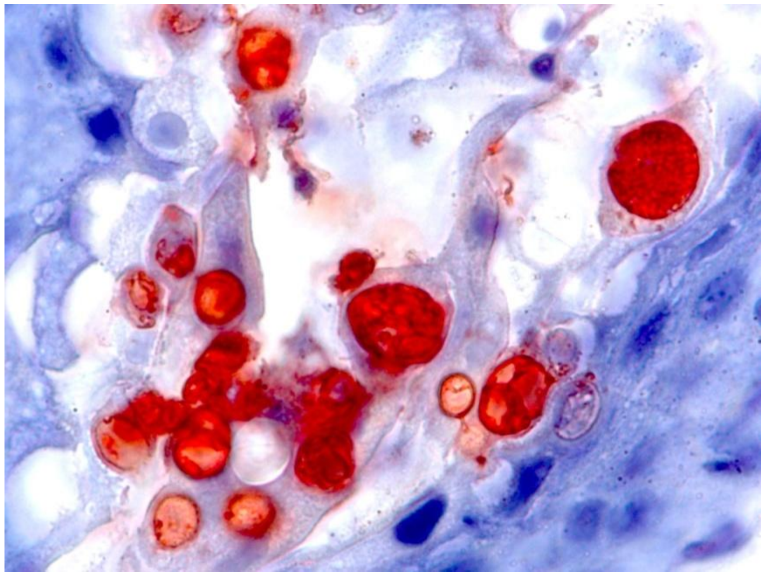
Curso-Seminario
Problemática de la Disminución de las Poblaciones de Anfibios y el Papel de la Medicina Veterinaria



22 y 23 de mayo, 2008

Servicio Nacional de Salud Animal

Heredia, Costa Rica

Informe Final

Organizado por

Financiado por

Arguedas, R., M. Baldi, F. Bolaños, D. Leandro, J. Rodríguez & Y. Matamoros (Eds.) 2008. Curso-Seminario: Problemática de la Disminución de las Poblaciones de Anfibios y el Papel de la Medicina Veterinaria. Informe Final. 22 y 23 de mayo de 2008. Servicio Nacional de Salud Animal, Heredia, Costa Rica.

Foto: *Batrachochytrium dendrobatidis.* Professor Rick Speare (BVSc, MBBS, PhD), James Cook University Townsville, Australia.

Una contribución del Grupo de Especialistas en Conservación y Reproducción (CBSG) SSC/UICN.

CBSG, SSC y UICN, promueven talleres y otros foros para el análisis y consideración de problemas relativos a la conservación, y considera que los informes de estas reuniones son de gran utilidad cuando son distribuidos extensamente.

Las opiniones y recomendaciones expresadas en este informe reflejan los asuntos discutidos y las ideas expresadas por los participantes del taller y no necesariamente refleja la opinión o la posición de CBSG, SSC o UICN.

Copias adicionales de esta publicación se pueden ordenar a través de: IUCN/SSC Conservation Breeding Specialist Group (CBSG), 12101 Johnny Cake Ridge Road, Apple Valley, MN 55124. E-mail: office@cbsg.org Website: www.cbsg.org

The CBSG Conservation Council

These generous contributors make the work of CBSG possible

$50,000 and above

Chicago Zoological Society
-Chairman Sponsor
SeaWorld/Busch Gardens

$20,000 and above

Evenson Design Group
Minnesota Zoological Garden
-Office Sponsor
Omaha's Henry Doorly Zoo
Toronto Zoo
Zoological Society of London

$15,000 and above

Columbus Zoo & Aquarium
Disney's Animal Kingdom
Saint Louis Zoo
Wildlife Conservation Society
World Association of Zoos and Aquariums (WAZA)

$7,000 and above

Australian Regional Association of Zoological Parks and Aquaria (ARAZPA)
Cleveland Zoological Society
Linda Malek
Nan Schaffer
San Diego Zoo
White Oak Conservation Center

$1,000 and above

African Safari Wildlife Park
Albuquerque Biological Park
Al Ain Zoo
Alice D. Andrews
Allwetterzoo Münster
Anne Baker
Association of Zoos and Aquariums (AZA)
Auckland Zoological Park
Audubon Zoo
Bristol Zoo Gardens
British and Irish Association of Zoos and Aquariums (BIAZA)
Calgary Zoological Society
Central Zoo Authority, India
Chester Zoo
Cincinnati Zoo
Colchester Zoo
Copenhagen Zoo
Cotswold Wildlife Park
Detroit Zoological Society
Dickerson Park Zoo
Durrell Wildlife Conservation Trust
El Paso Zoo
Everland Zoo
Fort Wayne Children's Zoo
Fort Worth Zoo
Fota Wildlife Park
Gladys Porter Zoo
Great Plains Zoo & Delbridge Museum
Hong Kong Zoological and Botanical Gardens
Japanese Association of Zoological Gardens and Aquariums (JAZA)
Kansas City Zoo
Laurie Bingaman Lackey
Los Angeles Zoo
Marwell Zoological Park
Milwaukee County Zoological Society
North Carolina Zoological Park
Ocean Park Conservation Foundation
Paignton Zoo
Palm Beach Zoo at Dreher Park
Parco Natura Viva - Italy
Perth Zoo
Philadelphia Zoo
Phoenix Zoo
Pittsburgh Zoo & PPG Aquarium
Point Defiance Zoo & Aquarium
Prudence P. Perry
Ringling Bros., Barnum & Bailey
Robert Lacy
Rotterdam Zoo
Royal Zoological Society Antwerp
Royal Zoological Society Scotland – Edinburgh Zoo
Saitama Children's Zoo
San Antonio Zoo
San Francisco Zoo
Sedgwick County Zoo
Schönbrunner Tiergarten-Zoo Vienna
Taipei Zoo
The Living Desert
Thrigby Hall Wildlife Gardens
Toledo Zoo
Twycross Zoo
Union of German Zoo Directors
Utah's Hogle Zoo
Wassenaar Wildlife Breeding Centre
Wilhelma Zoo
Woodland Park Zoo
Zoo Frankfurt
Zoo Zurich
Zoological Society of Wales-Welsh Mountain Zoo
Zoologischer Garten Köln
Zoologischer Garten Rostock
Zoos South Australia

$500 and above

Aalborg Zoo
Akron Zoological Park
Banham Zoo and Sanctuary
BioSolutions Division of SAIC
Fairchild Tropical Botanic Garden
Friends of the Rosamond Gifford Zoo
General Mills Foundation
Givskud Zoo
Jacksonville Zoo and Gardens
Katey & Mike Pelican
Kerzner International North America, Inc.
Knuthenborg Safaripark
Lincoln Park Zoo
Lisbon Zoo
Little Rock Zoo
Madrid Zoo-Parques Reunidos
Nancy & Pete Killilea
Naturzoo Rheine
Nordens Ark
Odense Zoo
Oregon Zoo
Ouwehands Dierenpark
Riverbanks Zoological Park
Svenska Djurparksföreningen
Wellington Zoo
Wildlife World Zoo
Zoo de Granby
Zoo de la Palmyre

$250 and above

Alice Springs Desert Park
Apenheul Zoo
Arizona - Sonora Desert Museum
Bramble Park Zoo
Brandywine Zoo
David Traylor Zoo of Emporia
Ed Asper
Edward & Marie Plotka
Lee Richardson Zoo
Mark Barone
Montgomery Zoo
Racine Zoological Gardens
Roger Williams Park Zoo
Rolling Hills Wildlife Adventure
Sacramento Zoo
Tautphaus Park Zoo
Tokyo Zoological Park Society
Topeka Zoological Park

$100 and above

African Safari-France
Aquarium of the Bay
Bighorn Institute
Chahinkapa Zoo
Elias Sadalla Filho
International Centre for Birds of Prey
James & Pamela Sebesta
Lincoln Children's Zoo
Lion Country Safari, Inc.
Miami Metrozoo
Miller Park Zoo
Steinhart Aquarium
Steven J. Olson

$50 and above

Alameda Park Zoo
Casey Schwarzkopf
Darmstadt Zoo
Margie Lindberg
Oglebay's Good Children's Zoo
Safari Parc de Peaugres - France
Stiftung Natur-und Artenschutz in den Tropen
Touro Parc - France

Thank you for your support!
30 June 2008

CONTENIDOS

Curso Seminario
Problemática de la Disminución de las Poblaciones de Anfibios y el Papel de la Medicina Veterinaria

INFORME FINAL

Servicio Nacional de Salud Animal
Heredia, Costa Rica
22 y 23 de mayo, 2008

Sección I
Agenda Desarrollada

Problemática de la disminución de las poblaciones de anfibios y el papel de la medicina veterinaria

Curso-Seminario

Auditorio SENASA-MAG

Barreal de Heredia

Costa Rica

22y 23 de mayo, 2008

Agenda

22 de mayo

8:30 a.m.	Registro de los participantes
9:00 a.m.	Palabras de bienvenida **Dr. Oscar Johanning** **Subdirector SENASA-MAG**
9:15 a.m.	Biología de anfibios **MSc. Federico Bolaños** **Escuela de Biología, Universidad de Costa Rica**
10:00 a.m.	Café
10:30 a.m.	Decline de anfibios a nivel global **Dr. Andrew Cunningham** **Sociedad Zoológica de Londres**

12:00 m.d.	Almuerzo
1:00 p.m.	Situación actual de los anfibios de Costa Rica **MSc. Federico Bolaños** **Escuela de Biología, Universidad de Costa Rica**
2:00 p.m.	Acciones de la Estrategia Nacional para la conservación de anfibios **Licda. Yolanda Matamoros** **FUNDAZOO-CBSG Mesoamérica**
2:45 p.m.	Café
3:00 p.m.	Enfermedades de anfibios y diagnóstico (*in situ y ex situ*) **Dr. Andrew Cunningham** **Sociedad Zoológica de Londres**
5:00 p.m.	Cierre

23 de mayo

9:00 a.m.	Enfermedades de anfibios diagnósticas de Costa Rica **Dr. Juan Alberto Morales** **Escuela de Medicina Veterinaria, Universidad Nacional**
10:00 a.m.	Café
10:30 a.m.	Métodos para la toma de muestras en campo y en cautiverio **Dr. Andrew Cunningham** **Sociedad Zoológica de Londres**
12:00 m.d.	Almuerzo

1:00 p.m.	Bioseguridad en condiciones de campo y cautiverio **Dr. Andrew Cunningham** **Sociedad Zoológica de Londres**
5:00 p.m.	Clausura

Curso Seminario
Problemática de la Disminución de las Poblaciones de Anfibios y el Papel de la Medicina Veterinaria

INFORME FINAL

Servicio Nacional de Salud Animal
Heredia, Costa Rica
22 y 23 de mayo, 2008

Sección II
Resumen Ejecutivo

Resumen Ejecutivo

Dada la problemática enfrentada por los anfibios en Costa Rica, cuya situación quedó bien definida en el Taller CAMP I del año 2002 y en el Análisis Global de Anfibios (GAA) de ese mismo año, se vio la necesidad de establecer una Estrategia Nacional de Conservación para el grupo. Esta iniciativa fue realizada por CBSG Mesoamérica y la Escuela de Biología de la Universidad de Costa Rica, contando con el apoyo de Kevin Zippel, especialista en anfibios de CBSG quien a su vez consiguió el apoyo de Sea World, el Zoológico de Chester, el Zoológico Twycross, el Zoológico de Denver y la Universidad de California en Berkely.

En este evento participaron 54 personas, costarricenses y extranjeros, que trabajan con anfibios en el país, tanto investigadores como personas que laboran en herpetarios; autoridades gubernamentales que trabajan en el área de los Recursos Naturales, control de enfermedades en animales y educación pública; estudiantes universitarios y representantes de diversas ONGs interesados en el tema, que representaban un total de 29 instituciones. También fueron invitados especialistas extranjeros que trabajan en proyectos para la conservación de anfibios en sus respectivos países. Cabe destacar la participación de Jay Savage quien dedicó gran parte de su vida académica al estudio se los anfibios y reptiles de Costa Rica, y de las dos personas con los mejores conocimientos en los dos órdenes de anfibios menos conocidos, David Wake quien ha estudiado las salamandras y Marvelee Wake los cecilios. La reunión tuvo lugar en el Parque Zoológico y Jardín Botánico Nacional Simón Bolívar del 28 al 30 de setiembre y el 1 de octubre del año 2006.

Con el fin de resolver los problemas encontrados, los participantes propusieron varios objetivos y acciones, de los cuales los siguientes estaban en relación directa con el campo de la medicina veterinaria:

Recopilar información sobre poblaciones de anfibios, manejo en cautiverio, nutrición, enfermedad, medicina veterinaria
Establecer una red de investigadores a nivel nacional e internacional
Establecer programas multidisciplinarios de investigación sobre nutrición, manejo en cautiverio, enfermedades y veterinaria en anfibios..
Desarrollar investigaciones de apoyo al programa de manejo *Ex situ*, especialmente a programas de reintroducción, traslocaciones y repoblaciones.
Establecer un programa de respuesta a las amenazas identificadas. Evaluar el impacto de factores de amenaza sobre las poblaciones de anfibios, principalmente toxicología, epidemiología,.
Definir protocolos de respuesta a los factores de amenaza para generar un plan de acción para cada especie.
Desarrollar planes de respuesta rápida para los taxones de estatus conocidos y disponibles.
Llevar a cabo el manejo de las especies creando programas como: salud, nutrición, cuarentena, manejo genético entre otros.

Formar un grupo entrenado y capacitado para detección de enfermedades, contaminantes y otros.
Capacitar al personal encargado del manejo en cautiverio. . Organizar un curso en Costa Rica. El curso se debe continuar dando periódicamente por capacitadores entrenados.
Organizar la comunidad que trabaja *ex situ* a nivel local, nacional, regional. Participar a los representantes de gobiernos, universidades y zoológicos.
Involucrar al gobierno en toda la estrategia. Presentación de la Estrategia. elaborar borrador de protocolo y que los protocolos sean avalados por las autoridades (MINAE y Universidades).
Fomentar a los estudiantes universitarios para que realicen estudios sobre especies de anfibios en instituciones autorizadas para el manejo *ex situ*.

Para darle continuidad a estas acciones de la Estrategia, los médicos veterinarios Randall Arguedas de la Fundación pro Zoológicos, Mario Baldi de la Escuela de Medicina Veterinaria de la Universidad Nacional, y Danilo Leandro del Servicio Nacional de Sanidad Animal del Ministerio de Agricultura y Ganadería, en conjunto con el herpetólogo Federico Bolaños de la Escuela de Biología de la Universidad de Costa rica y con el apoyo de CBSG Mesoamérica, propusieron el curso "Problemática de la disminución de anfibios y el papel del médico veterinario" dirigido a médicos veterinarios, estudiantes de medicina veterinaria, microbiólogos, nutricionistas y biólogos expertos en el tema, con el fin de informarles de las amenazas que tienen este tipo de animales, los problemas médicos que les afectan y las medidas de bioseguridad que hay que tomar cuando se mantienen en cautiverio.

Este curso se realizó en las instalaciones del Servicio Nacional de Sanidad Animal (SENASA) del Ministerio de Agricultura y Ganadería los días 22 y 23 de mayo del 2008 y fue financiado por SENASA y American Wildlife Conservation Foundation, INC. Participaron 50 personas provenientes de 10 instituciones nacionales.

Los expositores fueron el Dr. Andrew Cunnigham de la Academia Zoológica de Londres, el MSc. Federico Bolaños, herpetólogo de la Escuela de Biología de la Universidad de Costa Rica, el Dr. Juan Alberto Morales, patólogo de la Escuela de Medicina Veterinaria de la Universidad Nacional y la Licda. Yolanda Matamoros de FUNDAZOO-CBSGMesoamérica.

El Dr. Cunnigham habló sobre la perspectiva global de la declinación de las poblaciones de anfibios, agentes infecciosos y parasitarios que afectan a los anfibios, las técnicas de muestreo de la quitridiomicosis en anfibios, la bioseguridad y la prevención de la dispersión de enfermedad con especial consideración al hongo quitridio (*Batrachochytrium dendobatridis*) y de las enfermedades infecciosas de los anfibios.

El MSc. Federico Bolaños dio dos conferencias, una sobre la Diversidad de las poblaciones de los anfibios de Costa Rica, y otra sobre la Disminución de las poblaciones de los anfibios de Costa Rica.

El Dr Juan Alberto Morales expuso sus experiencias en la patología de los anfibios que habían llegado a su laboratorio de la Escuela de Medicina Veterinaria de la Universidad Nacional.

La Licda. Yolanda Matamoros habló sobre la Estrategia de Costa Rica para la Conservación de los anfibios y los avances en su implementación.

Los participantes concluyeron que la problemática de la disminución de las poblaciones de anfibios tiene un origen multifactorial, y como tal, debe ser abordada desde un plano multidisciplinario. Como resultado de esto, se reconoció la necesidad de establecer grupos interdisciplinarios con la intención de entender mejor las razones del decline de anfibios. Se destacó además la necesidad de que Costa Rica realice mayores esfuerzos en llevar a cabo más investigaciones sobre este tema y a la vez estimular una mayor participación de médicos veterinarios y estudiantes. Se reforzó la idea de una mayor capacitación en el uso de herramientas diagnósticas y de protocolos a seguir para evitar dispersión de agentes potencialmente peligrosos para los anfibios. Se hizo especial referencia a la necesidad de una mayor difusión de esta problemática entre la comunidad científica del país y el público en general.

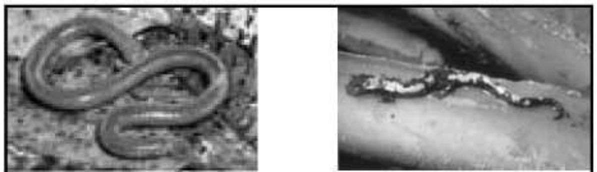
Al final de la actividad los participantes indicaron cuáles podrían ser sus aportes a la conservación de los anfibios, información que se retomará para establecer una estrategia de trabajo en el campo de la medicina veterinaria.

Curso Seminario
Problemática de la Disminución de las Poblaciones de Anfibios y el Papel de la Medicina Veterinaria

INFORME FINAL

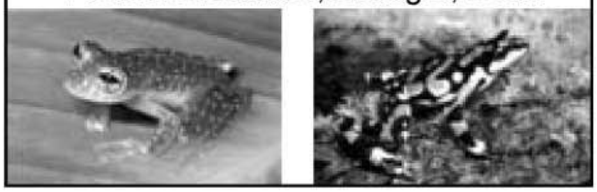
Servicio Nacional de Salud Animal
Heredia, Costa Rica
22 y 23 de mayo, 2008

Sección III
Presentaciones de Federico Bolaños



Anfibios de Costa Rica: Diversidad

Federico Bolaños, Biología, UCR



Gymnophiona: Caeciliidae [3](7)

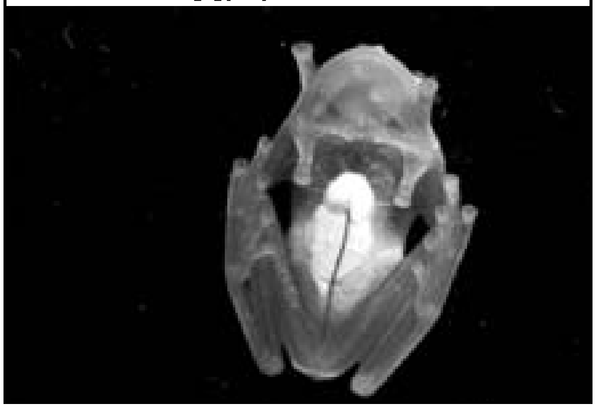
Caudata: Plethodontidae 1[3](42)

Anura 14[42](139)

Aromobatidae: *Allobates talamancae*

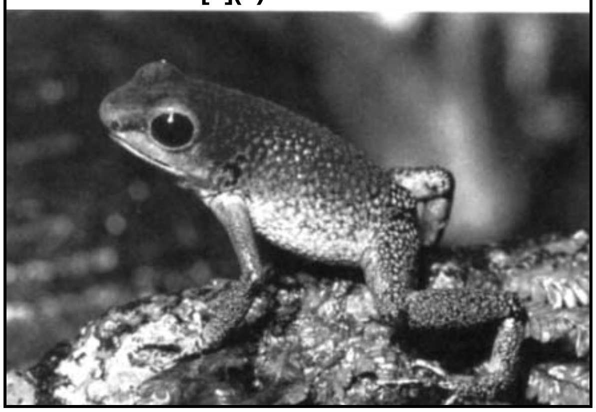
Bufonidae [5](17)

Centrolenidae [3](13)



Craugastoridae: *Craugastor* (28)

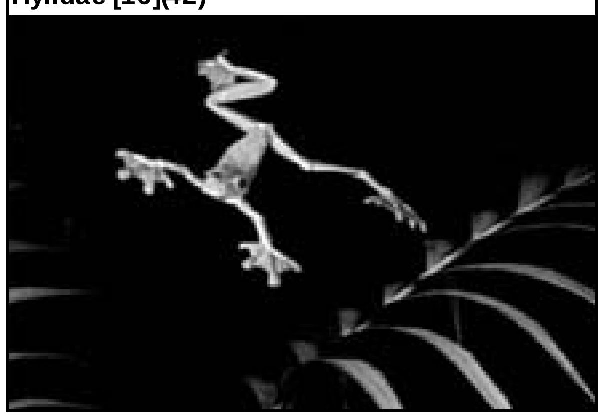
Dendrobatidae [5](8)



Eleutherodactylidae [2](5)

Hemiphractidae [1](1): *Gastrotheca cornuta*

Hylidae [16](42)



Leiuperidae [1](1): *Engystomops pustulosus*

Leptodactylidae [1](5): *Leptodactylus* (5)

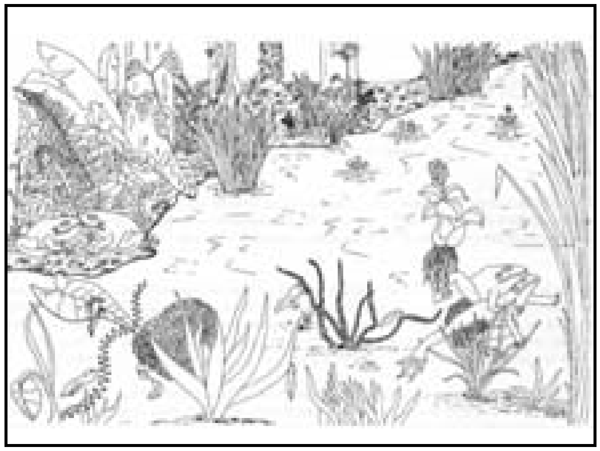
Microhylidae [3](3)

