group decided a number of indicator cies for intensive monitoring, chosen nclude as many families as reasonably sible, in a variety of habitats and ecoterns, and with a range of genetic and rphological variation.

The Canadian working group will be st active at the provincial level, with gional Coordinators. Details for each dy population and site will be commuated to Eastern and Western Coordiors and the Coordinator for Canada. o will communicate with the IUCN Task rce. this hierarchical setup should ep Coordinators in touch and allow the gions to act both individually and in coeration with each other and with comarable regional groups in the United tates. To facilitate communication to all rticipants, the CAH/ACH Bulletin was osen as the official news medium.

The complete final report is to be ublished in March of 1992 as a Canadian Wildlife Service Technical Report. For rther information contact Christine ishop, Canadian Wildlife Service, Box 050, Burlington, Ontario L7R 4AG, anada.

CANADIAN WORKING GROUP

National Co-ordinator — David M. Green (McGill University)

Regional Co-ordinators — Don McAlpine (New Brunswick Museum), for Eastern Canada. Stan Orchard (Royal B.C. Museum) for Western Canada.

Provincial Co-ordinators (to be confirmed) — John Gilhen (Nova Scotia Museum), Nova Scotia; Don McAlpine, New Brunswick and P.E.I; Joel Bonin and Roger Bider (MacDonald College, McGill University), Quebec; Wayne Weller and Mike Oldham (Ontario Ministry of Natural Resources), Ontario; Bill Koonz (Manitoba Department of Natural Resources), Manitoba; Wayne Roberts (University of Alberta), Alberta; Stan Orchard, British Columbia.

Historical Population Trends — Martyn Obbard, Fred Schueller, Wayne Weller, Mike Oldham.

Intensive Monitoring — Mike Berrill, Jim Bogart, Ron Brooks, Francis Cook.

Extensive Monitoring — Bill Freedman

Environmental Contaminants — Christine Bishop

Diseases — Graham Crawshaw

Netherlands Conference

Annie Zuiderwijk, Chair of the Western European Working Group, represented the DAPTF at the International Symposium on the "Impact of Climate Change on Ecosystems and Species", convened in Amersfoort, The Netherlands in December. Experts, invited from different parts of the world, prepared evaluations of regionally important ecosystems. Workshop sessions focused on identifying key factors affecting selected ecosystems, identifying the main responses and determining various rates of change. Publication of reports from the symposium, expected soon, are intended to provide assessments applicable to issues in conservation, species diversity and management of ecosystems.

In the United Kingdom

Tim Halliday, chair of the UK Working Group (and a Task Force Director), reports that action is being taken to establish liaison and collaborative activities with the Western European group. UK sites of amphibian populations known to be "healthy" 10-15 years ago are being identified so that they can again be surveyed during the coming breeding season. A grant proposal for DAP related research has been submitted. Halliday is also arranging an October/November planning meeting.

Australians Take Action

A $47,000 grant from the Australian government was awarded to Michael J. Tyler, a Director of the Task Force, to organize a meeting of amphibian scientists and produce an Action Plan for Australia as a framework for new legislation, and for developing conservation and management goals for the next five years. To obtain an information base for this endeavor, a "Frogwatch" survey is being conducted in which 150 conservation organizations are participating in distribution of 600.000 (sic) questionnaires.

An organizational workshop convened by Tyler met in Canberra, ACT, last July. This initial meeting was attended by a nucleus of 16 representatives from the several States and Territories. The first half of the program addressed broad overviews and individual species case histories, the status of distribution maps, current legislation and the character of native population cycles. The subsequent general discussions dealt with causal agents, the use of museum records, sampling strategies, pathological studies, etc.

As of the present date, the Action Plan has been partly completed. Formal establishment of the Australian Working Group and its participating members is underway.

Reports from U.S. Working Groups

CAL/NEVA

The California/Nevada Working Group met for the first time at Point Reyes National Seashore on February 4, 1992. The group, chaired by Gary Fellers, included 14 representatives from the U.S. National Park Service, U.S. Forest Service, University of Nevada - LasVegas, St. Mary's College, University of California - Davis, California Academy of Sciences, University of California - Los Angeles, California Department of Fish and Game, and U.S. Fish and Wildlife Service.

Each member of the Working Group provided a short summary of their research relating to amphibians. Most of these reports provided compelling evidence for dramatic declines in amphibian populations throughout all or part of a species' range. Though some of the losses resulted from obvious factors (e.g., habitat loss), numerous cases were noted in which declines occurred with no identifiable reason. There appears to be strong evidence that acid precipitation is not the cause of the declines, though it might be acting in concert with other environmental stressors.

The status of the U.S. National Museum of Natural History handbook on monitoring protocols was addressed at some length. Further discussions centered on the need to gather data that are compatible among studies of different species and/or habitats. A form designed for use by the U.S. Fish and Wildlife Service (see report from Rocky Mountains Working Group) was examined in detail with the goal of determining the minimum data that should be collected as part of any amphibian field study.

ROCKY MOUNTAINS

Stephen Corn and Bruce Bury, co-chairs of the Rocky Mountains Working Group, are compiling a database of research activity on amphibians throughout the region. The Working Group is being organized in two tiers: those with current or recently completed research or monitoring programs, and those with more general interests regarding conservation activities. No formal meeting has yet been scheduled; however, the co-chairs participated in the Cal/Neva meetings at Point Reyes, California in early February to coordinate activities of the contiguous regional groups.

Data forms from their recent publication (Bury, R.B. and P.S. Corn. 1991. *Sampling Methods for Amphibians in Streams in the Pacific Northwest*. U.S. Forest Service, Pacific Northwest Re-

search Station. Gen. Tech. Rpt. PNW-GTR-275.) were evaluated during the joint meetings for potential application to all monitoring procedures. The recommended changes will be incorporated in a revised form for further review and consideration of adoption by other Working Groups.

NORTHEAST

The first meeting of the Northeastern Working Group, chaired by Richard Wyman, was held at the Pennsylvania State University on August 9, 1991.

Following a brief introduction regarding the objectives of the DAPTF, the group discussion focused upon the regional organization and development of an action plan. Priorities to be addressed include a survey of all active herpetologists in the region; assembly of all available regional data relating to the status of amphibian populations, identification of particular characteristics of species that would make data as to their presence or absence environmentally significant, and establishing a mechanism for maintaining a long-term monitoring network in the NE region.

The group is also initiating a search for thesis and dissertations that may contain usable density data, and for relevant records that may have been maintained at biological field stations.

Wyman has also generated a questionnaire for a mail survey as to the status of amphibian populations in the region. Copies of this form, which may be applicable for use by other Working Groups, may be obtained by contacting him (see address and telecommunications number, on page 4).

SOUTHEAST

A network of 40 cooperators in Florida, Alabama, Georgia and South Carolina will serve as the communication resource for data on SE US amphibians. Lists of currently recognized taxa are being generated for a status review by the Working Group. Ken Dodd, chair of the Working Group, has assumed the presidency of the SE section of the ASIH and plans to enlarge attention of the herpetological community upon the Task Force activities.

Carolyn Sekerak (M.S. student, Univ. Florida) is finishing her thesis work on the structure of amphibian temporary pond breeding sites. She has taken a position with the U.S. Fish and Wildlife Service in Jackson, MS. Her responsibilities include monitoring the status of amphibians and preparing federal listing proposals for the dusky gopher frog and other amphibian species.

A habitat conservation plan is being developed for the Red Hills salamander. The plan will involve the U.S. Fish and Wildlife Service, The Alabama Natural Heritage Program) is conducting a survey of the Sipsey Fork waterdog (*Necturus* sp.) in Alabama.

Pablo Delis and Henry Mushinsky (Univ. South Florida) are analyzing data on amphibian population fluctuations in Florida sandhill habitats based on 6 years' data.

Carlos Camp (Piedmont College) reports declines in relict populations of *Rana sylvatica* and *Ambystoma maculatum* in northeast Georgia. Wetland habitat alteration is suspected as the cause.

Dodd's paper on the biotic diversity of amphibians and reptiles in a Florida sandhills temporary pond has been accepted in the new journal Biodiversity and Conservation. Population declines due to drought (best guess) are noted, but long-term effects cannot yet be demonstrated.

Amphibian Bioassay as Assessment Tool for Superfund Sites

The U.S. Department of Defense has initiated an interagency agreement (IAG) with the Environmental Research Laboratory - Corvallis and several others to evaluate test procedures involving the effects of several classes of chemicals on amphibians. Initial studies will employ the Frog Embryos Teratogenesis Assay: *Xenopus* (FETAX). The utility of this test in ecological site assessment has been demonstrated at some Superfund sites using in situ exposures of mature amphibian species. Applications of the test procedures may provide information as to possible factors involved in declines of indigenous amphibian species and the use of mature amphibians as bioindicators of the health of wetland ecosystems.

Recent Reports of Declines

The Estonian herpetofauna consists of ten species of amphibians and five species of reptiles, apparently the result of post-glacial immigration from southeast (*Bufo viridis*), south (majority of species) and south-west (*Bufo calamita*). Earlier recorded *Rana ridibunda* and *Emys orbicularis* have become extinct.

From the perspective of distribution and degree of commonness, three groups of herptiles can be identified: rare and vulnerable species (*Triturus cristatus, Bufo calamita, B. viridis, Pelobates fuscus, Lacerta agilis*), less common species with sporadic distribution (*Rana arvalis, R. lessonae, R. esculenta, Anguis fragilis, Natrix natrix*), and common, widely distributed species (*Triturus vulgaris, Rana temporaria, Bufo bufo, Lacerta vivipara, Vipera berus*).

The distributions of *Triturus cristatus, Rana esculenta* (complex), *Pelobates fuscus* and *Lacerta agilis* seem to be relict in nature; some Estonian amphibians represent the northernmost distribution limits of the species (*Bufo calamita, B. viridis, Pelobates fuscus*). Many local populations of herptiles are reported as declining during the past ten to twenty years. (Talvi, T. 1991. *Amphibians and Reptiles of Estonia: list, geographic relationships and current situation.* Abst. 6th Ord. Gen. Mtg. Soc. European Herp., Budapest)

W.S. Osborne, in a recent status report (in litt.) on frog populations in the Australian Capital Territory documents the decline of *Pseudophryne corroboree* and *P. bibroni*, although both species are relatively common in other parts of their ranges. In contrast, there has been a complete disappearance of *Litoria aurea* and *L. raniformis* in the region, while *L. verreauxii* has become rare. Prolonged dry seasons are believed to be a contributing factor; however, the magnitudes of declines are such that other, yet unknown, factors are possibly involved.

In their recent report (Herp. Rev. 22(4):125-128, 1991) E. La Marca and H.P. Reinthaler have noted "drastically diminished" populations among five species of *Atelopus* in the Venezuelan Andes. Deforestation and expanding agrofarming appear to be the dominant factors impacting upon *A. carbonerensis, A. mucubajiensis, A. oxyrhynchus, A. pinangoi* and *A. sorianoi*. Flooding has scoured the montane streamside vegetation, and a high percentage of road kills in other areas are reported. The extent to which collecting may have reduced endemic *Atelopus* is also discussed. This report states (as with many others) that climatic change, pollution, as well as introduced species of plants and fish, are potentially significant factors in these declines and recommends action for both research and conservation.

RIBBIT Croaks

An earlier newsletter, *Ribbit*, was pioneered in the late 1980's by Bruce Bury and Stephen Corn to report on the decline of amphibian populations in the western U.S. Because of administrative constraints but a single issue was released (January, 1989). It will be superseded by FROGLOG beginning with this number.

Donor Support for DAPTF

Operations of the DAPTF would not be possible without the generous sponsorship of numerous organizations and individuals.

Major financial contributions have been awarded by the International Union for the Conservation of Nature (IUCN), Chicago Zoological Society, U.S. Department of State (AID program), Center for Analysis of Environmental Change (CAEC), the Jacob Bleibereu Foundation, Inc. and Frog's Leap Winery.

Support staff, equipment and office space have been provided by the CAEC (a consortium represented by Batelle-Pacific Northwest Laboratorities, U.S. Forest Service-Pacific Northwest, Oregon State University, and the EPA Environmental Research Laboratory) at Corvallis, Oregon.

DAPTF Directors and Chairs

BOARD OF DIRECTORS

William R. Branch
Port Elizabeth Museum
P.O. Box 13147
Humewood, 6013
SOUTH AFRICA (Code 27)
Phone: (041) 561051 Fax: (041) 562175

Alain Dubois
Laboratoire des Reptiles et Amphibians
Musée National d'Histoire Naturelle
25, Rue Cuvier
75005 Paris
FRANCE (Code 33)
Phone: (1) 40 79 34 87 Fax: (1) 40 79 34 84

Timothy R. Halliday
Biology Department
The Open University
Walton Hall
Milton Keynes, MK7 6AA
UNITED KINGDOM (Code 44)
Phone: 0908-653831 Fax: 0908-654167

Sergius L. Kuzmin
Institute of Evolutionary Morphology
and Ecology of Animals
Academy of Sciences
Leninsky Prospekt, 33,
Moscow 117071
RUSSIA

Robert Johnson
Metropolitan Toronto Zoo
P.O. Box 280
West Hill, Ontario, M1E 4R5
CANADA
Phone: (416) 392-5968 Fax: (416) 392-4979

James B. Murphy
Dallas Zoo
621 E. Clarendon Dr.
Dallas, TX 75203, USA
Phone: (214) 670-7573 Fax: (214) 670-7450

*Jaime F. Péfauer
Departamento de Biologia
Facultad de Ciencias
Universidad de los Andes
Merida, VENEZUELA 5101 (Code 58)
Fax: 74 40 1286

George B. Rabb, IUCN/SSC
Chicago Zoological Society
Brookfield, IL 60513, USA
Phone: (708) 485-0263 Fax: (708) 485-3532

*Michael J. Tyler
Department of Zoology
University of Adelaide
GPO Box 498
Adelaide, South Australia 5001
AUSTRALIA (Code 61)
Fax: 8 223 5817

David B. Wake (Chair)
Museum of Vertebrate Zoology
University of California
Berkeley, CA 94720, USA
Phone: (510) 642-3567 Fax: (510) 643-8238

William Winner (Ex officio)
Center for Analysis of Environmental Change
Oregon State University
Corvallis, OR 97333, USA
Phone: (503) 737-1744 Fax: (503) 737-3399

John W. Wright
Museum of Natural History
Los Angeles, CA 90007, USA
Phone: (213) 744-3371

* Also serves as Organizational Chairperson for Regional Working Group.

WORKING GROUP CHAIRS

K.D. Arudpragasam, *Co-Chair, Sri Lanka*
University of Colombo
SRI LANKA

W.R. Breckenridge, *Co-Chair, Sri Lanka*
Department of Zoology
University of Peradeniya
Peradeniya
SRI LANKA

David M. Green, *National Coordinator, Canada*
Redpath Museum
McGill University
Montreal, PQ H3A 2K6
CANADA
Phone: (514) 398-4088

*Kuang-Yang Lue, *Taiwan*
Department of Biology
88 Roosevelt Rd., Sec. 5
11718, Taipei, Taiwan
REPUBLIC OF CHINA (Code 86)
Phone: (02) 932-5234 Fax: (02) 931-3904

Jay M. Savage, *Lower Central America-Costa Rica/Panama*
Department of Biology
University of Miami
Coral Gables, FL 33142, USA
Phone: (305) 284-2130 Fax: (305) 284-3039

Annie Zuiderwijk, *Western Europe*
Institute of Taxonomic Zoology
University of Amsterdam
P.O. Box 4766 - 1009 AT Amsterdam
THE NETHERLANDS (Code 31)
Phone: (20) 5256624 Fax: (20) 5257238

*(Acceptance pending)

U.S. Regions

Ronn Altig, *Mississippi Delta (AR,MS,LA)*
P.O. Drawer GY
Department of Biological Science
Mississippi State University
Mississippi State, MS 39762
Phone: (601) 325-7561 Fax: (601) 325-7939

Andrew R. Blaustein, *Co-Chair, Pacific Northwest* (WA,OR,AL)
Department of Zoology
Oregon State University
Corvallis, OR 97331-2914
Phone: (503) 737-5356

Ronald A. Brandon, *Central (IA,MO,IL,IN,OH)*
Department of Zoology
Southern Illinois University
Carbondale, IL 62901
Phone: (618) 536-2314

Richard C. Bruce, *Appalachian (KY,TN,NC,VA,WV)*
Highlands Biological Station
P.O. Box 580
Highlands, NC 28741
Phone: (704) 526-2602

R. Bruce Bury, *Co-Chair, Rocky Mountains (ID,MT,WY,CO,UT)*
National Ecology Research Center
4512 McMurray Ave.
Fort Collins, CO 80525-3400
Phone: (303) 226-9270

Janalee P. Caldwell, *Southern Plains (OK,TX)*
Oklahoma Museum of Natural History
1335 Asp Ave.
Norman, OK 73019-0606
Phone: (405) 325-4712 Fax: (405) 325-7771

P. Stephen Corn, *Co-Chair, Rocky Mountains (ID,MT,WY,CO,UT)*
National Ecology Research Center
4512 McMurray Ave.
Fort Collins, CO 80525-3400

C. Kenneth Dodd, Jr., *Southeast (AL,GA,FL,SC)*
National Ecology Research Center
412 NE 16th Avenue (Room 250)
Gainesville, FL 32601
Phone: (904) 372-2571 Fax: (904) 374-8080

Gary M. Fellers, *Cal/Neva (CA,NV)*
Point Reyes National Seashore
Point Reyes, CA 94956
Phone: (415) 663-8522

A. Ross Kiester, *Co-Chair, Pacific Northwest (WA,OR,AL)*
USDA Forest Service
3200 SW Jefferson Way
Corvallis, OR 97331
Phone: (503) 750-7269

James E. Platz, *Northern Plains (ND,SD,NE,KS)*
Department of Biology
Creighton University
2500 California St.
Omaha, NE 68178
Fax: (910) 622-0287

Norman J. Scott, Jr., *Southwest (AZ,NM)*
Department of Biology
University of New Mexico
Albuquerque, NM 87131
Phone: (505) 766-3903

Richard L. Wyman, *Northeast (ME,NH,VT,NY,PA,MD,DE,MA,RI,CT,NJ,DC)*
Edmund Niles Huyck Preserve, Inc.
P.O. Box 188
Rensselaerville, NY 12147
Phone: (518) 797-3440

Issue Specific

W. Ronald Heyer, *Monitoring Protocols*
Division of Amphibians and Reptiles
US National Museum of Natural History
(NHB Mail Stop 162)
Washington, DC 20560, USA
Phone: (202) 357-2195

Roy McDiarmid (Will succeed WRH)
National Fish and Wildlife Laboratory
U.S. National Museum of Natural History
(NHB Mail Stop 162)
Washington, D.C. 20560, USA
Phone: (202) 357-2780

James L. Vial, *Documentary Resources*
(Address given below)

FROGLOG

Newsletter of the IUCN/SSC Task Force on Declining Amphibians

Dr. James L. Vial
Task Force Coordinator, *Editor*
Center for Analysis of Environmental Change
EPA Environmental Research Laboratory
200 South West 35th Street
Corvallis, Oregon 97333, USA
(503) 754-4798
Telefax (503) 754-4799

Funding for FROGLOG is provided in part by a special donation from Frog's Leap Vineyards, St. Helena, California 95474, USA

It appears complex and intimidating, but actually can be completed in a short amount of time after a [illegible] to be circled, and the remaining variables are numerical and easy to determine. The data sheet is divided into four sections, divided by double lines. Each section describes a cohesive set of variables. In addition the back of the sheet includes a grid for a rough sketch of the site and space for additional comments. The map is optional, but the future value of the data is enhanced if it is supplied.

SECTION 1 – LOCALITY *These data are essential. Many amphibian surveys have been hampered by the inability to relocate exact locations in the historical record. Some of this information can be completed in the office after the survey.*

DATE: Use the format DD-MMM-YY (e.g., 05-APR-92).

BEGIN TIME: List the time survey of habitat for amphibians began in 24 hour format.

END TIME: List the time the survey ended in 24 hour format. (The total time (END TIME - BEGIN TIME) should reflect only the amount of time spent searching for amphibians. Total time plus number of observers may be used to assess relative abundance.)

OBSERVERS: List names or initials of all persons involved in searching.

LOCALITY: Describe the *specific* geographic location of the site. Use air distance in two directions (e.g., 5km N and 7.5 km W) of a map landmark that likely will not change (distance from a large town or city is not all that helpful).

STATE: Use the 2-letter abbreviation.

COUNTY:

MAP NAME: List the name of the U.S.G.S. quadrangle or other map used to locate the site.

OWNER: List the public land manager (e.g., Roosevelt Nat. Forest or Rocky Mtn NP), or name of the owner if the site is on private land (listing the owner's name will make it clear that you did not trespass to survey the site).

ELEVATION: Circle the scale used; meters are preferred.

T: township **R:** range **S:** section

SECTION DESCRIPTION: Describe the location of the site within the section (e.g., SE ¼ or NE ¼ of SE ¼)

UTM ZONE, NORTHING, EASTING: Universal Transverse Mercator coordinates are preferred over longitude and latitude. The UTM zone is listed on newer topographic maps. If you are using a map without the UTM grid, substitute latitude for Northing and longitude for Easting.

SECTION 2 – SPECIES DATA *List all amphibian species observed. If garter snakes are seen, list them here also.*

SPECIES: Use the scientific name. Convenient shorthand is to use a 4-letter code made up of the first 2 letters of the genus and species (e.g., *Rana sylvatica* would be RASY).

ADULTS/JUVENILES: Indicate presence with a check, but numbers seen are more valuable data

CALLING?: Circle Y if frogs are vocalizing in a breeding chorus, of if a breeding aggregation of species that don't call (e.g., *Bufo boreas*) is observed.

TADPOLES/LARVAE: Same as for adults/juveniles

EGG MASSES: Same as above. Numbers of egg masses are especially valuable data. If possible, describe the developmental stage of eggs in the space for additional notes on the back of the form.

METHOD: Circle how observations were made: **VISUAL/AURAL ID** – species identified without picking it up, either by sight or by recognition of the breeding call; **HAND COLLECTED** – animal was picked up and identified in the field (higher confidence than visual id); **DIP NET/SEINE** – the usual method of collection for larvae; **TRAPPED** – minnow-type traps are also used for larvae; **VOUCHER COLLECTED?** – circle yes or no (voucher specimens are recommended for every site, *especially if identification is uncertain and for larvae*). Indicate voucher status in addition to method used.

FISH PRESENT?: If yes, list species if you can. Circle the question marks if you are not certain, but suspect that fish are present.

ENTIRE SITE SEARCHED?: If no, list either the meters of shoreline or the area (m^2) of habitat (e.g., amount of wet meadow) searched.

SECTION 3 – PHYSICAL AND CHEMICAL DATA *Water chemistry data are difficult to collect accurately without thorough planning and quality equipment; these data are optional. Weather data are important for determining the quality of the observations (e.g., was an absence of amphibians due to observations made during a blizzard?)*

WEATHER, WIND: Indicate atmospheric conditions

AIR TEMPERATURE: Take at chest height in shade. The Celsius scale is preferred.

WATER TEMPERATURE: Take 1 meter from margin and at 2 cm depth, or where egg masses are observed.

COLOR: This is a qualitative assessment of whether the water clear or tea-colored from organic (humic) acids.

TURBIDITY: This is a qualitative assessment of whether the water clear or clouded from suspended particulate matter.

SECTION 4 – HABITAT DESCRIPTION *These data are important for developing hypotheses to explain changes in abundance of amphibians. This section needs to be filled out only once for each site (a reasonable amphibian survey should include at least 2 – 3 visits to each site in one season).*

ORIGIN: Decide whether the lake is a natural geologic formation or man-made. Bodies of water enlarged by a dam are problematic. List them as man-made, but add an explanation in the space for additional notes on the back of the form.

DRAINAGE: Circle whether the site has permanent drainage, no drainage, or occasional drainage. Determining the potential for occasional drainage requires judgement. Look for clues in the topography and vegetation.

DESCRIPTION: Decide how best to describe the site. If there is evidence of past or present beaver activity, circle one of these choices in addition to your choice.

LENGTH, WIDTH: Record the maximum length and width of lakes and ponds. For streams, record the length and average width of the reach searched.

MAXIMUM DEPTH: Most times, you will not have access to a boat, so estimate depth (deep lakes are usually not important to amphibians).

STREAM ORDER: This is an index of stream size, and you will need a topographic map to determine it. First-order streams have no tributaries, second-order streams are formed by the confluence of two 1st-order streams, third-order streams are formed by the confluence of two 2nd-order streams, and so on.

PRIMARY SUBSTRATE: Circle the type that covers the majority of the bottom of the site.

EMERGENT VEGETATION: Circle the percentage of the margin of the site with emergent vegetation present, and list the dominant species. If you are botanically-disadvantaged, list the categories of the dominant species (e.g., cattail, sedges, etc.).

NORTH SHORELINE CHARACTERS: Describe the north shore of a lake or pond in terms of shallow water and emergent vegetation. This is important in evaluating quality of breeding habitat in some mountain locations.

FOREST CHARACTERS: List the closest distance between the water and the surrounding forest, and list the most common tree species. Leave these fields blank if there is no forest. Describe other surrounding habitat types in the notes section on the back of the form.

References

Blaustein A.R., and D.B. Wake. 1990. Declining amphibians: a global trend? Trends in ecology and evolution. 5:203-204.

Carpenter, C.C. 1953. An ecological survey of the herpetofauna of the Grand Teton-Jackson Hole area of Wyoming. Copeia, 1953(3):170-174.

Corn, P.S. and R.B. Bury. 1989. Logging in Western Oregon: responses of headwater habitats and stream amphibians. Forest Ecology and Management, 29(1989):39-57.

Corn, P.S. and R.B. Bury. 1990. Sampling methods for terrestrial amphibians and reptiles. USDA Forest Service, Pacific Northwest Research Station, Portland, Oregon. General technical Report PNW-GTR-256.

Corn, P.S. and J.C. Fogleman. 1984. Extinction of montane populations of the northern leopard frog (*Rana pipiens*) in Colorado. Journal of Herpetology, 18(2):147-152.

Groves, C. 1989. Idaho's amphibians and reptiles. Nongame Wildlife Leaflet #7. Idaho Department of Fish and Game.

Halliday, T. and K. Adler. 1987. The encyclopedia of amphibians and reptiles. Facts on File Publications, New York. 143 pp.

Karns, D.R. 1986. Field herpetology: methods for the study of amphibians and reptiles in Minnesota. Occasional Paper No. 18, Division of Comparative Biology, James Ford Bell Museum of Natural History, University of Minnesota, Minneapolis. (Contains good descriptions of techniques for finding and monitoring populations).

Nussbaum, R.A. E.D. Brodie, and R.M. Storm. 1983. Amphibians and reptiles of the Pacific Northwest. University Press of Idaho, Moscow. 332 p. The best general source of information on the amphibians and reptiles of Idaho).