Ranidae [1](6): *Lithobates* (6)

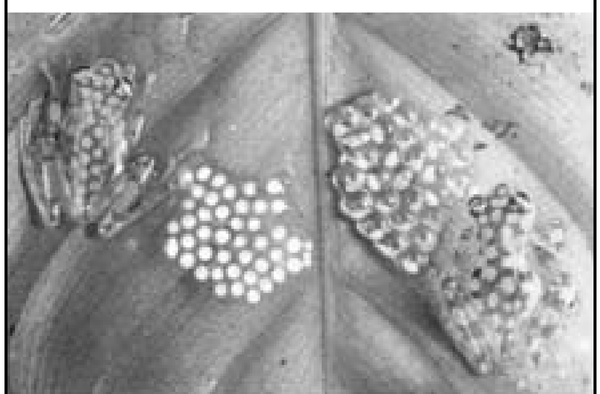
Rhinophrynidae [1](1): *Rhinophrynus dorsalis*

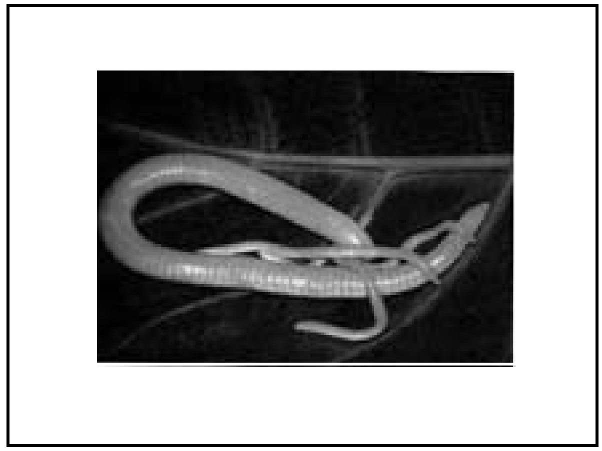
Strabomantidae [2](9)

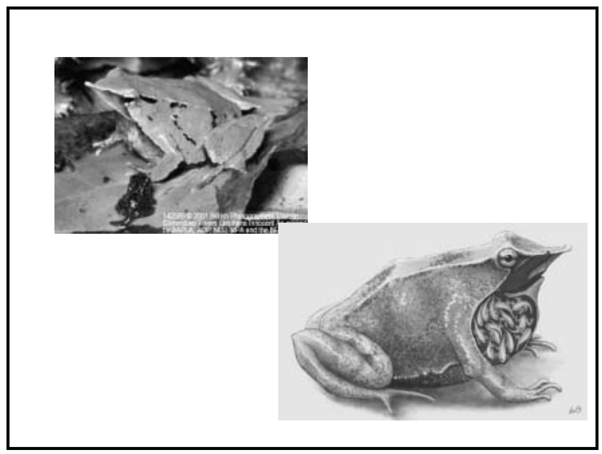
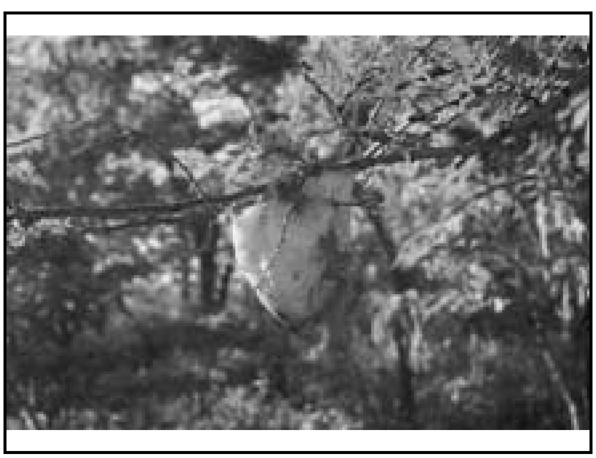
Taxa	Familias	Géneros	Especies
Caudata	1	3	42
Plethodontidae		3	42
Gymnophiona	1	3	7
Caeciliidae		3	7
Anura	14	42	139
Aromobatidae		1	1
Bufonidae		5	17
Centrolenidae		3	13
Craugastoridae		1	28
Dendrobatidae		4	7
Eleutherodactylidae		2	5
Hemiphractidae		1	1
Hylidae		16	42
Leiuperidae		1	1
Leptodactylidae		1	5
Microhylidae		3	3
Ranidae		1	6
Rhinophrynidae		1	1
Strabomantidae		2	9
Total	16	48	188

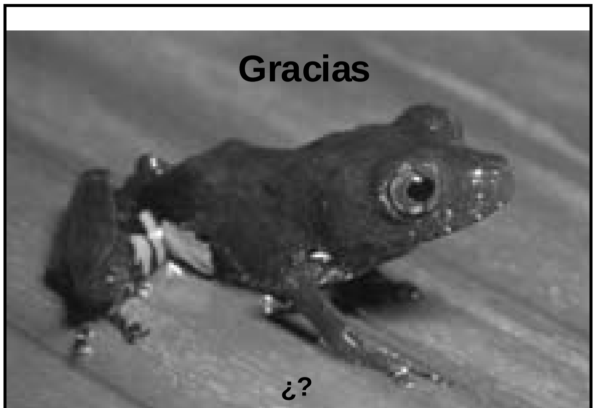


Taxón	Larva	Des. Dir	Vivíparo	Duda	Total
Anura	93	43		3	139
Aromobatidae	1				1
Bufonidae	14			3	17
Centrolenidae	13				13
Craugastoridae		28			28
Dendrobatidae	7				7
Eleutherodactylidae		5			5
Hemiphractidae		1			1
Hylidae	42				42
Leiuperidae	1				1
Leptodactylidae	5				5
Microhylidae	3				3
Ranidae	6				6
Rhinophrynidae	1				1
Strabomantidae		9			9
Caudata					
Plethodontidae		42			42
Gymnophiona					
Caeciliidae			7		7
Total	93	85	7	3	188

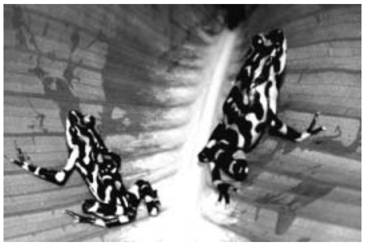


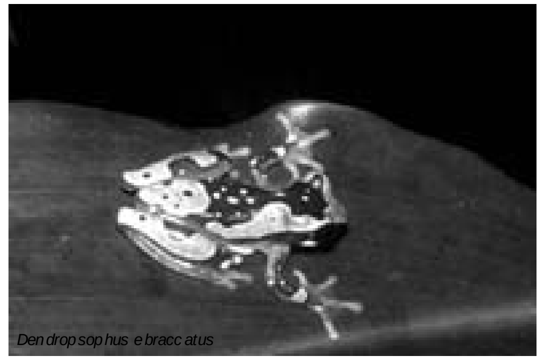


Gracias
¿?

Disminución de Poblaciones de Anfibios en Costa Rica



Federico Bolaños [1], Gerardo Chaves [1], Robert Puschendorf [1,2]
[1] Universidad de Costa Rica, [2] James Cook University

Ranas y Sapos (Anura)



Dendropsophus ebraccatus

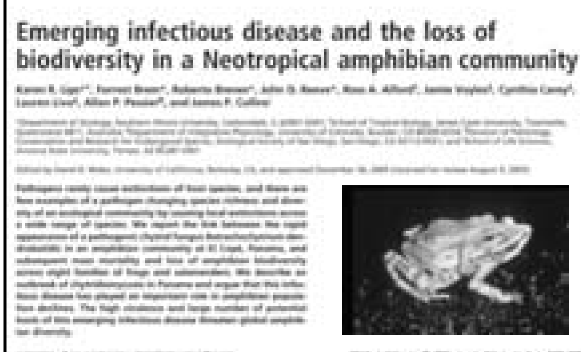
Salamandras (Caudata)

toglossa colonnea

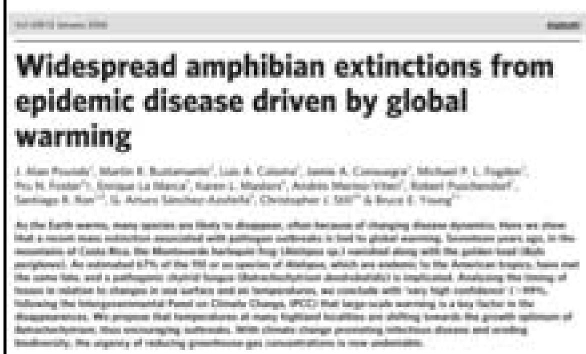
Cecilios (Gymnophiona)

Gymnopis multiplicata

- Disminuciones reportadas desde 1989
  - Monteverde (Crump *et al.* 1992)
  - San Ramón (Bolaños & Ehmcke 1992)
  - Las Tablas (Lips 1998)
- Causas
  - Climate (Pounds & Crump 1994, …)
  - Pathogens (Lips 2003)
  - Synergistic (Pounds *et al.* 2006)



Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community



Widespread amphibian extinctions from epidemic disease driven by global warming

The amphibian chytrid fungus along an altitudinal transect before the first reported declines in Costa Rica

Resultados Importantes

- El Chytridio se encontró en 5.94% de los ejemplares.
- en 30 % de la especies observadas.
- Se encontró en:
  - Especies con disminuciones: *Atelopus varius*, *Rana vibicaria*, *Duellmanohyla uranochroa*.
  - Sobrevivientes de zonas altas: *Hyla pseudopuma*, *Eleutherodactylus podiciferus*.
  - Especies comunes de zonas bajas: *Eleutherodactylus fitzingeri*, *Eleutherodactylus bransfordii*, *Eleutherodactylus talamancae*, *Dendrobates pumilio*

Cerro Chompipe (Río Las Vueltas)

- 1983
  - **Una visita de un día**
  - – 15 especies de ranas
  - – 1 especie de salamandra
- 1994-1995
  - **Visita Mensual**
  - – 3 especies de ranas
  - – Ninguna salamandra

Número de Ranas y Sapos en Costa Rica

	No. Especies
Sin Información	**27**
Extinciones Locales	**23**
Vistas con Frecuencia	**81**
Total	**131**

Especies con Extinciones Locales

BUFONIDAE	*Isthmohyla pictipes*
Atelopus chiriquiensis	*Isthmohyla rivularis*
Atelopus senex	*Isthmohyla tica*
Atelopus varius	*Hylomantis lemur*
Ollotis fastidiosus	LEPTODACTYLIDAE
Ollotis holdridgei	*Craugastor andi*
Ollotis periglenes	*Craugastor angelicus*
DENDROBATIDAE	*Craugastor escoses*
Silverstoneia nubicola	*Craugastor fleischmanni*
HYLIDAE	*Craugastor ranoides*
Agalychnis annae	*Pristimantis caryophyllaceus*
Duellmanohyla uranochroa	RANIDAE
Isthmohyla angustilineata	*Lithobates vibicarius*
Isthmohyla calypsa	*Lithobates warschewitschii*

Especies con Distribuciones Restringidas

BUFONIDAE	*Isthmohyla pictipes*
Atelopus chiriquiensis	*Isthmohyla rivularis*
Atelopus senex	*Isthmohyla tica*
Atelopus varius	*Hylomantis lemur*
Ollotis fastidiosus	LEPTODACTYLIDAE
Ollotis holdridgei	*Craugastor andi*
Ollotis periglenes	*Craugastor angelicus*
DENDROBATIDAE	*Craugastor escoses*
Silverstoneia nubicola	*Craugastor fleischmanni*
HYLIDAE	*Craugastor ranoides*
Agalychnis annae	*Pristimantis caryophyllaceus*
Duellmanohyla uranochroa	RANIDAE
Isthmohyla angustilineata	*Lithobates vibicarius*
Isthmohyla calypsa	*Lithobates warschewitschii*

Ollotis periglenes

Especies Posiblemente Extintas

BUFONIDAE	*Isthmohyla pictipes*
Atelopus chiriquiensis	*Isthmohyla rivularis*
Atelopus senex	*Isthmohyla tica*
Atelopus varius	*Hylomantis lemur*
Ollotis fastidiosus	LEPTODACTYLIDAE
Ollotis holdridgei	*Craugastor andi*
Ollotis periglenes	*Craugastor angelicus*
DENDROBATIDAE	*Craugastor escoses*
Silverstoneia nubicola	*Craugastor fleischmanni*
HYLIDAE	*Craugastor ranoides*
Agalychnis annae	*Pristimantis caryophyllaceus*
Duellmanohyla uranochroa	RANIDAE
Isthmohyla angustilineata	*Lithobates vibicarius*
Isthmohyla calypsa	*Lithobates warschewitschii*

Especies Posiblemente Extintas

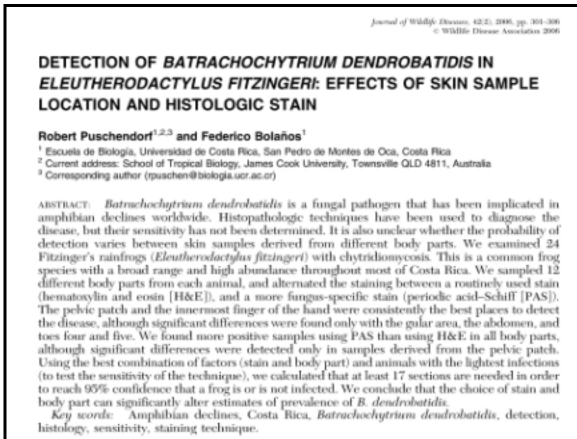
BUFONIDAE	*Isthmohyla pictipes*
Atelopus chiriquiensis	*Isthmohyla rivularis*
Atelopus senex	*Isthmohyla tica*
Atelopus varius	*Hylomantis lemur*
Ollotis fastidiosus	LEPTODACTYLIDAE
Ollotis holdridgei	*Craugastor andi*
Ollotis periglenes	*Craugastor angelicus*
DENDROBATIDAE	*Craugastor escoses*
Silverstoneia nubicola	*Craugastor fleischmanni*
HYLIDAE	*Craugastor ranoides*
Agalychnis annae	*Pristimantis caryophyllaceus*
Duellmanohyla uranochroa	RANIDAE
Isthmohyla angustilineata	*Lithobates vibicarius*
Isthmohyla calypsa	*Lithobates warschewitschii*

Atelopus varius

Silverstoneia nubicola

Isthmohyla angustilineata

Lithobates vibicarius



Journal of Wildlife Diseases, 42(2), 2006, pp. 301–306

DETECTION OF *BATRACHOCHYTRIUM DENDROBATIDIS* IN *ELEUTHERODACTYLUS FITZINGERI*: EFFECTS OF SKIN SAMPLE LOCATION AND HISTOLOGIC STAIN

Robert Puschendorf[1,2,3] and Federico Bolaños[1]
[1] Escuela de Biología, Universidad de Costa Rica, San Pedro de Montes de Oca, Costa Rica
[2] Current address: School of Tropical Biology, James Cook University, Townsville QLD 4811, Australia
[3] Corresponding author (rpuschen@biologia.ucr.ac.cr)

ABSTRACT: *Batrachochytrium dendrobatidis* is a fungal pathogen that has been implicated in amphibian declines worldwide. Histopathologic techniques have been used to diagnose the disease, but their sensitivity has not been determined. It is also unclear whether the probability of detection varies between skin samples derived from different body parts. We examined 24 Fitzinger's rainfrogs (*Eleutherodactylus fitzingeri*) with chytridiomycosis. This is a common frog species with a broad range and high abundance throughout most of Costa Rica. We sampled 12 different body parts from each animal, and alternated the staining between a routinely used stain (hematoxylin and eosin [H&E]), and a more fungus-specific stain (periodic acid–Schiff [PAS]). The pelvic patch and the innermost finger of the hand were consistently the best places to detect the disease, although significant differences were found only with the gular area, the abdomen, and toes four and five. We found more positive samples using PAS than using H&E in all body parts, although significant differences were detected only in samples derived from the pelvic patch. Using the best combination of factors (stain and body part) and animals with the lightest infections (to test the sensitivity of the technique), we calculated that at least 17 sections are needed in order to reach 95% confidence that a frog is or is not infected. We conclude that the choice of stain and body part can significantly alter estimates of prevalence of *B. dendrobatidis*.

Key words: Amphibian declines, Costa Rica, *Batrachochytrium dendrobatidis*, detection, histology, sensitivity, staining technique.

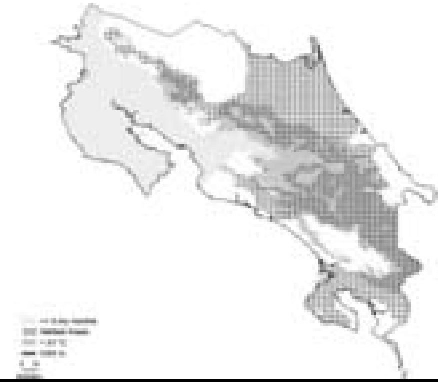
Distribution of *C. fitzingeri*

Métodos

- 349 especímenes de *C. fitzingeri*
  - 177 vertiente Pacífica
  - 172 vertiente Atlántica
  - Desde el nivel del mar hasta 2500 m
- La infección del hongo fue evaluada de acuerdo a humedad y altitud

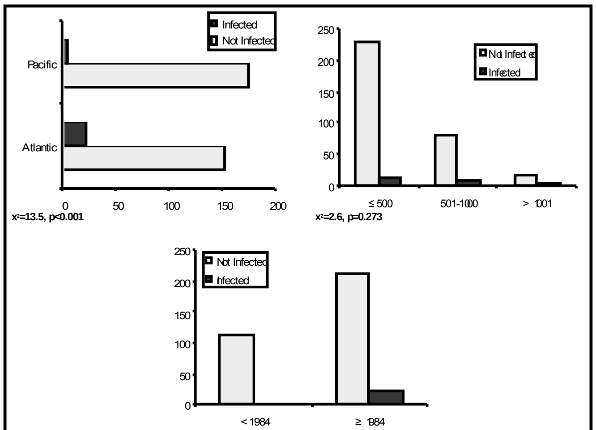
Esfuerzo de Muestreo en *C. fitzingeri*

Temperatura y Humedad

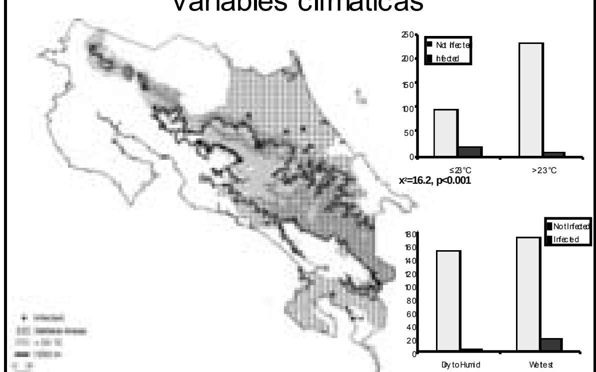


Distribución de la infección en *E. fitzingeri*

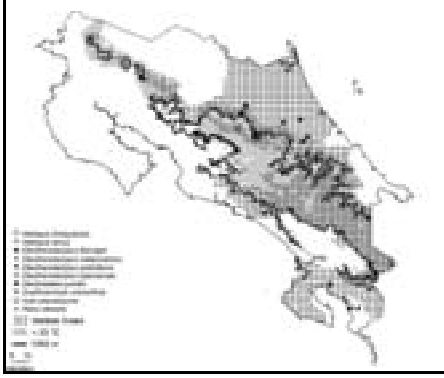
- 349 especímenes (24, 6.9% infectados)
- 132 localidades
  - –Atlántico 58 (44%)
    - 12 con el hongo (86%)
  - –Pacífico 74 (56 %)
    - 2 con el hongo (14 %)



Infección de *C. fitzingeri* de acuerdo a variables climáticas



Infección en todas las especies



Conclusiones

- El hongo en *C. fitzingeri* para Costa Rica se encuentra solo en lugares con mucha humedad, sin estación seca marcada.
- Incluyendo la información de otras especies donde se ha encontrado el hongo sigue restringido a sitios húmedos pero incluyendo ejemplos de sitios más fríos.