Pisani, G.R. 1973. A guide to preservation techniques for amphibians and reptiles. Herp. Circular. No. 1. 22 p. Society for the Study of Amphibians and reptiles.

Pough, F.H., J.B. Heiser, and W.N. McFarland. 1989. Vertebrate Life. Third edition. Macmillan Publishing Co., New York. (An excellent textbook on vertebrate zoology; especially good chapters on amphibians and reptiles).

Stebbins, R.C. 1985. A field guide to western reptiles and amphibians. Houghton Mifflin Co., Boston. 336 pp. (The best field guide to the amphibians and reptiles of the western United States).

Turner, F.B. 1958. Life history of the western spotted frog in Yellowstone National Park. Herpetologica, 14:96-100.

Turner, F.B. 1960. Population structure and dynamics of the western spotted frog, *Rana p. pretiosa* Baird & Girard, in Yellowstone Park, Wyoming. Ecological Monographs, 30:251-278.

Vial, J.L. (editor) 1992. Froglog - IUCN/SSC Declining Amphibians Task Force Newsletter. March, 1992, 1.

Welsh, H.H. 1987. Monitoring herpetofauna in woodland habitats of northwestern california and southwestern Oregon: A comprehensive strategy. Gen. Tech. Rep. PSW-100. Berkeley, CA. Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture.

Welsh, H.H., A.J. Lind, and D. L. Waters. 1991. Monitoring frogs and toads on Region 5 National Forests. R-5 Fish Habitat Relationship Technical Bulletin. Number 4, May 1991. 12 p.

Welsh, H.H. and A.J. Lind. *In press.* Structure of herpetofauanal assemblage of the Douglas-fir/hardwood forests of northern California and southwestern Oregon. Pages XX-XX *in* L.F. Ruggiero, K.B. Aubry, A.B. Carey, and M.H. Huff, tech. coords. Wildlife and vegetation of unmanaged Douglas-fir forests. USDA For. Serv. Gen Tech. Rep. PNW-GTR-XXX. (Contains a discussion of management strategies to maintain a viable herpetofauna)

References

Blaustein A.R., and D.B. Wake. 1990. Declining amphibians: a global trend? Trends in ecology and evolution. 5:203-204.

Carpenter, C.C. 1953. An ecological survey of the herpetofauna of the Grand Teton-Jackson Hole area of Wyoming. Copeia, 1953(3):170-174.

Corn, P.S. and R.B. Bury. 1989. Logging in Western Oregon: responses of headwater habitats and stream amphibians. Forest Ecology and Management, 29(1989):39-57.

Corn, P.S. and R.B. Bury. 1990. Sampling methods for terrestrial amphibians and reptiles. USDA Forest Service, Pacific Northwest Research Station, Portland, Oregon. General technical Report PNW-GTR-256.

Corn, P.S. and J.C. Fogleman. 1984. Extinction of montane populations of the northern leopard frog (*Rana pipiens*) in Colorado. Journal of Herpetology, 18(2):147-152.

Groves, C. 1989. Idaho's amphibians and reptiles. Nongame Wildlife Leaflet #7. Idaho Department of Fish and Game.

Halliday, T. and K. Adler. 1987. The encyclopedia of amphibians and reptiles. Facts on File Publications, New York. 143 pp.

Karns, D.R. 1986. Field herpetology: methods for the study of amphibians and reptiles in Minnesota. Occasional Paper No. 18, Division of Comparative Biology, James Ford Bell Museum of Natural History, University of Minnesota, Minneapolis. (Contains good descriptions of techniques for finding and monitoring populations).

Nussbaum, R.A. E.D. Brodie, and R.M. Storm. 1983. Amphibians and reptiles of the Pacific Northwest. University Press of Idaho, Moscow. 332 p. The best general source of information on the amphibians and reptiles of Idaho).

Pisani, G.R. 1973. A guide to preservation techniques for amphibians and reptiles. Herp. Circular. No. 1. 22 p. Society for the Study of Amphibians and reptiles.

Pough, F.H., J.B. Heiser, and W.N. McFarland. 1989. Vertebrate Life. Third edition. Macmillan Publishing Co., New York. (An excellent textbook on vertebrate zoology; especially good chapters on amphibians and reptiles).

Stebbins, R.C. 1985. A field guide to western reptiles and amphibians. Houghton Mifflin Co., Boston. 336 pp. (The best field guide to the amphibians and reptiles of the western United States).

Turner, F.B. 1958. Life history of the western spotted frog in Yellowstone National Park. Herpetologica, 14:96-100.

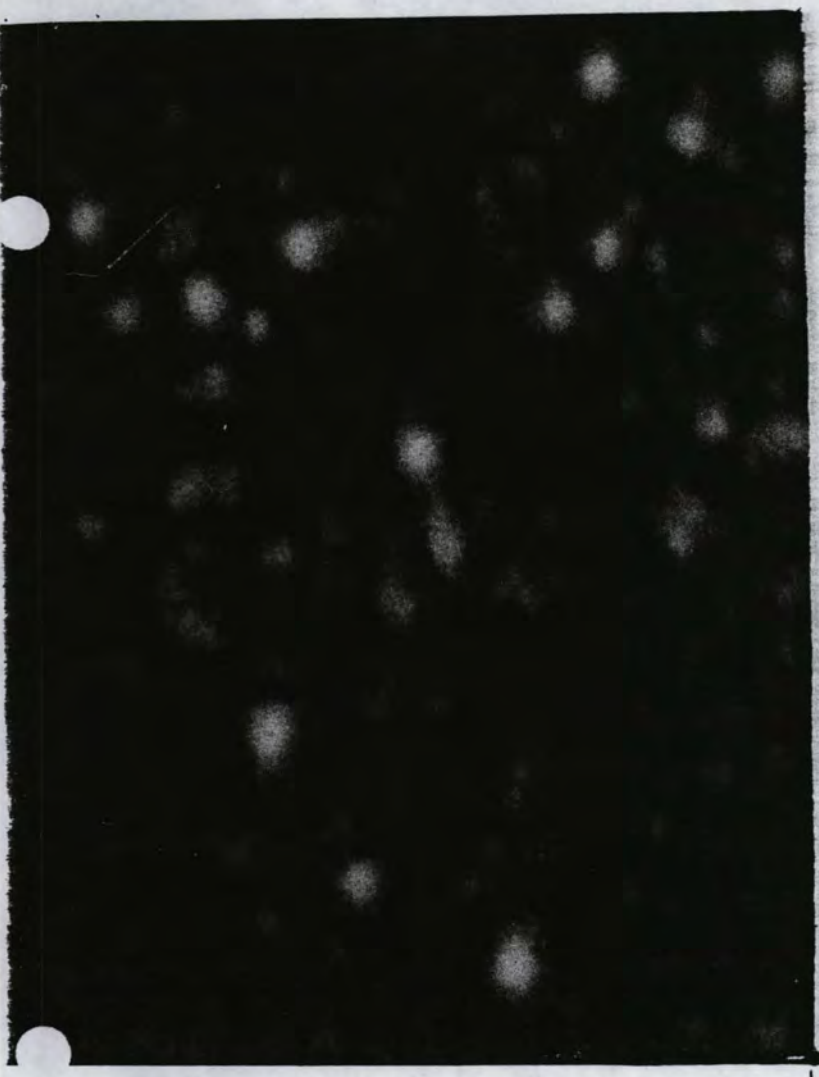
Turner, F.B. 1960. Population structure and dynamics of the western spotted frog, *Rana p. pretiosa* Baird & Girard, in Yellowstone Park, Wyoming. Ecological Monographs, 30:251-278.

Vial, J.L. (editor) 1992. Froglog - IUCN/SSC Declining Amphibians Task Force Newsletter. March, 1992, 1.

Welsh, H.H. 1987. Monitoring herpetofauna in woodland habitats of northwestern california and southwestern Oregon: A comprehensive strategy. Gen. Tech. Rep. PSW-100. Berkeley, CA. Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture.

Welsh, H.H., A.J. Lind, and D. L. Waters. 1991. Monitoring frogs and toads on Region 5 National Forests. R-5 Fish Habitat Relationship Technical Bulletin. Number 4, May 1991. 12 p.

Welsh, H.H. and A.J. Lind. *In press.* Structure of herpetofauanal assemblage of the Douglas-fir/hardwood forests of northern California and southwestern Oregon. Pages XX-XX *in* L.F. Ruggiero, K.B. Aubry, A.B. Carey, and M.H. Huff, tech. coords. Wildlife and vegetation of unmanaged Douglas-fir forests. USDA For. Serv. Gen Tech. Rep. PNW-GTR-XXX. (Contains a discussion of management strategies to maintain a viable herpetofauna)



A GUIDE TO PRESERVATION TECHNIQUES FOR AMPHIBIANS AND REPTILES

George R. Pisani
Museum of Natural History
University of Kansas
Lawrence, Kansas 66044

Illustrations
by

Patricia May and Tom Swearingen
Museum of Natural History
University of Kansas

Table of Contents

INTRODUCTION

Over the years, several informative works describing the preservation of amphibians and reptiles have been published. Most of these have been intended for relatively limited distribution by the institutions or individuals publishing them. This fact, coupled with new laws pertaining to syringes and certain drugs used for killing specimens, warrants an additional treatment of the subject. This article is an attempt to combine a complete survey of current techniques with a page size that the individual collector can conveniently carry in the field.

In an age when so many wild species and areas of suitable habitat are at the threshold of extermination, it seems advisable at the outset to include a plea for conservation in this booklet. Current museum collections contain excellent samples of various North American species of reptiles and amphibians from certain areas within their ranges. In these instances, it is a needless waste to collect and preserve additional material when this will not add appreciably to our knowledge of these creatures. I am not referring to such collecting as may be associated with the compiling of a synoptic teaching collection by a school or to collection of specimens needed for a particular aspect of research, but rather to the capture and preservation of animals simply to amass a collection which may never be used for scientific or educational purposes. There are numerous geographic areas, including several in North America, in which the amphibians and reptiles are poorly known. Collections from these areas can add measurably to our herpetological knowledge. Persons wishing to learn of the desirability of specimens from particular areas should consult with herpetologists at nearby universities, museums, zoos, etc.

I would like to express my thanks to Woodrow W. Barber, Hobart M. Smith, William E. Duellman, Joseph T. Collins, Clarence J. McCoy, and George Iannarone for furnishing helpful material and/or advice and to Phyllis Shaffer, Judy Hamilton, Leanne Johnson, and Ginger Stiggins for typing assistance. Thanks are also expressed to Jaime Villa for preparing much of the "International Shipment" section.

FIELD NOTES

Specimens not accompanied by data identifying the collection locality are virtually useless to scientific investigators. The more data available for a specimen, the greater its value in research. Hence, keeping accurate, complete field notes is necessary. Many times, data felt to be trivial at the time of collection may prove to be quite useful when many observations are pooled. Field notes should be written in waterproof ink ("Pelikan" brand is preferred by many) using only one side of each page. Several brands of waterproof ink will "run" if alcohol is accidentally spilled on the page, hence care should be used in selecting ink. A worthwhile technique is to carry a small notebook for on-the-spot data taking. Then transfer these data into the permanent field notes as soon afterward as possible.

The following is a representative outline of data included in field notes (numbers refer to those in figure 1a):

1. Locality- Do not record locality with reference to business establishments. Use towns or mapped roadways; determine distance from an automobile odometer (if available), or estimate distance carefully from maps. It is not unusual for roads to be rerouted, renumbered or both. It is therefore advisable to refer to roads indicated in a good atlas to which future reference can be made. In the U. S., the American Highway Atlas (Gousha Co., Chicago) is suitable. If collecting in areas remote from roadways, locate the collecting site as accurately as possible from U. S. Geological Survey topographic maps. Collectors in foreign countries should try to obtain accurate, detailed maps of the areas in which they are working. Elevation of the locality should also be recorded whenever possible.

2. Date- Always write out the name of the month, or indicate month by a Roman numeral; 6-10-71 could refer either to June 10th or October 6th.

3. Name(s) of all collector(s) present.

4. Time of collecting.

fig.1a

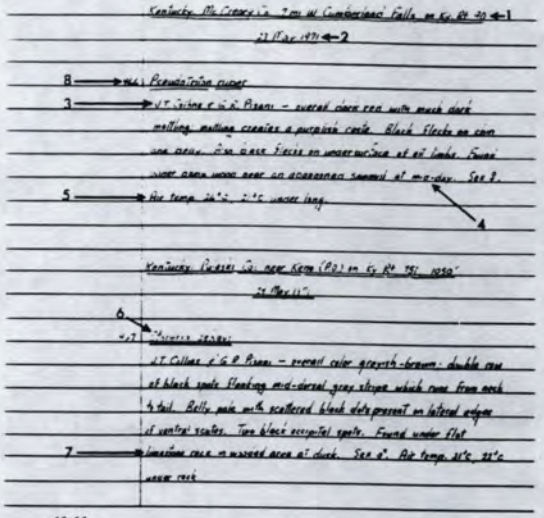
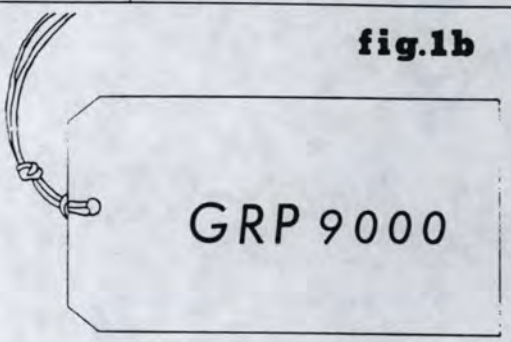


fig.1b



5. Air temperature and other appropriate weather notes- It is often useful to note existing cloud cover and moisture conditions, as well as general weather conditions preceding the collection.

6. Species- List all species collected plus the number collected of each, followed by species which may be observed but not collected. Accurate color notes are a worthwhile inclusion, especially when collecting in regions having a poorly known herpetofauna. It is also worthwhile to take accurate color notes when an atypically colored individual of a well-known species is encountered.

7. Microhabitat of species collected and any significant behavior (courtship, defensive display, etc.) observed.

8. Field number- It is useful to carry a series of numbered field tags on collecting trips. These should be printed on heavy paper in permanent ink. Satisfactory tags can also be made with one of the commercially available label makers that imprint plastic tape. Thread can be sewn through the numbered tags, but the backing on the tape should not be removed. Avoid using colored thread, or thread made from synthetics such as nylon, which may be destroyed by preservatives. White, cotton carpet thread is suitable for tagging. After threading the tag, tie a small knot in the string as shown in figure 1b. Each specimen should be assigned its own number, which greatly simplifies the task of keeping specimens and localities associated. Testes, stomach contents, photos, tape recordings, etc. are assigned the same number to increase efficiency in future analyses. Field tags should be securely tied (with a square knot) to specimens as illustrated in Plates 1 and 2. Lizards possessing femoral pores should be tagged by knotting the string below the knee; this avoids covering the pores with string. Tags in the field series should be numbered independently from the catalog series discussed in a later section.

Of the above data, numbers 1 (locality), 2 (date), 3 (name(s) of collector(s), and 8 (field number) represent the minimum data which should be recorded. If specimens are donated to an institution, the field notes should be donated with them. Do not include field notes in the same container used to hold specimens.

KILLING OF SPECIMENS

It is essential that live herpetological specimens be killed in such a manner as to leave the muscles in a relaxed state. Following this, they can be fixed, or hardened, in standardized positions which enables researchers to examine them conveniently and most accurately (refer to Plates 1 and 2). Many books recommend that reptiles be killed by hypodermic injection of aqueous sodium pentobarbital (Nembutal) into the heart. This technique is indeed excellent, but the reader should be aware that Nembutal is not a generally available drug, its possession being closely regulated by the Federal Bureau of Narcotics and Dangerous Drugs. It is possible for qualified persons to obtain a permit to purchase Nembutal, but the application procedure is best begun several months in advance of anticipated need. For additional details concerning the permit, the reader is urged to consult representatives of the above-mentioned Bureau at the Federal centers in most large cities. State and local regulations should also be checked. Commercial Nembutal is sold at a concentration of 50 mg/cc. The form of Nembutal sold as a syrupy elixer should be avoided. Commercial Nembutal may be used directly for larger specimens (over 5 pounds body weight), and diluted 1:5 with water for smaller reptiles; for very small specimens such as Typhlops or small Scincella it is possible to dilute to 1:10 and retain effectiveness. Nembutal diluted 1:10 can also be used on larger specimens, but death will be delayed. One cc (used commercial strength) injected into the heart is generally sufficient to quickly kill an animal of the bulk (volume) of a 3 foot timber rattlesnake (Crotalus horridus). Position of the heart in snakes can often be judged by closely watching the ventral plates on the anterior 1/3 of the body to detect heartbeat. Injection anywhere into the anterior 1/3 of the body cavity is also effective, but death is not as rapid as from heart injection. Other reptiles can be killed by injection into the heart region. Do not attempt to inject specimens which are so small or thin as to be heavily damaged by the needles at hand.

A number of other effective killing means are available. Turtles may be chloroformed if care is taken not to allow them to stiffen. Confining the turtle with a chloroform moistened rag or cotton wad in a closed container for 15-30 minutes (Cook, 1965) should suffice. The use of chloroform on other reptiles is definitely not recommended, as severe contortion usually results. Trichloroethylene or ether may be substituted for chloroform with good results, and can be used on most reptiles. Most specimens can be killed by confinement with either trichloroethylene or ether for 5 minutes beyond the time the animal loses the ability to right itself when turned over. These liquids are available to the public from either biological supply houses or certain drugstores. Their use may be superior to Nembutal when working with small, fragile animals like some tropical geckos. Caution should be observed with ether, as it is highly flammable and can, under certain storage conditions, explode. Read labels carefully.

All amphibians and a number of smaller reptiles (e.g. small, tropical geckos) are easily killed by immersing them in a solution of Chloretone (hydrous chlorobutanol). A stock supply is commonly prepared as a saturated solution of Chloretone in 95% ethanol. This stock solution may be conveniently carried in a small vial; 2 cc of it added to a pint of water is effective. The solution should be kept tightly covered when not in use, and can be used over and over; its strength will diminish with use.

Various other means are suitable for killing reptiles and amphibians. Securing the animal(s) in a cloth sack and immersing the sack in warm (110°-120°F; 43-47°C) water is effective, but specimens should be removed shortly after death. Specimens may also be immersed in alcohol (15-25% for amphibians; 50-60% for reptiles). Though the method is not recommended, bags containing reptiles may also be left exposed to direct sunlight until death from overheating occurs. Great care must be used however, as dehydration and accompanying contortion can happen quickly; amphibians should never be killed in this way. Both procaine hydrochloride (Livezey, 1958) and succinylcholine chloride (Anectine) (Lambert, 1967) have been used effectively as killing agents; however, their availability is usually restricted like that of Nembutal.

Recent drug laws have greatly increased the difficulty of obtaining syringes for preserving purposes. State laws may also vary in the regulation of the above-mentioned chemicals. It is often possible to obtain necessary supplies through institutions, particularly in return for depositing desired specimens.

FIXING

The purpose of fixation is to preserve the actual morphological state and color of the specimen, and to prepare the tissues for microscopic examination. Hence, the fixative should kill tissue quickly; penetrate it uniformly and rapidly; prevent postmortem decomposition; not distort the tissue; and should prepare the tissue for staining. No single fixative will do all of these things, so various compromises must be made.

The most widely accepted and suitable general fixatives for field use are:

1) Formalin ("Formol" or "Formalina" in Spanish; "das Formaldehyd" in German)- Sold commercially as a solution of approximately 40 percent formaldehyde gas in water, formalin is the most widely used field fixative. For purposes of dilution, commercial formalin is usually considered as 100%, and can be used in 10% strength (1 part formalin: 9 parts water) for fixation. Formalin may be buffered (which helps to reduce discoloration of specimens) by mixing 1 tablespoon of baking soda or borax with each pint of 10% formalin. Generally sold as a liquid (often in drugstores), it is also available as a solid polymer (paraformaldehyde), which is convenient for saving weight and space in transport. Huheey (1963) recommends sealing 16 grams of paraformaldehyde and 4 grams of anhydrous sodium carbonate in packets for field transport; 1 packet added to 400 ml (about 1/2 quart) of water makes a 10% solution of buffered formalin. Premixed, buffered paraformaldehyde powder is available from Carolina Biological Supply House. Paraformaldehyde alone can be obtained from Eastman Organic Chemicals, Rochester, New York. Formalin, while an excellent general fixative, is highly irritating to the user's skin and (as a vapor) to mucous membranes. It is not uncommon for users to develop strong allergies

to formalin. Also, formalin has a tendency to cause swelling of several types of tissue, rendering them unsuitable for some histological purposes.

2) FAA (formalin-alcohol-acetic acid)- Prepared by mixing 10 parts commercial formalin, 50 parts of 95% alcohol (ethyl or isopropyl), 40 parts water and 2 parts glacial acetic acid. FAA penetrates tissue far better than formalin alone, and has less tendency to cause cell distortion. The rapid tissue penetration can also be an aid to preserving valuable specimens found dead and, perhaps, partially decomposed. The primary disadvantages of FAA are: the need to mix several components; and, the necessary alcohol and acetic acid may not be available in certain localities. FAA is not available in powder form, but can be premixed without the water to reduce volume in transport; water may be added later. If FAA is to be used extensively in hot regions, it is recommended that the acetic acid be added just prior to actual use, as it quickly evaporates from the solution; containers may be cooled by wrapping them in wet rags and shading them to retard evaporation of acetic acid.

3) Alcohol- If neither formalin nor FAA are available, alcohol may be used as a fixative. Cook (1965) recommends ethanol (95% for reptiles; 70% for amphibians) or isopropanol to fix in the absence of other solutions, but the latter is not desirable.

4) Special- A large number of other fixatives exist, each being useful for different types of tissues, and studies. Bouin's solution (75 parts saturated aqueous picric acid, 25 parts commercial formalin, 5 parts glacial acetic acid) is especially useful for field preservation of testes to be used in spermatogenesis studies. Testes may be placed in vials of Bouin's and safely kept there for long periods of time without distortion of cells; the remainder of the specimen may be fixed with FAA or formalin. For a complete discussion of special fixatives, the reader is referred to Guyer (1961) and similar texts.

5) Miscellaneous- If a valuable specimen must be saved and no other solutions are available, a number of emergency measures are possible. The specimen may be frozen or packed in strong brine until preservative can be obtained. Liquor is generally not a suitable source of alcohol, as 110 proof liquor is only 55% ethanol. However, strong tequila (about 160 proof) may be useful; rubbing alcohol can also be used. These, however, are only desperation measures and it is usually more beneficial to get the specimen into a proper fixative (hospitals, local schools, etc. are suggested as possible sources).

It is always preferable to introduce fixative into the body cavity, as specimens (particularly reptiles and larger amphibians) can decompose internally if simply placed in fixative. Enough fixative should be injected to fill, but not distend, the animal. Care should also be taken not to damage the femoral pores of many lizards by puncturing them with the needle. The neck of turtles should be completely extended and the mouth held open with wood, cork, or tightly wadded paper prior to fixation. Excellent neck extension can be obtained by hooking the dead turtle's upper jaw over a nail or broken branch and letting the animal's hanging weight pull the neck out straight prior to injecting it. The upper jaw can also be hooked over a paper clip placed over the edge of the fixing tray, and the neck then drawn out. One hemipenis of male lizards and snakes should be partially everted with thumb pressure on the base of the tail, followed by injection to completely evert it as indicated on the front cover. The hemipenis should not be permitted to remain incompletely everted; thread may be tied around the base of the fully everted hemipenis to help retain fluid within it. It is also an acceptable practice to evert the hemipenis by injection of fixative alone. Typical sites for injection of preservative are starred in Plate 2. Tails of lizards and snakes should be slit lengthwise, being very careful not to break the tail off; sharp instruments are a "must." Large amounts of fixative can be conveniently handled in the injection apparatus designed by Jackson (1971), although the author has never felt at a disadvantage using larger syringes. If no injection apparatus is available, the specimen should be deeply slit in several places ventrally and placed belly-up in fixative. Spread the sides of the slits to admit fixative more easily. Avoid cutting the anal plates of snakes and lizards and femoral pores of lizards.

Once the animal is injected or slit, it is most conveniently fixed by placing it (after proper positioning) between pieces of white paper toweling moistened liberally with fixative. This can be done in shallow, covered plastic or rustproof metal pans. Surgical instrument pans with sliding metal covers are very handy for this. Avoid colored towels, as the colors dissolve in the fixative and stain the specimen.

Preferred positions for fixing and sites for field tag attachment are illustrated in Plates 1 and 2. Amphisbaenids and caecilians should be fixed in the same position as snakes; it is useful to fix these with the mouth open, as this greatly facilitates examination of oral characters later on. Lizards with long tails should be fixed with the tails bent as shown. Frogs and toads may be positioned with the sole of the foot down (Duellman, 1962). However, because this position obscures many hind limb and anal characters, others feel that anurans are best fixed with the hind limbs in the position shown in Plate 1c. Toes and fingers should always be straight and spread apart. Small amphibians need not be injected or slit prior to positioning, as the fixative will penetrate to the body cavity quite easily. Small amphibians and lizards may have the field tag tied around the body just anterior to the pelvic region.

Amphibian eggs and larvae are best fixed and stored by dropping them directly into jars of 10% formalin; preserve entire egg clutches whenever possible. Many amphibians attach their eggs to leaves, twigs, etc. Whenever it is practical, these items should be preserved with the eggs <u>in situ</u>, as the latter are often severely damaged by attempts to disengage them. Change the formalin on eggs and larvae after about 12 hours. Reptile eggs should be measured (length and width, in millimeters), then injected.

All specimens should be allowed to remain in fixative for 24 hours.