POLICYFORUM

Confronting Amphibian Declines and Extinctions

28 de Setiembre – 1 de Octubre 2006
Taller "La Estrategia de Conservación de Anfibios de Costa Rica"

Riesgo de Extinción	GAA	Taller
Extincto (EX)	1	3
En Peligro Crítico (CR)	20	22
En Peligro (EN)	22	22
Casi Amenazado (NT)	9	11
Vulnerable (VU)	20	15
Preocupación Menor (LC)	90	100
Datos Deficientes (DD)	22	15
Total	184	188

		GAA								
		EX	CR	EN	VU	NT	LC	DD	NE	Total
UCR	EX	1	2							3
	CR		18	2	1			1		22
	EN			18	1		1	2	1	23
	VU			1	9			3	2	15
	NT			1	5	2	2			10
	LC				4	6	87	3		100
	DD					1		13	1	15
	Total	1	20	22	20	9	90	22	4	188

Bufonidae	Leptodactylidae
Atelopus chiriquiensis	*Craugastor andi*
Atelopus senex	*Craugastor angelicus*
Atelopus varius	*Craugastor catalinae*
Ollotis fastidiosus	*Craugastor escoses*
Ollotis holdridgei	*Craugastor fleishmanni*
Ollotis periglenes	*Craugastor ranoides*
Hylidae	*Craugastor taurus*
Duellmanohyla uranochroa	**Ranidae**
Hylomantis lemur	*Lithobates vibicarius*
Hyloscirtus colymba	**Plethodontidae**
Isthmohyla angustilineata	*Nototriton major*
Isthmohyla calypsa	*Oedipina altura*
Isthmohyla debilis	*Oedipina paucidentata*
Isthmohyla rivularis	
Isthmohyla tica	

Especies Posiblemente Extintas

BUFONIDAE	*Isthmohyla calypsa*
Atelopus chiriquiensis	*Isthmohyla pictipes*
Atelopus senex	*Isthmohyla rivularis*
Atelopus varius	*Isthmohyla tica*
Ollotis fastidiosus	**LEPTODACTYLIDAE**
Ollotis holdridgei	*Craugastor andi*
Ollotis periglenes	*Craugastor angelicus*
DENDROBATIDAE	*Craugastor escoses*
Silverstoneia nubicola	*Craugastor fleischmanni*
HYLIDAE	*Craugastor ranoides*
Agalychnis annae	*Pristimantis caryophyllaceus*
Duellmanohyla uranochroa	**RANIDAE**
Hylomantis lemur	*Lithobates vibicarius*
Isthmohyla angustilineata	*Lithobates warschewitschii*

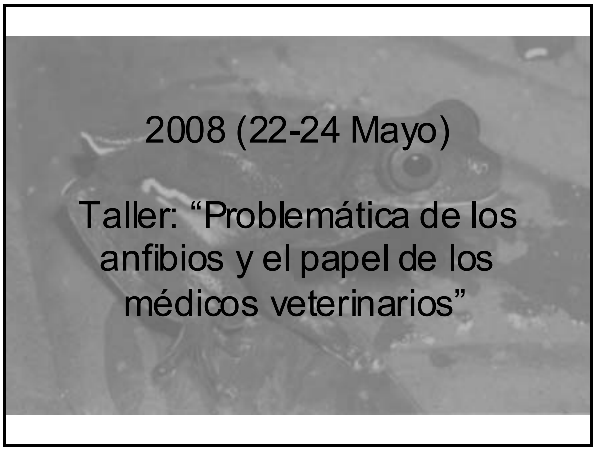
Bufonidae	Leptodactylidae
Atelopus chiriquiensis	*Craugastor andi*
Atelopus senex	*Craugastor angelicus*
Atelopus varius	*Craugastor catalinae*
Ollotis fastidiosus	*Craugastor escoses*
Ollotis holdridgei	*Craugastor fleishmanni*
Ollotis periglenes	*Craugastor ranoides*
Hylidae	*Craugastor taurus*
Duellmanohyla uranochroa	**Ranidae**
Hylomantis lemur	*Lithobates vibicarius*
Hyloscirtus colymba	**Plethodontidae**
Isthmohyla angustilineata	*Nototriton major*
Isthmohyla calypsa	*Oedipina altura*
Isthmohyla debilis	*Oedipina paucidentata*
Isthmohyla rivularis	
Isthmohyla tica	

Bufonidae	Leptodactylidae
Atelopus chiriquiensis	*Craugastor andi*
Atelopus senex	*Craugastor angelicus*
Atelopus varius	*Craugastor catalinae*
Ollotis fastidiosus	*Craugastor escoses*
Ollotis holdridgei	*Craugastor fleishmanni*
Ollotis periglenes	*Craugastor ranoides*
Hylidae	*Craugastor taurus*
Duellmanohyla uranochroa	**Ranidae**
Hylomantis lemur	*Lithobates vibicarius*
Hyloscirtus colymba	**Plethodontidae**
Isthmohyla angustilineata	*Nototriton major*
Isthmohyla calypsa	*Oedipina altura*
Isthmohyla debilis	*Oedipina paucidentata*
Isthmohyla rivularis	
Isthmohyla tica	

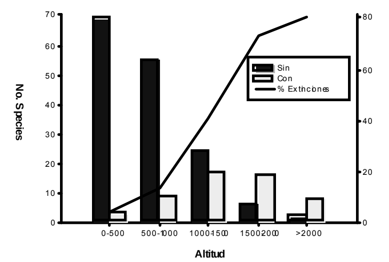
Hylidae
- *Duellmanohyla uranochroa*
- *Hylomantis lemur*
- *Isthmohyla angustilineata*
- *Isthmohyla pictipes*
- *Isthmohyla rivularis*
- *Ptychohyla legleri*

Plethodontidae
- *Bolitoglossa pesrubra*
- *Nototriton abscondens*
- *Oedipina uniformis*

Caeciliidae
- *Gymnopis multiplicata*



Número y Porcentage de Especies con Extinciones Locales según Altitud




Amphibian and reptile declines over 35 years at La Selva, Costa Rica

Curso Seminario
Problemática de la Disminución de las Poblaciones de Anfibios y el Papel de la Medicina Veterinaria

INFORME FINAL

Servicio Nacional de Salud Animal
Heredia, Costa Rica
22 y 23 de mayo, 2008

Sección IV
Presentación de Yolanda Matamoros

Estrategia de Conservación de Anfibios de Costa Rica

Yolanda Matamoros
CBSG Mesoamérica/FUNDAZOO

Este trabajo se ha realizado dentro de la iniciativa AARK

- Esta iniciativa se da en respuesta al Análisis Mundial de los Anfibios (GAA), en la que varias instituciones se unieron para tratar de alertar al público sobre la **crisis mundial de los anfibios** y proponer acciones para mitigarla.

Grupos de trabajo

- Conservación in situ
- Conservación ex situ
- Educación

Conservación *in situ*

- CAMP 2002
- GAA 2006
- Trabajo de campo, lo que permitió bajar de
- 76 especies con información desconocida (DD) en el CAMP I a 15 en el CAMP II,

y aumentar el conocimiento de la distribución de todas las 188 especies estudiadas.

- CAMP II 2007

Giras a Talamanca

- Escuela de Biología UCR
- INBIO
- British Museum
- Resultados:
- 7 especies nuevas de salamandras y 6 especies nuevas de ranas

Giras a Talamanca

- No se encontraron especies de *Atelopus*
- Se encontraron muy pocas especies de la familia Hylidae
- Tampoco especies del grupo rugulosus del género *Craugastor*, especies que deberían estar en esta zona.

Conservación *in situ*

- Finalización del informe del CAMP II.
- En un mes será distribuído
- El grupo continúa las giras de campo a Panamá.

Educación

- Alberto Solano, Eduardo Boza y Jairo Moya elaboraron 15 folletos con información sobre las especies de las diferentes familias de anfibios que hay en Costa Rica. Están buscando fondos para la publicación de 1000 ejemplares de cada folleto.

Educación

- FUNDAZOO está planeando en conjunto con la Asesoría Nacional de Biología del MEP, la Escuela de Biología de la UCR y el Instituto Metereológico Nacional tres cursos sobre Biología de anfibios y cambio climático. Este curso será dirigido a público adulto (mayores de 15 años) y habrán dos modalidades, uno regular los viernes de Agosto a Noviembre y dos intensivos durante las dos primeras semanas de diciembre. Este curso será apoyado también por el Colegio de Licenciados y Profesores.

Exhibición itinerante

- Ya está terminado el trabajo de diseño. Falta únicamente una revisión final por Federico Bolaños para tener la propuesta de financiamiento.

Banner de presentación

Conservación *ex situ*

- CBSG Mesoamérica y FUNDAZOO presentaron una propuesta a la AZA para buscar fondos para un Curso de capacitación y un Taller para establecer una estrategia de conservación de anfibios para Mesoamérica.
- El Zoológico de Santa Ana, California le dio el respaldo.

Curso sobre medicina veterinaria

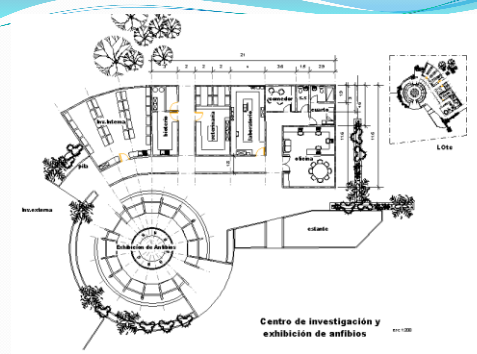
- Organización de este curso-taller.

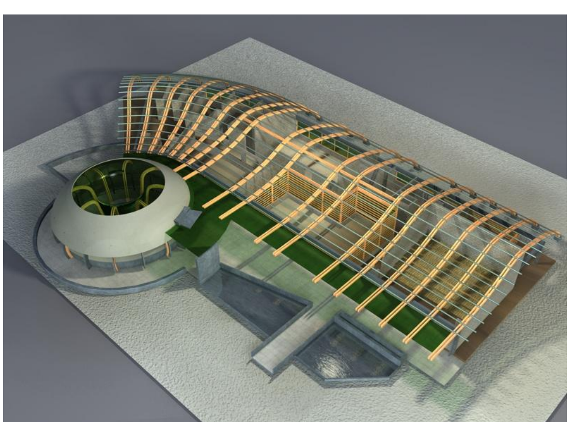
Taller para la priorización de especies

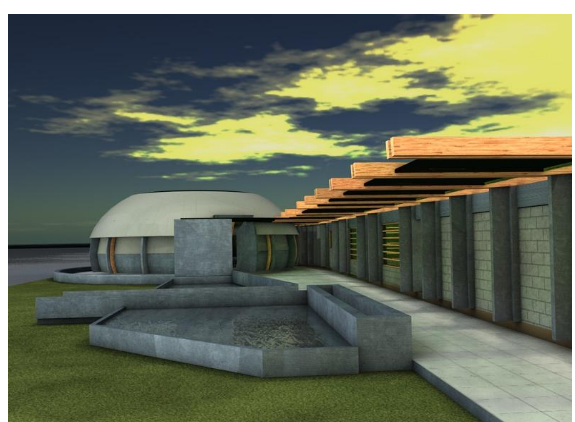
- En el mes de Noviembre del 2007, se realizó un taller en el que se priorizaron las 188 especies de anfibios para determinar cuales estaban listas para que se iniciara un programa en cautiverio.

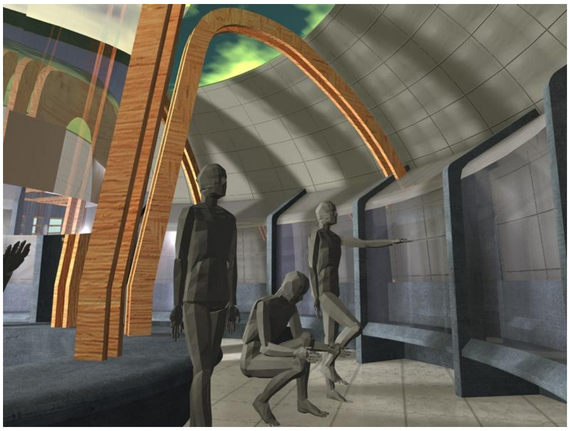
Planos del Centro Nacional de Conservación e Investigación de Anfibios

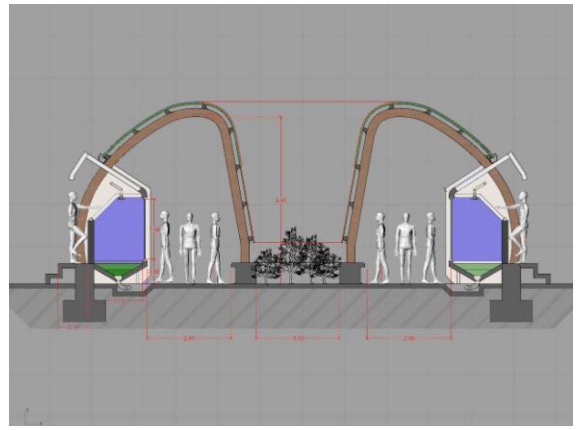
- En el Centro de Conservación Santa Ana se construirá el Centro Nacional de Conservación e Investigación de Anfibios.
- El anteproyecto lo desarrollaron estudiantes de TCU de la Universidad de Costa Rica, según los requerimientos de los integrantes del Grupo de trabajo.

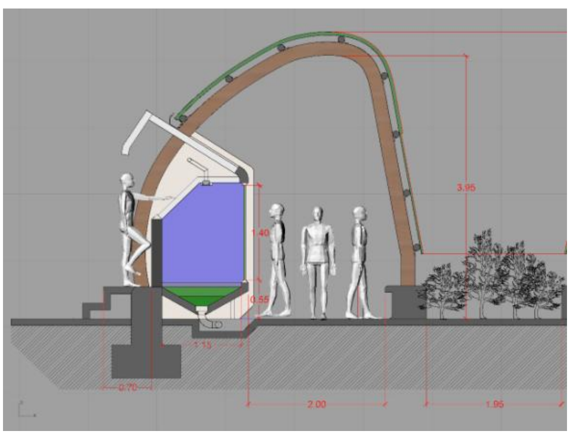


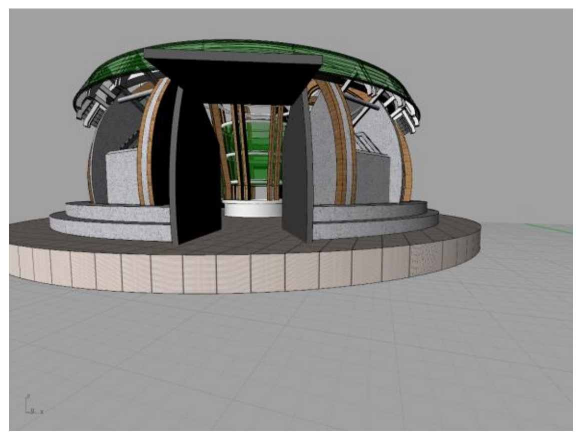


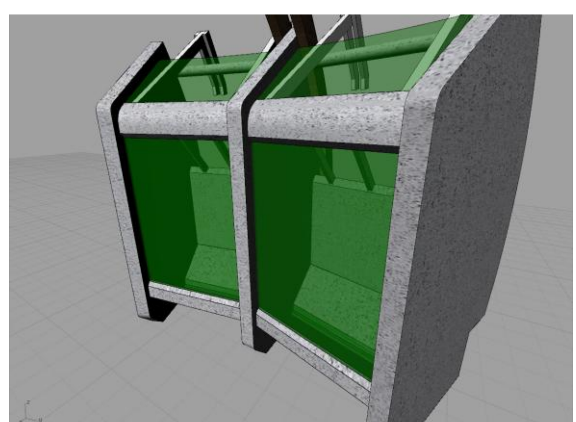


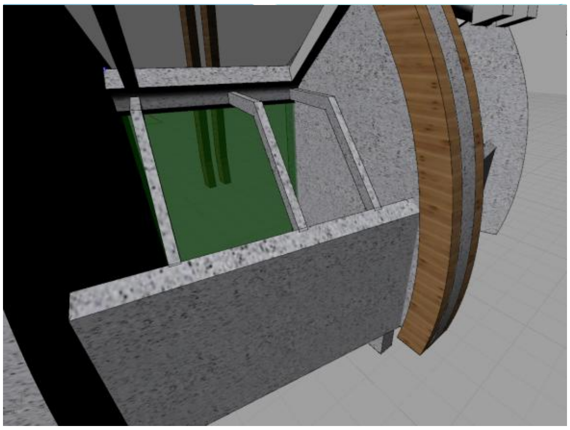


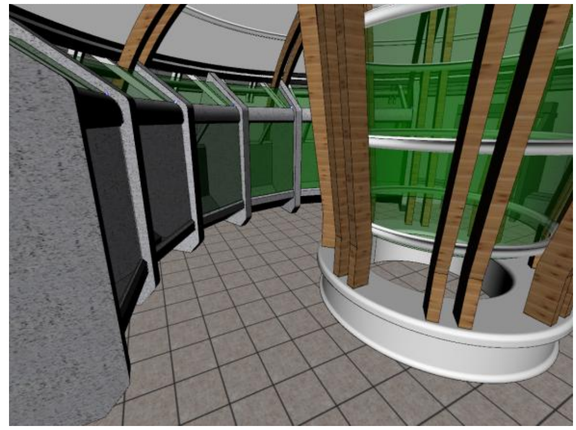



3.95
1.40
0.55
1.15
0.70
2.00
1.96









Instituciones participantes

- Ministerio de Agricultura y Ganadería
- SENASA
- Ministerio de Ambiente y Energía
- SINAC
- Instituto Metereológico Nacional
- Ministerio de Educación
- Asesorías Nacionales

Instituciones participantes

- Universidad de Costa Rica
- Escuela de Biología
- Escuela de Zootecnia
- Escuela de Ingienería Industrial
- Universidad Nacional
- Escuela de Medicina Veterinaria
- Fundación pro Zoológicos
- CBSG Mesoamérica

Nos han dado apoyo

- Sea World
- Zoologico de Denver, Colorado,USA
- Zoológico de Chester, Inglaterra
- ZoológicoTwycross, Inglaterra. The Universidad de California en Berkeley

Nos han dado apoyo

- AARK
- Grupo de Especialistas en Anfibios
- Grupo de Especialistas en Conservación Reproducción SSC UICN
- WAZA (Organización Mundial de Zoológicos).
- CBSG Mesoamérica

Nos han dado apoyo

- Nature Serve
- FUNDAZOO

Gracias

Curso Seminario
Problemática de la Disminución de las Poblaciones de Anfibios y el Papel de la Medicina Veterinaria

INFORME FINAL

Servicio Nacional de Salud Animal
Heredia, Costa Rica
22 y 23 de mayo, 2008

Sección V
Presentaciones de Andrew Cunningham

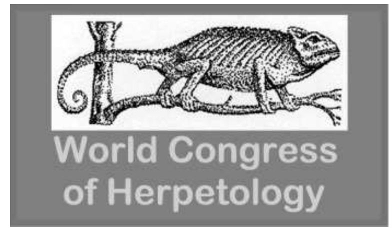
Amphibian Population Declines – a global perspective

Andrew A. Cunningham

ZSL Institute of Zoology
LIVING CONSERVATION

Global Amphibian Declines

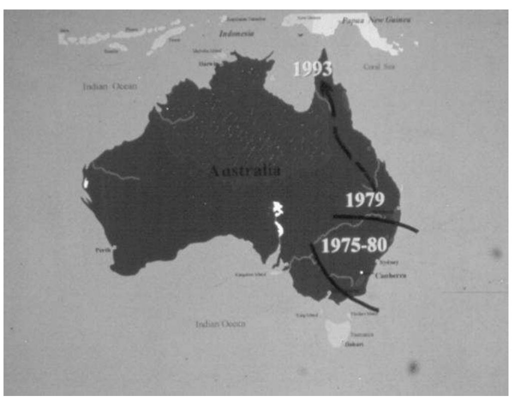
ZSL



- Amphibians are undergoing unprecedented declines around the world **<u>including</u>** in **protected areas** and in **pristine habitats**.
- Declines were initially ascribed to natural population cycles, habitat destruction, excessive UV-B irradiation, pesticide use, acid rain, other pollutants, etc…

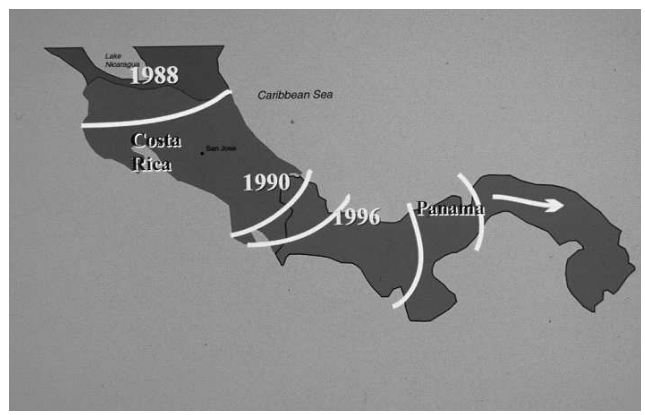
Amphibian declines - Australia

ZSL



Amphibian declines – Central America

ZSL



Amphibian declines – mortality driven

ZSL

Beginnings of a mass extinction

ZSL

- Nearly one-third (32 %) of the world's amphibian species—representing 1,856 species—are known to be threatened with extinction. (23% are data deficient)
- Up to 165 species may have gone extinct since 1980.
- At least 43 % of all species are declining in population size.
- The extent of these declines and extinctions is without precedent in any class of animal over the last few millennia.

Amphibians & Extinction Risk

ZSL

- Amphibians are much more poorly studied than mammals or birds (22.5% Data Deficient c.f. 5.3% mammals and 0.8% birds)
- Many species are poorly described, have a high level of taxonomic uncertainty, or are known purely from the holotype
- For many amphibian species the categorisation as DD means that we simply do not know where on the spectrum of extinction risk these species fit. (Whereas for mammals and birds assessed for the Red List it is likely that the majority of DD species are actually threatened. The assumption that all DD amphibians are threatened is unlikely to be an accurate reflection of their true conservation status.)

Amphibians & Extinction Risk

ZSL

- Many taxa, including amphibians, have been shown to have a taxonomically non-random distribution of threatened species
- Some families have significantly more threatened species than expected (e.g. Bufonidae) and others have fewer than expected (e.g. Ranidae), a pattern previously reported for mammals and birds
- Likewise, rapidly declining species are not evenly distributed between families
- Such a non-random pattern has been termed "taxonomic selectivity", and has been observed for species extinctions as well as extinction risk

Amphibians & Extinction Risk

ZSL

- There are three possible, non-exclusive reasons for this taxonomic selectivity:
    - non-random knowledge of species conservation status
    - clades endemic to different regions experiencing different intensities of threatening process
    - the effects of clade-specific biological attributes on the susceptibility of species to those processes.

Taxonomic Selectivity

ZSL

1. Non-random knowledge of species conservation status

- the distribution of threatened amphibians remains taxonomically non-random when species of unknown conservation status are omitted from analyses

Taxonomic Selectivity

ZSL

2. Clades endemic to different regions experience different intensities of threatening process

- significant selectivity is detected not only at a global geographic scale, but also in country- and site-specific data sets.
- the same families tend to be over- or under-threatened within different countries (e.g. bufonidae, leptodactylidae & Rhacophridae are consistently over-threatened; Hylidae, Microhylidae & Ranidae are consistently under-threatened)

Amphibians & Extinction Risk

ZSL

1. non-random knowledge of species conservation status
2. clades endemic to different regions experiencing different intensities of threatening process
3. the effects of clade-specific biological attributes on the susceptibility of species to those processes.

Therefore, this suggests that <u>biological</u> <u>differences</u> among amphibian families play an important role in determining species' susceptibility to threatening processes.

Amphibians & Extinction Risk

- Across a range of taxa, low fecundity, large body size and small range size are amongst the most commonly cited extinction risk correlates in the literature
- Each has been found to correlate with population declines in amphibians
- However, for amphibians, these studies focussed on small numbers of species in limited areas, so may not reflect global patterns
- In addition, only one of these studies controlled for the phylogenetic non-independence of species

Amphibians & Extinction Risk

Low fecundity => takes much longer to recover from any increased mortality or population decline, increasing the likelihood of stochastic extinction prior to recovery.

In many taxonomic groups large body size correlates with traits that promote extinction risk e.g. low population density and overexploitation.

Amphibian species with small geographic ranges will be more threatened than those with larger ranges because it is more likely that almost any threatening factor will affect their entire range, or a large proportion of it.

Species with small ranges also tend to have small population sizes so demographic stochasticity and inbreeding may further enhance extinction risk.

Small range size – a circular argument

- Small geographic range size is a correlate of extinction risk or rate of extirpation
- BUT, the IUCN Red List uses small geographic range size as one of its criteria for listing a species as threatened; this can lead to a circular relationship between extinction risk and geographic range.

Global Amphibian Assessment

The GAA (Stuart et al. *Science* 306:1783-1786, 2004)

- the first evaluation of all known amphibian species according to the Red List criteria
- described a total of 398 anuran species that had experienced rapid declines (RD) and a genuine increase in extinction risk over recent years
- The status changes of RD species were attributed to three causes:
  - habitat reduction
  - over-exploitation
  - enigmatic decline

Investigating "enigmatic declines"

- "Enigmatic decline" species have declined for reasons that are not fully understood.
- many of the species categorised as "enigmatic RDs" are thought to have declined due to chytridiomycosis
- We analysed RDs to find rules-of-thumb regarding which biological and environmental traits make species more susceptible to increased extinction risk in the future, and to form a basis for recommendations on future conservation efforts

Investigating "enigmatic declines"

- As 91% (398/435) of amphibian RD species are anuran, and the biology of the three amphibian orders are so different, we concentrated our analyses solely on frogs.
- In order to determine which biological, environmental and anthropogenic factors predicted species' RD status, we collected information on a number of life history, ecological and environmental variables.

Investigating "enigmatic declines"

A. Do RD species differ biologically from other threatened species?

B. Do Enigmatic RD species differ biologically from other threatened and RD species?

C. Do *Bd*+ RD species differ biologically from *Bd*+ species that have not suffered a rapid decline?

D. Do RD species that have been infected/diagnosed as *Bd*+ differ biologically from RD species not infected/diagnosed with *Bd*?

Investigating "enigmatic declines"

A. Do RD species differ biologically from other threatened species?

When RD species are compared with other threatened species, significant predictor:

- Aquatic for at least part of life cycle

Investigating "enigmatic declines"

B. Comparing enigmatic declining species with other threatened species, significant predictors:

- Aquatic life stage
- Live in locations with low annual temperature variation
- Low fecundity
- High altitude

Investigating "enigmatic declines"

C. Do *Bd*+ RD species differ biologically from *Bd*+ species that have not suffered a rapid decline?

When *Bd*+ve species that have undergone rapid decline are compared with *Bd*+ve species that have not declined, RD was significantly associated* with:

- High altitude
- Small geographic range
- Aquatic life stage

*This comparison gave the greatest degree of predictive ability of all comparisons tested.

Investigating "enigmatic declines"

D. Do RD species that have been infected/diagnosed as *Bd*+ differ biologically from RD species not infected/diagnosed with *Bd*?

No predictor variables were significantly associated with a species' *Bd* status, although RD species with large geographic ranges in areas of low actual evapotranspiration (temperate regions) were more likely to be *Bd*+.

(Perhaps wider ranging species are more likely to be infected with, and diagnosed with, *Bd* – but restricted range species would be more likely to suffer serious consequences of chytridiomycosis over a short time scale.)

Declines due to *Bd* are predictable (to a point)

We have reached a point where predictions can be made as to which species are most at risk from chytridiomycosis.

These tend to be species that:

- occupy high-altitude habitats
- breed in streams/aquatic phase
- occupy small ranges (restricted range)
- low fecundity

Complex Causes of Declines

The causes of amphibian declines and extinctions are multiple and include –

- habitat loss and degradation
- climate change
- chemical contamination
- infectious disease
- invasive species
- over-harvesting

In most cases where declines have been studied in detail, several of the causes have been shown to interact in complex ways.

Complex Causes of Declines

- while chytridiomycosis is a major cause for concern in amphibian conservation, the effects of other enigmatic factors, either alone or in combination with infectious diseases (e.g. climate change), must not be ignored.

Global distribution of anuran species with a predicted probability of *Bd*-related decline

These locations are where pre-emptive conservation policies and management actions should be made a high priority.

Bd – a particularly serious threat

Habitat loss is one of the greatest threats to amphibians, impacting almost 90% of threatened species.

However, chytridiomycosis causes catastrophic mortality in amphibian populations, and subsequent extinctions.

Also, many species are declining due to this disease in both unprotected and protected sites.

Chytridiomycosis is a threat that cannot be readily addressed through traditional conservation strategies.

Investigating “enigmatic declines”

C. Do *Bd*+ RD species differ biologically from *Bd*+ species that have not suffered a rapid decline?

When *Bd*+ve species that have undergone rapid decline are compared with *Bd*+ve species that have not declined, RD was significantly associated* with:

- High altitude
- Small geographic range
- Aquatic life stage

*This comparison gave the greatest degree of predictive ability of all comparisons tested.

Conservation Management Actions

- monitoring species population trends
- directing more detailed local studies
- screening for pathogens
- the introduction of legislation in order to reduce the possibility of pathogen introduction
- preventing the spread of *Bd* by establishing field hygiene protocols
- the establishment of *ex-situ* populations

Infectious Diseases of Amphibians

Andrew A. Cunningham

ZSL Institute of Zoology
LIVING CONSERVATION

Disease threats to biodiversity

ZSL

Disease can cause:

(1) Death

(2) Increased susceptibility to predation or further disease

(3) Lowered reproductive capacity

(4) Combinations of (1), (2) and (3)

Disease threats to biodiversity

ZSL

Thus pathogens:
(*i.e.* infectious disease)

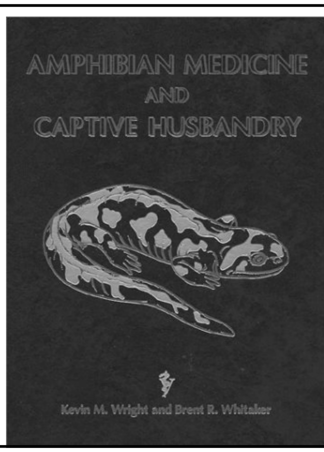
- drive evolution (*e.g.* red queen hypothesis)
- maintain biodiversity

Disease threats to biodiversity

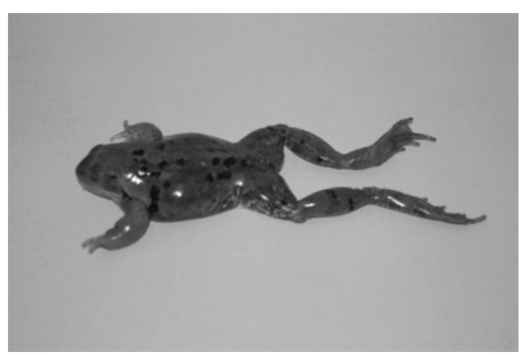
ZSL

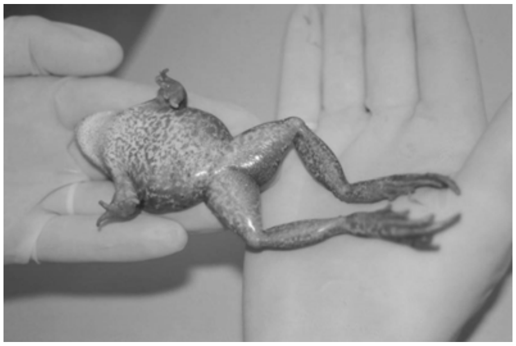
Therefore, changes in host-parasite ecology may have broad, long-term and unforeseeable effects on ecosystems.

ZSL



ZSL

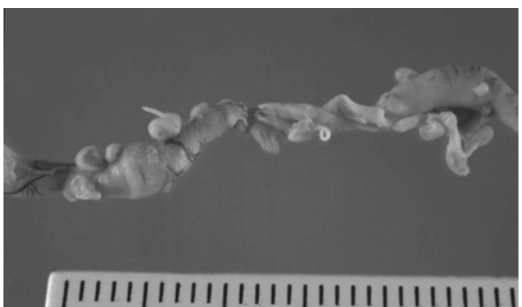




Infectious agents of amphibians

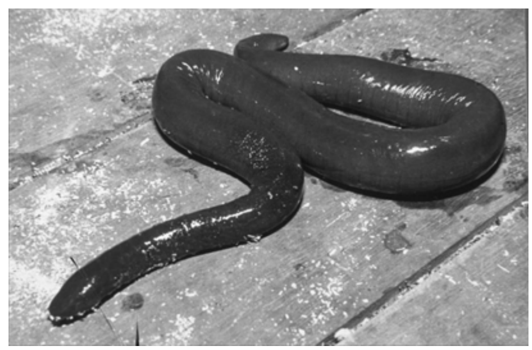
- METAZOAN PARASITES – e.g. *Rhabdias bufonis*
- PROTOZOAN PARASITES – e.g. *Trichodina* sp.
- FUNGI – e.g. *Batrachochytrium dendrobatidis*
- BACTERIA – e.g. *Mycobacterium* spp.
- VIRUSES – e.g. Ranaviruses

Not all parasites cause disease

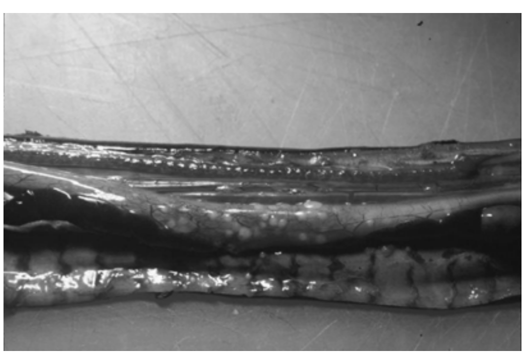


Physaloptera sp. in intermediate host

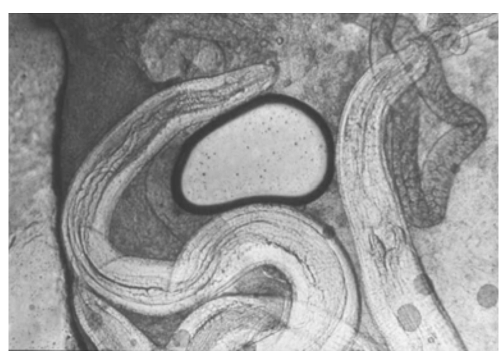
Caecilian (*Typhlonectes* sp.)



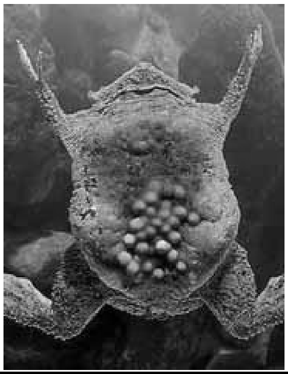
Caecilian – nodules over serosa



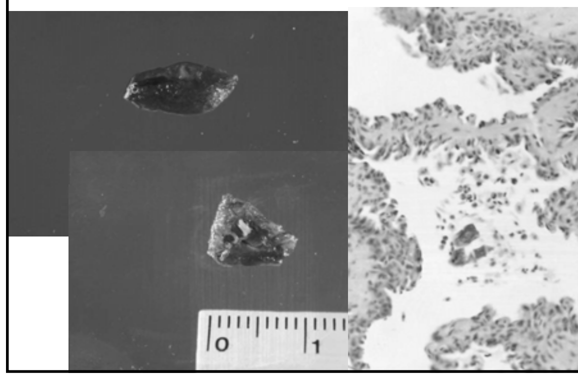
Ortleppascaris alata larvae



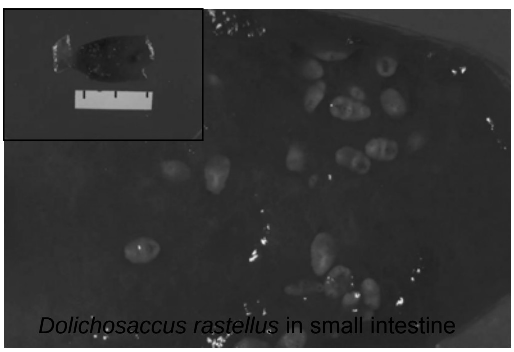
Surinam toad – another intermediate host



Common frog – lung worms



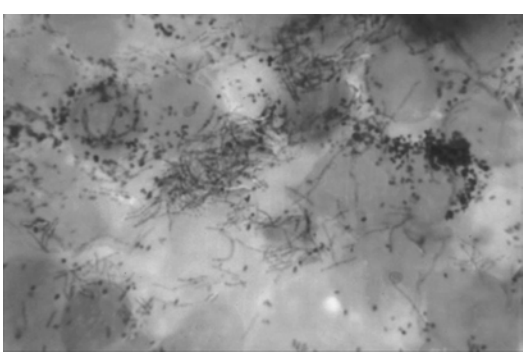
Association *vs* causation



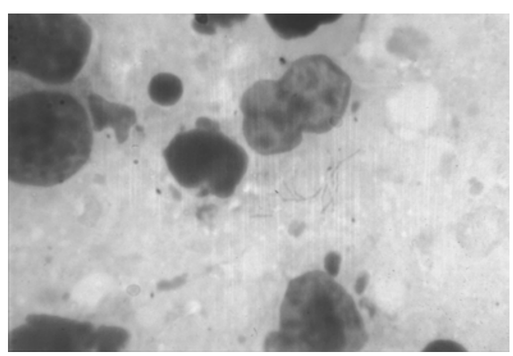
Dolichosaccus rastellus in small intestine

Some “parasites” may be important commensals

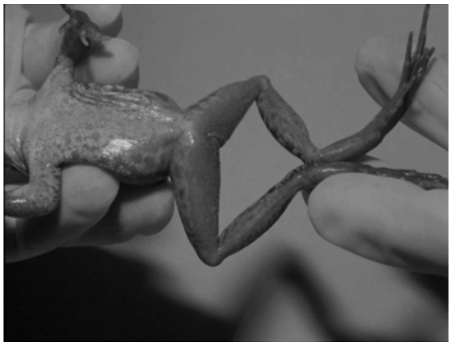
Potential zoonoses – e.g. *M. fortuitum*



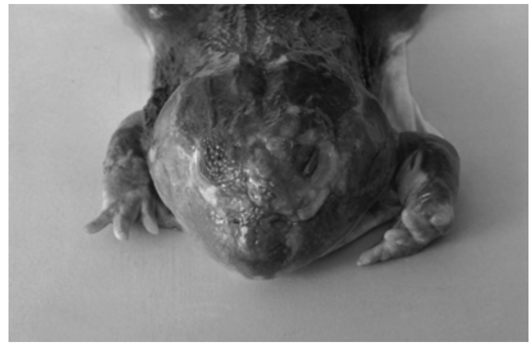
Mycobacteriosis – e.g. *M. fortuitum*



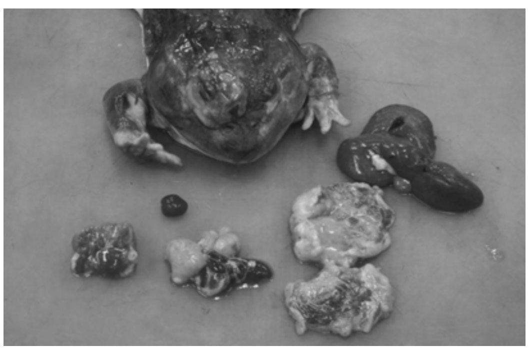
Redleg



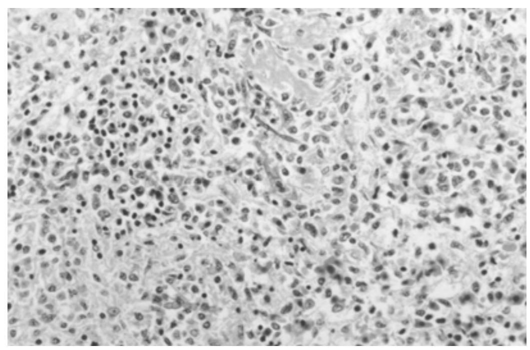
African bullfrog (*Pyxicephalus adspersus*)



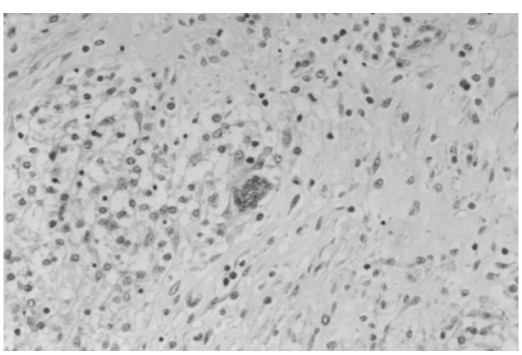
Chromomycosis (chromoblastomycosis)



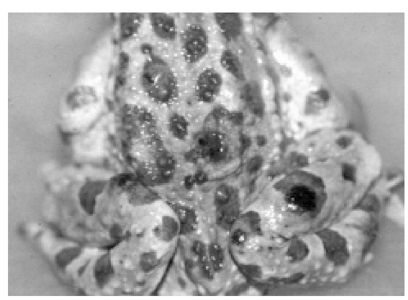
Chromomycosis (chromoblastomycosis)



Chromomycosis (granuloma in stomach)

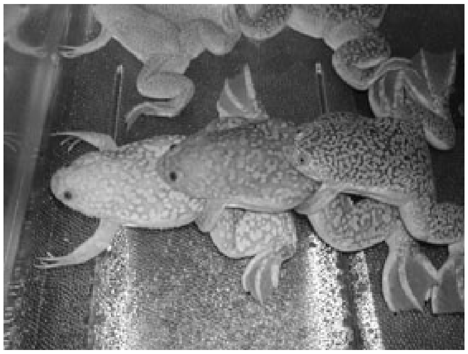


Black yeast infection

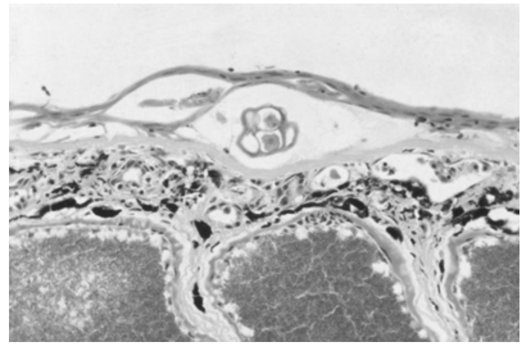


Exophiala sp. “black yeast” infection in a green toad from the Jordan valley

Xenopus laevis



Pseudocapillaroides xenopi



Pseudocapillaroides xenopi - treatment

A variety of treatments has been reported

- levamisole s/c 5mg/kg every 10 days for 3 doses
- thiabendazole 0.1g/litre of aquarium water repeated after two weeks
- Ivermectin – 2 mg/kg subcutaneously; 0.2 mg/kg i/m.

Families of viruses isolated from amphibians

Family

- Herpesvirus
- Iridovirus
- Adenovirus
- Papovavirus
- Togavirus
- Calicivirus

Herpeviruses

Leopard frog (*Rana pipiens*)

1938 Lucké *J. Exp. Med.* **68,** 457

1956 Fawcett *J. Biophys. & Biochem. Cytol.* **2,** 725

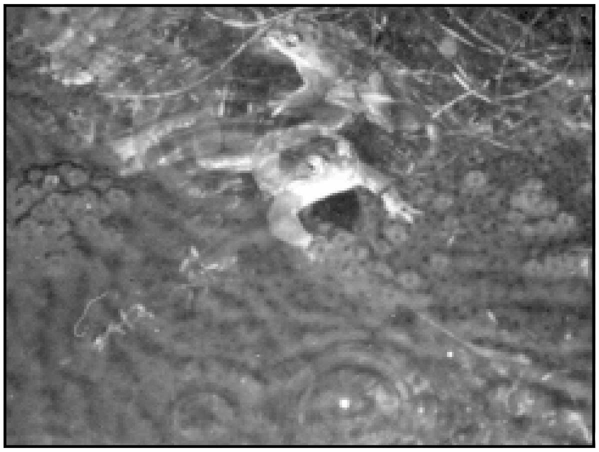
Lucké tumour herpesvirus (RaHV-1)

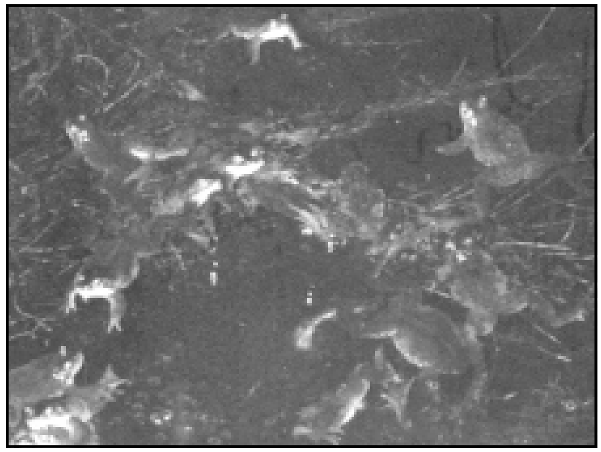
- causes renal adenocarcinoma

1965 Rafferty *Ann. N. Y. Acad. Sci.* **126,** 3 (FV-4)

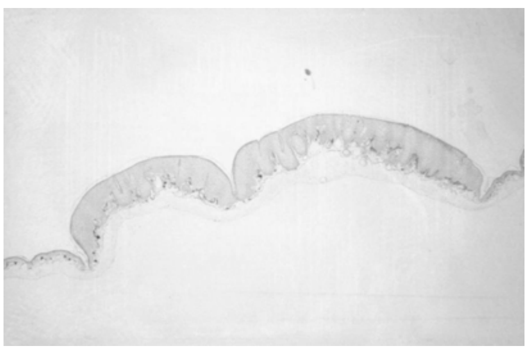
Agile frog (*Rana dalmatina*)

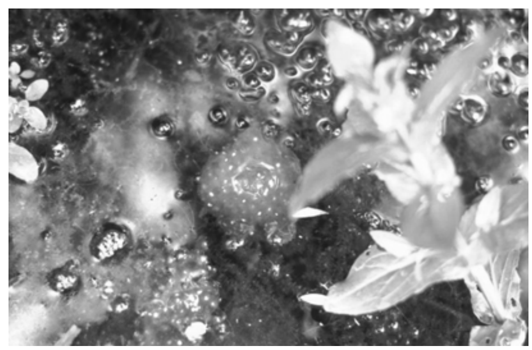
1994 Bennati *et al.* *Vet. Rec.* **135,** 625





Herpesvirus – epidermal hyperplasia





Disease and global amphibian declines

Recently, there has been growing interest in infectious diseases and their role in global amphibian declines.