<u>Very Large Specimens</u> (too large to be conveniently stored entire in liquid)

1. Snakes- Obtain the snout to vent and tail lengths (in mm). Then skin by making a long ventral incision to the side of the mid-line; leave the head and tail attached to the skin, severing these from the carcass (avoid cutting the anal plate), and then inject head and tail (evert hemipenis if male) with fixative. With boids, sever the hind part just

Plate 1. Amphibians

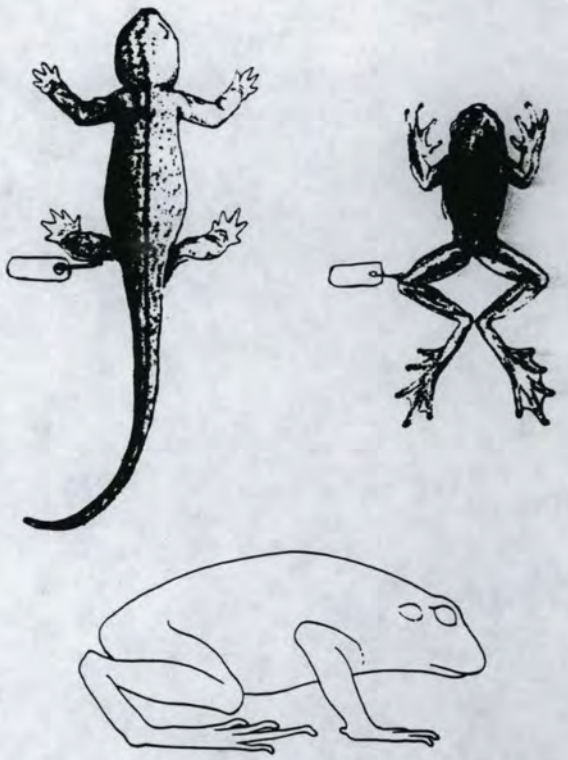
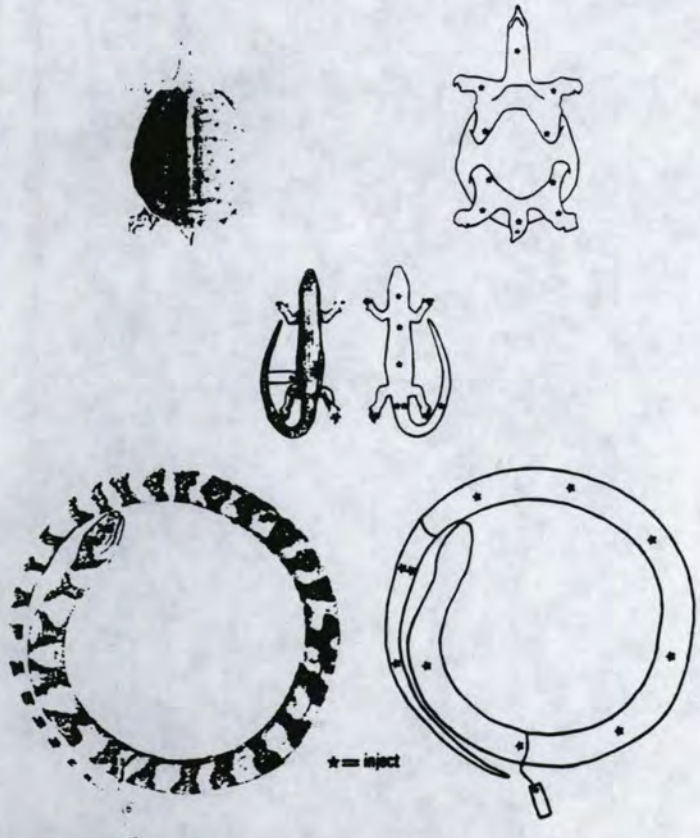


Plate 2. Reptiles



ahead of the bony, vestigial pelvic elements. The skin may now be preserved by covering the flesh side with cloth or absorbent paper, rolling loosely and immersing in fixative, or by rubbing with borax or arsenical soap, rolling and drying. In this latter instance, it is best to preserve the head and tail separately in liquid. If the specimen is a male, a testis should also be preserved. Reproductive condition of females should be noted (<u>i.e.</u>- number of ova present, size of the largest ovum, etc.). Embryos, especially those of poorly known species should be preserved in liquid; it is preferrable to do this by preserving the entire oviduct rather than by removing embryos.

2. Turtles- Avoid cutting the shell. It is preferable to cut the head, neck and forelegs out as one unit, the hind legs and tail as a second unit and preserve these in liquid. The stomach and reproductive organs should also be preserved in liquid. Carefully clean out and dry the shell.

3. Crocodilians- Measure and skin the specimen as for snakes, except that the tails should be skinned as well. Feet may be left attached (inject with fixative), instead of skinned out. Rub the skin with borax or arsenical soap and dry.

<u>SHIPPING</u>

Once they have been properly fixed, most herpetological specimens may conveniently be transported by wrapping them loosely in cheesecloth or white paper towel which has been liberally moistened with alcohol (70% ethanol or 40% isopropanol), or the fixative, then sealing them in two plastic bags (one within the other, individually closed by twisting the end and knotting or securing with a rubber band). Several small specimens may be wrapped in a single length of cheesecloth by laying the cloth out flat, spacing the specimens down the length of it, folding the sides over the animals and rolling the cloth loosely, like a rug.

Thus packed, the specimens occupy minimum space and weight, important factors when they must be transported any distance or mailed. Specimens will remain in

ondition for several weeks, so long as the bags are well sealed to retard
e and evaporation. Cotton in sheet form may be substituted for cheesecloth,
bulkier and may adhere to the scales of some rough-scaled species of lizards,
nen it is moist.

s being shipped (parcel post is a convenient means) should be carefully
ed and clearly marked "PRESERVED SCIENTIFIC SPECIMENS." Packages often are
t to much "wear and tear," so, effort in preparation pays off! Paint cans
ying sizes make leakproof, sturdy mailing containers. Plastic bags containing
ens may be simply placed in the can and extra space filled with wadded rags
er. Bags with heavy specimens should never be placed on top of lighter ones.
address labels should be typed or written with permanent ink. Place one
se in the can with the specimens, tape a second to the side of the can.
the third label to the paper used to wrap the parcel.

aint cans are not too costly, and a source of supply can generally be found
sulting local paint stores. Watching auction notices sometimes turns up a
store that is going out of business and may have cans. Remove handles from
efore use. In lieu of cans, specimens may be packed in any durable container.
postal regulations for size and weight restrictions before packing extremely
or unwieldy parcels. Specimens such as large turtle shells or the skins of
crocodilians may have to be sent via freight, and again, secure packing is
. Shipment of all crocodilian specimens is subject to stringent regulation,
y of these species are endangered animals. Collectors planning to take
should carefully check customs regulations for import restrictions as well
ecking capture laws in countries where the animals occur. Proper arrangements
ften be made through the institution where one proposes to deposit the specimens.

Generally, specimens should never be sent in glass containers. Obvious
tions to this are ampnibian eggs (and sometimes, larvae) and very fragile
ens. These should be placed in the smallest containers needed to hold the
ens plus fluid to maintain them; fluid should fill the containers, which
be heavily padded with cardboard or cotton. If rigid plastic tubing of
ient diameter is available, break resistant containers may be fashioned
it by cutting an appropriate length, stoppering one end, enclosing specimens
luid, then sealing the other end. The tube may be wrapped lengthwise with

wire to secure the stoppers. Plastic vials are available from some biological supply houses; larger drugstores may also furnish the names of suppliers of these. Be sure to only use vials which can be securely closed (screw-on or snap-on lid). Again, pay special attention to wrapping such containers.

INTERNATIONAL SHIPMENTS

Collectors should be aware of proper methods for shipping specimens internationally. Donation of all or part of a collection to institutions outside one's own country serves to:

1. make synoptic herpetofaunal collections of different areas available to as many researchers as possible,

2. prevent the loss (through war, neglect, earthquakes and other damage) of valuable collections deposited entirely in a single institution, and

3. place the herpetologist in contact with colleagues in foreign institutions; this frequently leads to a most beneficial exchange of ideas and data, thus advancing herpetology as a field of study.

The private hoarding of specimens by any person is a waste of valuable biological data, and can lead to overcollecting (i.e.--researchers may gather specimens from areas already represented, though inaccessible, in private collections). It is with the above points in mind, and the hope that more collectors will decide to enter into donation, exchange or loan relationships with foreign institutions, that the following guidelines are presented.

The methods of packing described in the preceding section are adequate for international shipment. Generally, mail is the most convenient means of sending packages which are not too heavy or bulky. Parcels sent via surface ("ordinary") mail should have extra preservative added to the specimen bags, as they may take as long as 4 months to reach their destination. Persons

ing specimens internationally should check local mail regulations on
el size, weight and any special packing provisions. The shipper may also
equired to affix various postal and customs "declaration tags" to parcels.
e tags vary with parcel destination and are generally provided by the
al service.

Very large or heavy packages will have to be sent via freight (air,
n, ship). The sender will be required to complete a "waybill" (avail-
e from the carrier) listing, among other things, the nature and value of
contents. To avoid excess charges, package and waybill should be marked
Commercial Value." Postal services in all countries have the legal right
inspect all packages. Intensive efforts to curtail the traffic of narco-
s and other restricted drugs has led to the extensive exercising of this
nt, and the fact that several persons have attempted to smuggle drugs with-
specimen containers has not aided the situation. Inspectors often open
stic bags of specimens, and may be unaware of the need to reseal them.
s causes loss of fluid and dehydration and probable loss of the specimens.
is therefore advisable to include two copies of the following statement with
parcel (one pasted on the outside and one sealed within):

SPECTION OFFICER: This package contains dead, preserved amphibians and/or
ptiles packed in plastic bags. As the specimens have great scientific
ue and will be ruined if not kept moist in their preservative, it is
perative that the bags be tightly resealed after inspection to avoid
poration or leakage of preservative. Thank you.

SPECTOR POSTAL: Este paquete contiene ejemplares de anfibios y/o reptiles
ertos, preservados y empacados en bolsas plasticas. Puesto que los
emplares son de valor cientifico y se arruinan si no permanecen en su
qui servativo, se suplica que, despues de abrir las bolsas para
sp rlas, las cierre hermeticamente para evitar que el liquido se
apore o se derrame. Gracias.

AUTORIDADES ALSANDEGARIAS: Este volume contem anfibios e repteis mortos,
nservados em sacos plasticos. Como o conteudo tem valor cientifico e se
tragara se nao for mantido humido no preservativo, pedese que apos abrir
sacos para inspecao os mesmos sejam firmemente fechados para evitar
aporacao ou derramamento do liquido. Obrigado.

Biologists should also be aware that the international shipment of specimens (alive or preserved) is being ever more closely regulated for conservation reasons. Shipments of preserved animals sent to the USA must be accompanied by a list bearing the number and scientific name of all specimens included. The importer (in the USA) must obtain a special permit from the Bureau of Sport Fisheries and Wildlife (Dept. of the Interior) in order to receive foreign shipments of preserved or live specimens.

Live shipments are additionally regulated by the Dept. of Agriculture and the Public Health Service. In all cases, endangered species are covered by regulations separate from species not currently considered endangered. You are urged to carefully investigate all legal aspects of international shipment before preparing to send animals.

STORAGE AND LABELLING

This section is not intended to be a complete guide to curatorial technique. Rather, it is meant to serve as a set of capsule directions for those wishing to start a preserved herpetological collection. A detailed discussion of curatorial technique may be found in Slevin (1927).

Preserved collections are best maintained in alcohol. Suitable alcohol generally costs about the same (per gallon) as formaldehyde, and alcohol-stored specimens are far easier to work with. Formaldehyde also tends to corrode metal lids and containers. Most collectors will be deterred from using ethanol by the high tax imposed upon its sales. Isopropanol is far cheaper, and is entirely satisfactory for storage of specimens. Methanol should never be used. Concentrati of 50% is suitable for reptiles, while 40% is better for amphibians. Both ethanol and isopropanol are generally sold at 95% concentration; 526 ml of this plus 474 ml of water make one liter of 50% concentration (421 ml alcohol + 479 ml water for 40%). Specimens being transferred from formalin or FAA fixation to alcohol must first be soaked in water for 48 hours. Failure to soak the specimen often results in its being severely dehydrated by the alcohol. Properly fixed specimens will not be harmed by this method. If material is desired for use in histological work, selected pieces of tissue should remain in 30% alcohol

for 24 hours, then 24 hours of 50% alcohol before going to final storage (omit the water soak). Do not pack specimens tightly in the jar. Snakes fixed in the position illustrated earlier will readily coil in jars for storage.

Each specimen retained in the collection should be assigned a catalog (in addition to the aforementioned "field number"). Amphibian eggs and larvae and reptile eggs may be cataloged with a single tag designating one clutch or lot. This number should be entered in a permanent catalog (using waterproof ink), along with the species name, date of capture/preservation, sex, locality, ecological notes and name of collector. Tags may be tied in the same region as the field tag. Collector's field number should also be entered. As a cross-reference, it is useful to maintain a card file (by taxonomic family) in which a single card is used for each species. On this card may be entered numbers from the catalog that apply to these species.

It is convenient to place a label bearing species name, catalog numbers and locality data with each container. These should be written in permanent ink on heavy, durable paper. That produced by Byron-Weston Mills under the name "Linen Record Ledger", 100% cotton and linen fiber, 36 lb. and Dennison Paper Company's product "Resistall Index Bristol", 100% rag, 110 lb. wt. are both excellent and are available through printing shops. The label may either be placed within transparent containers, or attached to the outside of opaque ones with masking tape. If moderate cost can be withstood, external labels can be placed within tie-on, plastic label-holders. A typical museum label is shown in Figure 2.

Specimen jars should be stored in cool places to help retard evaporation of preservative, and should never be exposed to sunlight, as specimen colors are rapidly faded by such exposure. Placing a piece of Parafilm sheet (available from Carolina Biological Supply House, Burlington, North Carolina) over jar mouths before screwing on the cap will also reduce evaporation. Containers should be checked periodically and fluid level maintained. Well preserved and cared for collections make valuable teaching and research tools.

COLOR PRESERVATION

While preserving the morphological state of herpetological specimens has never presented any severe hurdles to collectors, preservation of color is quite another matter. All currently used, popular preserving fluids are alcoholic and/or acidic to some degree. Therefore, it usually is not too long before most pigments are dissolved by such fluids and extracted from the specimens. Amphibians seem particularly vulnerable in this regard, though the effect on reptiles is noticeable.

Previously, the only acceptable method of retaining amphibian skin color was that described in Cook (1965). Basically, this consists of skinning the specimen, confining all cuts to the ventral surfaces of body and limbs. The skin is then floated flesh-side up in a pan of water and remaining particles of tissue are removed. The skin next is floated flesh-side down and spread out in a second pan. A wet piece of cardboard may then be brought up beneath the skin, which is rubbed lightly to flatten it and remove trapped air. The cardboard-skin preparation may be dried on blotting paper until moist, then placed between layers of blotting paper and thoroughly dried with heavy weights (such as books) on top of it; it may also be placed in a plant press. Reptiles may be similarly prepared. In all cases, the carcass should be preserved in fluid and tagged with the same number as the skin. Skins thus prepared should be stored in the dark and not exposed to prolonged light.

The above technique, while useful, is tedious. Windsor (1971) has described a technique for using 50% saturated, aqueous ammonium sulfate solution as a preservative of frogs. As the compound is an aqueous, neutral salt, no pigment was dissolved and natural color was still evident in the specimens 6 months after preparation. Total fixing time should be at least 36 hours.

Specimens may be stored in buffered formalin (10%) or isopropanol (40%) to which liquid Ionol-40 R has been added (White and Peters, 1969). Storage should be in dark places which are not subjected to heat much above 70°F. The formalin/Ionol method has been successfully used with herpetological material by Mr. Woodrow Barber, Biology Department, University of Kentucky at Morehead, and by Dr. George Iannarone, Chicago Academy of Sciences (personal communication).

Powdered Ionol should not be used, as it is difficult to prepare a stable solution of it in preservative. Ionol is sold by the Shell Oil Company (Chemicals Division).

While the two chemical methods discussed above have not been widely used with herpetological material, their success on a limited scale coupled with the value of accurate color preservation suggests that they should be more thoroughly investigated.

LITERATURE CITED

Cook, Francis R. 1965. Collecting and preserving amphibians and reptiles, p. 128-151, In R. M. Anderson (ed)., Methods of collecting and preserving vertebrate animals. Nat. Mus. Canada, Bull. No. 69, Biol. Ser.: 1-199.

Duellman, William E. 1962. Directions for preserving amphibians and reptiles, p. 37-40.In E. Raymond Hall (ed)., Collecting and preparing study specimens of vertebrates. Misc. Publ. Univ. Kansas Mus. Nat. Hist., (30): 1-46.

Guyer, Michael F. 1961. Animal micrology, 5th impression. Univ. Chicago Press, Ill.; xviii + 327 pp.

Huheey, James E. 1963. Concerning the use of paraformaldehyde as a field preservative. Copeia, 1963(1):192-193.

Jackson, Morris K. 1971. A new "syringe" for injecting herptiles. Herpetol. Rev., 3(4):75.

Lambert, Lewis H., Jr. 1967. 'Anectine': a herpetological killing agent. Herpetologica, 23(2):135-136.

Livezey, Robert L. 1958. Procaine hydrochloride as a killing agent for reptiles and amphibians. Herpetologica, 13(4):280.

Slevin, Joseph R. 1927. The making of a scientific collection of reptiles and amphibians. Proc. Calif. Acad. Sci., 16(9):231-259.

White, David A. and Edward J. Peters. 1969. A method of preserving color in aquatic vertebrates and invertebrates. Turtox News, 47(9):296-297.

Windsor, Donald A. 1971. Ammonium sulfate as a preservative which does not remove color from frogs. Copeia, 1971(2):356-357.

INDEX

References

Blaustein A.R., and D.B. Wake. 1990. Declining amphibians: a global trend? Trends in ecology and evolution. 5:203-204.

Carpenter, C.C. 1953. An ecological survey of the herpetofauna of the Grand Teton-Jackson Hole area of Wyoming. Copeia, 1953(3):170-174.

Corn, P.S. and R.B. Bury. 1989. Logging in Western Oregon: responses of headwater habitats and stream amphibians. Forest Ecology and Management, 29(1989):39-57.

Corn, P.S. and R.B. Bury. 1990. Sampling methods for terrestrial amphibians and reptiles. USDA Forest Service, Pacific Northwest Research Station, Portland, Oregon. General technical Report PNW-GTR-256.

Corn, P.S. and J.C. Fogleman. 1984. Extinction of montane populations of the northern leopard frog (*Rana pipiens*) in Colorado. Journal of Herpetology, 18(2):147-152.

Groves, C. 1989. Idaho's amphibians and reptiles. Nongame Wildlife Leaflet #7. Idaho Department of Fish and Game.

Halliday, T. and K. Adler. 1987. The encyclopedia of amphibians and reptiles. Facts on File Publications, New York. 143 pp.

Karns, D.R. 1986. Field herpetology: methods for the study of amphibians and reptiles in Minnesota. Occasional Paper No. 18, Division of Comparative Biology, James Ford Bell Museum of Natural History, University of Minnesota, Minneapolis. (Contains good descriptions of techniques for finding and monitoring populations).

Nussbaum, R.A. E.D. Brodie, and R.M. Storm. 1983. Amphibians and reptiles of the Pacific Northwest. University Press of Idaho, Moscow. 332 p. The best general source of information on the amphibians and reptiles of Idaho).

Pisani, G.R. 1973. A guide to preservation techniques for amphibians and reptiles. Herp. Circular. No. 1. 22 p. Society for the Study of Amphibians and reptiles.

Pough, F.H., J.B. Heiser, and W.N. McFarland. 1989. Vertebrate Life. Third edition. Macmillan Publishing Co., New York. (An excellent textbook on vertebrate zoology; especially good chapters on amphibians and reptiles).

Stebbins, R.C. 1985. A field guide to western reptiles and amphibians. Houghton Mifflin Co., Boston. 336 pp. (The best field guide to the amphibians and reptiles of the western United States).

Turner, F.B. 1958. Life history of the western spotted frog in Yellowstone National Park. Herpetologica, 14:96-100.

Turner, F.B. 1960. Population structure and dynamics of the western spotted frog, *Rana p. pretiosa* Baird & Girard, in Yellowstone Park, Wyoming. Ecological Monographs, 30:251-278.

Vial, J.L. (editor) 1992. Froglog - IUCN/SSC Declining Amphibians Task Force Newsletter. March, 1992, 1.

Welsh, H.H. 1987. Monitoring herpetofauna in woodland habitats of northwestern california and southwestern Oregon: A comprehensive strategy. Gen. Tech. Rep. PSW-100. Berkeley, CA. Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture.

Welsh, H.H., A.J. Lind, and D. L. Waters. 1991. Monitoring frogs and toads on Region 5 National Forests. R-5 Fish Habitat Relationship Technical Bulletin. Number 4, May 1991. 12 p.

Welsh, H.H. and A.J. Lind. *In press.* Structure of herpetofauanal assemblage of the Douglas-fir/hardwood forests of northern California and southwestern Oregon. Pages XX-XX *in* L.F. Ruggiero, K.B. Aubry, A.B. Carey, and M.H. Huff, tech. coords. Wildlife and vegetation of unmanaged Douglas-fir forests. USDA For. Serv. Gen Tech. Rep. PNW-GTR-XXX. (Contains a discussion of management strategies to maintain a viable herpetofauana)

FROGLOG

IUCN/SSC Declining Amphibian Populations Task Force

March, 1992, No. 1

Coordinator's Column

The DAPTF has been established by the International Union for the Conservation of Nature (IUCN), Species Survival Commission (SSC) to organize a global monitoring program for (1) determining the status of amphibian populations (2) assessing the implications of any declines (3) studying potential causative factors and (4) making appropriate policy recommendations based upon these findings. The Coordinating Council, administered by the Coordinator, includes researchers, liaison officers of societies and agencies as well as other interested parties, all of whom serve as communicators.

As of the last week in January, the Task Force became equipped and manned at the projected level when it occupied its present facilities at the Environmental Research Laboratory in Corvallis, Oregon. In addition to the recent acquisition of computer hardware, we now have a full time information systems manager in the person of Tony Clem. Once our system is interfaced we shall initiate an electronic database and other activities designed to serve as a viable communications network.

We are still in need of Regional Working Group Chairperson for the U.S. Great Lakes area (WI, MIN, MI).

Priority has been given to organization of a Working Group to assist in compilation of a comprehensive bibliography of reports relating to amphibian populations that will be generated and maintained at the Coordinator's office. We wish to include titles of primary and secondary literature, thesis and dissertations, as well as names of earlier investigators who have archived their field notes at a repository. This resource will be freely available to those wishing to make comparisons with contemporary studies.

Anyone interested in these or related studies are invited to join the DAPTF. Please send your name, address and telecommunications number(s), indicating your interest or participation, to the Coordinator's address.

Canada Launches Major Initiative

(The following is edited and condensed from a report by Hinrich Kaiser, *Redpath Museum, McGill University, on the workshop "Declines in Canadian amphibian populations: designing a national monitoring strategy" held at the Canada Centre for Inland Waters in Burlington, Ontario, on October 5 and 6, 1991.* Bull. CAH/ACH 5(2):1-4.)

The workshop in Burlington, organized by Christine Bishop (Canadian Wildlife Service) and Bob Johnson (Metro Toronto Zoo), constituted the first comprehensive attempt to address the declining amphibian phenomenon from a Canadian viewpoint. The problem of amphibian declines has become an urgent concern among Canadian herpetologists. Participation of researchers in universities, governments and private organizations was truly exceptional. This meeting was the largest gathering of Canadian herpetologists in memory.

In her opening remarks, Bishop stated that the aim of the meeting was to create a framework to monitor Canadian amphibian declines and the factors causing them. Johnson, a DAPTF Board of Directors member, highlighted the problem. Speakers representing the various provinces gave depressing status reports on amphibian populations throughout Canada. In many cases, these were anecdotal accounts, although causal relationships between declines and anthropogenic events can be confirmed in all too many cases. Invariably, each speaker referred to the basic lack of knowledge about the amphibians in question: distributions are insufficiently known, causes behind disappearances are uncertain, and habitat surveys are insufficiently detailed.

The introduction of non-native amphibian species and sports fish, mismanagement of wetlands, human intrusion, and logging, have all been identified as damaging to amphibian populations in more than one province. All were cited as being at least partly responsible for population declines in British Columbia. In Nova Scotia fragmented habitats and the resulting inbreeding within many species have produced increased frequencies of albinism and extra-limbed individuals. A well-documented problem is shown by *Rana pipiens*, stemming from the sale of over a million frogs to biological supply companies in the U.S. until die-offs began in 1975. In the middle 1970s, the famous Manitoba frog holes were empty, and despite an eight-year ban on picking frogs their numbers have not much increased.

Natural events, such as droughts, may be in part responsible for declines observed in populations in Saskatchewan. An outbreak of red leg disease in 1976 resulted in many deaths of *Rana pipiens* in Alberta. Recent observations on *Rana catesbeiana* in the Algonquin area showed that the average weight of calling bullfrogs at two separate sites differed significantly. It is unknown whether life history, social structure or harvesting contributed to this phenomenon.

In Ontario and Quebec, amphibian monitoring has been going on for some time. Since 1984, Ontario has received a total of 52,000 records from 2,700 volunteers and has also compiled a bibliography of herpetology including ca. 1,400 references. In Quebec, 5,400 records are reported.

However, it is puzzling that some species seem entirely unaffected. It has been suggested that certain ones may be rebounding from natural, cyclical events and that there may be positive changes observed in many areas within the next few years.

The afternoon talks centered on the monitoring of amphibian populations, including reports of projects that have produced quantitative data. Data show the best estimate is gained by intensive study. This method has actually been employed in a four-year study of Fowler's toads at Long Point . These toads have dramatically increased in numbers since the study began, likely an effect of the water level rise in Lake Erie.

Among other concerns presented was the importance of: experimental design, timing and length of study; preservations of natural conditions of the habitat; measuring both natural and anthropogenic environmental factors; generating a genetic database during monitoring; larval stages in relation to reproductive success and gene flow; pathological conditions present in the populations; and determining the effects of contaminants upon entire populations.

Open discussions began on the second day. It was first determined that the Working Group will be a research coordinating body for investigating the hypothesis that amphibian populations are in decline. If this hypothesis is supported, the group should then seek ways to reverse the declines. It was agreed that

goal is best served by separately sidering historical data, intensive monig studies, and extensive monitoring ects.

The intensive monitoring group dised how to approach the monitoring cess. Life history research must be current with the monitoring process. group decided a number of indicator cies for intensive monitoring, chosen clude as many families as reasonably sible, in a variety of habitats and ecotems, and with a range of genetic and rphological variation.

The Canadian working group will be st active at the provincial level, with gional Coordinators. Details for each dy population and site will be commuated to Eastern and Western Coordiors and the Coordinator for Canada. o will communicate with the IUCN Task rce. this hierarchical setup should ep Coordinators in touch and allow the gions to act both individually and in coeration with each other and with comrable regional groups in the United ates. To facilitate communication to all rticipants, the CAH/ACH Bulletin was osen as the official news medium.

The complete final report is to be blished in March of 1992 as a Canadian ildlife Service Technical Report. For rther information contact Christine ishop, Canadian Wildlife Service, Box 050, Burlington, Ontario L7R 4AG, anada.

CANADIAN WORKING GROUP

National Co-ordinator — David M. Green (McGill University)

Regional Co-ordinators — Don McAlpine (New Brunswick Museum), for Eastern Canada. Stan Orchard (Royal B.C. Museum) for Western Canada.

Provincial Co-ordinators (to be confirmed) — John Gilhen (Nova Scotia Museum), Nova Scotia: Don McAlpine, New Brunswick and P.E.I; Joel Bonin and Roger Bider (MacDonald College, McGill University), Quebec; Wayne Weller and Mike Oldham (Ontario Ministry of Natural Resources), Ontario; Bill Koonz (Manitoba Department of Natural Resources), Manitoba; Wayne Roberts (University of Alberta), Alberta; Stan Orchard, British Columbia.

Historical Population Trends — Martyn Obbard, Fred Schueller, Wayne Weller, Mike Oldham.

Intensive Monitoring — Mike Berrill, Jim Bogart, Ron Brooks, Francis Cook.

Extensive Monitoring — Bill Freedman

Environmental Contaminants — Christine Bishop

Diseases — Graham Crawshaw

Netherlands Conference

Annie Zuiderwijk, Chair of the Western European Working Group, represented the DAPTF at the International Symposium on the "Impact of Climate Change on Ecosystems and Species", convened in Amersfoort, The Netherlands in December. Experts, invited from different parts of the world, prepared evaluations of regionally important ecosystems. Workshop sessions focused on identifying key factors affecting selected ecosystems, identifying the main responses and determining various rates of change. Publication of reports from the symposium, expected soon, are intended to provide assessments applicable to issues in conservation, species diversity and management of ecosystems.

In the United Kingdom

Tim Halliday, chair of the UK Working Group (and a Task Force Director), reports that action is being taken to establish liaison and collaborative activities with the Western European group. UK sites of amphibian populations known to be "healthy" 10-15 years ago are being identified so that they can again be surveyed during the coming breeding season. A grant proposal for DAP related research has been submitted. Halliday is also arranging an October/November planning meeting.

Australians Take Action

A $47,000 grant from the Australian government was awarded to Michael J. Tyler, a Director of the Task Force, to organize a meeting of amphibian scientists and produce an Action Plan for Australia as a framework for new legislation, and for developing conservation and management goals for the next five years. To obtain an information base for this endeavor, a "Frogwatch" survey is being conducted in which 150 conservation organizations are participating in distribution of 600.000 (sic) questionnaires.

An organizational workshop convened by Tyler met in Canberra, ACT, last July. This initial meeting was attended by a nucleus of 16 representatives from the several States and Territories. The first half of the program addressed broad overviews and individual species case histories, the status of distribution maps, current legislation and the character of native population cycles. The subsequent general discussions dealt with causal agents; the use of museum records, sampling strategies, pathological studies, etc.

As of the present date, the Action Plan has been partly completed. Formal establishment of the Australian Working Group and its participating members is underway.

Reports from U.S. Working Groups

CAL/NEVA

The California/Nevada Working Group met for the first time at Point Reyes National Seashore on February 4, 1992. The group, chaired by Gary Fellers, included 14 representatives from the U.S. National Park Service, U.S. Forest Service, University of Nevada - LasVegas, St. Mary's College, University of California - Davis, California Academy of Sciences, University of California - Los Angeles, California Department of Fish and Game, and U.S. Fish and Wildlife Service.

Each member of the Working Group provided a short summary of their research relating to amphibians. Most of these reports provided compelling evidence for dramatic declines in amphibian populations throughout all or part of a species' range. Though some of the losses resulted from obvious factors (e.g., habitat loss), numerous cases were noted in which declines occurred with no identifiable reason. There appears to be strong evidence that acid precipitation is not the cause of the declines, though it might be acting in concert with other environmental stressors.

The status of the U.S. National Museum of Natural History handbook on monitoring protocols was addressed at some length. Further discussions centered on the need to gather data that are compatible among studies of different species and/or habitats. A form designed for use by the U.S. Fish and Wildlife Service (see report from Rocky Mountains Working Group) was examined in detail with the goal of determining the minimum data that should be collected as part of any amphibian field study.

ROCKY MOUNTAINS

Stephen Corn and Bruce Bury, co-chairs of the Rocky Mountains Working Group, are compiling a database of research activity on amphibians throughout the region. The Working Group is being organized in two tiers: those with current or recently completed research or monitoring programs, and those with more general interests regarding conservation activities. No formal meeting has yet been scheduled; however, the co-chairs participated in the Cal/Neva meetings at Point Reyes, California in early February to coordinate activities of the contiguous regional groups.

Data forms from their recent publication (Bury, R.B. and P.S. Corn. 1991. *Sampling Methods for Amphibians in Streams in the Pacific Northwest*. U.S. Forest Service, Pacific Northwest Re-

search Station. Gen. Tech. Rpt. PNW-GTR-275.) were evaluated during the joint meetings for potential application to all monitoring procedures. The recommended changes will be incorporated in a revised form for further review and consideration of adoption by other Working Groups.

NORTHEAST

The first meeting of the Northeastern Working Group, chaired by Richard Wyman, was held at the Pennsylvania State University on August 9, 1991.

Following a brief introduction regarding the objectives of the DAPTF, the group discussion focused upon the regional organization and development of an action plan. Priorities to be addressed include a survey of all active herpetologists in the region; assembly of all available regional data relating to the status of amphibian populations, identification of particular characteristics of species that would make data as to their presence or absence environmentally significant, and establishing a mechanism for maintaining a long-term monitoring network in the NE region.

The group is also initiating a search for thesis and dissertations that may contain usable density data, and for relevant records that may have been maintained at biological field stations.

Wyman has also generated a questionnaire for a mail survey as to the status of amphibian populations in the region. Copies of this form, which may be applicable for use by other Working Groups, may be obtained by contacting him (see address and telecommunications number, on page 4).

SOUTHEAST

A network of 40 cooperators in Florida, Alabama, Georgia and South Carolina will serve as the communication resource for data on SE US amphibians. Lists of currently recognized taxa are being generated for a status review by the Working Group. Ken Dodd, chair of the Working Group, has assumed the presidency of the SE section of the ASIH and plans to enlarge attention of the herpetological community upon the Task Force activities.

Carolyn Sekerak (M.S. student, Univ. Florida) is finishing her thesis work on the structure of amphibian temporary pond breeding sites. She has taken a position with the U.S. Fish and Wildlife Service in Jackson, MS. Her responsibilities include monitoring the status of amphibians and preparing federal listing proposals for the dusky gopher frog and other amphibian species.

A habitat conservation plan is being developed for the Red Hills salamander. The plan will involve the U.S. Fish and Wildlife Service. The Alabama Natural Heritage Program) is conducting a survey of the Sipsey Fork waterdog (*Necturus* sp.) in Alabama.

Pablo Delis and Henry Mushinsky (Univ. South Florida) are analyzing data on amphibian population fluctuations in Florida sandhill habitats based on 6 years' data.

Carlos Camp (Piedmont College) reports declines in relict populations of *Rana sylvatica* and *Ambystoma maculatum* in northeast Georgia. Wetland habitat alteration is suspected as the cause.

Dodd's paper on the biotic diversity of amphibians and reptiles in a Florida sandhills temporary pond has been accepted in the new journal Biodiversity and Conservation. Population declines due to drought (best guess) are noted, but long-term effects cannot yet be demonstrated.

Amphibian Bioassay as Assessment Tool for Superfund Sites

The U.S. Department of Defense has initiated an interagency agreement (IAG) with the Environmental Research Laboratory - Corvallis and several others to evaluate test procedures involving the effects of several classes of chemicals on amphibians. Initial studies will employ the Frog Embryos Teratogenesis Assay: *Xenopus* (FETAX). The utility of this test in ecological site assessment has been demonstrated at some Superfund sites using in situ exposures of mature amphibian species. Applications of the test procedures may provide information as to possible factors involved in declines of indigenous amphibian species and the use of mature amphibians as bioindicators of the health of wetland ecosystems.

Recent Reports of Declines

The Estonian herpetofauna consists of ten species of amphibians and five species of reptiles, apparently the result of post-glacial immigration from southeast (*Bufo viridis*), south (majority of species) and south-west (*Bufo calamita*). Earlier recorded *Rana ridibunda* and *Emys orbicularis* have become extinct.

From the perspective of distribution and degree of commonness, three groups of herptiles can be identified: rare and vulnerable species (*Triturus cristatus, Bufo calamita, B. viridis, Pelobates fuscus, Lacerta agilis*), less common species with sporadic distribution (*Rana arvalis, R. lessonae, R. esculenta, Anguis fragilis, Natrix natrix*), and common, widely distributed species (*Triturus vulgaris, Rana temporaria, Bufo bufo, Lacerta vivipara, Vipera berus*).

The distributions of *Triturus cristatus, Rana esculenta* (complex), *Pelobates fuscus* and *Lacerta agilis* seem to be relict in nature; some Estonian amphibians represent the northernmost distribution limits of the species (*Bufo calamita, B. viridis, Pelobates fuscus*). Many local populations of herptiles are reported as declining during the past ten to twenty years. (Talvi, T. 1991. *Amphibians and Reptiles of Estonia: list, geographic relationships and current situation.* Abst. 6th Ord. Gen. Mtg. Soc. European Herp., Budapest)

W.S. Osborne, in a recent status report (in litt.) on frog populations in the Australian Capital Territory documents the decline of *Pseudophryne corroboree* and *P. bibroni*, although both species are relatively common in other parts of their ranges. In contrast, there has been a complete disappearance of *Litoria aurea* and *L. raniformis* in the region, while *L. verreauxii* has become rare. Prolonged dry seasons are believed to be a contributing factor; however, the magnitudes of declines are such that other, yet unknown, factors are possibly involved.

In their recent report (Herp. Rev. 22(4):125-128, 1991) E. La Marca and H.P. Reinthaler have noted "drastically diminished" populations among five species of *Atelopus* in the Venezuelan Andes. Deforestation and expanding agrofarming appear to be the dominant factors impacting upon *A. carbonerensis, A. mucubajiensis, A. oxyrhynchus, A. pinangoi* and *A. sorianoi*. Flooding has scoured the montane streamside vegetation, and a high percentage of road kills in other areas are reported. The extent to which collecting may have reduced endemic *Atelopus* is also discussed. This report states (as with many others) that climatic change, pollution, as well as introduced species of plants and fish, are potentially significant factors in these declines and recommends action for both research and conservation.

RIBBIT Croaks

An earlier newsletter, *Ribbit*, was pioneered in the late 1980's by Bruce Bury and Stephen Corn to report on the decline of amphibian populations in the western U.S. Because of administrative constraints but a single issue was released (January, 1989). It will be superseded by FROGLOG beginning with this number.

Donor Support for DAPTF

Operations of the DAPTF would not be possible without the generous sponsorship of numerous organizations and individuals.

Major financial contributions have been awarded by the International Union for the Conservation of Nature (IUCN), Chicago Zoological Society, U.S. Department of State (AID program), Center for Analysis of Environmental Change (CAEC), the Jacob Bleibereu Foundation, Inc. and Frog's Leap Winery.

Support staff, equipment and office space have been provided by the CAEC (a consortium represented by Batelle-Pacific Northwest Laboratorities, U.S. Forest Service-Pacific Northwest , Oregon State University, and the EPA Environmental Research Laboratory) at Corvallis, Oregon.

DAPTF Directors and Chairs

BOARD OF DIRECTORS

Iliam R. Branch
t Elizabeth Museum
). Box 13147
mewood, 6013
UTH AFRICA (Code 27)
one: (041) 561051 Fax: (041) 562175

ain Dubois
oratoire des Reptiles et Amphibians
sée National d'Histoire Naturelle
. Rue Cuvier
005 Paris
RANCE (Code 33)
one: (1) 40 79 34 87 Fax: (1) 40 79 34 84

Timothy R. Halliday
ology Department
e Open University
alton Hall
lton Keynes, MK7 6AA
NITED KINGDOM (Code 44)
one: 0908-653831 Fax: 0908-654167

ergius L. Kuzmin
stitute of Evolutionary Morphology
and Ecology of Animals
cademy of Sciences
eninsky Prospekt, 33.
loscow 117071
RUSSIA

Robert Johnson
Metropolitan Toronto Zoo
P.O. Box 280
West Hill, Ontario, M1E 4R5
CANADA
Phone: (416) 392-5968 Fax: (416) 392-4979

James B. Murphy
Dallas Zoo
621 E. Clarendon Dr.
Dallas, TX 75203, USA
Phone: (214) 670-7573 Fax: (214) 670-7450

*Jaime F. Péfauer
Departamento de Biologia
Facultad de Ciencias
Universidad de los Andes
Merida, VENEZUELA 5101 (Code 58)
Fax: 74 40 1286

George B. Rabb, IUCN/SSC
Chicago Zoological Society
Brookfield, IL 60513, USA
Phone: (708) 485-0263 Fax: (708) 485-3532

*Michael J. Tyler
Department of Zoology
University of Adelaide
GPO Box 498
Adelaide, South Australia 5001
AUSTRALIA (Code 61)
Fax: 8 223 5817

David B. Wake (Chair)
Museum of Vertebrate Zoology
University of California
Berkeley, CA 94720, USA
Phone: (510) 642-3567 Fax: (510) 643-8238

William Winner (Ex officio)
Center for Analysis of Environmental Change
Oregon State University
Corvallis, OR 97333, USA
Phone: (503) 737-1744 Fax: (503) 737-3399

John W. Wright
Museum of Natural History
Los Angeles, CA 90007, USA
Phone: (213) 744-3371

* Also serves as Organizational Chairperson for Regional Working Group.

WORKING GROUP CHAIRS

K.D. Arudpragasam, *Co-Chair, Sri Lanka*
University of Colombo
SRI LANKA

W.R. Breckenridge, *Co-Chair, Sri Lanka*
Department of Zoology
University of Peradeniya
Peradeniya
SRI LANKA

David M. Green, *National Coordinator, Canada*
Redpath Museum
McGill University
Montreal, PQ H3A 2K6
CANADA
Phone: (514) 398-4088

*Kuang-Yang Lue, *Taiwan*
Department of Biology
88 Roosevelt Rd., Sec. 5
11718, Taipei, Taiwan
REPUBLIC OF CHINA (Code 86)
Phone: (02) 932-5234 Fax: (02) 931-3904

Jay M. Savage, *Lower Central America-Costa Rica/Panama*
Department of Biology
University of Miami
Coral Gables, FL 33142, USA
Phone: (305) 284-2130 Fax: (305) 284-3039

Annie Zuiderwijk, *Western Europe*
Institute of Taxonomic Zoology
University of Amsterdam
P.O. Box 4766 - 1009 AT Amsterdam
THE NETHERLANDS (Code 31)
Phone: (20) 5256624 Fax: (20) 5257238

*(Acceptance pending)

U.S. Regions

Ronn Altig, *Mississippi Delta (AR,MS,LA)*
P.O. Drawer GY
Department of Biological Science
Mississippi State University
Mississippi State, MS 39762
Phone: (601) 325-7561 Fax: (601) 325-7939

Andrew R. Blaustein, *Co-Chair, Pacific Northwest* (WA,OR,AL)
Department of Zoology
Oregon State University
Corvallis, OR 97331-2914
Phone: (503) 737-5356

Ronald A. Brandon, *Central (IA,MO,IL,IN,OH)*
Department of Zoology
Southern Illinois University
Carbondale, IL 62901
Phone: (618) 536-2314

Richard C. Bruce, *Appalachian (KY,TN,NC,VA,WV)*
Highlands Biological Station
P.O. Box 580
Highlands, NC 28741
Phone: (704) 526-2602

R. Bruce Bury, *Co-Chair, Rocky Mountains (ID,MT,WY,CO,UT)*
National Ecology Research Center
4512 McMurray Ave.
Fort Collins, CO 80525-3400
Phone: (303) 226-9270

Janalee P. Caldwell, *Southern Plains (OK,TX)*
Oklahoma Museum of Natural History
1335 Asp Ave.
Norman, OK 73019-0606
Phone: (405) 325-4712 Fax: (405) 325-7771

P. Stephen Corn, *Co-Chair, Rocky Mountains (ID,MT,WY,CO,UT)*
National Ecology Research Center
4512 McMurray Ave.
Fort Collins, CO 80525-3400

C. Kenneth Dodd, Jr., *Southeast (AL,GA,FL,SC)*
National Ecology Research Center
412 NE 16th Avenue (Room 250)
Gainesville, FL 32601
Phone: (904) 372-2571 Fax: (904) 374-8080

Gary M. Fellers, *Cal/Neva (CA,NV)*
Point Reyes National Seashore
Point Reyes, CA 94956
Phone: (415) 663-8522

A. Ross Kiester, *Co-Chair, Pacific Northwest (WA,OR,AL)*
USDA Forest Service
3200 SW Jefferson Way
Corvallis, OR 97331
Phone: (503) 750-7269

James E. Platz, *Northern Plains (ND,SD,NE,KS)*
Department of Biology
Creighton University
2500 California St.
Omaha, NE 68178
Fax: (910) 622-0287

Norman J. Scott, Jr., *Southwest (AZ,NM)*
Department of Biology
University of New Mexico
Albuquerque, NM 87131
Phone: (505) 766-3903

Richard L. Wyman, *Northeast (ME,NH,VT,NY,PA,MD,DE,MA,RI,CT,NJ,DC)*
Edmund Niles Huyck Preserve, Inc.
P.O. Box 188
Rensselaerville, NY 12147
Phone: (518) 797-3440

Issue Specific

W. Ronald Heyer, *Monitoring Protocols*
Division of Amphibians and Reptiles
US National Museum of Natural History
(NHB Mail Stop 162)
Washington, DC 20560, USA
Phone: (202) 357-2195

Roy McDiarmid (Will succeed WRH)
National Fish and Wildlife Laboratory
U.S. National Museum of Natural History
(NHB Mail Stop 162)
Washington, D.C. 20560, USA
Phone: (202) 357-2780

James L. Vial, *Documentary Resources*
(Address given below)

FROGLOG

Newsletter of the IUCN/SSC Task Force on Declining Amphibians

Dr. James L. Vial
Task Force Coordinator, *Editor*
Center for Analysis of Environmental Change
EPA Environmental Research Laboratory
200 South West 35th Street
Corvallis, Oregon 97333, USA
(503) 754-4798
Telefax (503) 754-4799

Funding for FROGLOG is provided in part by a special donation from Frog's Leap Vineyards, St. Helena, California 95474, USA

It appears complex and intimidating, but actually can be completed in a short amount of [illegible]

to be circled, and the remaining variables are numerical and easy to determine. The data sheet is divided into four sections, divided by double lines. Each section describes a cohesive set of variables. In addition the back of the sheet includes a grid for a rough sketch of the site and space for additional comments. The map is optional, but the future value of the data is enhanced if it is supplied.

SECTION 1 – LOCALITY *These data are essential. Many amphibian surveys have been hampered by the inability to relocate exact locations in the historical record. Some of this information can be completed in the office after the survey.*

DATE: Use the format DD-MMM-YY (e.g., 05-APR-92).

BEGIN TIME: List the time survey of habitat for amphibians began in 24 hour format.

END TIME: List the time the survey ended in 24 hour format. (The total time (END TIME - BEGIN TIME) should reflect only the amount of time spent searching for amphibians. Total time plus number of observers may be used to assess relative abundance.)

OBSERVERS: List names or initials of all persons involved in searching.

LOCALITY: Describe the *specific* geographic location of the site. Use air distance in two directions (e.g., 5km N and 7.5 km W) of a map landmark that likely will not change (distance from a large town or city is not all that helpful).

STATE: Use the 2-letter abbreviation.

COUNTY:

MAP NAME: List the name of the U.S.G.S. quadrangle or other map used to locate the site.

OWNER: List the public land manager (e.g., Roosevelt Nat. Forest or Rocky Mtn NP), or name of the owner if the site is on private land (listing the owner's name will make it clear that you did not trespass to survey the site).

ELEVATION: Circle the scale used; meters are preferred.

T: township **R:** range **S:** section

SECTION DESCRIPTION: Describe the location of the site within the section (e.g., SE ¼ or NE ¼ of SE ¼)

UTM ZONE, NORTHING, EASTING: Universal Transverse Mercator coordinates are preferred over longitude and latitude. The UTM zone is listed on newer topographic maps. If you are using a map without the UTM grid, substitute latitude for Northing and longitude for Easting.

SECTION 2 – SPECIES DATA *List all amphibian species observed. If garter snakes are seen, list them here also.*

SPECIES: Use the scientific name. Convenient shorthand is to use a 4-letter code made up of the first 2 letters of the genus and species (e.g., *Rana sylvatica* would be RASY).

ADULTS/JUVENILES: Indicate presence with a check, but numbers seen are more valuable data

CALLING?: Circle Y if frogs are vocalizing in a breeding chorus, of if a breeding aggregation of species that don't call (e.g., *Bufo boreas*) is observed.

TADPOLES/LARVAE: Same as for adults/juveniles

EGG MASSES: Same as above. Numbers of egg masses are especially valuable data. If possible, describe the developmental stage of eggs in the space for additional notes on the back of the form.

METHOD: Circle how observations were made: **VISUAL/AURAL ID** – species identified without picking it up, either by sight or by recognition of the breeding call; **HAND COLLECTED** – animal was picked up and identified in the field (higher confidence than visual id); **DIP NET/SEINE** – the usual method of collection for larvae; **TRAPPED** – minnow-type traps are also used for larvae; **VOUCHER COLLECTED?** – circle yes or no (voucher specimens are recommended for every site, *especially if identification is uncertain and for larvae*). Indicate voucher status in addition to method used.

FISH PRESENT?: If yes, list species if you can. Circle the question marks if you are not certain, but suspect that fish are present.

ENTIRE SITE SEARCHED?: If no, list either the meters of shoreline or the area (m^2) of habitat (e.g., amount of wet meadow) searched.

SECTION 3 – PHYSICAL AND CHEMICAL DATA *Water chemistry data are difficult to collect accurately without thorough planning and quality equipment; these data are optional. Weather data are important for determining the quality of the observations (e.g., was an absence of amphibians due to observations made during a blizzard?)*

WEATHER, WIND: Indicate atmospheric conditions.

AIR TEMPERATURE: Take at chest height in shade. The Celsius scale is preferred.

WATER TEMPERATURE: Take 1 meter from margin and at 2 cm depth, or where egg masses are observed.

COLOR: This is a qualitative assessment of whether the water clear or tea-colored from organic (humic) acids.

TURBIDITY: This is a qualitative assessment of whether the water clear or clouded from suspended particulate matter.

SECTION 4 – HABITAT DESCRIPTION *These data are important for developing hypotheses to explain changes in abundance of amphibians. This section needs to be filled out only once for each site (a reasonable amphibian survey should include at least 2 – 3 visits to each site in one season).*

ORIGIN: Decide whether the lake is a natural geologic formation or man-made. Bodies of water enlarged by a dam are problematic. List them as man-made, but add an explanation in the space for additional notes on the back of the form.

DRAINAGE: Circle whether the site has permanent drainage, no drainage, or occasional drainage. Determining the potential for occasional drainage requires judgement. Look for clues in the topography and vegetation.

DESCRIPTION: Decide how best to describe the site. If there is evidence of past or present beaver activity, circle one of these choices in addition to your choice.

LENGTH, WIDTH: Record the maximum length and width of lakes and ponds. For streams, record the length and average width of the reach searched.

MAXIMUM DEPTH: Most times, you will not have access to a boat, so estimate depth (deep lakes are usually not important to amphibians).

STREAM ORDER: This is an index of stream size, and you will need a topographic map to determine it. First-order streams have no tributaries, second-order streams are formed by the confluence of two 1st-order streams, third-order streams are formed by the confluence of two 2nd-order streams, and so on.

PRIMARY SUBSTRATE: Circle the type that covers the majority of the bottom of the site.

EMERGENT VEGETATION: Circle the percentage of the margin of the site with emergent vegetation present, and list the dominant species. If you are botanically-disadvantaged, list the categories of the dominant species (e.g., cattail, sedges, etc.).

NORTH SHORELINE CHARACTERS: Describe the north shore of a lake or pond in terms of shallow water and emergent vegetation. This is important in evaluating quality of breeding habitat in some mountain locations.

FOREST CHARACTERS: List the closest distance between the water and the surrounding forest, and list the most common tree species. Leave these fields blank if there is no forest. Describe other surrounding habitat types in the notes section on the back of the form.

AMPHIBIAN WORKSHOP

Taylor Ranch
18 July 1992

Charles R. Peterson
Department of Biological Sciences, Idaho State University
Idaho Museum of Natural History
Campus Box 8007
Pocatello, Idaho 83209
(208) 236-3922

I. Introduction

II. Checklist of Possible Amphibians

III. Species Accounts (slides)
- A. Range map
- B. Adults
- C. Eggs
- D. Larvae
- E. Juveniles
- F. Habitat
- G. Activity patterns
- H. Calls

IV. Sampling Techniques
- A. Incidental observations (forms)
- B. Seize and capture
- C. Time constrained Searches
- D. Area constrained searches
- E. Trapping (pit and funnel traps with drift fences)
- F. Seining
- G. Calling surveys
- H. Road driving

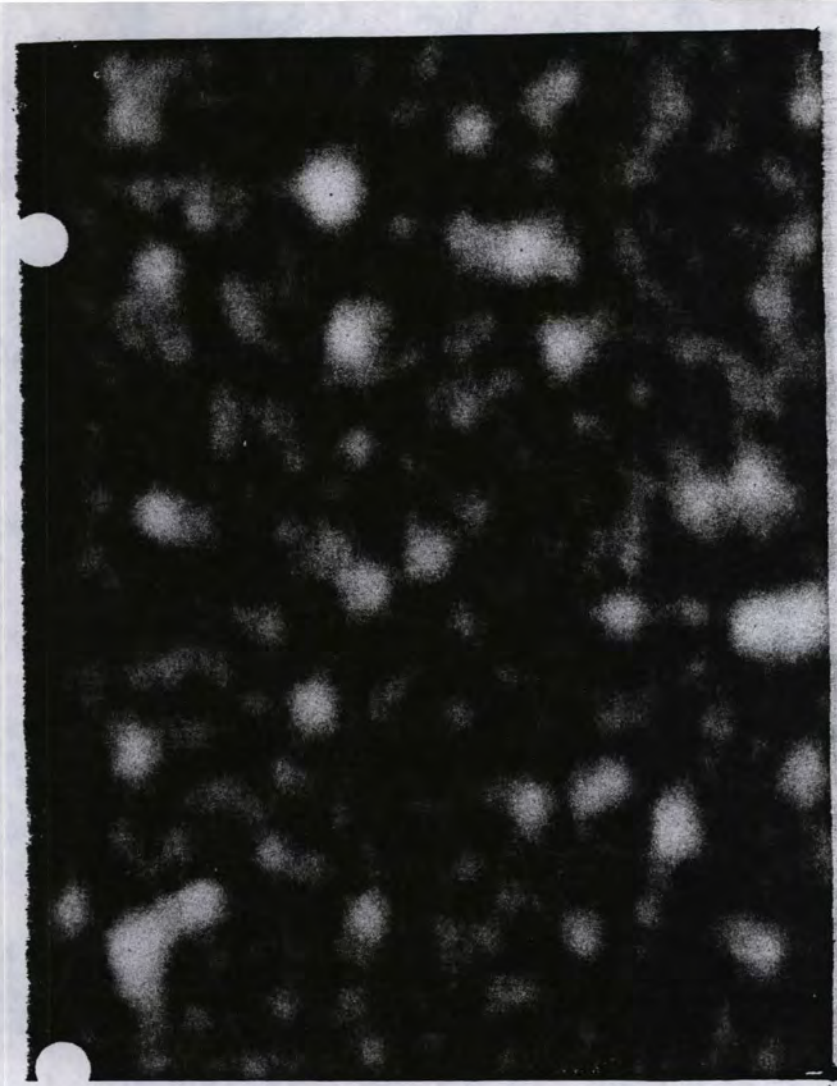
V. Surveys - USFWS Sample Site Data Form

VI. Monitoring Programs

VII. Preservation Techniques

VIII. Examination of Preserved and Living Specimens

IX. References



A GUIDE TO PRESERVATION TECHNIQUES FOR AMPHIBIANS AND REPTILES

George R. Pisani
Museum of Natural History
University of Kansas
Lawrence, Kansas 66044

Illustrations
by

Patricia May and Tom Swearingen
Museum of Natural History
University of Kansas

Table of Contents

INTRODUCTION

Over the years, several informative works describing the preservation of amphibians and reptiles have been published. Most of these have been intended for relatively limited distribution by the institutions or individuals publishing them. This fact, coupled with new laws pertaining to syringes and certain drugs used for killing specimens, warrants an additional treatment of the subject. This article is an attempt to combine a complete survey of current techniques with a page size that the individual collector can conveniently carry in the field.