In particular, the following infections have been implicated as a cause of increased mortality leading to amphibian population declines:

- Saprolegniosis (*Saprolegnia ferax*)
- *Ribeiroia ondatrae* infection
- Ranavirus disease
- chytridiomycosis (*Batrachochytrium dendrobatidis*)

.

Disease and global amphibian declines

Demonstration of a link between declines and these infectious diseases is a challenging task that requires:

1. Fulfilment of Koch's postulates for pathogens hypothesized to cause disease in amphibians.

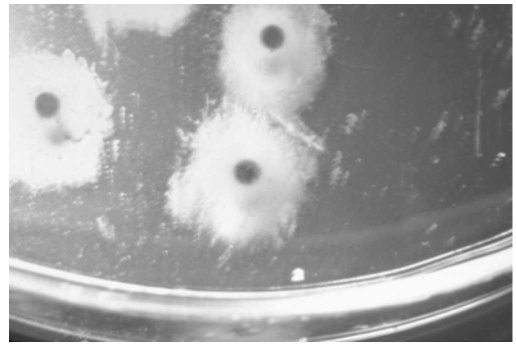
2. Identification of the causative pathogen in carcasses from mortality events that constitute part of a decline in population (mortality events may consist of mass die-offs or subtle increases in mortality rates).

Disease and global amphibian declines

3. Pathological evidence that the disease caused death in a significant number of cases within these mortality events (i.e. demonstration of gross, cellular or tissue damage concomitant with infection and with a valid mechanism for a cause of death proposed).

4. Clear evidence that the mortalities are the cause of declines.

Saprolegnia infection – chicken or egg?

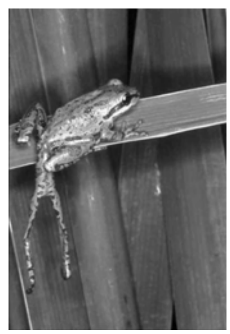


Malformations

Rana catesbeiana – extra forelimbs

leopard frog – missing hindlimb

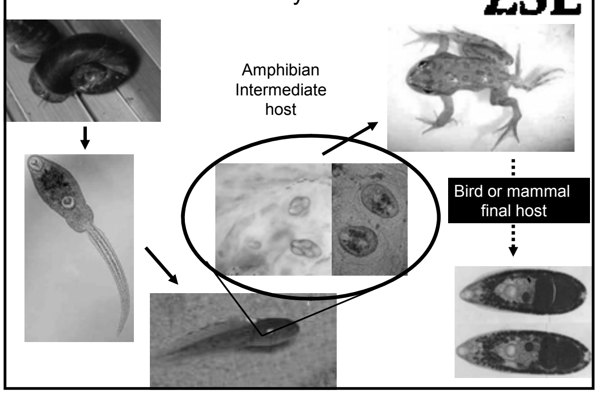
Malformations



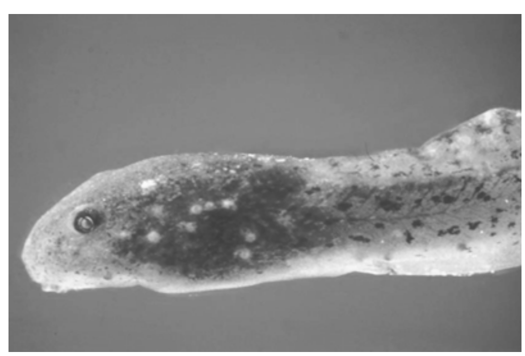
Pacific treefrog – extra hindlimbs

Oregon spotted frog – extra hindlimbs

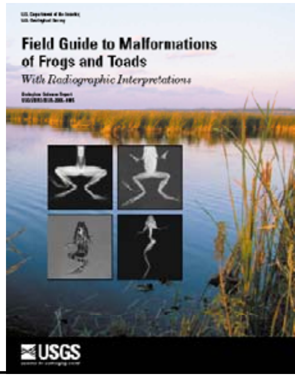
Ribeiroia ondatrae life-cycle

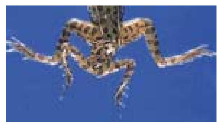


Trematode metacercarial infection of tadpole



http://www.nwhc.usgs.gov/publications/fact_sheets/pdfs/frog.pdf

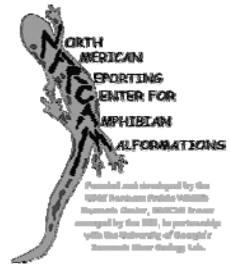




Changing incidence of amphibian malformations

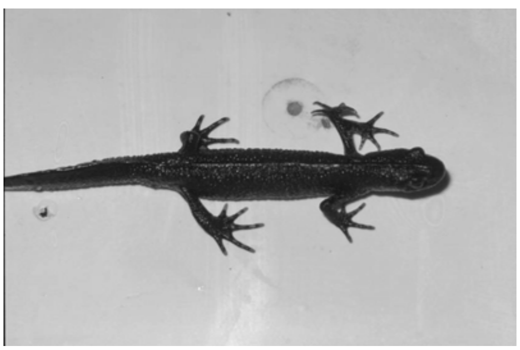
- Amphibian malformations documented in 44 States
- Almost 60 species affected
- Up to 60% prevalence for some species
- Huge increase in number of reports over recent years

- But recently there has been a marked increase in surveillance effort
- Most cases are one-off individuals
- Very few cases of mass-malformations

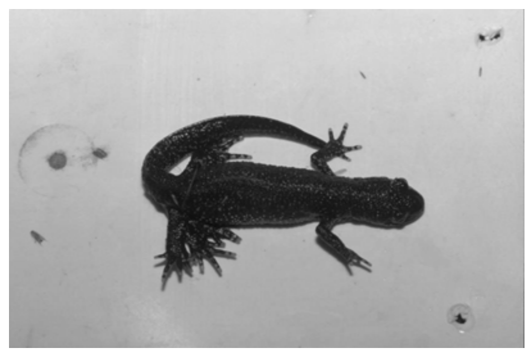


BUT there does seem to be a true increase in incidence, possibly due to changes in the ecology of waterbodies, such as eutrophication, increasing snail populations….

Limb deformity in *Triturus cristatus*, U.K.



Limb deformity in *Triturus cristatus*, U.K.

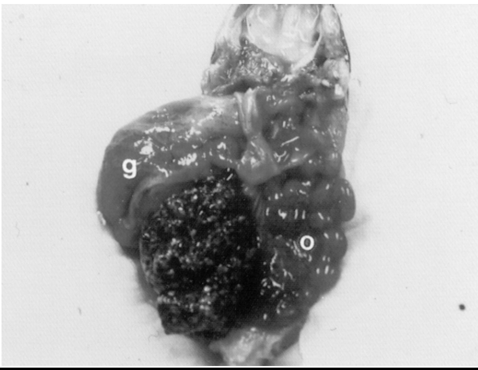


Iridoviruses (Ranaviruses) from amphibians

1965	leopard frog	U.S.A.	incidental finding (captive)
1968	N. A. bullfrog	U.S.A.	tadpole mortality
1991	edible frog	Croatia	? mortality of captive animals
1992	ornate burrowing frog	Australia	mortality of captive animals
1992	common frog	U.K.	mass mortality
1994	red-legged frog	U.S.A.	mortality
1995	common toad	U.K.	mortality
1997	tiger salamander	U.S.A. (Arizona)	mass mortality
1999	tiger salamander	Canada	mass mortality
1999	spotted salamander	U.S.A. (Maine)	mortality
1999	Ranid frog	China	mortality

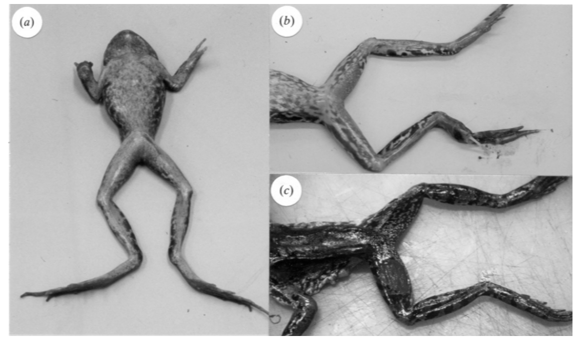
Haemorrhagic disease syndrome

ZSL



Ulcerative skin disease syndrome

ZSL

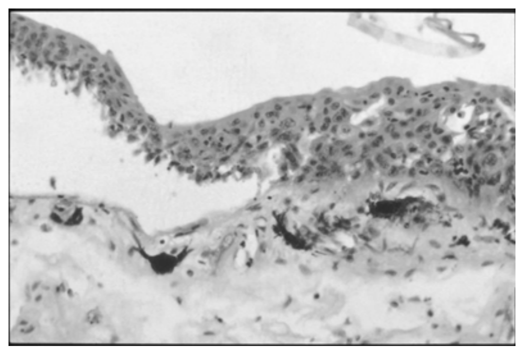


Ulcerative skin disease syndrome

ZSL

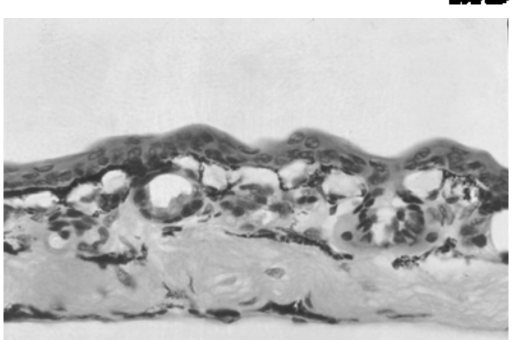
Skin ulcer formation

ZSL



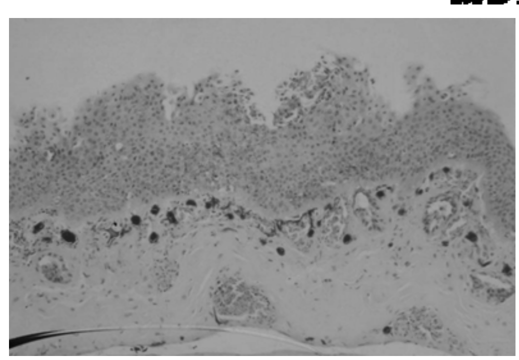
Common frog – normal skin

ZSL

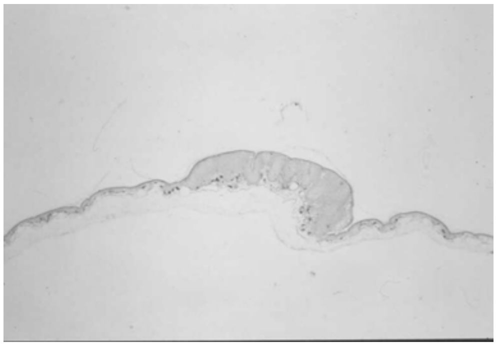


Skin of frog with haemorrhagic syndrome

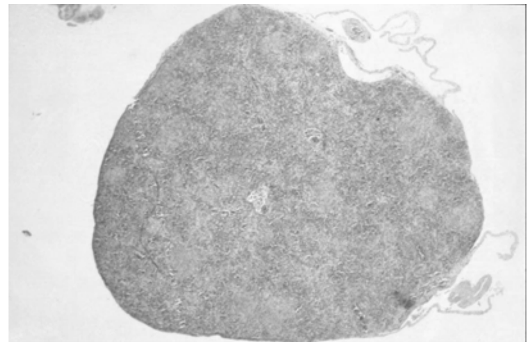
ZSL



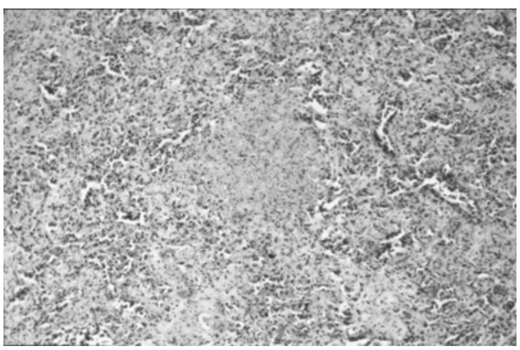
Agile frog – epidermal hyperplasia



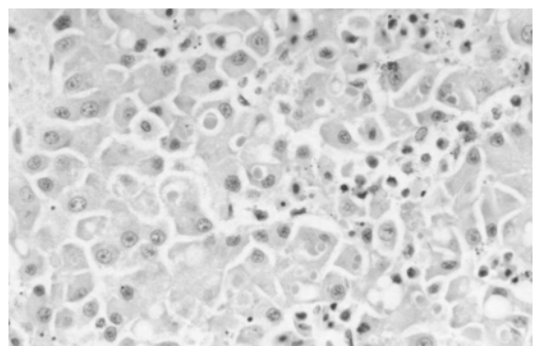
Frog with HS - spleen



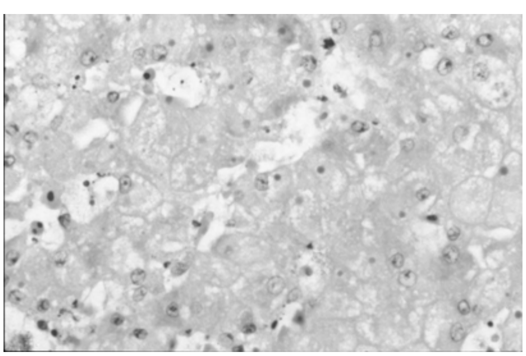
Frog with HS - spleen



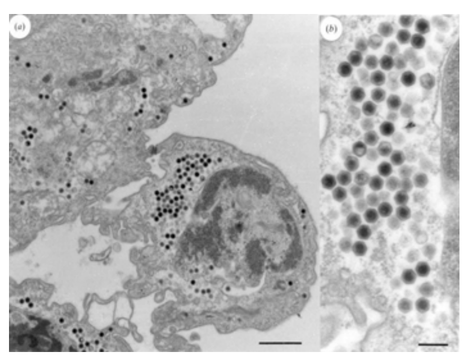
Liver – eosinophilic i/c inclusion bodies



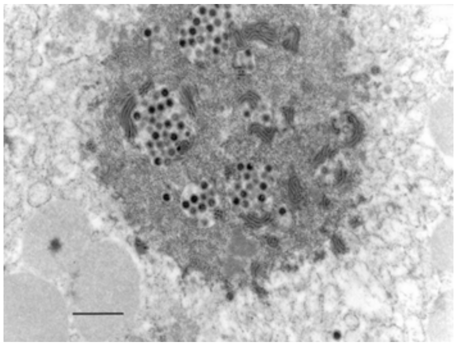
Liver – basophilic i/c inclusion bodies



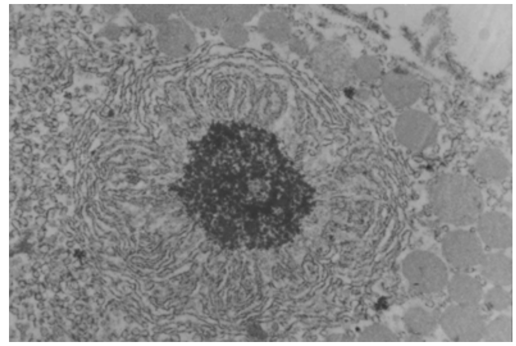
Epidermis - EM of edge of skin ulcer

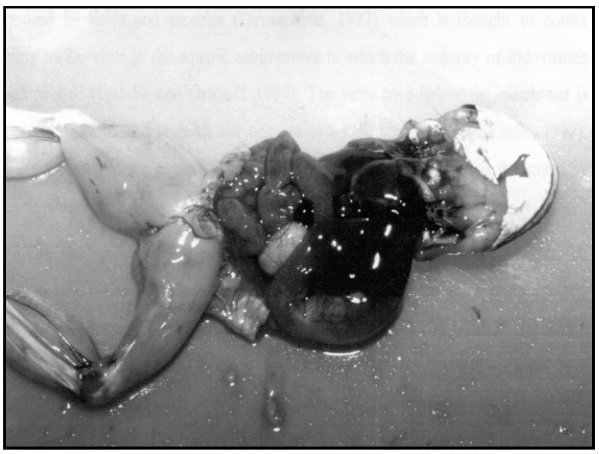


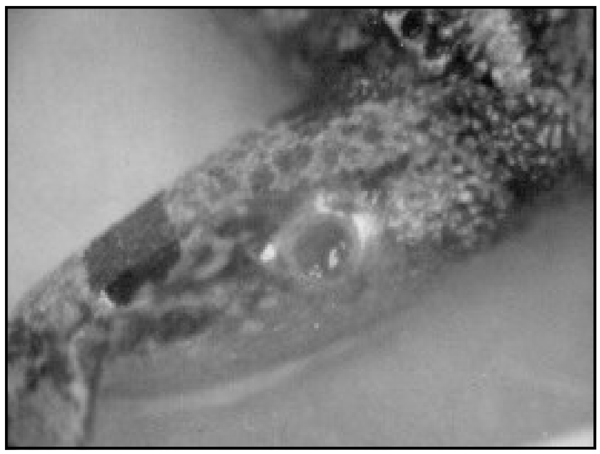
Liver - EM of basophilic i/c inclusion **ZSL**

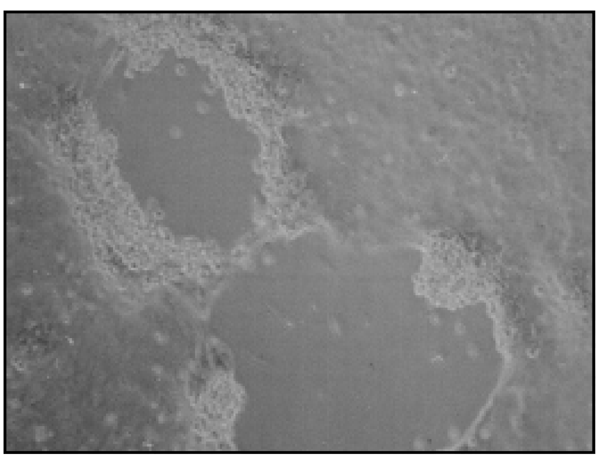


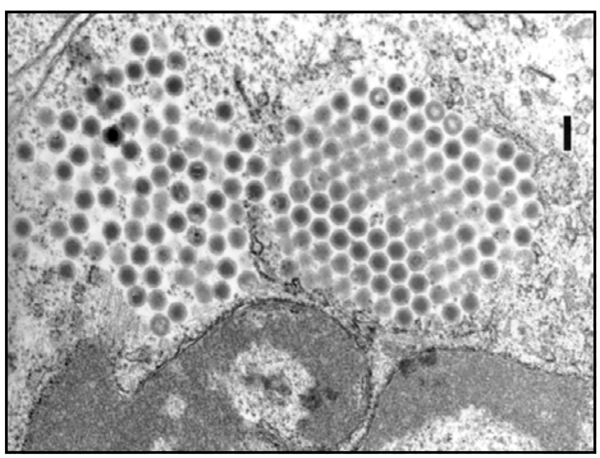
Liver - EM of eosinophilic i/c inclusion **ZSL**





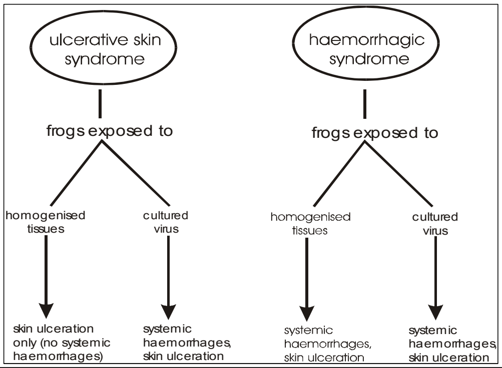






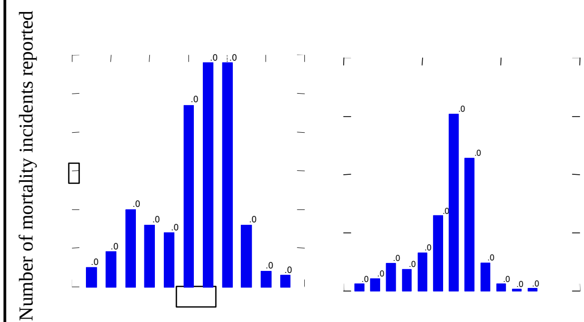
Outcomes of experimental exposure

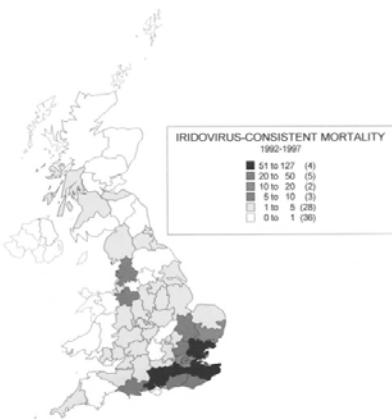
ZSL



Temporal incidence of frog mortality

ZSL





Ranaviruses and amphibian declines?

ZSL

There has been a large increase in the number of new ranaviruses discovered in recent years, but only two cases where they have been associated consistently significant levels of mortality in populations of wild amphibians:

(i) tiger salamanders (*Ambystoma tigrinum*) in Arizona, U.S.A. and in Saskatchewan, Canada

(ii) common frogs (*Rana temporaria*) in Britain

Species naturally affected by ranavirus infection in Britain

ZSL

AMPHIBIA

Common frog *Rana temporaria*

Common toad *Bufo bufo*

REPTILIA

Hermann's tortoise *Testudo hermanni*

Impact on amphibian populations - UK

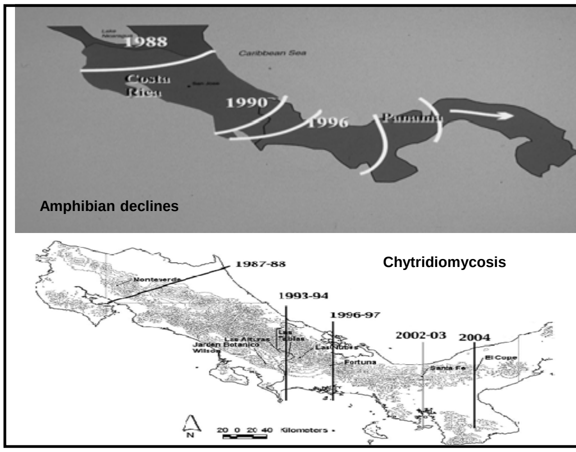
ZSL

ANNUAL MONITORING OF TEN INDEX SITES OF FROG MORTALITY, 1992 - 1996

- Recurrent annual mortalities at 3 sites
- Decline in number of adult frogs seen at all ten sites
- Complete loss of adult frogs at one site
- Decline in amount of spawn seen at 8 sites
- Complete loss of 2 sites as a breeding pond

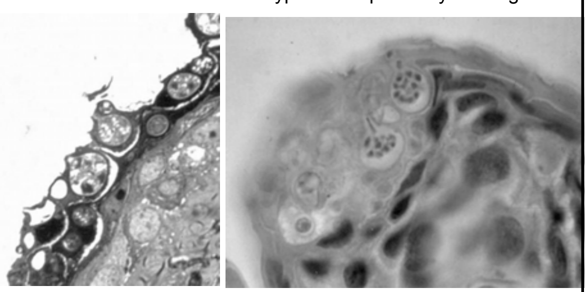
Impact on other species?