In an age when so many wild species and areas of suitable habitat are at the threshold of extermination, it seems advisable at the outset to include a plea for conservation in this booklet. Current museum collections contain excellent samples of various North American species of reptiles and amphibians from certain areas within their ranges. In these instances, it is a needless waste to collect and preserve additional material when this will not add appreciably to our knowledge of these creatures. I am not referring to such collecting as may be associated with the compiling of a synoptic teaching collection by a school or to collection of specimens needed for a particular aspect of research, but rather to the capture and preservation of animals simply to amass a collection which may never be used for scientific or educational purposes. There are numerous geographic areas, including several in North America, in which the amphibians and reptiles are poorly known. Collections from these areas can add measurably to our herpetological knowledge. Persons wishing to learn of the desirability of specimens from particular areas should consult with herpetologists at nearby universities, museums, zoos, etc.

I would like to express my thanks to Woodrow W. Barber, Hobart M. Smith, William E. Duellman, Joseph T. Collins, Clarence J. McCoy, and George Iannarone for furnishing helpful material and/or advice and to Phyllis Shaffer, Judy Hamilton, Leanne Johnson, and Ginger Stiggins for typing assistance. Thanks are also expressed to Jaime Villa for preparing much of the "International Shipment" section.

FIELD NOTES

Specimens not accompanied by data identifying the collection locality are virtually useless to scientific investigators. The more data available for a specimen, the greater its value in research. Hence, keeping accurate, complete field notes is necessary. Many times, data felt to be trivial at the time of collection may prove to be quite useful when many observations are pooled. Field notes should be written in waterproof ink ("Pelikan" brand is preferred by many) using only one side of each page. Several brands of waterproof ink will "run" if alcohol is accidentally spilled on the page, hence care should be used in selecting ink. A worthwhile technique is to carry a small notebook for on-the-spot data taking. Then transfer these data into the permanent field notes as soon afterward as possible.

The following is a representative outline of data included in field notes (numbers refer to those in figure 1a):

1. Locality- Do not record locality with reference to business establishments. Use towns or mapped roadways; determine distance from an automobile odometer (if available), or estimate distance carefully from maps. It is not unusual for roads to be rerouted, renumbered or both. It is therefore advisable to refer to roads indicated in a good atlas to which future reference can be made. In the U. S., the American Highway Atlas (Gousha Co., Chicago) is suitable. If collecting in areas remote from roadways, locate the collecting site as accurately as possible from U. S. Geological Survey topographic maps. Collectors in foreign countries should try to obtain accurate, detailed maps of the areas in which they are working. Elevation of the locality should also be recorded whenever possible.

2. Date- Always write out the name of the month, or indicate month by a Roman numeral; 6-10-71 could refer either to June 10th or October 6th.

3. Name(s) of all collector(s) present.

4. Time of collecting.

fig.1a

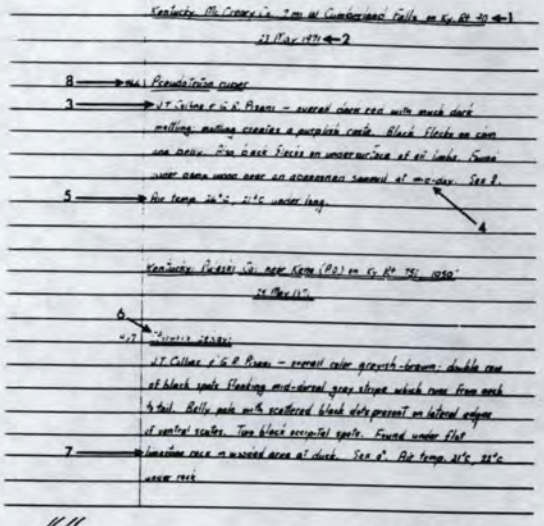
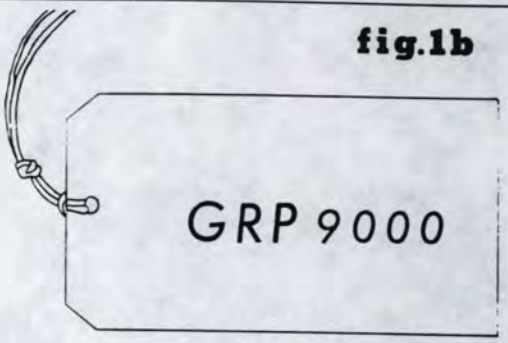


fig.1b



5. Air temperature and other appropriate weather notes- It is often useful to note existing cloud cover and moisture conditions, as well as general weather conditions preceding the collection.

6. Species- List all species collected plus the number collected of each, followed by species which may be observed but not collected. Accurate color notes are a worthwhile inclusion, especially when collecting in regions having a poorly known herpetofauna. It is also worthwhile to take accurate color notes when an atypically colored individual of a well-known species is encountered.

7. Microhabitat of species collected and any significant behavior (courtship, defensive display, etc.) observed.

8. Field number- It is useful to carry a series of numbered field tags on collecting trips. These should be printed on heavy paper in permanent ink. Satisfactory tags can also be made with one of the commercially available label makers that imprint plastic tape. Thread can be sewn through the numbered tags, but the backing on the tape should not be removed. Avoid using colored thread, or thread made from synthetics such as nylon, which may be destroyed by preservatives. White, cotton carpet thread is suitable for tagging. After threading the tag, tie a small knot in the string as shown in figure 1b. Each specimen should be assigned its own number, which greatly simplifies the task of keeping specimens and localities associated. Testes, stomach contents, photos, tape recordings, etc. are assigned the same number to increase efficiency in future analyses. Field tags should be securely tied (with a square knot) to specimens as illustrated in Plates 1 and 2. Lizards possessing femoral pores should be tagged by knotting the string below the knee; this avoids covering the pores with string. Tags in the field series should be numbered independently from the catalog series discussed in a later section.

Of the above data, numbers 1 (locality), 2 (date), 3 (name(s) of collector(s), and 8 (field number) represent the minimum data which should be recorded. If specimens are donated to an institution, the field notes should be donated with them. Do not include field notes in the same container used to hold specimens.

KILLING OF SPECIMENS

It is essential that live herpetological specimens be killed in such a manner as to leave the muscles in a relaxed state. Following this, they can be fixed, or hardened, in standardized positions which enables researchers to examine them conveniently and most accurately (refer to Plates 1 and 2). Many books recommend that reptiles be killed by hypodermic injection of aqueous sodium pentobarbital (Nembutal) into the heart. This technique is indeed excellent, but the reader should be aware that Nembutal is not a generally available drug, its possession being closely regulated by the Federal Bureau of Narcotics and Dangerous Drugs. It is possible for qualified persons to obtain a permit to purchase Nembutal, but the application procedure is best begun several months in advance of anticipated need. For additional details concerning the permit, the reader is urged to consult representatives of the above-mentioned Bureau at the Federal centers in most large cities. State and local regulations should also be checked. Commercial Nembutal is sold at a concentration of 50 mg/cc. The form of Nembutal sold as a syrupy elixer should be avoided. Commercjal Nembutal may be used directly for larger specimens (over 5 pounds body weight), and diluted 1:5 with water for smaller reptiles; for very small specimens such as Typhlops or small Scincella it is possible to dilute to 1:10 and retain effectiveness. Nembutal diluted 1:10 can also be used on larger specimens, but death will be delayed. One cc (used commercial strength) injected into the heart is generally sufficient to quickly kill an animal of the bulk (volume) of a 3 foot timber rattlesnake (Crotalus horridus). Position of the heart in snakes can often be judged by closely watching the ventral plates on the anterior 1/3 of the body to detect heartbeat. Injection anywhere into the anterior 1/3 of the body cavity is also effective, but death is not as rapid as from heart injection. Other reptiles can be killed by injection into the heart region. Do not attempt to inject specimens which are so small or thin as to be heavily damaged by the needles at hand.

A number of other effective killing means are available. Turtles may be chloroformed if care is taken not to allow them to stiffen. Confining the turtle with a chloroform moistened rag or cotton wad in a closed container for 15-30 minutes (Cook, 1965) should suffice. The use of chloroform on other reptiles is definitely not recommended, as severe contortion usually results. Trichloroethylene or ether may be substituted for chloroform with good results, and can be used on most reptiles. Most specimens can be killed by confinement with either trichloroethylene or ether for 5 minutes beyond the time the animal loses the ability to right itself when turned over. These liquids are available to the public from either biological supply houses or certain drugstores. Their use may be superior to Nembutal when working with small, fragile animals like some tropical geckos. Caution should be observed with ether, as it is highly flammable and can, under certain storage conditions, explode. Read labels carefully.

All amphibians and a number of smaller reptiles (e.g. small, tropical geckos) are easily killed by immersing them in a solution of Chloretone (hydrous chlorobutanol). A stock supply is commonly prepared as a saturated solution of Chloretone in 95% ethanol. This stock solution may be conveniently carried in a small vial; 2 cc of it added to a pint of water is effective. The solution should be kept tightly covered when not in use, and can be used over and over; its strength will diminish with use.

Various other means are suitable for killing reptiles and amphibians. Securing the animal(s) in a cloth sack and immersing the sack in warm (110°-120°F; 43-47°C) water is effective, but specimens should be removed shortly after death. Specimens may also be immersed in alcohol (15-25% for amphibians; 50-60% for reptiles). Though the method is not recommended, bags containing reptiles may also be left exposed to direct sunlight until death from overheating occurs. Great care must be used however, as dehydration and accompanying contortion can happen quickly; amphibians should never be killed in this way. Both procaine hydrochloride (Livezey, 1958) and succinylcholine chloride (Anectine) (Lambert, 1967) have been used effectively as killing agents; however, their availability is usually restricted like that of Nembutal.

Recent drug laws have greatly increased the difficulty of obtaining syringes for preserving purposes. State laws may also vary in the regulation of the above-mentioned chemicals. It is often possible to obtain necessary supplies through institutions, particularly in return for depositing desired specimens.

FIXING

The purpose of fixation is to preserve the actual morphological state and color of the specimen, and to prepare the tissues for microscopic examination. Hence, the fixative should kill tissue quickly; penetrate it uniformly and rapidly; prevent postmortem decomposition; not distort the tissue; and should prepare the tissue for staining. No single fixative will do all of these things, so various compromises must be made.

The most widely accepted and suitable general fixatives for field use are:

1) Formalin ("Formol" or "Formalina" in Spanish; "das Formaldehyd" in German)- Sold commercially as a solution of approximately 40 percent formaldehyde gas in water, formalin is the most widely used field fixative. For purposes of dilution, commercial formalin is usually considered as 100%, and can be used in 10% strength (1 part formalin: 9 parts water) for fixation. Formalin may be buffered (which helps to reduce discoloration of specimens) by mixing 1 tablespoon of baking soda or borax with each pint of 10% formalin. Generally sold as a liquid (often in drugstores), it is also available as a solid polymer (paraformaldehyde), which is convenient for saving weight and space in transport. Huheey (1963) recommends sealing 16 grams of paraformaldehyde and 4 grams of anhydrous sodium carbonate in packets for field transport; 1 packet added to 400 ml (about 1/2 quart) of water makes a 10% solution of buffered formalin. Premixed, buffered paraformaldehyde powder is available from Carolina Biological Supply House. Paraformaldehyde alone can be obtained from Eastman Organic Chemicals, Rochester, New York. Formalin, while an excellent general fixative, is highly irritating to the user's skin and (as a vapor) to mucous membranes. It is not uncommon for users to develop strong allergies

to formalin. Also, formalin has a tendency to cause swelling of several types of tissue, rendering them unsuitable for some histological purposes.

2) FAA (formalin-alcohol-acetic acid)- Prepared by mixing 10 parts commercial formalin, 50 parts of 95% alcohol (ethyl or isopropyl), 40 parts water and 2 parts glacial acetic acid. FAA penetrates tissue far better than formalin alone, and has less tendency to cause cell distortion. The rapid tissue penetration can also be an aid to preserving valuable specimens found dead and, perhaps, partially decomposed. The primary disadvantages of FAA are: the need to mix several components; and, the necessary alcohol and acetic acid may not be available in certain localities. FAA is not available in powder form, but can be premixed without the water to reduce volume in transport; water may be added later. If FAA is to be used extensively in hot regions, it is recommended that the acetic acid be added just prior to actual use, as it quickly evaporates from the solution; containers may be cooled by wrapping them in wet rags and shading them to retard evaporation of acetic acid.

3) Alcohol- If neither formalin nor FAA are available, alcohol may be used as a fixative. Cook (1965) recommends ethanol (95% for reptiles; 70% for amphibians) or isopropanol to fix in the absence of other solutions, but the latter is not desirable.

4) Special- A large number of other fixatives exist, each being useful for different types of tissues, and studies. Bouin's solution (75 parts saturated aqueous picric acid, 25 parts commercial formalin, 5 parts glacial acetic acid) is especially useful for field preservation of testes to be used in spermatogenesis studies. Testes may be placed in vials of Bouin's and safely kept there for long periods of time without distortion of cells; the remainder of the specimen may be fixed with FAA or formalin. For a complete discussion of special fixatives, the reader is referred to Guyer (1961) and similar texts.

5) Miscellaneous- If a valuable specimen must be saved and no other solutions are available, a number of emergency measures are possible. The specimen may be frozen or packed in strong brine until preservative can be obtained. Liquor is generally not a suitable source of alcohol, as 110 proof liquor is only 55% ethanol. However, strong tequila (about 160 proof) may be useful; rubbing alcohol can also be used. These, however, are only desperation measures and it is usually more beneficial to get the specimen into a proper fixative (hospitals, local schools, etc. are suggested as possible sources).

It is always preferable to introduce fixative into the body cavity, as specimens (particularly reptiles and larger amphibians) can decompose internally if simply placed in fixative. Enough fixative should be injected to fill, but not distend, the animal. Care should also be taken not to damage the femoral pores of many lizards by puncturing them with the needle. The neck of turtles should be completely extended and the mouth held open with wood, cork, or tightly wadded paper prior to fixation. Excellent neck extension can be obtained by hooking the dead turtle's upper jaw over a nail or broken branch and letting the animal's hanging weight pull the neck out straight prior to injecting it. The upper jaw can also be hooked over a paper clip placed over the edge of the fixing tray, and the neck then drawn out. One hemipenis of male lizards and snakes should be partially everted with thumb pressure on the base of the tail, followed by injection to completely evert it as indicated on the front cover. The hemipenis should not be permitted to remain incompletely everted; thread may be tied around the base of the fully everted hemipenis to help retain fluid within it. It is also an acceptable practice to evert the hemipenis by injection of fixative alone. Typical sites for injection of preservative are starred in Plate 2. Tails of lizards and snakes should be slit lengthwise, being very careful not to break the tail off; sharp instruments are a "must." Large amounts of fixative can be conveniently handled in the injection apparatus designed by Jackson (1971), although the author has never felt at a disadvantage using larger syringes. If no injection apparatus is available, the specimen should be deeply slit in several places ventrally and placed belly-up in fixative. Spread the sides of the slits to admit fixative more easily. Avoid cutting the anal plates of snakes and lizards and femoral pores of lizards.

Once the animal is injected or slit, it is most conveniently fixed by placing it (after proper positioning) between pieces of white paper toweling moistened liberally with fixative. This can be done in shallow, covered plastic or rustproof metal pans. Surgical instrument pans with sliding metal covers are handy for this. Avoid colored towels, as the colors dissolve in the fixative and stain the specimen.

Preferred positions for fixing and sites for field tag attachment are illustrated in Plates 1 and 2. Amphisbaenids and caecilians should be fixed in the same position as snakes; it is useful to fix these with the mouth open, as this greatly facilitates examination of oral characters later on. Lizards with long tails should be fixed with the tails bent as shown. Frogs and toads may be positioned with the sole of the foot down (Duellman, 1962). However, because this position obscures many hind limb and anal characters, others feel that anurans are best fixed with the hind limbs in the position shown in Plate 1c. Toes and fingers should always be straight and spread apart. Small amphibians need not be injected or slit prior to positioning, as the fixative will penetrate to the body cavity quite easily. Small amphibians and lizards may have the field tag tied around the body just anterior to the pelvic region.

Amphibian eggs and larvae are best fixed and stored by dropping them directly into jars of 10% formalin; preserve entire egg clutches whenever possible. Many amphibians attach their eggs to leaves, twigs, etc. Whenever it is practical, these items should be preserved with the eggs in situ, as the latter are often severely damaged by attempts to disengage them. Change the formalin on eggs and larvae after about 12 hours. Reptile eggs should be measured (length and width, in millimeters), then injected.

All specimens should be allowed to remain in fixative for 24 hours.

Very Large Specimens (too large to be conveniently stored entire in liquid)

1. Snakes- Obtain the snout to vent and tail lengths (in mm). Then skin by making a long ventral incision to the side of the mid-line; leave the head and tail attached to the skin, severing these from the carcass (avoid cutting the anal plate), and then inject head and tail (evert hemipenis if male) with fixative. With boids, sever the hind part just

Plate 1. Amphibians

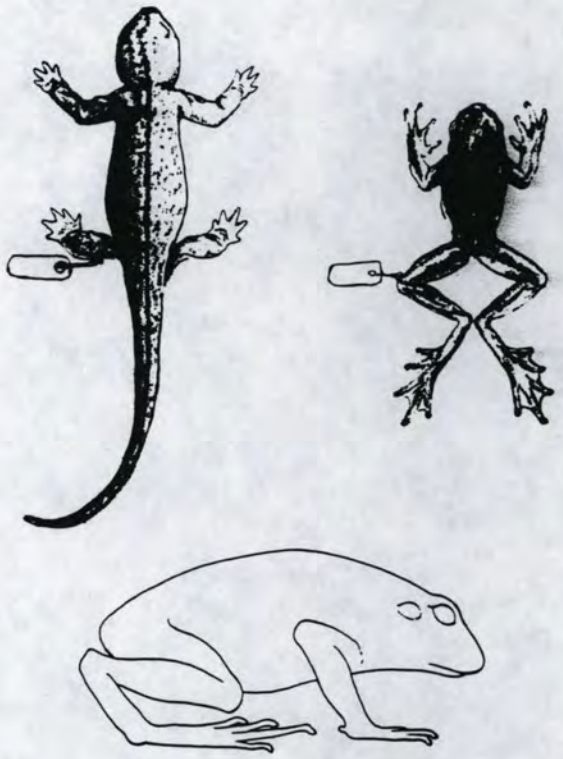
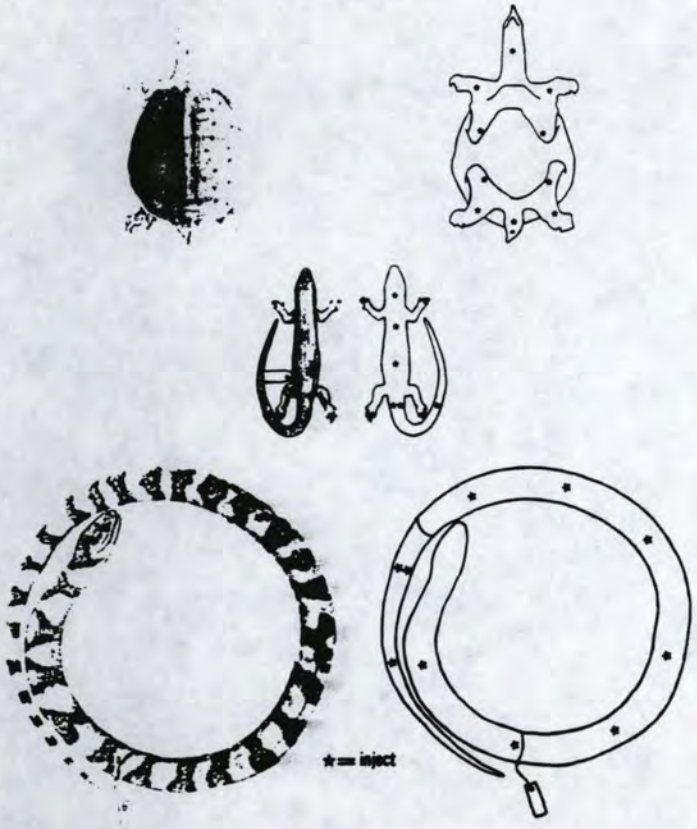


Plate 2. Reptiles



ahead of the bony, vestigial pelvic elements. The skin may now be preserved by covering the flesh side with cloth or absorbent paper, rolling loosely and immersing in fixative, or by rubbing with borax or arsenical soap, rolling and drying. In this latter instance, it is best to preserve the head and tail separately in liquid. If the specimen is a male, a testis should also be preserved. Reproductive condition of females should be noted (i.e.- number of ova present, size of the largest ovum, etc.). Embryos, especially those of poorly known species should be preserved in liquid; it is preferrable to do this by preserving the entire oviduct rather than by removing embryos.

2. Turtles- Avoid cutting the shell. It is preferable to cut the head, neck and forelegs out as one unit, the hind legs and tail as a second unit and preserve these in liquid. The stomach and reproductive organs should also be preserved in liquid. Carefully clean out and dry the shell.

3. Crocodilians- Measure and skin the specimen as for snakes, except that the tails should be skinned as well. Feet may be left attached (inject with fixative), instead of skinned out. Rub the skin with borax or arsenical soap and dry.

SHIPPING

Once they have been properly fixed, most herpetological specimens may conveniently be transported by wrapping them loosely in cheesecloth or white paper towel which has been liberally moistened with alcohol (70% ethanol or 40% isopropanol), or the fixative, then sealing them in two plastic bags (one within the other, individually closed by twisting the end and knotting or securing with a rubber band). Several small specimens may be wrapped in a single length of cheesecloth by laying the cloth out flat, spacing the specimens down the length of it, folding the sides over the animals and rolling the cloth loosely, like a rug.

Thus packed, the specimens occupy minimum space and weight, important factors when they must be transported any distance or mailed. Specimens will remain in

ndition for several weeks, so long as the bags are well sealed to retard
e and evaporation. Cotton in sheet form may be substituted for cheesecloth,
bulkier and may adhere to the scales of some rough-scaled species of lizards,
nen it is moist.

s being shipped (parcel post is a convenient means) should be carefully
ed and clearly marked "PRESERVED SCIENTIFIC SPECIMENS." Packages often are
to much "wear and tear," so, effort in preparation pays off! Paint cans
ying sizes make leakproof, sturdy mailing containers. Plastic bags containing
ens may be simply placed in the can and extra space filled with wadded rags
ar. Bags with heavy specimens should never be placed on top of lighter ones.
address labels should be typed or written with permanent ink. Place one
se in the can with the specimens, tape a second to the side of the can.
the third label to the paper used to wrap the parcel.

aint cans are not too costly, and a source of supply can generally be found
sulting local paint stores. Watching auction notices sometimes turns up a
store that is going out of business and may have cans. Remove handles from
efore use. In lieu of cans, specimens may be packed in any durable container.
postal regulations for size and weight restrictions before packing extremely
or unwieldy parcels. Specimens such as large turtle shells or the skins of
crocodilians may have to be sent via freight, and again, secure packing is
. Shipment of all crocodilian specimens is subject to stringent regulation,
y of these species are endangered animals. Collectors planning to take
should carefully check customs regulations for import restrictions as well
ecking capture laws in countries where the animals occur. Proper arrangements
ften be made through the institution where one proposes to deposit the specimens.

Generally, specimens should never be sent in glass containers. Obvious
tions to this are amphibian eggs (and sometimes, larvae) and very fragile
ens. These should be placed in the smallest containers needed to hold the
ens plus fluid to maintain them; fluid should fill the containers, which
e heavily padded with cardboard or cotton. If rigid plastic tubing of
ient diameter is available, break resistant containers may be fashioned
t by cutting an appropriate length, stoppering one end, enclosing specimens
luid, then sealing the other end. The tube may be wrapped lengthwise with
wire to secure the stoppers. Plastic vials are available from some biological supply houses; larger drugstores may also furnish the names of suppliers of these. Be sure to only use vials which can be securely closed (screw-on or snap-on lid). Again, pay special attention to wrapping such containers.

INTERNATIONAL SHIPMENTS

Collectors should be aware of proper methods for shipping specimens internationally. Donation of all or part of a collection to institutions outside one's own country serves to:

1. make synoptic herpetofaunal collections of different areas available to as many researchers as possible,

2. prevent the loss (through war, neglect, earthquakes and other damage) of valuable collections deposited entirely in a single institution, and

3. place the herpetologist in contact with colleagues in foreign institutions; this frequently leads to a most beneficial exchange of ideas and data, thus advancing herpetology as a field of study.

The private hoarding of specimens by any person is a waste of valuable biological data, and can lead to overcollecting (i.e.--researchers may gather specimens from areas already represented, though inaccessible, in private collections). It is with the above points in mind, and the hope that more collectors will decide to enter into donation, exchange or loan relationships with foreign institutions, that the following guidelines are presented.

The methods of packing described in the preceding section are adequate for international shipment. Generally, mail is the most convenient means of sending packages which are not too heavy or bulky. Parcels sent via surface ("ordinary") mail should have extra preservative added to the specimen bags, as they may take as long as 4 months to reach their destination. Persons

ing specimens internationally should check local mail regulations on
el size, weight and any special packing provisions. The shipper may also
equired to affix various postal and customs "declaration tags" to parcels.
e tags vary with parcel destination and are generally provided by the
al service.

Very large or heavy packages will have to be sent via freight (air,
n, ship). The sender will be required to complete a "waybill" (avail-
e from the carrier) listing, among other things, the nature and value of
contents. To avoid excess charges, package and waybill should be marked
Commercial Value." Postal services in all countries have the legal right
inspect all packages. Intensive efforts to curtail the traffic of narco-
s and other restricted drugs has led to the extensive exercising of this
nt, and the fact that several persons have attempted to smuggle drugs with-
specimen containers has not aided the situation. Inspectors often open
stic bags of specimens, and may be unaware of the need to reseal them.
s causes loss of fluid and dehydration and probable loss of the specimens.
is therefore advisable to include two copies of the following statement with
parcel (one pasted on the outside and one sealed within):

PECTION OFFICER: This package contains dead, preserved amphibians and/or
tiles packed in plastic bags. As the specimens have great scientific
ue and will be ruined if not kept moist in their preservative, it is
erative that the bags be tightly resealed after inspection to avoid
poration or leakage of preservative. Thank you.

SPECTOR POSTAL: Este paquete contiene ejemplares de anfibios y/o reptiles
ertos, preservados y empacados en bolsas plasticas. Puesto que los
emplares son de valor cientifico y se arruinan si no permanecen en su
qui servativo, se suplica que, despues de abrir las bolsas para
sp rlas, las cierre hermeticamente para evitar que el liquido se
apore o se derrame. Gracias.

AUTORIDADES ALSANDEGARIAS: Este volume contem anfibios e repteis mortos,
nservados em sacos plasticos. Como o conteudo tem valor cientifico e se
tragara se nao for mantido humido no preservativo, pedese que apos abrir
sacos para inspecao os mesmos sejam firmemente fechados para evitar
aporacao ou derramamento do liquido. Obrigado.

Biologists should also be aware that the international shipment of specimens (alive or preserved) is being ever more closely regulated for conservation reasons. Shipments of preserved animals sent to the USA must be accompanied by a list bearing the number and scientific name of all specimens included. The importer (in the USA) must obtain a special permit from the Bureau of Sport Fisheries and Wildlife (Dept. of the Interior) in order to receive foreign shipments of preserved or live specimens.

Live shipments are additionally regulated by the Dept. of Agriculture and the Public Health Service. In all cases, endangered species are covered by regulations separate from species not currently considered endangered. You are urged to carefully investigate all legal aspects of international shipment before preparing to send animals.

STORAGE AND LABELLING

This section is not intended to be a complete guide to curatorial technique. Rather, it is meant to serve as a set of capsule directions for those wishing to start a preserved herpetological collection. A detailed discussion of curatorial technique may be found in Slevin (1927).

Preserved collections are best maintained in alcohol. Suitable alcohol generally costs about the same (per gallon) as formaldehyde, and alcohol-stored specimens are far easier to work with. Formaldehyde also tends to corrode metal lids and containers. Most collectors will be deterred from using ethanol by the high tax imposed upon its sales. Isopropanol is far cheaper, and is entirely satisfactory for storage of specimens. Methanol should never be used. Concentrati of 50% is suitable for reptiles, while 40% is better for amphibians. Both ethanol and isopropanol are generally sold at 95% concentration; 526 ml of this plus 474 ml of water make one liter of 50% concentration (421 ml alcohol + 479 ml water for 40%). Specimens being transferred from formalin or FAA fixation to alcohol must first be soaked in water for 48 hours. Failure to soak the specimen often results in its being severely dehydrated by the alcohol. Properly fixed specimens will not be harmed by this method. If material is desired for use in histological work, selected pieces of tissue should remain in 30% alcohol

for 24 hours, then 24 hours of 50% alcohol before going to final storage (omit the water soak). Do not pack specimens tightly in the jar. Snakes fixed in the position illustrated earlier will readily coil in jars for storage.

Each specimen retained in the collection should be assigned a catalog (in addition to the aforementioned "field number"). Amphibian eggs and larvae and reptile eggs may be cataloged with a single tag designating one clutch or lot. This number should be entered in a permanent catalog (using waterproof ink), along with the species name, date of capture/preservation, sex, locality, ecological notes and name of collector. Tags may be tied in the same region as the field tag. Collector's field number should also be entered. As a cross-reference, it is useful to maintain a card file (by taxonomic family) in which a single card is used for each species. On this card may be entered numbers from the catalog that apply to these species.

It is convenient to place a label bearing species name, catalog numbers and locality data with each container. These should be written in permanent ink on heavy, durable paper. That produced by Byron-Weston Mills under the name "Linen Record Ledger", 100% cotton and linen fiber, 36 lb. and Dennison Paper Company's product "Resistall Index Bristol", 100% rag, 110 lb. wt. are both excellent and are available through printing shops. The label may either be placed within transparent containers, or attached to the outside of opaque ones with masking tape. If moderate cost can be withstood, external labels can be placed within tie-on, plastic label-holders. A typical museum label is shown in Figure 2.

Specimen jars should be stored in cool places to help retard evaporation of preservative, and should never be exposed to sunlight, as specimen colors are rapidly faded by such exposure. Placing a piece of Parafilm sheet (available from Carolina Biological Supply House, Burlington, North Carolina) over jar mouths before screwing on the cap will also reduce evaporation. Containers should be checked periodically and fluid level maintained. Well preserved and cared for collections make valuable teaching and research tools.

COLOR PRESERVATION

While preserving the morphological state of herpetological specimens has never presented any severe hurdles to collectors, preservation of color is quite another matter. All currently used, popular preserving fluids are alcoholic and/or acidic to some degree. Therefore, it usually is not too long before most pigments are dissolved by such fluids and extracted from the specimens. Amphibians seem particularly vulnerable in this regard, though the effect on reptiles is noticeable.

Previously, the only acceptable method of retaining amphibian skin color was that described in Cook (1965). Basically, this consists of skinning the specimen, confining all cuts to the ventral surfaces of body and limbs. The skin is then floated flesh-side up in a pan of water and remaining particles of tissue are removed. The skin next is floated flesh-side down and spread out in a second pan. A wet piece of cardboard may then be brought up beneath the skin, which is rubbed lightly to flatten it and remove trapped air. The cardboard-skin preparation may be dried on blotting paper until moist, then placed between layers of blotting paper and thoroughly dried with heavy weights (such as books) on top of it; it may also be placed in a plant press. Reptiles may be similarily prepared. In all cases, the carcass should be preserved in fluid and tagged with the same number as the skin. Skins thus prepared should be stored in the dark and not exposed to prolonged light.

The above technique, while useful, is tedious. Windsor (1971) has described a technique for using 50% saturated, aqueous ammonium sulfate solution as a preservative of frogs. As the compound is an aqueous, neutral salt, no pigment was dissolved and natural color was still evident in the specimens 6 months after preparation. Total fixing time should be at least 36 hours.

Specimens may be stored in buffered formalin (10%) or isopropanol (40%) to which liquid Ionol-40 R has been added (White and Peters, 1969). Storage should be in dark places which are not subjected to heat much above 70°F. The formalin/Ionol method has been successfully used with herpetological material by Mr. Woodrow Barber, Biology Department, University of Kentucky at Morehead, and by Dr. George Iannarone, Chicago Academy of Sciences (personal communication).

Powdered Ionol should not be used, as it is difficult to prepare a stable solution of it in preservative. Ionol is sold by the Shell Oil Company (Chemicals Division).

While the two chemical methods discussed above have not been widely used with herpetological material, their success on a limited scale coupled with the value of accurate color preservation suggests that they should be more thoroughly investigated.

LITERATURE CITED

Cook, Francis R. 1965. Collecting and preserving amphibians and reptiles, p. 128-151, In R. M. Anderson (ed)., Methods of collecting and preserving vertebrate animals. Nat. Mus. Canada, Bull. No. 69, Biol. Ser.: 1-199.

Duellman, William E. 1962. Directions for preserving amphibians and reptiles, p. 37-40, In E. Raymond Hall (ed)., Collecting and preparing study specimens of vertebrates. Misc. Publ. Univ. Kansas Mus. Nat. Hist., (30): 1-46.

Guyer, Michael F. 1961. Animal micrology, 5th impression. Univ. Chicago Press, Ill.; xviii + 327 pp.

Huheey, James E. 1963. Concerning the use of paraformaldehyde as a field preservative. Copeia, 1963(1):192-193.

Jackson, Morris K. 1971. A new "syringe" for injecting herptiles. Herpetol. Rev., 3(4):75.

Lambert, Lewis H., Jr. 1967. 'Anectine': a herpetological killing agent. Herpetologica, 23(2):135-136.

Livezey, Robert L. 1958. Procaine hydrochloride as a killing agent for reptiles and amphibians. Herpetologica, 13(4):280.

Slevin, Joseph R. 1927. The making of a scientific collection of reptiles and amphibians. Proc. Calif. Acad. Sci., 16(9):231-259.

White, David A. and Edward J. Peters. 1969. A method of preserving color in aquatic vertebrates and invertebrates. Turtox News, 47(9):296-297.

Windsor, Donald A. 1971. Ammonium sulfate as a preservative which does not remove color from frogs. Copeia, 1971(2):356-357.

INDEX

A GUIDE TO PRESERVATION TECHNIQUES FOR AMPHIBIANS AND REPTILES

George R. Pisani
Museum of Natural History
University of Kansas
Lawrence, Kansas 66044

Illustrations
by

Patricia May and Tom Swearingen
Museum of Natural History
University of Kansas

Table of Contents

INTRODUCTION

Over the years, several informative works describing the preservation of amphibians and reptiles have been published. Most of these have been intended for relatively limited distribution by the institutions or individuals publishing them. This fact, coupled with new laws pertaining to syringes and certain drugs used for killing specimens, warrants an additional treatment of the subject. This article is an attempt to combine a complete survey of current techniques with a page size that the individual collector can conveniently carry in the field.

In an age when so many wild species and areas of suitable habitat are at the threshold of extermination, it seems advisable at the outset to include a plea for conservation in this booklet. Current museum collections contain excellent samples of various North American species of reptiles and amphibians from certain areas within their ranges. In these instances, it is a needless waste to collect and preserve additional material when this will not add appreciably to our knowledge of these creatures. I am not referring to such collecting as may be associated with the compiling of a synoptic teaching collection by a school or to collection of specimens needed for a particular aspect of research, but rather to the capture and preservation of animals simply to amass a collection which may never be used for scientific or educational purposes. There are numerous geographic areas, including several in North America, in which the amphibians and reptiles are poorly known. Collections from these areas can add measurably to our herpetological knowledge. Persons wishing to learn of the desirability of specimens from particular areas should consult with herpetologists at nearby universities, museums, zoos, etc.

I would like to express my thanks to Woodrow W. Barber, Hobart M. Smith, William E. Duellman, Joseph T. Collins, Clarence J. McCoy, and George Iannarone for furnishing helpful material and/or advice and to Phyllis Shaffer, Judy Hamilton, Leanne Johnson, and Ginger Stiggins for typing assistance. Thanks are also expressed to Jaime Villa for preparing much of the "International Shipment" section.

FIELD NOTES

Specimens not accompanied by data identifying the collection locality are virtually useless to scientific investigators. The more data available for a specimen, the greater its value in research. Hence, keeping accurate, complete field notes is necessary. Many times, data felt to be trivial at the time of collection may prove to be quite useful when many observations are pooled. Field notes should be written in waterproof ink ("Pelikan" brand is preferred by many) using only one side of each page. Several brands of waterproof ink will "run" if alcohol is accidentally spilled on the page, hence care should be used in selecting ink. A worthwhile technique is to carry a small notebook for on-the-spot data taking. Then transfer these data into the permanent field notes as soon afterward as possible.

The following is a representative outline of data included in field notes (numbers refer to those in figure 1a):

1. Locality- Do not record locality with reference to business establishments. Use towns or mapped roadways; determine distance from an automobile odometer (if available), or estimate distance carefully from maps. It is not unusual for roads to be rerouted, renumbered or both. It is therefore advisable to refer to roads indicated in a good atlas to which future reference can be made. In the U. S., the American Highway Atlas (Gousha Co., Chicago) is suitable. If collecting in areas remote from roadways, locate the collecting site as accurately as possible from U. S. Geological Survey topographic maps. Collectors in foreign countries should try to obtain accurate, detailed maps of the areas in which they are working. Elevation of the locality should also be recorded whenever possible.

2. Date- Always write out the name of the month, or indicate month by a Roman numeral; 6-10-71 could refer either to June 10th or October 6th.

3. Name(s) of all collector(s) present.

4. Time of collecting.

fig.1a

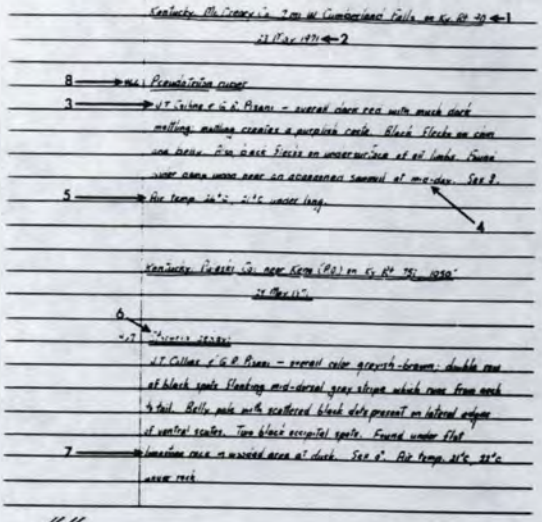
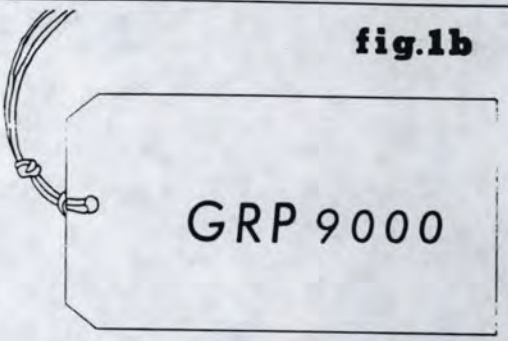


fig.1b



5. Air temperature and other appropriate weather notes- It is often useful to note existing cloud cover and moisture conditions, as well as general weather conditions preceding the collection.

6. Species- List all species collected plus the number collected of each, followed by species which may be observed but not collected. Accurate color notes are a worthwhile inclusion, especially when collecting in regions having a poorly known herpetofauna. It is also worthwhile to take accurate color notes when an atypically colored individual of a well-known species is encountered.

7. Microhabitat of species collected and any significant behavior (courtship, defensive display, etc.) observed.

8. Field number- It is useful to carry a series of numbered field tags on collecting trips. These should be printed on heavy paper in permanent ink. Satisfactory tags can also be made with one of the commercially available label makers that imprint plastic tape. Thread can be sewn through the numbered tags, but the backing on the tape should not be removed. Avoid using colored thread, or thread made from synthetics such as nylon, which may be destroyed by preservatives. White, cotton carpet thread is suitable for tagging. After threading the tag, tie a small knot in the string as shown in figure 1b. Each specimen should be assigned its own number, which greatly simplifies the task of keeping specimens and localities associated. Testes, stomach contents, photos, tape recordings, etc. are assigned the same number to increase efficiency in future analyses. Field tags should be securely tied (with a square knot) to specimens as illustrated in Plates 1 and 2. Lizards possessing femoral pores should be tagged by knotting the string below the knee; this avoids covering the pores with string. Tags in the field series should be numbered independently from the catalog series discussed in a later section.

Of the above data, numbers 1 (locality), 2 (date), 3 (name(s) of collector(s), and 8 (field number) represent the minimum data which should be recorded. If specimens are donated to an institution, the field notes should be donated with them. Do not include field notes in the same container used to hold specimens.

KILLING OF SPECIMENS

It is essential that live herpetological specimens be killed in such a manner as to leave the muscles in a relaxed state. Following this, they can be fixed, or hardened, in standardized positions which enables researchers to examine them conveniently and most accurately (refer to Plates 1 and 2). Many books recommend that reptiles be killed by hypodermic injection of aqueous sodium pentobarbital (Nembutal) into the heart. This technique is indeed excellent, but the reader should be aware that Nembutal is not a generally available drug, its possession being closely regulated by the Federal Bureau of Narcotics and Dangerous Drugs. It is possible for qualified persons to obtain a permit to purchase Nembutal, but the application procedure is best begun several months in advance of anticipated need. For additional details concerning the permit, the reader is urged to consult representatives of the above-mentioned Bureau at the Federal centers in most large cities. State and local regulations should also be checked. Commercial Nembutal is sold at a concentration of 50 mg/cc. The form of Nembutal sold as a syrupy elixer should be avoided. Commercial Nembutal may be used directly for larger specimens (over 5 pounds body weight), and diluted 1:5 with water for smaller reptiles; for very small specimens such as Typhlops or small Scincella it is possible to dilute to 1:10 and retain effectiveness. Nembutal diluted 1:10 can also be used on larger specimens, but death will be delayed. One cc (used commercial strength) injected into the heart is generally sufficient to quickly kill an animal of the bulk (volume) of a 3 foot timber rattlesnake (Crotalus horridus). Position of the heart in snakes can often be judged by closely watching the ventral plates on the anterior 1/3 of the body to detect heartbeat. Injection anywhere into the anterior 1/3 of the body cavity is also effective, but death is not as rapid as from heart injection. Other reptiles can be killed by injection into the heart region. Do not attempt to inject specimens which are so small or thin as to be heavily damaged by the needles at hand.

A number of other effective killing means are available. Turtles may be chloroformed if care is taken not to allow them to stiffen. Confining the turtle with a chloroform moistened rag or cotton wad in a closed container for 15-30 minutes (Cook, 1965) should suffice. The use of chloroform on other ·tiles is definitely not recommended, as severe contortion usually results. :hloroethylene or ether may be substituted for chloroform with good results, and can be used on most reptiles. Most specimens can be killed by confinement with either trichloroethylene or ether for 5 minutes beyond the time the animal loses the ability to right itself when turned over. These liquids are available to the public from either biological supply houses or certain drugstores. Their use may be superior to Nembutal when working with small, fragile animals like some tropical geckos. Caution should be observed with ether, as it is highly flammable and can, under certain storage conditions, explode. Read labels carefully.

All ampnibians and a number of smaller reptiles (e.g. small, tropical geckos) are easily killed by immersing them in a solution of Chloretone (hydrous chlorobutanol). A stock supply is commonly prepared as a saturated solution of Chloretone in 95% ethanol. This stock solution may be conveniently carried in a small vial; 2 cc of it added to a pint of water is effective. The solution should be kept tightly covered when not in use, and can be used over and over; its strength will diminish with use.

Various other means are suitable for killing reptiles and amphibians. Securing the animal(s) in a cloth sack and immersing the sack in warm (110°-120°F; 43-47°C) water is effective, but specimens should be removed shortly after death. Specimens may also be immersed in alcohol (15-25% for amphibians; 50-60% for reptiles). Though the method is not recommended, bags containing reptiles may also be left exposed to direct sunlight until death from overheating occurs. Great care must be used however, as dehydration and accompanying contortion can happen quickly; ampnibians should never be killed in this way. Both procaine hydrochloride (Livezey, 1958) and succinylcholine chloride (Anectine) (Lambert, 1967) have been used effectively as killing agents; however, their availability is usually restricted like that of Nembutal.

Recent drug laws have greatly increased the difficulty of obtaining syringes for preserving purposes. State laws may also vary in the regulation of the above-mentioned chemicals. It is often possible to obtain necessary supplies through institutions, particularly in return for depositing desired specimens.

FIXING

The purpose of fixation is to preserve the actual morphological state and color of the specimen, and to prepare the tissues for microscopic examination. Hence, the fixative should kill tissue quickly; penetrate it uniformly and rapidly; prevent postmortem decomposition; not distort the tissue; and should prepare the tissue for staining. No single fixative will do all of these things, so various compromises must be made.

The most widely accepted and suitable general fixatives for field use are:

1) Formalin ("Formol" or "Formalina" in Spanish; "das Formaldehyd" in German)- Sold commercially as a solution of approximately 40 percent formaldehyde gas in water, formalin is the most widely used field fixative. For purposes of dilution, commercial formalin is usually considered as 100%, and can be used in 10% strength (1 part formalin: 9 parts water) for fixation. Formalin may be buffered (which helps to reduce discoloration of specimens) by mixing 1 tablespoon of baking soda or borax with each pint of 10% formalin. Generally sold as a liquid (often in drugstores), it is also available as a solid polymer (paraformaldehyde), which is convenient for saving weight and space in transport. Huheey (1963) recommends sealing 16 grams of paraformaldehyde and 4 grams of anhydrous sodium carbonate in packets for field transport; 1 packet added to 400 ml (about 1/2 quart) of water makes a 10% solution of buffered formalin. Premixed, buffered paraformaldehyde powder is available from Carolina Biological Supply House. Paraformaldehyde alone can be obtained from Eastman Organic Chemicals, Rochester, New York. Formalin, while an excellent general fixative, is highly irritating to the user's skin and (as a vapor) to mucous membranes. It is not uncommon for users to develop strong allergies

to formalin. Also, formalin has a tendency to cause swelling of several types of tissue, rendering them unsuitable for some histological purposes.

2) FAA (formalin-alcohol-acetic acid)- Prepared by mixing 10 parts commercial formalin, 50 parts of 95% alcohol (ethyl or isopropyl), 40 parts water and 2 parts glacial acetic acid. FAA penetrates tissue far better than formalin alone, and has less tendency to cause cell distortion. The rapid tissue penetration can also be an aid to preserving valuable specimens found dead and, perhaps, partially decomposed. The primary disadvantages of FAA are: the need to mix several components; and, the necessary alcohol and acetic acid may not be available in certain localities. FAA is not available in powder form, but can be premixed without the water to reduce volume in transport; water may be added later. If FAA is to be used extensively in hot regions, it is recommended that the acetic acid be added just prior to actual use, as it quickly evaporates from the solution; containers may be cooled by wrapping them in wet rags and shading them to retard evaporation of acetic acid.

3) Alcohol- If neither formalin nor FAA are available, alcohol may be used as a fixative. Cook (1965) recommends ethanol (95% for reptiles; 70% for amphibians) or isopropanol to fix in the absence of other solutions, but the latter is not desirable.

4) Special- A large number of other fixatives exist, each being useful for different types of tissues, and studies. Bouin's solution (75 parts saturated aqueous picric acid, 25 parts commercial formalin, 5 parts glacial acetic acid) is especially useful for field preservation of testes to be used in spermatogenesis studies. Testes may be placed in vials of Bouin's and safely kept there for long periods of time without distortion of cells; the remainder of the specimen may be fixed with FAA or formalin. For a complete discussion of special fixatives, the reader is referred to Guyer (1961) and similar texts.

5) Miscellaneous- If a valuable specimen must be saved and no other solutions are available, a number of emergency measures are possible. The specimen may be frozen or packed in strong brine until preservative can be obtained. Liquor is generally not a suitable source of alcohol, as 110 proof liquor is only 55% ethanol. However, strong tequila (about 160 proof) may be useful; rubbing alcohol can also be used. These, however, are only desperation measures and it is usually more beneficial to get the specimen into a proper fixative (hospitals, local schools, etc. are suggested as possible sources).

It is always preferable to introduce fixative into the body cavity, as specimens (particularly reptiles and larger amphibians) can decompose internally if simply placed in fixative. Enough fixative should be injected to fill, but not distend, the animal. Care should also be taken not to damage the femoral pores of many lizards by puncturing them with the needle. The neck of turtles should be completely extended and the mouth held open with wood, cork, or tightly wadded paper prior to fixation. Excellent neck extension can be obtained by hooking the dead turtle's upper jaw over a nail or broken branch and letting the animal's hanging weight pull the neck out straight prior to injecting it. The upper jaw can also be hooked over a paper clip placed over the edge of the fixing tray, and the neck then drawn out. One hemipenis of male lizards and snakes should be partially everted with thumb pressure on the base of the tail, followed by injection to completely evert it as indicated on the front cover. The hemipenis should not be permitted to remain incompletely everted; thread may be tied around the base of the fully everted hemipenis to help retain fluid within it. It is also an acceptable practice to evert the hemipenis by injection of fixative alone. Typical sites for injection of preservative are starred in Plate 2. Tails of lizards and snakes should be slit lengthwise, being very careful not to break the tail off; sharp instruments are a "must." Large amounts of fixative can be conveniently handled in the injection apparatus designed by Jackson (1971), although the author has never felt at a disadvantage using larger syringes. If no injection apparatus is available, the specimen should be deeply slit in several places ventrally and placed belly-up in fixative. Spread the sides of the slits to admit fixative more easily. Avoid cutting the anal plates of snakes and lizards and femoral pores of lizards.

Once the animal is injected or slit, it is most conveniently fixed by placing it (after proper positioning) between pieces of white paper toweling moistened liberally with fixative. This can be done in shallow, covered plastic or rustproof metal pans. Surgical instrument pans with sliding metal covers are handy for this. Avoid colored towels, as the colors dissolve in the fixative and stain the specimen.

Preferred positions for fixing and sites for field tag attachment are illustrated in Plates 1 and 2. Amphisbaenids and caecilians should be fixed in the same position as snakes; it is useful to fix these with the mouth open, as this greatly facilitates examination of oral characters later on. Lizards with long tails should be fixed with the tails bent as shown. Frogs and toads may be positioned with the sole of the foot down (Duellman, 1962). However, because this position obscures many hind limb and anal characters, others feel that anurans are best fixed with the hind limbs in the position shown in Plate 1c. Toes and fingers should always be straight and spread apart. Small amphibians need not be injected or slit prior to positioning, as the fixative will penetrate to the body cavity quite easily. Small amphibians and lizards may have the field tag tied around the body just anterior to the pelvic region.

Amphibian eggs and larvae are best fixed and stored by dropping them directly into jars of 10% formalin; preserve entire egg clutches whenever possible. Many amphibians attach their eggs to leaves, twigs, etc. Whenever it is practical, these items should be preserved with the eggs in situ, as the latter are often severely damaged by attempts to disengage them. Change the formalin on eggs and larvae after about 12 hours. Reptile eggs should be measured (length and width, in millimeters), then injected.

All specimens should be allowed to remain in fixative for 24 hours.