- "Concurrent fish deaths" was significantly ($p < 0.001$) associated with iridovirus mortality of frogs in the U.K.
- Fish killed experimentally by BIV (Australia) and naturally by RCV (U.S.A.)
- Tortoises killed by U.K. frog ranavirus – what about native reptiles?



Cutaneous chytridiomycosis - histology

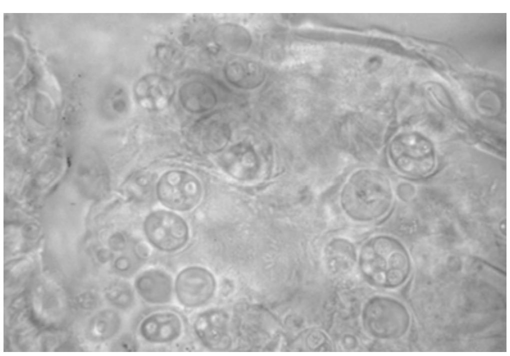
Causative agent: *Batrachochytrium dendrobatidis*, a non-hyphal zoosporic chytrid fungus



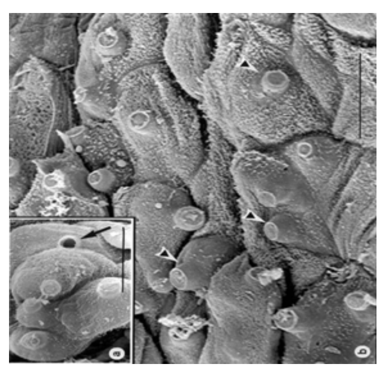
Berger *et al.* (1998) *P.N.A.S.* 95, 9031-9036.

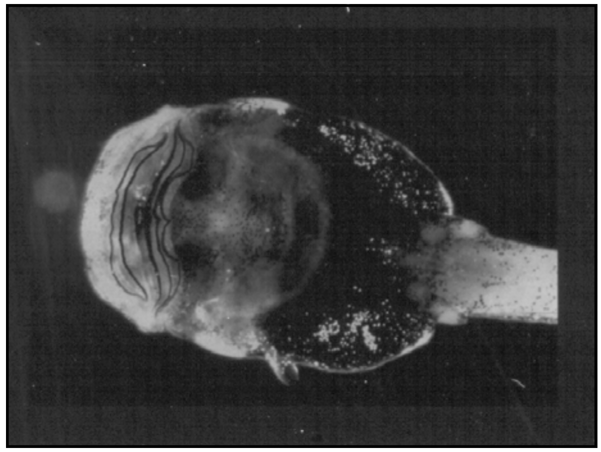
Mountain chicken with chytridiomycosis

B. dendrobatidis - cytology



Cutaneous chytridiomycosis - SEM





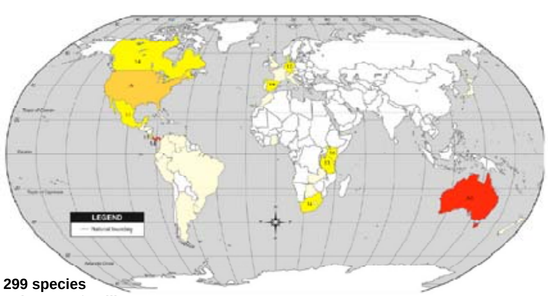
larval *Rana muscosa* mouthparts

- illustration of two patterns of pigment loss over time. Upper (anterior) tooth rows (t), upper and lower (posterior) jaw sheaths (j), and four lower tooth rows (t) are shown. Black is pigmentation and gray is loss of pigment.
- a-d represents the pattern of loss associated with *Bd*
- e-h represents the pattern of pigment loss associated with low overwintering temperature.

Examples of epidemics of chytridiomycosis in wild amphibian populations

E. & S. AUSTRALIA 1993 - 1999	Multiple montane rain forest and temperate species, local and global extinctions
W. AUSTRALIA 1998 - on-going	Multiple species, predominantly *Litoria moorei*, with marked population declines
COSTA RICA & PANAMA 1994-1999	Multiple montane rain forest and temperate species, local and global(?) extinctions
ECUADOR 1999 - On-going	Multiple species; unknown impact
U.S.A. 1970s – on-going	Bufo canorus (CA) & Rana pipiens (CO) Bufo boreas (CO) & Rana spp. (Arizona) other species?
SPAIN 1998 – on-going	*Alytes obstetricans*, *Salamandra salamandra* *Bufo bufo*

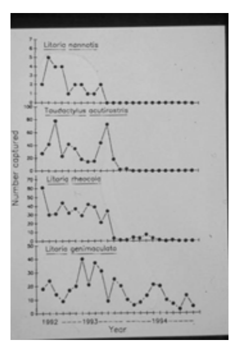
Known global distribution of *Bd*



- **299 species**
- **at least 14 families**
- **42 countries**
- **6 continents**

Global declines of amphibians

Mixophyes fasciolatus

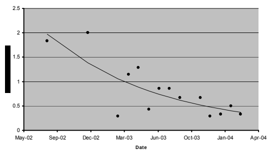


Amphibian declines, 1993-1998

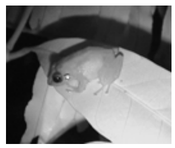
Disease threat to population

ZSL

- Estimate mountain chicken population decline of ~80% between December 2002 and March 2004



- We do not know what effect the fungus is having on Dominica's other amphibians…..



Extinction due to chytridiomycosis?

ZSL

Ollotis (Bufo) periglenes

Rheobatrachus vitellinus
Rheobatrachus silus

ZSL

Ready to croak

A fungal disease is wiping out New Zealand's ancient frogs

Kihansi spray toad, Tanzania

How does chytridiomycosis cause population extinctions?

ZSL

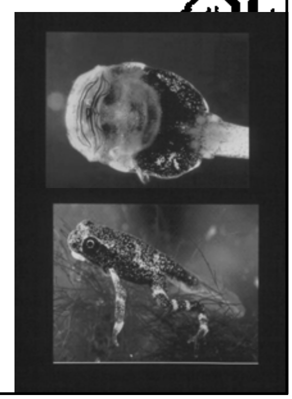
Many endangered species have small populations below the N_T of most pathogens

Reservoir hosts raise overall population size and enable extinction

e.g. African wild dogs

Chytridiomycosis

- **Larvae**
- **Introduced species**
- **Saprobic stages (8 weeks)**

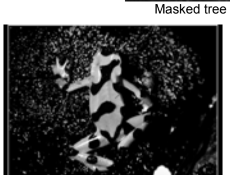


Differences in susceptibility

ZSL

Masked tree frog, *Smilisca phaeota*

- host
- pathogen
- environment



Panamanian golden frog, *Atelopus zeteki*

Harlequin frog, *Atelopus varius*

Chytrid in the environment

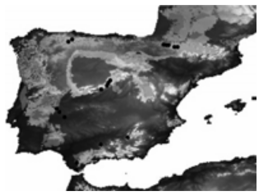
ZSL

- Experimental evidence that zoospores can live in lake water for up to 7 weeks & in moist keratin (e.g. damp feathers) for up to 8 weeks.
- Sampling for *Bd* from water collected at ponds in Spain where amphibians have died and declined due to chytrid has yielded *Bd*-positives
- In some cases, 200 mL of pond water yields easily detectable levels of *Bd* DNA
- Evidence that *Bd* can survive in pond sediment for at least three months (possibly much longer) in the absence of amphibians.

Chytrid in the environment - Water

ZSL

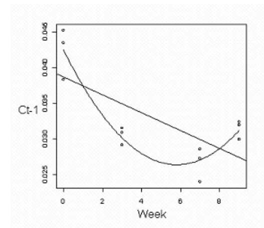
- Experimental evidence from Australia that zoospores can live in lake water for up to 7 weeks & in keratin (e.g. feathers) for up to 8 weeks.
- Sampling for *Bd* from water collected at ponds in Spain where amphibians have died and declined due to chytrid has yielded *Bd*-positives
- In some cases, 200 mL of pond water yielded easily-detectable levels of *Bd* DNA



Chytrid in the environment - Sediment

ZSL

- Experimental evidence in the UK that *Bd* DNA concentration (and therefore number of Bd organisms) increases in pond sediment over time (under temperate conditions in the laboratory).
- Evidence that *Bd* can survive and possibly even reproduce outside of the host?



Chytridiomycosis – a true global emerging disease

ZSL

- Histological surveys of museum specimens up to 10 years prior to epidemics and associated catastrophic population declines (Australia, Central & South America) have revealed no evidence of infection
- The disease was discovered independently, and contemporaneously, on separate continents
- The epidemic mortality and rapid population declines associated with infection are characteristic of the declines observed when novel pathogens infect a population.

Chytridiomycosis

ZSL

- is the only amphibian disease which is clearly linked to population declines and extinctions.
- is emerging – in new species, regions and populations (~299 species, at least 14 families, 6 continents)
- causes rapid-onset outbreaks with high case fatality rates in a range of amphibian species
- across the class, it has caused mass mortality and declines in a number of regions, and has been linked to multiple extinctions.

Treatment

ZSL

Variety of treatments attempted, varying degrees of success:

	Chytrid	Frog
• Trimethoprim-sulphadiazine	X	√
• Miconazole	√	X
• Itraconazole	√	√
• Fluconazole	X?	√
• Formalin/malachite green combination	√	X
• Benzalkonium chloride	X	√
• Heat treatment (37°C for 8 hours), repeated		

Treatment

ZSL

The treatment that consistently gives the best results:

Itraconazole – 0.01% baths for 5 mins/day for 11 days
(Ensure the entire frog is bathed)

Then 10 days "rest", then repeat 11 days of baths

May need to repeat for a third or fourth time

Monitor with repeated swabbing for up to 6 months post-treatment

What's driving the emergence of chytridiomycosis?

Factors proposed as underlying disease spread or "emergence"

- Environmental changes or "stress", such as increased levels of pollutants, habitat loss or change, acid rain…
- Stress due to increasing proximity to human beings, including hikers
- Ozone depletion leading to increased exposure to UV radiation
- Climate change, such as global warming and changes in rainfall patterns
- Globalisation, increased movement of people, animals, animal products and fomites

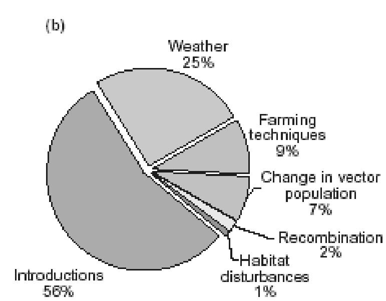
All have the potential to alter host-parasite ecology

Drivers of chytridiomycosis emergence?

Two Hypotheses

1. Endemic disease. Emerged due to global changes (UV-B, climate, pollution) increasing virulence or decreasing host immunity
2. Panzootic. Emerging due to anthropogenic introduction

Drivers of plant EIDs



Anderson *et al.* (2004) *Trends in Ecology and Evolution* **19,** 535-544.

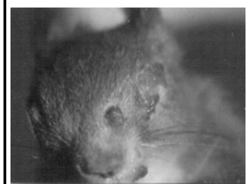
Drivers of Disease Threats

ZSL

- Rinderpest in Africa – pathogen co-introduction with domestic livestock
- PDV – pathogen introduction via forced migration of harp seals
- Crayfish plague – pathogen co-introduction with American signal crayfish
- Dutch elm disease – pathogen introduction from Asia to USA & Europe
- Squirrelpoxvirus – introduction of reservoir host +/- pathogen from North America

Squirrelpox and red squirrel declines

ZSL



Apparent competition *a.k.a*
Parasite-mediated competition

Sainsbury et al. (2000) *Animal Conservation* **3,** 229-233
Tompkins et al. (2002) *Proc. Roy. Soc. B* **269,** 529-533
Thomas et al. (2003) *J. Gen. Virol.* in press

Seroprevalence
reds: 2.9%, with disease
greys: 61%, without disease

Pathogen Pollution

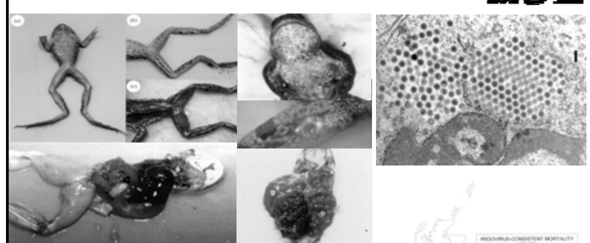
ZSL

The anthropogenic introduction of a parasite, host or vector allowing the crossing of an evolutionary boundary, such as geographic or ecological separation.

Cunningham et al. (2003) *J. Parasitol.* **89,** S78-S83.
Daszak & Cunningham (2003). *J. Parasitol.* **89,** S37-S41.

Frog mortality in Britain

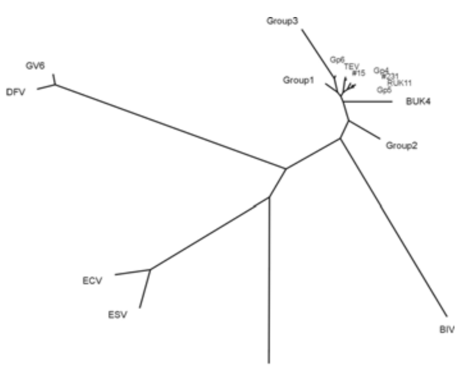
ZSL



Cunningham *et al.* (1996) *Phil. Trans. Roy. Soc. B* **351,** 1539-1557.

What is the origin of the UK ranavirus?

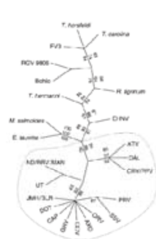
ZSL



Group 1:	FV3, UK frog
Group 2:	UK frog UK toad
Group 3:	S. Am. frog
Group 4:	UK frog
Group 5:	UK toad
Group 6:	UK tortoise

Ambystoma tigrinum virus in N. America

ZSL



Jancovich *et al.* (2005) *Molecular Ecology* **14:**213-224

What are the drivers of emergence of *Bd* ?

ZSL

Is trade to blame?


France learns to swallow its pride along with imported frogs' legs

- pet industry
- food industry
- biological materials
- other (scientific, zoos, education, etc.)

Anthropogenic spread

ZSL

Chytridiomycosis confirmed in:

- Pet trade (e.g. dendrobatid frogs)
- Food trade e.g. bullfrog farms in Uruguay & Brazil
  (> 1 million p.a. enter USA)
- Lab animal trade (*Xenopus* spp.)
- Zoo animal trade
- Introduced species e.g. bullfrogs, alpine newts

Extent of Amphibian Trade

ZSL

- Food trade (> 1 million bullfrogs p.a. USA)
- US official trade
  > 5 million live amphibians imported p.a. (majority wild caught)
- > 2500 tons of frog legs exported annually from China

Live amphibian trade in the U.S.A. 1996-2001

ZSL

IMPORTS: 26.5 million live amphibians

- 74% of which were wild-caught
- 23% captive-bred
- 3% unknown source
- > 6 million were *Rana catesbeiana*
- only 1 of the 50 most imported species was CITES listed
- Approx. 0.1% of trade is in CITES-listed species

Source: M.A. Schlaepfer, Cornell; US Fish & Wildlife Service

Live amphibian trade in the U.S.A. 1996-2001

ZSL

EXPORTS: 1.9 million live amphibians

- 72% of which were wild-caught or of unknown source
- 28% captive-bred
- Most were re-exports

Source: M.A. Schlaepfer, Cornell; US Fish & Wildlife Service

Live amphibian trade

ZSL

"The worldwide trade in live reptiles and amphibians is huge."

"Unlike the trade in live birds and mammals, the live reptile and amphibian trade is largely unregulated, with comparatively few species listed on CITES."

TRAFFIC
The wildlife trade monitoring programmes of WWF & IUCN www.traffic.org

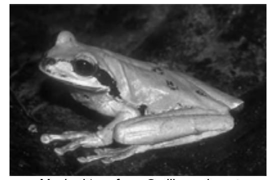
Chytrid infection in introduced bullfrogs

ZSL

Location	% positive
Canada (Vancouver Island)	**65**
U.S.A. (Arizona)	**45**
Brazil	**14**
Uruguay	**100**
Britain	**14**
France	**62**
Italy	**3**
Japan	**0**
Venezuela	**100**

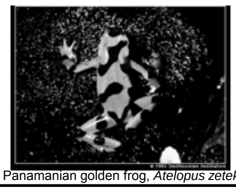
Differences in susceptibility

ZSL



Masked tree frog, *Smilisca phaeota*

- host
- pathogen
- environment



Panamanian golden frog, *Atelopus zeteki*

Harlequin frog, *Atelopus varius*

Chytrid has now reached the U.K.

ZSL

- Detected in two adult American bullfrogs in south east England

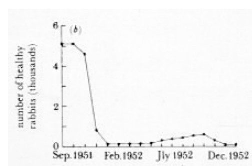
Rana catesbeiana

- Wiped out a captive population of *Bufo calamita* reared for restocking the wild (this species has been part of a conservation action plan in Britain for over a decade)

Bufo calamita

Rabbits, myxomatosis and a butterfly extinction

ZSL



1950 - myxomatosis introduced to UK

Changes in host-parasite ecology may have broad, long-term and unforeseeable effects on ecosystems.

1979 - large blue butterfly (*Maculina arion*) becomes extinct in UK

Amphibian Chytridiomycosis – sampling techniques

Andrew A. Cunningham

ZSL Institute of Zoology
LIVING CONSERVATION

Study Design

ZSL

Ensure study design and sampling strategy maximises your chances of obtaining meaningful and useful results.

For example: If there is a 5% prevalence of infection, at least 60 animals within the same "epidemiological unit" must be sampled in order to have a 95% chance of detecting at least one infected animal.

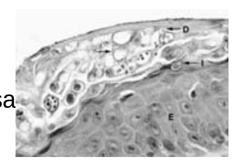
If possible, employ a random stratified sampling methodology. If not possible, make sure you are aware of any sampling biases and take these into account when interpreting your results.

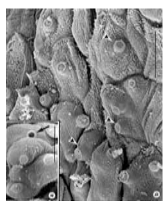
Employ an epidemiologist to advise you on your sampling methodology.

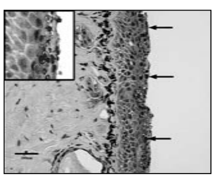
Diagnostic techniques

ZSL

- Histopathology
  - –H&E stain
  - –Immunoperoxidase assa
- Electron microscopy
- PCR (real time or nested)
- Culture







Toe-clipping - advantages

ZSL

- Individual or cohort identification
- Provides data on survival rate and movements of individuals
- Skeletochronology for estimating age
- Provides material for genetic analysis of the amphibian (individual or population)
- Provides material for pathogen detection (via detection of pathogen genetic material and/or via the microscopic detection of pathogens and/or lesions)

Toe-clipping - disadvantages

ZSL

- Infection of the foot +/- localised spread
- Systemic infection
- Inflammation
- Reduced mobility
- Reduced climbing ability (e.g. for tree frogs)
- Reduced survival

McCarthy & Parris (2004) Journal of Applied Ecology **41:**780-786

Effects of toe-clipping

ZSL

McCarthy & Parris (2004) Journal of Applied Ecology **41:**780-786

The effect of toe-clipping on the recapture rate (and hence implied survival) of amphibians increases with the number of toes removed.

Therefore, as more toes are removed, the survival of amphibians is increasingly decreased, such that:

- The recapture rate of Frogs with two toes removed was 96% that of frogs with one toe removed
- The recapture rate of frogs with eight toes removed was 28% that of frogs with one toe removed

The removal of one toe is likely to have substantially smaller impact on individuals and populations than the removal of multiple toes.

In defence of toe-clipping

Sir:

In News and Views ("Ethics and amphibians" Nature 431, 403; 2004), Robert M. May discusses a study by M. A. McCarthy and K. M. Parris on the effects of toe-clipping on amphibians. This is a standard technique for uniquely marking animals in ecological research (see guidelines at http://www.asih.org/pubs/ASIH_HACC_Final.pdf). McCarthy and Parris show that return rates decreased as the number of toes clipped increased in four frog species; they and May raise ethical and practical questions about the technique. There is, however, a fuller picture to be considered.

Several studies have found no negative effects of toe-clipping. McCarthy and Parris suggest that this may be due to low statistical power, but this has not been shown in all cases in which no effect was observed (see, for example, J. J. van Gelder and H. Strijbosch *Amphibia-Reptilia* **17**, 169–174, 1996; and J. A. Ott and D. E. Scott *J. Herpetol.* **33**, 344–348, 1999). Effects of toe-clipping evidently vary among species and so must be assessed accordingly.

Alternative marking techniques often cannot be used. Many adult frogs are less than 20 mm long, as are juveniles of larger species. The smallest passive integrated transponder (PIT) tags are about 10 mm long, too large for small frogs. Many species cannot be marked using other approaches, such as elastomers, alphanumeric tags or freeze-branding, and do not have consistent, identifiable markings.

Other techniques may have worse effects, as M. Schlaepfer has shown (*Herpetol. Rev.* **29**, 25–26; 1998). Implantation of PIT tags often involves surgery on the body cavity; even implantation under the skin carries a risk of more serious infection than in an extremity. Many ecological studies require trading the risks of a marking technique against the gains in understanding. More research evaluating these risks is needed.

Far from acting with 'casual barbarity', biologists who use the toe-clipping technique do so after considering alternative techniques and with the approval of institutional animal care and use committees. The resulting data are essential for managing threatened populations, as well as for other purposes: toe clippings are sources of DNA for genetic studies, and act as samples for identifying the diseases implicated in catastrophic amphibian declines.

We believe it is less ethical to sit back and watch species slip into extinction than it is to use the best available methods to help to conserve them.