Very Large Specimens (too large to be conveniently stored entire in liquid)

1. Snakes- Obtain the snout to vent and tail lengths (in mm). Then skin by making a long ventral incision to the side of the mid-line; leave the head and tail attached to the skin, severing these from the carcass (avoid cutting the anal plate), and then inject head and tail (evert hemipenis if male) with fixative. With boids, sever the hind part just

Plate 1. Amphibians

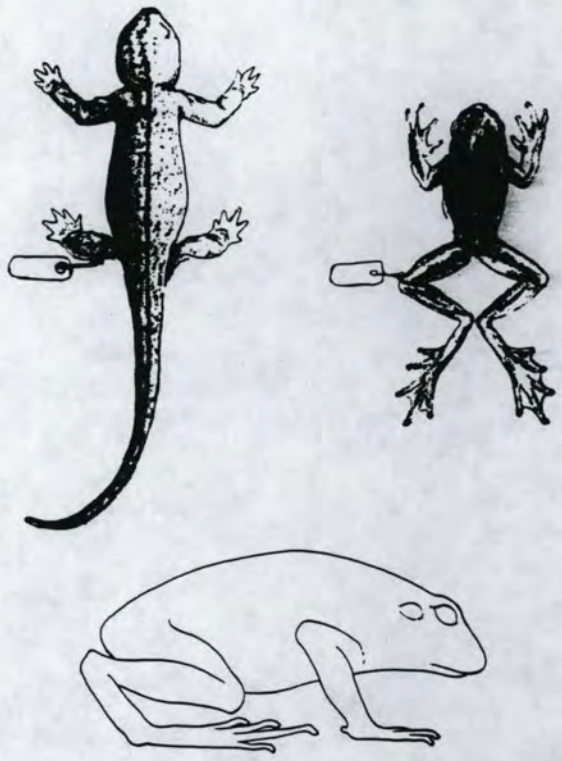
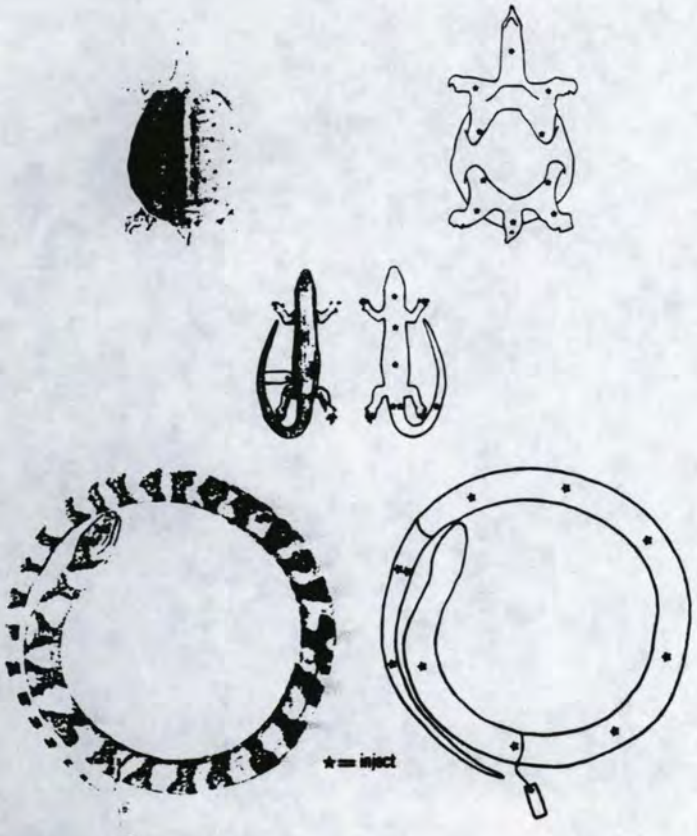


Plate 2. Reptiles



ahead of the bony, vestigial pelvic elements. The skin may now be preserved by covering the flesh side with cloth or absorbent paper, rolling loosely and immersing in fixative, or by rubbing with borax or arsenical soap, rolling and drying. In this latter instance, it is best to preserve the head and tail separately in liquid. If the specimen is a male, a testis should also be preserved. Reproductive condition of females should be noted (i.e.- number of ova present, size of the largest ovum, etc.). Embryos, especially those of poorly known species should be preserved in liquid; it is preferrable to do this by preserving the entire oviduct rather than by removing embryos.

2. Turtles- Avoid cutting the shell. It is preferable to cut the head, neck and forelegs out as one unit, the hind legs and tail as a second unit and preserve these in liquid. The stomach and reproductive organs should also be preserved in liquid. Carefully clean out and dry the shell.

3. Crocodilians- Measure and skin the specimen as for snakes, except that the tails should be skinned as well. Feet may be left attached (inject with fixative), instead of skinned out. Rub the skin with borax or arsenical soap and dry.

SHIPPING

Once they have been properly fixed, most herpetological specimens may conveniently be transported by wrapping them loosely in cheesecloth or white paper towel which has been liberally moistened with alcohol (70% ethanol or 40% isopropanol), or the fixative, then sealing them in two plastic bags (one within the other, individually closed by twisting the end and knotting or securing with a rubber band). Several small specimens may be wrapped in a single length of cheesecloth by laying the cloth out flat, spacing the specimens down the length of it, folding the sides over the animals and rolling the cloth loosely, like a rug.

Thus packed, the specimens occupy minimum space and weight, important factors when they must be transported any distance or mailed. Specimens will remain in

ondition for several weeks, so long as the bags are well sealed to retard
e and evaporation. Cotton in sheet form may be substituted for cheesecloth,
bulkier and may adhere to the scales of some rough-scaled species of lizards,
nen it is moist.

s being shipped (parcel post is a convenient means) should be carefully
ed and clearly marked "PRESERVED SCIENTIFIC SPECIMENS." Packages often are
to much "wear and tear," so, effort in preparation pays off! Paint cans
ying sizes make leakproof, sturdy mailing containers. Plastic bags containing
ens may be simply placed in the can and extra space filled with wadded rags
er. Bags with heavy specimens should never be placed on top of lighter ones.
address labels should be typed or written with permanent ink. Place one
se in the can with the specimens, tape a second to the side of the can.
the third label to the paper used to wrap the parcel.

aint cans are not too costly, and a source of supply can generally be found
sulting local paint stores. Watching auction notices sometimes turns up a
store that is going out of business and may have cans. Remove handles from
efore use. In lieu of cans, specimens may be packed in any durable container.
postal regulations for size and weight restrictions before packing extremely
or unwieldy parcels. Specimens such as large turtle shells or the skins of
crocodilians may have to be sent via freight, and again, secure packing is
. Shipment of all crocodilian specimens is subject to stringent regulation,
y of these species are endangered animals. Collectors planning to take
should carefully check customs regulations for import restrictions as well
ecking capture laws in countries where the animals occur. Proper arrangements
ften be made through the institution where one proposes to deposit the specimens.

Generally, specimens should never be sent in glass containers. Obvious
tions to this are amphibian eggs (and sometimes, larvae) and very fragile
ens. These should be placed in the smallest containers needed to hold the
ens plus fluid to maintain them; fluid should fill the containers, which
be heavily padded with cardboard or cotton. If rigid plastic tubing of
ient diameter is available, break resistant containers may be fashioned
t by cutting an appropriate length, stoppering one end, enclosing specimens
luid, then sealing the other end. The tube may be wrapped lengthwise with
wire to secure the stoppers. Plastic vials are available from some biological supply houses; larger drugstores may also furnish the names of suppliers of these. Be sure to only use vials which can be securely closed (screw-on or snap-on lid). Again, pay special attention to wrapping such containers.

INTERNATIONAL SHIPMENTS

Collectors should be aware of proper methods for shipping specimens internationally. Donation of all or part of a collection to institutions outside one's own country serves to:

1. make synoptic herpetofaunal collections of different areas available to as many researchers as possible,

2. prevent the loss (through war, neglect, earthquakes and other damage) of valuable collections deposited entirely in a single institution, and

3. place the herpetologist in contact with colleagues in foreign institutions; this frequently leads to a most beneficial exchange of ideas and data, thus advancing herpetology as a field of study.

The private hoarding of specimens by any person is a waste of valuable biological data, and can lead to overcollecting (i.e.--researchers may gather specimens from areas already represented, though inaccessible, in private collections). It is with the above points in mind, and the hope that more collectors will decide to enter into donation, exchange or loan relationships with foreign institutions, that the following guidelines are presented.

The methods of packing described in the preceding section are adequate for international shipment. Generally, mail is the most convenient means of sending packages which are not too heavy or bulky. Parcels sent via surface ("ordinary") mail should have extra preservative added to the specimen bags, as they may take as long as 4 months to reach their destination. Persons

ing specimens internationally should check local mail regulations on
el size, weight and any special packing provisions. The shipper may also
equired to affix various postal and customs "declaration tags" to parcels.
e tags vary with parcel destination and are generally provided by the
al service.

Very large or heavy packages will have to be sent via freight (air,
n, ship). The sender will be required to complete a "waybill" (avail-
e from the carrier) listing, among other things, the nature and value of
contents. To avoid excess charges, package and waybill should be marked
Commercial Value." Postal services in all countries have the legal right
inspect all packages. Intensive efforts to curtail the traffic of narco-
s and other restricted drugs has led to the extensive exercising of this
nt, and the fact that several persons have attempted to smuggle drugs with-
specimen containers has not aided the situation. Inspectors often open
stic bags of specimens, and may be unaware of the need to reseal them.
s causes loss of fluid and dehydration and probable loss of the specimens.
is therefore advisable to include two copies of the following statement with
parcel (one pasted on the outside and one sealed within):

PECTION OFFICER: This package contains dead, preserved amphibians and/or
tiles packed in plastic bags. As the specimens have great scientific
ue and will be ruined if not kept moist in their preservative, it is
erative that the bags be tightly resealed after inspection to avoid
poration or leakage of preservative. Thank you.

SPECTOR POSTAL: Este paquete contiene ejemplares de anfibios y/o reptiles
ertos, preservados y empacados en bolsas plasticas. Puesto que los
emplares son de valor cientifico y se arruinan si no permanecen en su
qui servativo, se suplica que, despues de abrir las bolsas para
sp rlas, las cierre hermeticamente para evitar que el liquido se
apore o se derrame. Gracias.

AUTORIDADES ALSANDEGARIAS: Este volume contem anfibios e repteis mortos,
nservados em sacos plasticos. Como o conteudo tem valor cientifico e se
tragara se nao for mantido humido no preservativo, pedese que apos abrir
sacos para inspecao os mesmos sejam firmemente fechados para evitar
aporacao ou derramamento do liquido. Obrigado.

Biologists should also be aware that the international shipment of specimens (alive or preserved) is being ever more closely regulated for conservation reasons. Shipments of preserved animals sent to the USA must be accompanied by a list bearing the number and scientific name of all specimens included. The importer (in the USA) must obtain a special permit from the Bureau of Sport Fisheries and Wildlife (Dept. of the Interior) in order to receive foreign shipments of preserved or live specimens.

Live shipments are additionally regulated by the Dept. of Agriculture and the Public Health Service. In all cases, endangered species are covered by regulations separate from species not currently considered endangered. You are urged to carefully investigate all legal aspects of international shipment before preparing to send animals.

STORAGE AND LABELLING

This section is not intended to be a complete guide to curatorial technique. Rather, it is meant to serve as a set of capsule directions for those wishing to start a preserved herpetological collection. A detailed discussion of curatorial technique may be found in Slevin (1927).

Preserved collections are best maintained in alcohol. Suitable alcohol generally costs about the same (per gallon) as formaldehyde, and alcohol-stored specimens are far easier to work with. Formaldehyde also tends to corrode metal lids and containers. Most collectors will be deterred from using ethanol by the high tax imposed upon its sales. Isopropanol is far cheaper, and is entirely satisfactory for storage of specimens. Methanol should never be used. Concentrati of 50% is suitable for reptiles, while 40% is better for amphibians. Both ethanol and isopropanol are generally sold at 95% concentration; 526 ml of this plus 474 ml of water make one liter of 50% concentration (421 ml alcohol + 479 ml water for 40%). Specimens being transferred from formalin or FAA fixation to alcohol must first be soaked in water for 48 hours. Failure to soak the specimen often results in its being severely dehydrated by the alcohol. Properly fixed specimens will not be harmed by this method. If material is desired for use in histological work, selected pieces of tissue should remain in 30% alcohol

for 24 hours, then 24 hours of 50% alcohol before going to final storage (omit the water soak). Do not pack specimens tightly in the jar. Snakes fixed in the position illustrated earlier will readily coil in jars for storage.

Each specimen retained in the collection should be assigned a catalog (in addition to the aforementioned "field number"). Amphibian eggs and larvae and reptile eggs may be cataloged with a single tag designating one clutch or lot. This number should be entered in a permanent catalog (using waterproof ink), along with the species name, date of capture/preservation, sex, locality, ecological notes and name of collector. Tags may be tied in the same region as the field tag. Collector's field number should also be entered. As a cross-reference, it is useful to maintain a card file (by taxonomic family) in which a single card is used for each species. On this card may be entered numbers from the catalog that apply to these species.

It is convenient to place a label bearing species name, catalog numbers and locality data with each container. These should be written in permanent ink on heavy, durable paper. That produced by Byron-Weston Mills under the name "Linen Record Ledger", 100% cotton and linen fiber, 36 lb. and Dennison Paper Company's product "Resistall Index Bristol", 100% rag, 110 lb. wt. are both excellent and are available through printing shops. The label may either be placed within transparent containers, or attached to the outside of opaque ones with masking tape. If moderate cost can be withstood, external labels can be placed within tie-on, plastic label-holders. A typical museum label is shown in Figure 2.

Specimen jars should be stored in cool places to help retard evaporation of preservative, and should never be exposed to sunlight, as specimen colors are rapidly faded by such exposure. Placing a piece of Parafilm sheet (available from Carolina Biological Supply House, Burlington, North Carolina) over jar mouths before screwing on the cap will also reduce evaporation. Containers should be checked periodically and fluid level maintained. Well preserved and cared for collections make valuable teaching and research tools.

COLOR PRESERVATION

While preserving the morphological state of herpetological specimens has never presented any severe hurdles to collectors, preservation of color is quite another matter. All currently used, popular preserving fluids are alcoholic and/or acidic to some degree. Therefore, it usually is not too long before most pigments are dissolved by such fluids and extracted from the specimens. Amphibians seem particularly vulnerable in this regard, though the effect on reptiles is noticeable.

Previously, the only acceptable method of retaining amphibian skin color was that described in Cook (1965). Basically, this consists of skinning the specimen, confining all cuts to the ventral surfaces of body and limbs. The skin is then floated flesh-side up in a pan of water and remaining particles of tissue are removed. The skin next is floated flesh-side down and spread out in a second pan. A wet piece of cardboard may then be brought up beneath the skin, which is rubbed lightly to flatten it and remove trapped air. The cardboard-skin preparation may be dried on blotting paper until moist, then placed between layers of blotting paper and thoroughly dried with heavy weights (such as books) on top of it; it may also be placed in a plant press. Reptiles may be similarly prepared. In all cases, the carcass should be preserved in fluid and tagged with the same number as the skin. Skins thus prepared should be stored in the dark and not exposed to prolonged light.

The above technique, while useful, is tedious. Windsor (1971) has described a technique for using 50% saturated, aqueous ammonium sulfate solution as a preservative of frogs. As the compound is an aqueous, neutral salt, no pigment was dissolved and natural color was still evident in the specimens 6 months after preparation. Total fixing time should be at least 36 hours.

Specimens may be stored in buffered formalin (10%) or isopropanol (40%) to which liquid Ionol-40 R has been added (White and Peters, 1969). Storage should be in dark places which are not subjected to heat much above 70°F. The formalin/Ionol method has been successfully used with herpetological material by Mr. Woodrow Barber, Biology Department, University of Kentucky at Morehead, and by Dr. George Iannarone, Chicago Academy of Sciences (personal communication).

Powdered Ionol should not be used, as it is difficult to prepare a stable solution of it in preservative. Ionol is sold by the Shell Oil Company (Chemicals Division).

While the two chemical methods discussed above have not been widely used with herpetological material, their success on a limited scale coupled with the value of accurate color preservation suggests that they should be more thoroughly investigated.

LITERATURE CITED

Cook, Francis R. 1965. Collecting and preserving amphibians and reptiles, p. 128-151, In R. M. Anderson (ed)., Methods of collecting and preserving vertebrate animals. Nat. Mus. Canada, Bull. No. 69, Biol. Ser.: 1-199.

Duellman, William E. 1962. Directions for preserving amphibians and reptiles, p. 37-40,In E. Raymond Hall (ed)., Collecting and preparing study specimens of vertebrates. Misc. Publ. Univ. Kansas Mus. Nat. Hist., (30): 1-46.

Guyer, Michael F. 1961. Animal micrology, 5th impression. Univ. Chicago Press, Ill.; xviii + 327 pp.

Huheey, James E. 1963. Concerning the use of paraformaldehyde as a field preservative. Copeia, 1963(1):192-193.

Jackson, Morris K. 1971. A new "syringe" for injecting herptiles. Herpetol. Rev., 3(4):75.

Lambert, Lewis H., Jr. 1967. 'Anectine': a herpetological killing agent. Herpetologica, 23(2):135-136.

Livezey, Robert L. 1958. Procaine hydrochloride as a killing agent for reptiles and amphibians. Herpetologica, 13(4):280.

Slevin, Joseph R. 1927. The making of a scientific collection of reptiles and amphibians. Proc. Calif. Acad. Sci., 16(9):231-259.

White, David A. and Edward J. Peters. 1969. A method of preserving color in aquatic vertebrates and invertebrates. Turtox News, 47(9):296-297.

Windsor, Donald A. 1971. Ammonium sulfate as a preservative which does not remove color from frogs. Copeia, 1971(2):356-357.

INDEX

It appears complex and intimidating, but actually can be completed in a short amount of time after a [illegible]. Most variables are simply to be circled, and the remaining variables are numerical and easy to determine. The data sheet is divided into four sections, divided by double lines. Each section describes a cohesive set of variables. In addition the back of the sheet includes a grid for a rough sketch of the site and space for additional comments. The map is optional, but the future value of the data is enhanced if it is supplied.

SECTION 1 – LOCALITY *These data are essential. Many amphibian surveys have been hampered by the inability to relocate exact locations in the historical record. Some of this information can be completed in the office after the survey.*

DATE: Use the format DD-MMM-YY (e.g., 05-APR-92).

BEGIN TIME: List the time survey of habitat for amphibians began in 24 hour format.

END TIME: List the time the survey ended in 24 hour format. (The total time (END TIME - BEGIN TIME) should reflect only the amount of time spent searching for amphibians. Total time plus number of observers may be used to assess relative abundance.)

OBSERVERS: List names or initials of all persons involved in searching.

LOCALITY: Describe the *specific* geographic location of the site. Use air distance in two directions (e.g., 5km N and 7.5 km W) of a map landmark that likely will not change (distance from a large town or city is not all that helpful).

STATE: Use the 2-letter abbreviation.

COUNTY:

MAP NAME: List the name of the U.S.G.S. quadrangle or other map used to locate the site.

OWNER: List the public land manager (e.g., Roosevelt Nat. Forest or Rocky Mtn NP), or name of the owner if the site is on private land (listing the owner's name will make it clear that you did not trespass to survey the site).

ELEVATION: Circle the scale used; meters are preferred.

T: township **R:** range **S:** section

SECTION DESCRIPTION: Describe the location of the site within the section (e.g., SE ¼ or NE ¼ of SE ¼)

UTM ZONE, NORTHING, EASTING: Universal Transverse Mercator coordinates are preferred over longitude and latitude. The UTM zone is listed on newer topographic maps. If you are using a map without the UTM grid, substitute latitude for Northing and longitude for Easting.

SECTION 2 – SPECIES DATA *List all amphibian species observed. If garter snakes are seen, list them here also.*

SPECIES: Use the scientific name. Convenient shorthand is to use a 4-letter code made up of the first 2 letters of the genus and species (e.g., *Rana sylvatica* would be RASY).

ADULTS/JUVENILES: Indicate presence with a check, but numbers seen are more valuable data

CALLING?: Circle Y if frogs are vocalizing in a breeding chorus, of if a breeding aggregation of species that don't call (e.g., *Bufo boreas*) is observed.

TADPOLES/LARVAE: Same as for adults/juveniles

EGG MASSES: Same as above. Numbers of egg masses are especially valuable data. If possible, describe the developmental stage of eggs in the space for additional notes on the back of the form.

METHOD: Circle how observations were made: **VISUAL/AURAL ID** – species identified without picking it up, either by sight or by recognition of the breeding call; **HAND COLLECTED** – animal was picked up and identified in the field (higher confidence than visual id); **DIP NET/SEINE** – the usual method of collection for larvae; **TRAPPED** – minnow-type traps are also used for larvae; **VOUCHER COLLECTED?** – circle yes or no (voucher specimens are recommended for every site, *especially if identification is uncertain and for larvae*). Indicate voucher status in addition to method used.

FISH PRESENT?: If yes, list species if you can. Circle the question marks if you are not certain, but suspect that fish are present.

ENTIRE SITE SEARCHED?: If no, list either the meters of shoreline or the area (m^2) of habitat (e.g., amount of wet meadow) searched.

SECTION 3 – PHYSICAL AND CHEMICAL DATA *Water chemistry data are difficult to collect accurately without thorough planning and quality equipment; these data are optional. Weather data are important for determining the quality of the observations (e.g., was an absence of amphibians due to observations made during a blizzard?)*

WEATHER, WIND: Indicate atmospheric conditions

AIR TEMPERATURE: Take at chest height in shade. The Celsius scale is preferred.

WATER TEMPERATURE: Take 1 meter from margin and at 2 cm depth, or where egg masses are observed.

COLOR: This is a qualitative assessment of whether the water clear or tea-colored from organic (humic) acids.

TURBIDITY: This is a qualitative assessment of whether the water clear or clouded from suspended particulate matter.

SECTION 4 – HABITAT DESCRIPTION *These data are important for developing hypotheses to explain changes in abundance of amphibians. This section needs to be filled out only once for each site (a reasonable amphibian survey should include at least 2 – 3 visits to each site in one season).*

ORIGIN: Decide whether the lake is a natural geologic formation or man-made. Bodies of water enlarged by a dam are problematic. List them as man-made, but add an explanation in the space for additional notes on the back of the form.

DRAINAGE: Circle whether the site has permanent drainage, no drainage, or occasional drainage. Determining the potential for occasional drainage requires judgement. Look for clues in the topography and vegetation.

DESCRIPTION: Decide how best to describe the site. If there is evidence of past or present beaver activity, circle one of these choices in addition to your choice.

LENGTH, WIDTH: Record the maximum length and width of lakes and ponds. For streams, record the length and average width of the reach searched.

MAXIMUM DEPTH: Most times, you will not have access to a boat, so estimate depth (deep lakes are usually not important to amphibians).

STREAM ORDER: This is an index of stream size, and you will need a topographic map to determine it. First-order streams have no tributaries, second-order streams are formed by the confluence of two 1st-order streams, third-order streams are formed by the confluence of two 2nd-order streams, and so on.

PRIMARY SUBSTRATE: Circle the type that covers the majority of the bottom of the site.

EMERGENT VEGETATION: Circle the percentage of the margin of the site with emergent vegetation present, and list the dominant species. If you are botanically-disadvantaged, list the categories of the dominant species (e.g., cattail, sedges, etc.).

NORTH SHORELINE CHARACTERS: Describe the north shore of a lake or pond in terms of shallow water and emergent vegetation. This is important in evaluating quality of breeding habitat in some mountain locations.

FOREST CHARACTERS: List the closest distance between the water and the surrounding forest, and list the most common tree species. Leave these fields blank if there is no forest. Describe other surrounding habitat types in the notes section on the back of the form.

References

Blaustein A.R., and D.B. Wake. 1990. Declining amphibians: a global trend? Trends in ecology and evolution. 5:203-204.

Carpenter, C.C. 1953. An ecological survey of the herpetofauna of the Grand Teton-Jackson Hole area of Wyoming. Copeia, 1953(3):170-174.

Corn, P.S. and R.B. Bury. 1989. Logging in Western Oregon: responses of headwater habitats and stream amphibians. Forest Ecology and Management, 29(1989):39-57.

Corn, P.S. and R.B. Bury. 1990. Sampling methods for terrestrial amphibians and reptiles. USDA Forest Service, Pacific Northwest Research Station, Portland, Oregon. General technical Report PNW-GTR-256.

Corn, P.S. and J.C. Fogleman. 1984. Extinction of montane populations of the northern leopard frog (*Rana pipiens*) in Colorado. Journal of Herpetology, 18(2):147-152.

Groves, C. 1989. Idaho's amphibians and reptiles. Nongame Wildlife Leaflet #7. Idaho Department of Fish and Game.

Halliday, T. and K. Adler. 1987. The encyclopedia of amphibians and reptiles. Facts on File Publications, New York. 143 pp.

Karns, D.R. 1986. Field herpetology: methods for the study of amphibians and reptiles in Minnesota. Occasional Paper No. 18, Division of Comparative Biology, James Ford Bell Museum of Natural History, University of Minnesota, Minneapolis. (Contains good descriptions of techniques for finding and monitoring populations).

Nussbaum, R.A. E.D. Brodie, and R.M. Storm. 1983. Amphibians and reptiles of the Pacific Northwest. University Press of Idaho, Moscow. 332 p. The best general source of information on the amphibians and reptiles of Idaho).

Pisani, G.R. 1973. A guide to preservation techniques for amphibians and reptiles. Herp. Circular. No. 1. 22 p. Society for the Study of Amphibians and reptiles.

Pough, F.H., J.B. Heiser, and W.N. McFarland. 1989. Vertebrate Life. Third edition. Macmillan Publishing Co., New York. (An excellent textbook on vertebrate zoology; especially good chapters on amphibians and reptiles).

Stebbins, R.C. 1985. A field guide to western reptiles and amphibians. Houghton Mifflin Co., Boston. 336 pp. (The best field guide to the amphibians and reptiles of the western United States).

Turner, F.B. 1958. Life history of the western spotted frog in Yellowstone National Park. Herpetologica, 14:96-100.

Turner, F.B. 1960. Population structure and dynamics of the western spotted frog, *Rana p. pretiosa* Baird & Girard, in Yellowstone Park, Wyoming. Ecological Monographs, 30:251-278.

Vial, J.L. (editor) 1992. Froglog - IUCN/SSC Declining Amphibians Task Force Newsletter. March, 1992, 1.

Welsh, H.H. 1987. Monitoring herpetofauna in woodland habitats of northwestern california and southwestern Oregon: A comprehensive strategy. Gen. Tech. Rep. PSW-100. Berkeley, CA. Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture.

Welsh, H.H., A.J. Lind, and D. L. Waters. 1991. Monitoring frogs and toads on Region 5 National Forests. R-5 Fish Habitat Relationship Technical Bulletin. Number 4, May 1991. 12 p.

Welsh, H.H. and A.J. Lind. *In press.* Structure of herpetofauanal assemblage of the Douglas-fir/hardwood forests of northern California and southwestern Oregon. Pages XX-XX *in* L.F. Ruggiero, K.B. Aubry, A.B. Carey, and M.H. Huff, tech. coords. Wildlife and vegetation of unmanaged Douglas-fir forests. USDA For. Serv. Gen Tech. Rep. PNW-GTR-XXX. (Contains a discussion of management strategies to maintain a viable herpetofauna)

AMPHIBIAN WORKSHOP

Taylor Ranch
18 July 1992

Charles R. Peterson
Department of Biological Sciences, Idaho State University
Idaho Museum of Natural History
Campus Box 8007
Pocatello, Idaho 83209
(208) 236-3922

I. Introduction

II. Checklist of Possible Amphibians

III. Species Accounts (slides)
- A. Range map
- B. Adults
- C. Eggs
- D. Larvae
- E. Juveniles
- F. Habitat
- G. Activity patterns
- H. Calls

IV. Sampling Techniques
- A. Incidental observations (forms)
- B. Seize and capture
- C. Time constrained Searches
- D. Area constrained searches
- E. Trapping (pit and funnel traps with drift fences)
- F. Seining
- G. Calling surveys
- H. Road driving

V. Surveys - USFWS Sample Site Data Form

VI. Monitoring Programs

VII. Preservation Techniques

VIII. Examination of Preserved and Living Specimens

IX. References

CHECKLIST OF IDAHO AMPHIBIANS*

Order Urodela

Family Ambystomatidae

Ambystoma tigrinum	Tiger Salamander
Ambystoma macrodactylum	Long-Toed Salamander
Dicamptodon aterrimus	Idaho Giant Salamander

Family Plethodontidae

Plethodon idahoensis	Couer d'Alene Salamander

Family Salamandridae

Taricha granulosa	Roughskin Newt

Order Anura

Family Bufonidae

Bufo boreas	Western Toad
Bufo woodhousii	Woodhouse's Toad

Family Hylidae

Pseudacris (= *Hyla*) *regilla*	Pacific Chorus Frog
Pseudacris triseriata	Western Chorus Frog

Family Leiopelmatidae

Ascaphus truei	Tailed Frog

Family Pelobatidae

Spea (= *Scaphiopus*) *intermontana*	Great Basin Spadefoot

Family Ranidae

Rana catesbeiana	Bullfrog
Rana pipiens	Northern Leopard Frog
Rana pretiosa	Spotted Frog
Rana sylvatica	Wood Frog

* Names are consistent with Collins, J.T. (1990). Standard Common and Current Scientific Names for North American Amphibians and Reptiles. Third Edition. SSAR Herpetological Circular No. 19: 1-41.

CHECKLIST OF IDAHO REPTILES*

Order Testudines - Turtles

Family Emydidae

Chrysemys picta	Painted Turtle
Clemmys marmorata	Western Pond Turtle

Order Squamata
Suborder Lacertilia - Lizards

Family Anguidae

Elgaria coerulea (= *Gerrhonotus*)	Northern Alligator Lizard

Family Iguanidae

Crotaphytus bicinctores	Mojave Black-collared Lizard
Gambelia wislizeni	Longnose Leopard Lizard
Phrynosoma douglassi	Short-horned Lizard
Phrynosoma platyrhinos	Desert Horned Lizard
Sceloporus graciosus	Sagebrush Lizard
Sceloporus occidentalis	Western Fence Lizard
Uta stansburiana	Side-blotched Lizard

Family Scincidae

Eumeces skiltonianus	Western Skink

Family Teiidae

Cnemidophorus tigris	Western Whiptail

Suborder Ophidia - Snakes
Family Boidae

Charina bottae	Rubber Boa

Family Colubridae

Coluber constrictor	Racer
Diadophis punctatus	Ringneck Snake
Hypsiglena torquata	Night Snake
Masticophis taeniatus	Striped Whipsnake
Pituophis catenifer	Gopher Snake
Rhinocheilus lecontei	Longnose Snake
Sonora semiannulata	Ground Snake
Thamnophis elegans	Western Terrestrial Garter Snake
Thamnophis sirtalis	Common Garter Snake

Family Viperidae

Crotalus viridis	Western Rattlesnake

*Names are consistent with Collins, J.T. (1990). Standard Common and Current Scientific Names for North American Amphibians and Reptiles. Third Edition. SSAR Herpetological Circular No. 19: 1-41.

FROGLOG

IUCN/SSC Declining Amphibian Populations Task Force

March, 1992, No. 1

Coordinator's Column

The DAPTF has been established by the International Union for the Conservation of Nature (IUCN), Species Survival Commission (SSC) to organize a global monitoring program for (1) determining the status of amphibian populations (2) assessing the implications of any declines (3) studying potential causative factors and (4) making appropriate policy recommendations based upon these findings. The Coordinating Council, administered by the Coordinator, includes researchers, liaison officers of societies and agencies as well as other interested parties, all of whom serve as communicators.

As of the last week in January, the Task Force became equipped and manned at the projected level when it occupied its present facilities at the Environmental Research Laboratory in Corvallis, Oregon. In addition to the recent acquisition of computer hardware, we now have a full time information systems manager in the person of Tony Clem. Once our system is interfaced we shall initiate an electronic database and other activities designed to serve as a viable communications network.

We are still in need of Regional Working Group Chairperson for the U.S. Great Lakes area (WI, MIN, MI).

Priority has been given to organization of a Working Group to assist in compilation of a comprehensive bibliography of reports relating to amphibian populations that will be generated and maintained at the Coordinator's office. We wish to include titles of primary and secondary literature, thesis and dissertations, as well as names of earlier investigators who have archived their field notes at a repository. This resource will be freely available to those wishing to make comparisons with contemporary studies.

Anyone interested in these or related studies are invited to join the DAPTF. Please send your name, address and telecommunications number(s), indicating your interest or participation, to the Coordinator's address.

Canada Launches Major Initiative

(The following is edited and condensed from a report by Hinrich Kaiser, *Redpath Museum, McGill University, on the workshop "Declines in Canadian amphibian populations: designing a national monitoring strategy" held at the Canada Centre for Inland Waters in Burlington, Ontario, on October 5 and 6, 1991.* Bull. CAH/ACH 5(2):1-4.)

The workshop in Burlington, organized by Christine Bishop (Canadian Wildlife Service) and Bob Johnson (Metro Toronto Zoo), constituted the first comprehensive attempt to address the declining amphibian phenomenon from a Canadian viewpoint. The problem of amphibian declines has become an urgent concern among Canadian herpetologists. Participation of researchers in universities, governments and private organizations was truly exceptional. This meeting was the largest gathering of Canadian herpetologists in memory.

In her opening remarks, Bishop stated that the aim of the meeting was to create a framework to monitor Canadian amphibian declines and the factors causing them. Johnson, a DAPTF Board of Directors member, highlighted the problem. Speakers representing the various provinces gave depressing status reports on amphibian populations throughout Canada. In many cases, these were anecdotal accounts, although causal relationships between declines and anthropogenic events can be confirmed in all too many cases. Invariably, each speaker referred to the basic lack of knowledge about the amphibians in question: distributions are insufficiently known, causes behind disappearances are uncertain, and habitat surveys are insufficiently detailed.

The introduction of non-native amphibian species and sports fish, mismanagement of wetlands, human intrusion, and logging, have all been identified as damaging to amphibian populations in more than one province. All were cited as being at least partly responsible for population declines in British Columbia. In Nova Scotia fragmented habitats and the resulting inbreeding within many species have produced increased frequencies of albinism and extra-limbed individuals. A well-documented problem is shown by *Rana pipiens*, stemming from the sale of over a million frogs to biological supply companies in the U.S. until die-offs began in 1975. In the middle 1970s, the famous Manitoba frog holes were empty, and despite an eight-year ban on picking frogs their numbers have not much increased.

Natural events, such as droughts, may be in part responsible for declines observed in populations in Saskatchewan. An outbreak of red leg disease in 1976 resulted in many deaths of *Rana pipiens* in Alberta. Recent observations on *Rana catesbeiana* in the Algonquin area showed that the average weight of calling bullfrogs at two separate sites differed significantly. It is unknown whether life history, social structure or harvesting contributed to this phenomenon.

In Ontario and Quebec, amphibian monitoring has been going on for some time. Since 1984, Ontario has received a total of 52,000 records from 2,700 volunteers and has also compiled a bibliography of herpetology including ca. 1,400 references. In Quebec, 5,400 records are reported.

However, it is puzzling that some species seem entirely unaffected. It has been suggested that certain ones may be rebounding from natural, cyclical events and that there may be positive changes observed in many areas within the next few years.

The afternoon talks centered on the monitoring of amphibian populations, including reports of projects that have produced quantitative data. Data show the best estimate is gained by intensive study. This method has actually been employed in a four-year study of Fowler's toads at Long Point. These toads have dramatically increased in numbers since the study began, likely an effect of the water level rise in Lake Erie.

Among other concerns presented was the importance of: experimental design, timing and length of study; preservations of natural conditions of the habitat; measuring both natural and anthropogenic environmental factors; generating a genetic database during monitoring; larval stages in relation to reproductive success and gene flow; pathological conditions present in the populations; and determining the effects of contaminants upon entire populations.

Open discussions began on the second day. It was first determined that the Working Group will be a research coordinating body for investigating the hypothesis that amphibian populations are in decline. If this hypothesis is supported, the group should then seek ways to reverse the declines. It was agreed that

is goal is best served by separately onsidering historical data, intensive monitring studies, and extensive monitoring rojects.

The intensive monitoring group discussed how to approach the monitoring rocess. Life history research must be oncurrent with the monitoring process. he group decided a number of indicator pecies for intensive monitoring, chosen o include as many families as reasonably ossible, in a variety of habitats and ecoystems, and with a range of genetic and norphological variation.

The Canadian working group will be nost active at the provincial level, with Regional Coordinators. Details for each study population and site will be communicated to Eastern and Western Coordinators and the Coordinator for Canada, who will communicate with the IUCN Task Force. this hierarchical setup should keep Coordinators in touch and allow the regions to act both individually and in cooperation with each other and with comparable regional groups in the United States. To facilitate communication to all participants, the CAH/ACH Bulletin was chosen as the official news medium.

The complete final report is to be published in March of 1992 as a Canadian Wildlife Service Technical Report. For further information contact Christine Bishop, Canadian Wildlife Service, Box 5050, Burlington, Ontario L7R 4AG, Canada.

CANADIAN WORKING GROUP

National Co-ordinator — David M. Green (McGill University)

Regional Co-ordinators — Don McAlpine (New Brunswick Museum), for Eastern Canada. Stan Orchard (Royal B.C. Museum) for Western Canada.

Provincial Co-ordinators (to be confirmed) — John Gilhen (Nova Scotia Museum), Nova Scotia; Don McAlpine, New Brunswick and P.E.I; Joel Bonin and Roger Bider (MacDonald College, McGill University), Quebec; Wayne Weller and Mike Oldham (Ontario Ministry of Natural Resources), Ontario; Bill Koonz (Manitoba Department of Natural Resources), Manitoba; Wayne Roberts (University of Alberta), Alberta; Stan Orchard, British Columbia.

Historical Population Trends — Martyn Obbard, Fred Schueller, Wayne Weller, Mike Oldham.

Intensive Monitoring — Mike Berrill, Jim Bogart, Ron Brooks, Francis Cook.

Extensive Monitoring — Bill Freedman

Environmental Contaminants — Christine Bishop

Diseases — Graham Crawshaw

Netherlands Conference

Annie Zuiderwijk, Chair of the Western European Working Group, represented the DAPTF at the International Symposium on the "Impact of Climate Change on Ecosystems and Species", convened in Amersfoort, The Netherlands in December. Experts, invited from different parts of the world, prepared evaluations of regionally important ecosystems. Workshop sessions focused on identifying key factors affecting selected ecosystems, identifying the main responses and determining various rates of change. Publication of reports from the symposium, expected soon, are intended to provide assessments applicable to issues in conservation, species diversity and management of ecosystems.

In the United Kingdom

Tim Halliday, chair of the UK Working Group (and a Task Force Director), reports that action is being taken to establish liaison and collaborative activities with the Western European group. UK sites of amphibian populations known to be "healthy" 10-15 years ago are being identified so that they can again be surveyed during the coming breeding season. A grant proposal for DAP related research has been submitted. Halliday is also arranging an October/November planning meeting.

Australians Take Action

A $47,000 grant from the Australian government was awarded to Michael J. Tyler, a Director of the Task Force, to organize a meeting of amphibian scientists and produce an Action Plan for Australia as a framework for new legislation, and for developing conservation and management goals for the next five years. To obtain an information base for this endeavor, a "Frogwatch" survey is being conducted in which 150 conservation organizations are participating in distribution of 600,000 (sic) questionnaires.

An organizational workshop convened by Tyler met in Canberra, ACT, last July. This initial meeting was attended by a nucleus of 16 representatives from the several States and Territories. The first half of the program addressed broad overviews and individual species case histories, the status of distribution maps, current legislation and the character of native population cycles. The subsequent general discussions dealt with causal agents, the use of museum records, sampling strategies, pathological studies, etc.

As of the present date, the Action Plan has been partly completed. Formal establishment of the Australian Working Group and its participating members is underway.

Reports from U.S. Working Groups

CAL/NEVA

The California/Nevada Working Group met for the first time at Point Reyes National Seashore on February 4, 1992. The group, chaired by Gary Fellers, included 14 representatives from the U.S. National Park Service, U.S. Forest Service, University of Nevada - LasVegas, St. Mary's College, University of California - Davis, California Academy of Sciences, University of California - Los Angeles, California Department of Fish and Game, and U.S. Fish and Wildlife Service.

Each member of the Working Group provided a short summary of their research relating to amphibians. Most of these reports provided compelling evidence for dramatic declines in amphibian populations throughout all or part of a species' range. Though some of the losses resulted from obvious factors (e.g., habitat loss), numerous cases were noted in which declines occurred with no identifiable reason. There appears to be strong evidence that acid precipitation is not the cause of the declines, though it might be acting in concert with other environmental stressors.

The status of the U.S. National Museum of Natural History handbook on monitoring protocols was addressed at some length. Further discussions centered on the need to gather data that are compatible among studies of different species and/or habitats. A form designed for use by the U.S. Fish and Wildlife Service (see report from Rocky Mountains Working Group) was examined in detail with the goal of determining the minimum data that should be collected as part of any amphibian field study.

ROCKY MOUNTAINS

Stephen Corn and Bruce Bury, co-chairs of the Rocky Mountains Working Group, are compiling a database of research activity on amphibians throughout the region. The Working Group is being organized in two tiers: those with current or recently completed research or monitoring programs, and those with more general interests regarding conservation activities. No formal meeting has yet been scheduled; however, the co-chairs participated in the Cal/Neva meetings at Point Reyes, California in early February to coordinate activities of the contiguous regional groups.

Data forms from their recent publication (Bury, R.B. and P.S. Corn. 1991. *Sampling Methods for Amphibians in Streams in the Pacific Northwest*. U.S. Forest Service, Pacific Northwest Re-

search Station. Gen. Tech. Rpt. PNW-GTR-275.) were evaluated during the joint meetings for potential application to all monitoring procedures. The recommended changes will be incorporated in a revised form for further review and consideration of adoption by other Working Groups.

NORTHEAST

The first meeting of the Northeastern Working Group, chaired by Richard Wyman, was held at the Pennsylvania State University on August 9, 1991.

Following a brief introduction regarding the objectives of the DAPTF, the group discussion focused upon the regional organization and development of an action plan. Priorities to be addressed include a survey of all active herpetologists in the region; assembly of all available regional data relating to the status of amphibian populations, identification of particular characteristics of species that would make data as to their presence or absence environmentally significant, and establishing a mechanism for maintaining a long-term monitoring network in the NE region.

The group is also initiating a search for thesis and dissertations that may contain usable density data, and for relevant records that may have been maintained at biological field stations.

Wyman has also generated a questionnaire for a mail survey as to the status of amphibian populations in the region. Copies of this form, which may be applicable for use by other Working Groups, may be obtained by contacting him (see address and telecommunications number, on page 4).

SOUTHEAST

A network of 40 cooperators in Florida, Alabama, Georgia and South Carolina will serve as the communication resource for data on SE US amphibians. Lists of currently recognized taxa are being generated for a status review by the Working Group. Ken Dodd, chair of the Working Group, has assumed the presidency of the SE section of the ASIH and plans to enlarge attention of the herpetological community upon the Task Force activities.

Carolyn Sekerak (M.S. student, Univ. Florida) is finishing her thesis work on the structure of amphibian temporary pond breeding sites. She has taken a position with the U.S. Fish and Wildlife Service in Jackson, MS. Her responsibilities include monitoring the status of amphibians and preparing federal listing proposals for the dusky gopher frog and other amphibian species.

A habitat conservation plan is being developed for the Red Hills salamander. The plan will involve the U.S. Fish and Wildlife Service, The Alabama Natural Heritage Program) is conducting a survey of the Sipsey Fork waterdog (*Necturus* sp.) in Alabama.

Pablo Delis and Henry Mushinsky (Univ. South Florida) are analyzing data on amphibian population fluctuations in Florida sandhill habitats based on 6 years' data.

Carlos Camp (Piedmont College) reports declines in relict populations of *Rana sylvatica* and *Ambystoma maculatum* in northeast Georgia. Wetland habitat alteration is suspected as the cause.

Dodd's paper on the biotic diversity of amphibians and reptiles in a Florida sandhills temporary pond has been accepted in the new journal Biodiversity and Conservation. Population declines due to drought (best guess) are noted, but long-term effects cannot yet be demonstrated.

Amphibian Bioassay as Assessment Tool for Superfund Sites

The U.S. Department of Defense has initiated an interagency agreement (IAG) with the Environmental Research Laboratory - Corvallis and several others to evaluate test procedures involving the effects of several classes of chemicals on amphibians. Initial studies will employ the Frog Embryos Teratogenesis Assay: *Xenopus* (FETAX). The utility of this test in ecological site assessment has been demonstrated at some Superfund sites using in situ exposures of mature amphibian species. Applications of the test procedures may provide information as to possible factors involved in declines of indigenous amphibian species and the use of mature amphibians as bioindicators of the health of wetland ecosystems.

Recent Reports of Declines

The Estonian herpetofauna consists of ten species of amphibians and five species of reptiles, apparently the result of post-glacial immigration from southeast (*Bufo viridis*), south (majority of species) and south-west (*Bufo calamita*). Earlier recorded *Rana ridibunda* and *Emys orbicularis* have become extinct.

From the perspective of distribution and degree of commonness, three groups of herptiles can be identified: rare and vulnerable species (*Triturus cristatus, Bufo calamita, B. viridis, Pelobates fuscus, Lacerta agilis*), less common species with sporadic distribution (*Rana arvalis, R. lessonae, R. esculenta, Anguis fragilis, Natrix natrix*), and common, widely distributed species (*Triturus vulgaris, Rana temporaria, Bufo bufo, Lacerta vivipara, Vipera berus*).

The distributions of *Triturus cristatus, Rana esculenta* (complex), *Pelobates fuscus* and *Lacerta agilis* seem to be relict in nature; some Estonian amphibians represent the northernmost distribution limits of the species (*Bufo calamita, B. viridis, Pelobates fuscus*). Many local populations of herptiles are reported as declining during the past ten to twenty years. (Talvi, T. 1991. *Amphibians and Reptiles of Estonia: list, geographic relationships and current situation.* Abst. 6th Ord. Gen. Mtg. Soc. European Herp., Budapest)

W.S. Osborne, in a recent status report (in litt.) on frog populations in the Australian Capital Territory documents the decline of *Pseudophryne corroboree* and *P. bibroni*, although both species are relatively common in other parts of their ranges. In contrast, there has been a complete disappearance of *Litoria aurea* and *L. raniformis* in the region, while *L. verreauxii* has become rare. Prolonged dry seasons are believed to be a contributing factor; however, the magnitudes of declines are such that other, yet unknown, factors are possibly involved.

In their recent report (Herp. Rev. 22(4):125-128, 1991) E. La Marca and H.P. Reinthaler have noted "drastically diminished" populations among five species of *Atelopus* in the Venezuelan Andes. Deforestation and expanding agrofarming appear to be the dominant factors impacting upon *A. carbonerensis, A. mucubajiensis, A. oxyrhynchus, A. pinangoi* and *A. sorianoi*. Flooding has scoured the montane streamside vegetation, and a high percentage of road kills in other areas are reported. The extent to which collecting may have reduced endemic *Atelopus* is also discussed. This report states (as with many others) that climatic change, pollution, as well as introduced species of plants and fish, are potentially significant factors in these declines and recommends action for both research and conservation.

RIBBIT Croaks

An earlier newsletter, *Ribbit*, was pioneered in the late 1980's by Bruce Bury and Stephen Corn to report on the decline of amphibian populations in the western U.S. Because of administrative constraints but a single issue was released (January, 1989). It will be superseded by FROGLOG beginning with this number.

Donor Support for DAPTF

Operations of the DAPTF would not be possible without the generous sponsorship of numerous organizations and individuals.

Major financial contributions have been awarded by the International Union for the Conservation of Nature (IUCN), Chicago Zoological Society, U.S. Department of State (AID program), Center for Analysis of Environmental Change (CAEC), the Jacob Bleibereu Foundation, Inc. and Frog's Leap Winery.

Support staff, equipment and office space have been provided by the CAEC (a consortium represented by Batelle-Pacific Northwest Laboratorities, U.S. Forest Service-Pacific Northwest , Oregon State University, and the EPA Environmental Research Laboratory) at Corvallis, Oregon.

DAPTF Directors and Chairs

BOARD OF DIRECTORS

William R. Branch
Port Elizabeth Museum
P.O. Box 13147
Humewood, 6013
SOUTH AFRICA (Code 27)
Phone: (041) 561051 Fax: (041) 562175

Alain Dubois
Laboratoire des Reptiles et Amphibians
Musée National d'Histoire Naturelle
25, Rue Cuvier
75005 Paris
FRANCE (Code 33)
Phone: (1) 40 79 34 87 Fax: (1) 40 79 34 84

Timothy R. Halliday
Biology Department
The Open University
Walton Hall
Milton Keynes, MK7 6AA
UNITED KINGDOM (Code 44)
Phone: 0908-653831 Fax: 0908-654167

Sergius L. Kuzmin
Institute of Evolutionary Morphology
and Ecology of Animals
Academy of Sciences
Leninsky Prospekt, 33.
Moscow 117071
RUSSIA

Robert Johnson
Metropolitan Toronto Zoo
P.O. Box 280
West Hill, Ontario, M1E 4R5
CANADA
Phone: (416) 392-5968 Fax: (416) 392-4979

James B. Murphy
Dallas Zoo
621 E. Clarendon Dr.
Dallas, TX 75203. USA
Phone: (214) 670-7573 Fax: (214) 670-7450

*Jaime F. Péfauer
Departamento de Biologia
Facultad de Ciencias
Universidad de los Andes
Merida, VENEZUELA 5101 (Code 58)
Fax: 74 40 1286

George B. Rabb, IUCN/SSC
Chicago Zoological Society
Brookfield, IL 60513, USA
Phone: (708) 485-0263 Fax: (708) 485-3532

*Michael J. Tyler
Department of Zoology
University of Adelaide
GPO Box 498
Adelaide, South Australia 5001
AUSTRALIA (Code 61)
Fax: 8 223 5817

David B. Wake (Chair)
Museum of Vertebrate Zoology
University of California
Berkeley, CA 94720, USA
Phone: (510) 642-3567 Fax: (510) 643-8238

William Winner (Ex officio)
Center for Analysis of Environmental Change
Oregon State University
Corvallis, OR 97333, USA
Phone: (503) 737-1744 Fax: (503) 737-3399

John W. Wright
Museum of Natural History
Los Angeles, CA 90007, USA
Phone: (213) 744-3371

* Also serves as Organizational Chairperson for Regional Working Group.

WORKING GROUP CHAIRS

K.D. Arudpragasam, *Co-Chair, Sri Lanka*
University of Colombo
SRI LANKA

W.R. Breckenridge, *Co-Chair, Sri Lanka*
Department of Zoology
University of Peradeniya
Peradeniya
SRI LANKA

David M. Green, *National Coordinator, Canada*
Redpath Museum
McGill University
Montreal, PQ H3A 2K6
CANADA
Phone: (514) 398-4088

*Kuang-Yang Lue, *Taiwan*
Department of Biology
88 Roosevelt Rd., Sec. 5
11718, Taipei, Taiwan
REPUBLIC OF CHINA (Code 86)
Phone: (02) 932-5234 Fax: (02) 931-3904

Jay M. Savage, *Lower Central America-Costa Rica/Panama*
Department of Biology
University of Miami
Coral Gables, FL 33142, USA
Phone: (305) 284-2130 Fax: (305) 284-3039

Annie Zuiderwijk, *Western Europe*
Institute of Taxonomic Zoology
University of Amsterdam
P.O. Box 4766 - 1009 AT Amsterdam
THE NETHERLANDS (Code 31)
Phone: (20) 5256624 Fax: (20) 5257238

*(Acceptance pending)

U.S. Regions

Ronn Altig, *Mississippi Delta (AR,MS,LA)*
P.O. Drawer GY
Department of Biological Science
Mississippi State University
Mississippi State, MS 39762
Phone: (601) 325-7561 Fax: (601) 325-7939

Andrew R. Blaustein, *Co-Chair, Pacific Northwest* (WA,OR,AL)
Department of Zoology
Oregon State University
Corvallis, OR 97331-2914
Phone: (503) 737-5356

Ronald A. Brandon, *Central (IA,MO,IL,IN,OH)*
Department of Zoology
Southern Illinois University
Carbondale, IL 62901
Phone: (618) 536-2314

Richard C. Bruce, *Appalachian (KY,TN,NC,VA,WV)*
Highlands Biological Station
P.O. Box 580
Highlands, NC 28741
Phone: (704) 526-2602

R. Bruce Bury, *Co-Chair, Rocky Mountains (ID,MT,WY,CO,UT)*
National Ecology Research Center
4512 McMurray Ave.
Fort Collins, CO 80525-3400
Phone: (303) 226-9270

Janalee P. Caldwell, *Southern Plains (OK,TX)*
Oklahoma Museum of Natural History
1335 Asp Ave.
Norman, OK 73019-0606
Phone: (405) 325-4712 Fax: (405) 325-7771

P. Stephen Corn, *Co-Chair, Rocky Mountains (ID,MT,WY,CO,UT)*
National Ecology Research Center
4512 McMurray Ave.
Fort Collins, CO 80525-3400

C. Kenneth Dodd, Jr., *Southeast (AL,GA,FL,SC)*
National Ecology Research Center
412 NE 16th Avenue (Room 250)
Gainesville, FL 32601
Phone: (904) 372-2571 Fax: (904) 374-8080

Gary M. Fellers, *Cal/Neva (CA,NV)*
Point Reyes National Seashore
Point Reyes, CA 94956
Phone: (415) 663-8522

A. Ross Kiester, *Co-Chair, Pacific Northwest (WA,OR,AL)*
USDA Forest Service
3200 SW Jefferson Way
Corvallis, OR 97331
Phone: (503) 750-7269

James E. Platz, *Northern Plains (ND,SD,NE,KS)*
Department of Biology
Creighton University
2500 California St.
Omaha, NE 68178
Fax: (910) 622-0287

Norman J. Scott, Jr., *Southwest (AZ,NM)*
Department of Biology
University of New Mexico
Albuquerque, NM 87131
Phone: (505) 766-3903

Richard L. Wyman, *Northeast (ME,NH,VT,NY,PA,MD,DE,MA,RI,CT,NJ,DC)*
Edmund Niles Huyck Preserve, Inc.
P.O. Box 188
Rensselaerville, NY 12147
Phone: (518) 797-3440

Issue Specific

W. Ronald Heyer, *Monitoring Protocols*
Division of Amphibians and Reptiles
US National Museum of Natural History
(NHB Mail Stop 162)
Washington, DC 20560, USA
Phone: (202) 357-2195

Roy McDiarmid (Will succeed WRH)
National Fish and Wildlife Laboratory
U.S. National Museum of Natural History
(NHB Mail Stop 162)
Washington, D.C. 20560, USA
Phone: (202) 357-2780

James L. Vial, *Documentary Resources*
(Address given below)

FROGLOG

Newsletter of the IUCN/SSC Task Force on Declining Amphibians

Dr. James L. Vial
Task Force Coordinator, *Editor*
Center for Analysis of Environmental Change
EPA Environmental Research Laboratory
200 South West 35th Street
Corvallis, Oregon 97333, USA
(503) 754-4798
Telefax (503) 754-4799

Funding for FROGLOG is provided in part by a special donation from Frog's Leap Vineyards, St. Helena, California 95474, USA