Nature **433**, 193 (20 January 2005) | doi: 10.1038/433193a

Alternative views of amphibian toe-clipping

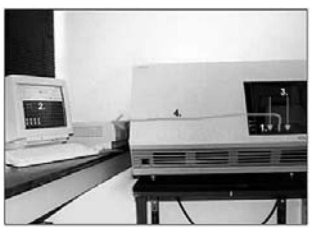
W. Chris Funk[1], Maureen A. Donnelly[2] and Karen R. Lips[3]

Minimising adverse effects of toe-clipping

- Remove as few toes as possible
  - Same foot vs different feet
- Use care on capturing the animal and minimise handling time (have everything ready before-hand)
- Use sterile, sharp instruments
- Cleanly cut through inter-phalangeal joint – do not leave bone exposed (push back skin prior to toe removal)
- Remove medial or lateral toes, but not middle (long) toes
- Do not remove toes required for special functions, such as amplexus, burrowing, climbing or nest excavation

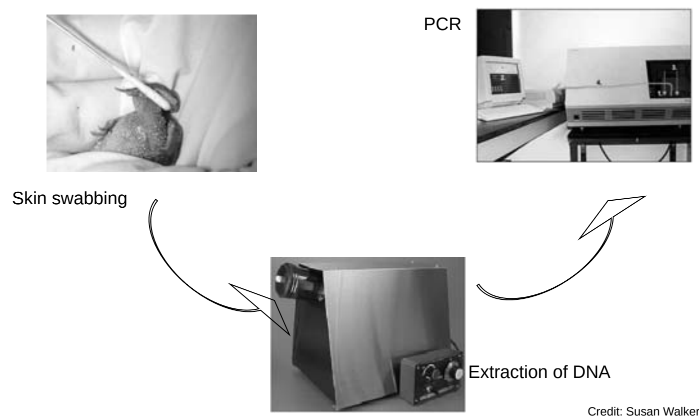
Polymerase chain reaction (PCR)

- Sensitive technique
- Amplifies the amount of genetic material present
- Uses probes to check for chytrid DNA
- Confirms presence or absence of fungus



Credit: Susan Walker

Polymerase chain reaction (PCR)



Credit: Susan Walker

Advantages of swabbing

- Non-destructive protocol
- Easy to use
- Long shelf life (<6 months)
- Assay not susceptible to bacterial contamination

Medical Wire & Equipment Co (UK) MW 100-100

Wear gloves

- Prevent transmission of chytrid fungus or other diseases between frogs
- Help protect delicate amphibian skin on handling
- Good personal hygiene practise

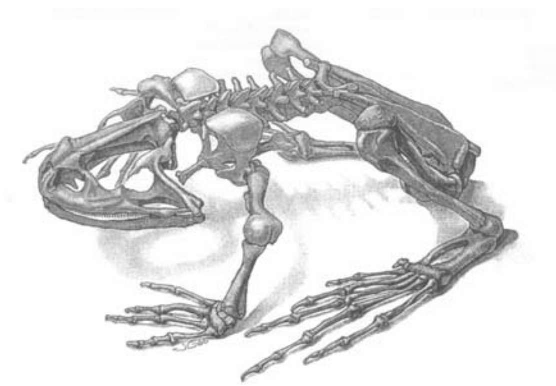
Which type of gloves do we use?

- 100% nitrile gloves
  - ant-chytrid action
- Powder free
  - No irritation
- Blue colour
  - easy to see!

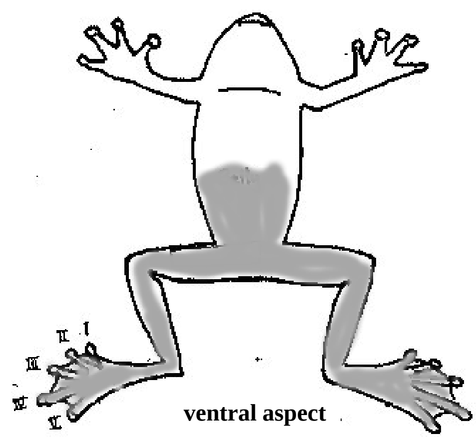
Handling

- Delicate skin
  - Osmoregulation
  - Cutaneous respiration
- Firm to prevent escape
- Careful to prevent damage
- Do not hold by feet – risk of fractures
- Handle close to ground

Anatomy – no rib cage – Care!



Where do we swab?



How to sample

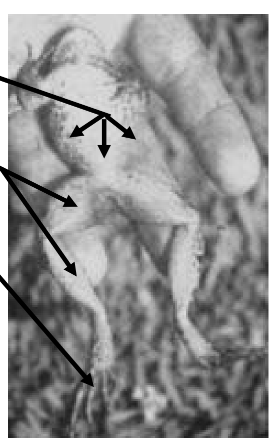
1. Swab drink patch (x3).

2. Swab inside leg(x3).

3. Swab inside foot (x3).

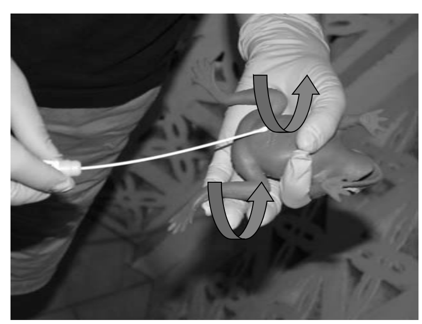
4. Replace swab into tube and notate.

5. Store on ice/at 4ºC.



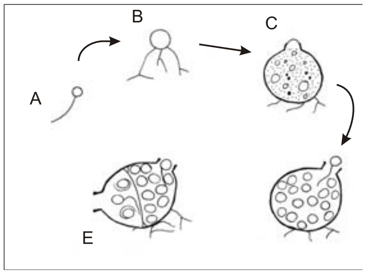
Swabbing protocol

- Swab thoroughly
- Rub and roll the swab back and forth over the skin



Why do we swab the underside?

- *Bd* transmission is associated with water
- Drink patch function to absorb water
- Underside has most contact with water in environment
- Drink patch, plantar aspect of hind feet and hind legs are predilection sites for *Bd* infection

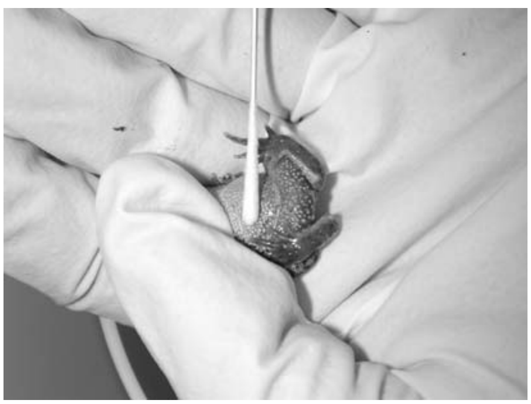


Lifecycle of *B .dendrobatidis*. A: zoospore B: germling C: Immature sporangium D: monocentric zoosporangium E: colonial thallus. (from Speare and Berger 2003)

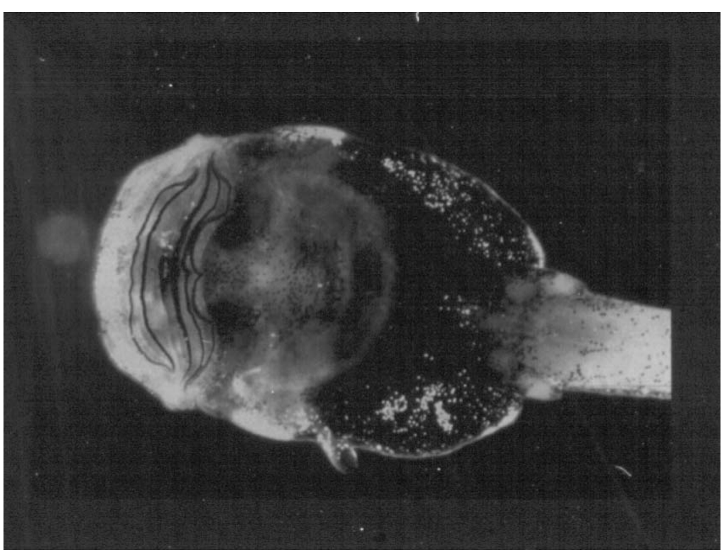
Swabbing protocol

- Fresh pair of gloves to handle EACH frog
- Remove the swab from the tube immediately before use
- Do not, at any stage, allow the tip of the swab to touch *anything* except the site being sampled

Swabbing regions 1. Drink patch



Swabbing areas 2. Legs and digits



Recording and archiving samples

- Label swab with permanent marker
- Record
  - –Species
  - –Sex (where known)
  - –Date
  - –Survey location
  - –Additional details as required

Recording and archiving samples

ZSL

On return from field

- Check and complete label details
- Check information is legible

Recording and archiving samples

ZSL

- Store each batch of swabs from the same survey together in a plastic bag

- Store swabs in freezer (or in 70-80% ethanol) until testing
- Complete record form with details of samples taken per survey

Disinfection

ZSL

- Essential to disinfect between sites
- Must avoid spreading disease through field surveys

Biosecurity and prevention of chytrid spread

Andrew A. Cunningham

ZSL Institute of Zoology
LIVING CONSERVATION

Main risks of chytrid spread

ZSL

(1) Spread of disease to new countries

(2) Spread of disease within a country

Spread to new countries

ZSL

- Risk of *B. dendrobatidis* arriving to new countries can be broken down into specific areas. Risk of introduction through:
- Movement of people (contaminated footwear, equipment)
- Movement of produce (contaminated soil, water; infected amphibian stow-aways).
- Intentional movement of amphibians (e.g. pet trade, conservation actions).

Risk Assessment: People

ZSL

- Water and soil which remains moist should be considered infective for at least 7 weeks after contact with an infected amphibian.
- Two examples of wet fomites that could spread *B. dendrobatidis* to new countries are damp soil transported with agricultural produce, and damp soil or water on the footwear or clothes of visitors.
- The persistence of small amounts of water on soil and sand particles means that soil may not need to be visibly damp to harbour viable zoospores.

Risk Assessment: People

ZSL

- This includes local people and tourists
- For most countries, there are no compulsory hygiene precautions for people undertaking international travel.
- the risk of infection arriving via this route, although likely to be small, cannot be ignored.
- Simple precautions that could be put in place are ensuring footwear is cleaned prior to, or on, arrival into a country (e.g. education of visitors to clean their footwear and equipment).

Educational Leaflet - Dominica

ZSL

Educational Poster - Dominica

ZSL

Risk Assessment: Trade

ZSL

Agricultural produce

- fresh agricultural produce is regularly traded internationally.
- The trade in bananas has been identified as particularly high risk as a method of spreading chytrid as they frequently harbour stow-away frogs.
- Soil contamination on produce must also be considered a risk.
- produce moved between countries by visitors and locals must also be considered.

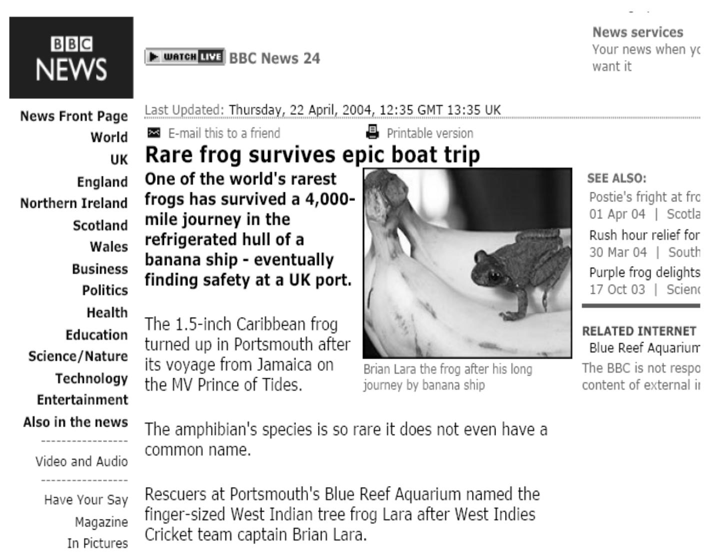
Risk Assessment: Trade

ZSL

- A potential method of spread is the accidental movement of infected amphibians within agricultural produce.
- It has been estimated that 50,000 frogs are accidentally translocated within Australia annually - the majority within banana bunches

For example:

- Montserrat receives approximately 78 deliveries of bananas annually from Dominica.
- Frogs have been found within the boxes but there is no active surveillance for them or any set protocols for dealing with them when they are found.



BBC NEWS

WATCH LIVE BBC News 24

News services
Your news when yo
want it

Last Updated: Thursday, 22 April, 2004, 12:35 GMT 13:35 UK

E-mail this to a friend | Printable version

News Front Page
World
UK
England
Northern Ireland
Scotland
Wales
Business
Politics
Health
Education
Science/Nature
Technology
Entertainment
Also in the news
Video and Audio
Have Your Say
Magazine
In Pictures

Rare frog survives epic boat trip

One of the world's rarest frogs has survived a 4,000-mile journey in the refrigerated hull of a banana ship - eventually finding safety at a UK port.

Brian Lara the frog after his long journey by banana ship

The 1.5-inch Caribbean frog turned up in Portsmouth after its voyage from Jamaica on the MV Prince of Tides.

The amphibian's species is so rare it does not even have a common name.

Rescuers at Portsmouth's Blue Reef Aquarium named the finger-sized West Indian tree frog Lara after West Indies Cricket team captain Brian Lara.

SEE ALSO:
Postie's fright at fro
01 Apr 04 | Scotla
Rush hour relief for
30 Mar 04 | South
Purple frog delights
17 Oct 03 | Scienc

RELATED INTERNET
Blue Reef Aquarium
The BBC is not respo
content of external i

Recommendations for prevention of chytridiomycosis spreading between countries - 1

Imported agricultural produce poses a clear threat. Strategies to reduce this risk which have been shown to work in Australia include:

- removing amphibians found on produce
- storage of boxes of produce in chillers overnight
- raising awareness of those who work with bananas
- In Australia, a publicity campaign has reduced the number translocated frogs that are released by supermarkets

Recommendations for prevention of chytridiomycosis spreading between countries - 2

- An additional strategy is to wash bananas on arrival
- Bananas should be immersed in water as soon as they arrive
- frogs which are displaced from the bunches on immersion should then be caught and euthanased

- The import of live amphibians for the pet trade, scientific study and food trade presents variable degrees of risk depending on the country.

Anthropogenic spread

Chytridiomycosis confirmed in:

- Pet trade (e.g. dendrobatid frogs)
- Food trade (e.g. bullfrog farms in Uruguay & Brazil)
  (> 1 million p.a. enter USA)
- Lab animal trade (*Xenopus* spp.)
- Zoo animal trade
- Invasive species of amphibian

Preventing spread within a country

- Don't take chytrid **in** to new sites

Don't take chytrid **out** to new sites

Disinfection

- Essential to disinfect between sites
- Must avoid spreading disease through field surveys

Disinfection

- Foot wear
  - –Always disinfect between sites
  - –Rubber boots
  - –Remove XS mud in field
- Equipment
  - –if it contacts frogs
- Vehicle tyres
  - –if on infrequently accessed roads or tracks?

Handling of frogs in the field

Protocols to prevent transmission of pathogens by non-invasive procedures such as weighing and measuring should be followed:

- Implements should be physically cleaned of any secretions or body fomites between use
- Implements should be sterilised by spraying or wiping with 70% alcohol or 0.4% bleach after cleaning and leaving the disinfectant on the surface for at least 30 seconds.
- If possible, each specimen should be weighed in its own plastic bag without coming into contact with scales or measuring implements.

Vehicles

Where necessary, vehicle tyres should be sprayed/flushed with a disinfecting solution in high-risk areas.

- Transmission of disease from vehicles is unlikely. However, if a vehicle is used to traverse a known frog site, which could result in mud and water being transferred to other bodies of water or frog sites, then wheels and tyres should undergo cleaning and disinfection. This should be carried out at a safe distance from water bodies, so that the disinfecting solution can infiltrate soil rather than run-off into a nearby water body.
- Spraying with hospital grade 'toilet duck' (active ingredient benzalkonium chloride) is recommended to disinfect car wheels and tyres. 'Toilet Duck' is also suitable for disinfecting footwear. Cleaning of footwear will prevent the transfer of pathogens from/to vehicle floor and control pedals.

NSW NATIONAL PARKS AND WILDLIFE SERVICE

Disinfection

- Follow manufacturers instructions
- Wear rubber gloves
- Care with eyes
- Keep away from frogs
- Keep away from water bodies

Disinfection strategies

- Correct dilution
- Sufficient time

- Kill chytrid & ranavirus:
- Sodium hypochlorite (household bleach)
  - 4% for 15 minute
- Virkon
  - 1 mg/ml for 1 minute

Berger 2001, Johnson et al 2003, Langdon 1989, Miocevic et al 1993

Disinfection

Correct equipment

e.g. rubber gloves, buckets, disinfectant solution, spray bottles, scrubbing brushes, water bottles

- Dedicated rubber boots for survey work
- Equipment
- Vehicle tyres

Cleaning footwear

- First - remove all mud from footwear and equipment using clean water

- Use water from containers *or*
- Use water from local streams *but*
- Do not wash boots in running water

- Dispose of dirty water in an area where waste soaks into ground
- Second – disinfect the cleaned footwear and equipment before visiting the next site.

Disinfection protocols

ZSL

Purpose	Disinfectant	Concentration	Time	Pathogen killed
Disinfecting surgical equipment and other instruments (eg. scales)	Ethanol	70%	1 min	*B. dendrobatidis* Ranaviruses
	Vircon	1 mg/ml	1 min	*B. dendrobatidis* Ranaviruses
	Benzalkonium chloride	1 mg/ml	1 min	*B. dendrobatidis*
Disinfecting collection equipment and containers	Sodium hypochlorite (bleach)	1%	1 min	*B. dendrobatidis*
	Sodium hypochlorite (bleach)	4%	15 min	Ranaviruses
	Didecyl dimethyl ammonium chloride	1 in 1000 dilution	0.5 min	*B. dendrobatidis*
	Complete drying		3 hrs or greater	*B. dendrobatidis*
	Heat	60°C	5 min	*B. dendrobatidis*
			15 min	Ranaviruses
	Heat	37°C	4 hrs	*B. dendrobatidis*
	Sterilising UV light		1 min	Ranaviruses only
Disinfecting footwear	Sodium hypochlorite (bleach)	1%	1 min	*B. dendrobatidis*
	Sodium hypochlorite (bleach)	4%	15 min	Ranaviruses
	Didecyl dimethyl ammonium chloride	1 in 1000 dilution	1 min	*B. dendrobatidis*
	Complete drying		3 hrs or greater	*B. dendrobatidis*
Disinfecting cloth (eg. bags, clothes)	Hot wash	60°C or greater	5 min	*B. dendrobatidis*
			15 min	Ranaviruses

HYGIENE PROTOCOL FOR HANDLING AMPHIBIANS IN FIELD STUDIES

Handling of frogs in the field

ZSL

- Frogs should only be handled if absolutely necessary.
- Protocols to prevent transmission of pathogens by procedures such as weighing and measuring should be followed:
  - Implements should be cleaned between use.
  - Implements should be disinfected between sites.
  - If possible, each frog should be weighed in its own plastic bag without coming into contact with scales or measuring implements.

Why do we wear gloves?

ZSL

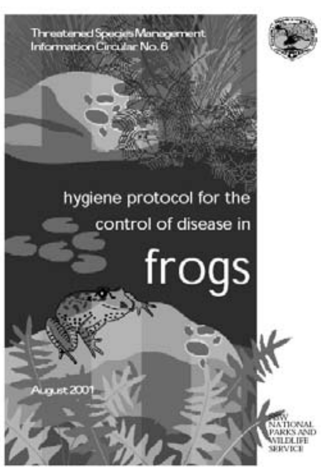
- Prevent transmission of chytrid fungus or other diseases between frogs
- Help protect delicate amphibian skin on handling
- Good personal hygiene practice

Handling & catching

ZSL

Further information

ZSL



Amphibian extinction crisis

ZSL

According to Amphibian ARK (www.amphibianark.org), 165 species believed to have already gone extinct in recent times:

- 34 known to be extinct
- 1 extinct in the wild, but extant in captivity
- 130 have not been found in recent years

Amphibian extinction crisis

500 species considered to be in imminent danger of extinction and for which mitigation of the threat is not possible in the wild in time to prevent extinction.

Therefore, rescue via captive breeding is required to save these species.

BUT…

Amphibian extinction crisis

…. only 10 species have been identified by zoos for captive assurance populations

It is likely that zoos globally will only have capacity for approx. 50 species

(e.g. only 10 species in North American zoos)

Therefore, in-country initiatives are vital if these species are to be saved!

Capacity building in Dominica

- 3 year collaborative project
- Began 1st April 2005

Partners

- Forestry & Wildlife Division
- Veterinary Services Division
- Zoological Society of London

Collaborators

- Fauna & Flora International
- Chester Zoo
- Durrell Wildlife Conservation Trust

Funded by the Darwin Initiative

Credit: DWCT/ Richard Gibson

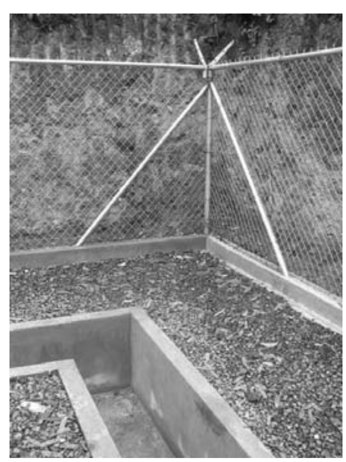
Project aims

- Public awareness campaign
- Amphibian population monitoring
- Disease screening
- Molecular laboratory construction & training
- Regional disease surveillance
- Regional management plan for disease
- Captive breeding
- Fund raising strategy for future

Credit: DWCT/ Richard Gibson

Captive breeding – biosecurity

Captive breeding – biosecurity



Captive breeding – biosecurity

ZSL

Captive breeding – biosecurity

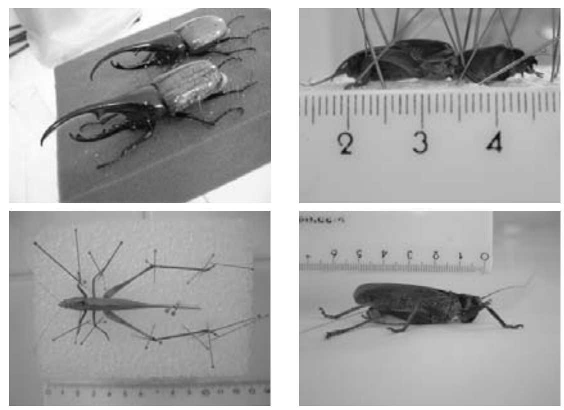
ZSL

Captive breeding – biosecurity

ZSL

Priority No. 2 – Food Source

ZSL

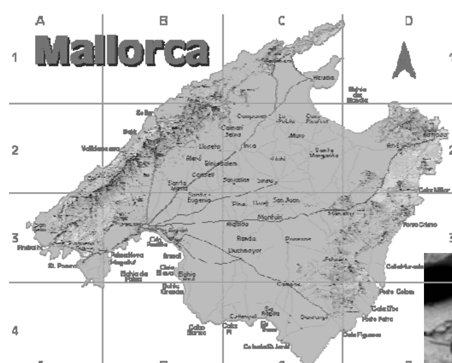


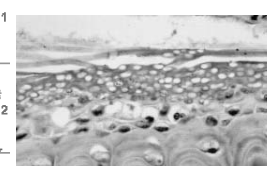
The primary prey of the mountain chicken is crickets (*Amphiacusta* sp.)?

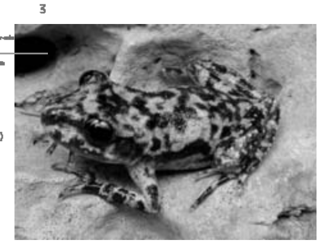
ZSL

Mallorcan midwife toad (*Alytes muletensis*)

ZSL







Loss of parasites

It may appear to be desirable for animals in captivity to be kept parasite-free, but this is not necessarily the case provided the parasites they harbour are those they would be exposed to in their natural habitat.

The maintenance of such a parasite burden, and hence the maintenance of genetic and other adaptations to these parasites, may help to ensure the survival of animals once they are reintroduced to the wild.

This also will allow the biodiversity of the parasites themselves to be conserved.

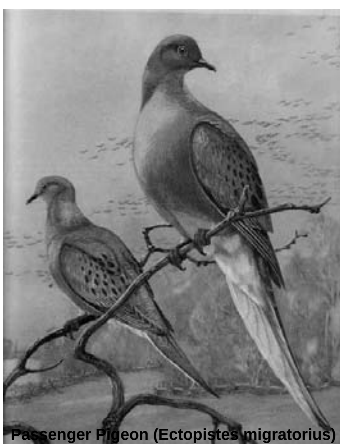
Loss of parasites

It may appear to be desirable for animals in captivity to be kept parasite-free, but this is not necessarily the case provided the parasites they harbour are those they would be exposed to in their natural habitat.

The maintenance of such a parasite burden, and hence the maintenance of genetic and other adaptations to these parasites, may help to ensure the survival of animals once they are reintroduced to the wild.

This also will allow the biodiversity of the parasites themselves to be conserved.

Co-extinction



Passenger Pigeon (Ectopistes migratorius)

Columbicola extinctus

Diplaegidia gladiator

Feather lice can be highly host-specific

Summary - 1

- Raise awareness (Government, commerce) with public awareness campaigns to target both locals and tourists.
- Introduce regulations to control import and export of amphibians and amphibian products
- Introduce control at ports of entry for detection of accidentally and deliberately introduced amphibians, accompanied by a policy of what to do with individuals once identified.
- Steps to minimise the risk of accidental introduction of chytrid by people (eg. clean footwear of tourists and locals)

Summary - 2

ZSL

- Training in amphibian survey techniques (transects and surveillance)
- Improved legal protection for amphibian species
- Implement early disease surveillance measure for amphibians
- Establish a network of contacts across the region for amphibian health and conservation
- Communication (of results, successes, failures)

Curso-Seminario
Problemática de la Disminución de las Poblaciones de Anfibios y el Papel de la Medicina Veterinaria

INFORME FINAL

Servicio Nacional de Salud Animal
Heredia, Costa Rica
22 y 23 de mayo, 2008

Sección VI
Contribuciones que ofrecen dar los participantes

Contribuciones que ofrecen dar los participantes

Alfaro Rojas, Christian
Escuela de Medicina Veterinaria
Universidad Nacional

Podría ayudar en cualquier tipo de asistencia y tengo un poco de experiencia en necropsia y microscopia.

Alvarado Barboza, Gilbert
Escuela de Biología
Universidad de Costa Rica
Escuela de Medicina Veterinaria
Universidad Nacional

Estoy desarrollando mi tesis en el tema.

Arroyo Luis Mariano
SENASA-MAG

Como voluntario en cualquier trabajo o investigación.

Elías Barquero Calvo
Escuela de Medicina Veterinaria
Universidad Nacional

Debido a que trabajo en el Laboratorio de Bacteriología de la Escuela de Veterinaria, es parte de mi labor e interés, colaborar en resolver los problemas infecciosos bacterianos tanto en animales domésticos como silvestres.

Bernal Valle, Sofía
Escuela de Medicina Veterinaria
Universidad Nacional

Investigación, generación y divulgación de información, trabajo de campo.
No tengo experiencia, pero estoy muy interesada en involucrarme en esta red en pro de la conservación de los anfibios, por lo que, en lo que pueda ayudar y aprender estoy dispuesta.

Brenes Soto, Andrea
Escuela de Zootecnia
Universidad de Costa Rica
FUNDAZOO

Mi campo de trabajo es la nutrición de animales silvestres en cautiverio, área en la que he trabajado más de 10 años.
Mi aporte a la conservación de los anfibios sería en la parte de nutrición animal, para programas de conservación en cautiverio.
La Escuela de Zootecnia está desarrollando un Programa de especies alternativas, dentro de las cuales se encuentran los animales silvestres, por lo que está muy interesada en apoyar estas iniciativas a través de proyectos de investigación y desarrollo de programas de nutrición animal en anfibios.

Brown Centeno, Michelle
Escuela de Medicina Veterinaria
Universidad Nacional

Trabajo voluntario

Canet, Cristian
EARTH

Yo como Ingeniero Agrónomo y como facilitador de proyectos de la EARTH, podría coordinar giras de muestreo a zonas indígenas de Alto Chirripó, la cual es la zona de impacto de los proyectos de la EARTH. También podría unirme a giras a otros lugares en días no laborables.
Me gustaría tener en mi poder información acerca de la problemática, la cual vía correo electrónico podría transferir a personas interesadas en vida silvestre, además de comunicar a instancias mayores de la EARTH, acerca de la problemática y en conjunto unir esfuerzos para apoyar la iniciativa con entes importantes que si podrían hacer campañas a favor de los anfibios, como parte de todo un ecosistema.

Carranza Cubero, Félix
SENASA-MAG

Redacción de protocolos y procedimientos sanitarios oficiales para la movilización de anfibios *in-situ* y *ex-situ* para Costa Rica.

Chaves Ramírez, Andrea
Escuela de Medicina Veterinaria
Universidad Nacional

Biología molecular, implementación y estandarización de técnicas moleculares, PCR, PCR en tiempo real, extracciones y más. Otras técnicas serológicas de diagnóstico. Virología.

Durán Salazar, Alexander
SENASA-MAG

A través de la educación y el apoyo que se pueda continuar dando a las Áreas Protegidas del SINAC-MINAE de la provincia de Limón, y a la investigación a través de proyecto personal de conservación en un centro de rescate de especies.

González Barrientos, Rocío
Escuela de Medicina Veterinaria
Universidad Nacional

Colaborando en proyectos presentes o futuros sobre las enfermedades que afectan a los anfibios de nuestro país, especialmente en el área de patología veterinaria.

Guevara Brenes, Arianna
Escuela de Medicina Veterinaria
Universidad Nacional

Ayudar en investigación de campo.
Divulgar información.
En todo lo que me sea posible colaborar, tomando en cuenta que tengo poca experiencia en el campo y que estoy relativamente empezando la carrera.

Hernández, Gabriela
Escuela de Medicina Veterinaria
Universidad Nacional

Colaborar en futuros talleres o trabajo requerido en laboratorios (toma de muestras, recolecta de especimenes, estandarización de alguna prueba diagnóstica para anfibios).

Leandro, Danilo
SENASA-MAG

Como voluntario en cualquier trabajo o investigación.

León, Bernal
SENASA-MAG

Como voluntario en cualquier trabajo o investigación.

Marchena Piña, Luis Enrique
Escuela de Medicina Veterinaria
Universidad Nacional

De cualquier manera que sea posible, soy estudiante.
Me gustaría participar de investigaciones, trabajo de campo, charlas y cualquier otra actividad que se requiera.

Martín, María Pía
Escuela de Medicina Veterinaria
Universidad Nacional

Puedo contribuir asistiendo a giras y muestreando o divulgando información.

Mora, Ronald
SENASA-MAG

Como voluntario en cualquier trabajo o investigación.

Morales Acuña, Juan Alberto
Servicio de Patología
Escuela de Medicina Veterinaria
Universidad Nacional

Integrar Laboratorio de Diagnóstico
Diagnóstico: disección e histopatología
Colecta de muestras para otros laboratorios de diagnóstico como Bacteriología, Parasitología, Virología, Micología.
Preservar muestras para PCR.

Pizarro Castillo, Marco Andrei
SENASA-MAG

Trabajo en la zona del Caribe, por lo tanto creo que podría colaborar con hospedaje y además acompañar en giras a esta zona.
También con equipo veterinario para trabajo de campo como microscopio y equipo de disección.

Quesada Jiménez, Angie Elizabeth
Escuela de Medicina Veterinaria
Universidad Nacional

Voluntariado para educación, investigación, trabajos de conservación.

Quesada Díaz, Fabiola
Escuela de Medicina Veterinaria
Universidad Nacional

Contribuyendo en proyectos que actualmente se están realizando o en proyectos futuros. Como estudiante de tesis, estoy anuente en todo lo que respecta a este tema y como futuro profesional contribuir en áreas de clínica o trabajo de campo.

Rodríguez Guarín, Carolina
Escuela de Medicina Veterinaria
Universidad Nacional

Participación en estudios de campo y toma de muestras para evaluar la salud y presencia de enfermedades.
Elaborando propuestas de investigación.

Rodríguez Matamoros, Jorge
CBSG Mesoamérica

Haciendo conecciones para realizar talleres.
Manejando base de datos o nueva información.
Solicitando herramientas para manejar información.

Salas Rojas, Carolina

Investigación en problemática anfibios-plaguicidas y otras sustancias tóxicas, ecotoxicología, evaluación de riesgo ambiental asociado a plaguicidas.

Sánchez Picado, Sergio
SENASA-MAG

Investigación. Trabajo en el Departamento de Patología. Por ahora una de mis capacitaciones se lleva a cabo en el Laboratorio de Patología de la Escuela de Medicina Veterinaria con el Dr. Juan Alberto Morales.

Solís Alfaro, Karolina
Escuela de Medicina Veterinaria
Universidad Nacional

Como médica veterinaria trabajar en conjunto con herpetólogos para reconocer enfermedades y saber como tratar los anfibios.
Tratar de tomar medidas de conservación y concientización para evitar la contaminación ambiental.
Trabajar también en conjunto con veterinarios con más conocimiento en este campo, así como en el de los reptiles, tanto en cursos aislados como en posibles proyectos.

Vargas Leitón, Rodolfo
Escuela de Biología
Universidad Latina

Trabajando en programas de zoocría en cautiverio.

Zumbado Ulate, Héctor
Escuela de Bilogía
Universidad de Costa Rica

Monitoreos periódicos.
Investigación en detección de B.D.
Informando a estudiantes sobre la problemática de los anfibios.

Curso-Seminario
Problemática de la Disminución de las Poblaciones de Anfibios y el Papel de la Medicina Veterinaria

INFORME FINAL

Servicio Nacional de Salud Animal
Heredia, Costa Rica
22 y 23 de mayo, 2008

Sección VII
Observaciones de los Participantes

Observaciones de los participantes al taller

Este taller estuvo muy interesante ya que se enfocó en lineamientos importantes para mejorar la toma de muestras de campo y la manipulación de anfibios. Además mostró datos sobre otras enfermedades que afectan a los anfibios.

Es importante tener un ejemplo de la forma de trabajo de personas como el Dr. Cunningham, el es una persona de conocimiento global. Es importante la lucha de traer otras personas a la investigación de los anfibios para no ser los mismos de siempre.

La experiencia del Dr. Cunningham ha sido muy enriquecedora pues permitió conocer el área de manejo sanitario de estas especies. Definitivamente en Costa Rica hay mucho por desarrollar, sobre todo los protocolos médicos y de bioseguridad tanto para los ranarios existentes como para los proyectos venideros, como el Centro Nacional que se construirá en Santa Ana. Es importante que este trabajo sea interdisciplinario, pues el manejo en cautiverio de los anfibios debe abarcar profesionales en el campo de la medicina veterinaria, manejo en cautiverio, nutrición animal y biología.

La problemática de la disminución de poblaciones de anfibios tiene un origen multifactorial, y como tal debe ser abordada desde un plano multidisciplinario. Es una gran necesidad aumentar el conocimiento veterinario de anfibios, para reconocer los individuos saludables y poder así identificar aquellos enfermos, todo esto para que sea de ayuda a la hora de hacer evaluaciones de los especimenes de vida libre, así como futuras poblaciones reproducidas en cautiverio.

Esta problemática requiere mucha investigación y estudios de campo, donde resulta urgente propuestas y participación activa de veterinarios y epidemiólogos.

Ojalá se siguiera con este tipo de talleres, incluyendo otras especies silvestres.

Me parece muy importante que se realicen actividades como estas para los estudiantes que estamos muy interesados en trabajar con vida silvestre y desconocemos la variedad de posibilidades que existen.

Este taller es un importante esfuerzo por informar a la población interesada en la conservación de la vida silvestre sobre la situación actual de los anfibios en nuestro país y en el mundo. Proporcionó información muy importante sobre la medicina en anfibios que difícilmente estaría disponible.

Considero de suma importancia actividades como esta, con profesionales de la calidad como los que asistieron y compartieron su conocimiento con nosotros, ya que de esta forma nos enteramos de los problemas y situaciones que están sufriendo los anfibios, en este caso y de los esfuerzos que se están realizando para evitar su extinción, o propagación de enfermedades, entre otras cosas. Además nos abre los ojos y las puertas a nuevas ideas de investigación y puntos en los que se puede ayudar o contribuir en la conservación de estos animales, y la vida silvestre en general.

Necesitamos más educación continua acerca de estos temas. Poder realizar prácticas de manejo, pruebas diagnósticas, toma de muestras entre otros sería muy apropiado.
.
Ojalá continúen haciendo este tipo de talleres porque nos involucran a nosotros los que en el futuro podemos hacer algo al respecto, y a aquellos que ya pueden hacer algo.

Es importante tener más conocimientos sobre técnicas de diagnóstico en virus que afectan los anfibios, su transmisión y zoonosis existentes

Al final de la actividad los participantes indicaron cuales podrían ser sus aportes a la conservación de los anfibios, información que se retomará para establecer una estrategia en el campo de la medicina veterinaria.

Curso-Seminario
Problemática de la Disminución de las Poblaciones de Anfibios y el Papel de la Medicina Veterinaria

INFORME FINAL

Servicio Nacional de Salud Animal
Heredia, Costa Rica
22 y 23 de mayo, 2008

Sección VIII
Lista de Participantes

Curso-Seminario:
Problemática de la Disminución de las Poblaciones de Anfibios y el Papel de la Medicina Veterinaria

Arianna Guevara ***UNA-Escuela de Medicina Veterinaria*** *Teléfono:88130664* *Fax:* *E-mail: arigb@gmail.com* *Dirección postal:*	***Andrea Brenes*** ***UCR-Escuela de Zootecnia*** *Teléfono: 22073441* *Fax:22245527* *E-mail: andrea.brenessoto@ucr.ac.cr* *Dirección postal:*
Bernal León ***SENASA-MAG*** *Teléfono: 22608300 Ext. 2106* *Fax: 22608300 Ext. 2106* *E-mail: bleon@senasa.go.cr* *Dirección postal:*2299-3000 Heredia	***Carolina Salas*** ***UCR-Escuela de Biología*** *Teléfono:* *Fax:* *E-mail: caros_r@yahoo.com* *Dirección postal:*
Caterina Guzmán ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 22380761* *Fax: 22381298* *E-mail: catguz@medvet.una.ac.cr* *Dirección postal:* 309-3000 Heredia	***Cristian Alfaro*** ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 88223566* *Fax: 24633086* *E-mail: alfarojas@gmail.com* *Dirección postal:*
Danilo Leandro ***SENASA-MAG*** *Teléfono: 22608300 Ext. 2110* *Fax: 22608300 Ext. 2020* *E-mail: dleandro@senasa.go.cr* *Dirección postal:* 3-3006 Cenada, Heredia	***Elías Barquero Calvo*** ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 83883233* *Fax: 22610049* *E-mail: eliasb@medvet.una.ac.cr* *Dirección postal:* 3-3006 Cenada, Heredia
Gabriela Hernández ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 88937609* *Fax: 22381298* *E-mail: gabbytica@gmail.com* *Dirección postal:*	***Gilbert Alvarado*** ***UCR-Escuela de Biología/UNA-Escuela de Medicina Veterinaria*** *Teléfono: 88366562* *Fax:* *E-mail: gilbert.alba@gmail.com* *Dirección postal:* 3-3006 Cenada, Heredia
Gilbert Contreras ***SENASA-MAG*** *Teléfono: 83517643* *Fax: 22608648* *E-mail: gcontreras@senasa.go.cr* *Dirección postal:*	***Héctor Zumbado*** ***UCR-Escuela de Biología*** *Teléfono: 83903193* *Fax: 22249661* *E-mail: hugozu1@yahoo.com* *Dirección postal:* 13255-1000 San José
Jorge Rodríguez ***CBSG Mesoamerica*** *Teléfono: 8372817* *Fax: 22231817* *E-mail: cbsg.ma@gmail.com* *Dirección postal: 04-4050 Alajuela*	***Juan Alberto Morales*** ***Servicio de Patología*** ***Escuela de Medicina Veterinaria*** ***Universidad Nacional*** *Teléfono: 2 260 08 49* *Fax: 2 260 08 49* *Dirección postal: 744-3000 Heredia*

Luis Marchena ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 88606142/22852854* *Fax:* *E-mail: luismapi26@hotmail.com* *Dirección postal:* Barrio el Carmen de Guadalupe. Residencial Las Hortensias Casa 115	***Maricruz Guevara*** ***SENASA-MAG*** *Teléfono: 83263927* *Fax: 22288300 Ext. 212* *E-mail: mguevara@senasa.go.cr* *Dirección postal:*8545-1000 San José
Mario Baldi ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 22380761* *Fax: 22381298* *E-mail: mbaldi@medvet.una.ac.cr* *ma.baldi1@gmail.com* *Dirección postal: 3265-3011 Barva, Heredia*	***Melissa Blandón*** ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 25624553* *Fax: 25624553* *E-mail: meli_blandon@hotmail.com* *Dirección postal:*
Rafael Vindas ***SENASA-MAG*** *Teléfono: 22620229* *Fax:22620231* *E-mail: rvindas@senasa.go.cr* *Dirección postal:*	***Randall Arguedas*** ***FUNDAZOO*** *Teléfono: 22550012* *Fax: 22231817* *E-mail: fundazoo.zsb@gmail.com* *Dirección postal: 11594*-1000 San José
Gerardo Chaves ***UCR-Escuela de Biología*** *Teléfono: 22075966* *Fax: 22074216* *E-mail: cachi@biologia.ucr.ac.cr* *Dirección postal:* Universidad de Costa Rica, Escuela de Biología, San Pedro de Montes de Oca.	***Rocío González*** ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 88298436* *Fax:* *E-mail: cristyrocio@yahoo.com* *Dirección postal:* 25 mts. este del costado de la Iglesia de Curridabat
Ronald Mora ***SENASA-MAG*** *Teléfono: 26663985* *Fax: 26663985* *E-mail: rmora@senasa.go.cr* *Dirección postal: 3-3006 Cenada, Heredia*	***Sergio Sánchez*** ***SENASA-MAG*** *Teléfono: 88349749* *Fax: 22279338* *E-mail: sanchez@senasa.go.cr* *Dirección postal:* 3-3006 Cenada, Heredia
Sofía Bernal ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 83732255* *Fax: 22262430* *E-mail:* sofiabv05@gmail.com *Dirección postal:*	***Viviana Herrera*** ***SENASA-MAG*** *Teléfono: 22609046* *Fax: 22609046* *E-mail: vherrera@senasa.go.cr* *Dirección postal:* 3-3006 Cenada, Heredia
Yolanda Matamoros ***FUNDAZOO-CBSG Mesoamerica*** *Teléfono: 22236701* *Fax: 22231817* *E-mail: fundazoo.zsb@gmail.com* *Dirección postal:* 11594-1000 San José	***Cristian Canet*** ***EARTH*** *Teléfono: 27130000* *Fax: 22359856* *E-mail: criscanet@gmail.com* *Dirección postal:* 499-1100 Tibás
Gonzalo Carmona ***DOS PINOS*** *Teléfono:24373565* *Fax:* *Correoelectrónico:gcarmona@dospinos.com* *Dirección postal:* 1(2400) Desamparados Centro	***Francisco Calvo*** ***SENASA-MAG*** *Teléfono: 83976422* *Fax:* *E-mail: francalvo_vet@yahoo.com* *Dirección postal:* 3-3006 Cenada, Heredia

Gerardo Rivera ***SENASA-MAG*** *Teléfono:22866000* *Fax: 22628642* *E-mail: grivera@senasa.go.cr* *Dirección postal: 3-3006 Cenada, Heredia*	***José Rojas*** ***SENASA-MAG*** *Teléfono: 22608300* *Fax: 22608364* *E-mail: jrojas@senasa.go.cr* *Dirección postal: 3-3006 Cenada, Heredia*
Félix Carranza ***SENASA-MAG*** *Teléfono: 22608300 Ext. 2110* *Fax: 22609049* *E-mail: anfife2050@gmail.com* *Dirección postal: 3-3006 Cenada, Heredia*	***Federico Bolaños*** ***UCR-Escuela de Biología*** *Teléfono: 22075959* *Fax: 22074216* *E-mail: bolanosv@biologia.ucr.ac.cr* *Dirección postal:* Universidad de Costa Rica, Escuela de Biología.
Ricardo Jiménez ***Universidad San Francisco de Asís*** *Teléfono:88760714* *Fax:* *E-mail: rik_rdo30@hotmail.com* *Dirección postal:*	***Rodolfo Vargas*** ***INBio Parque*** *Teléfono: 88280324* *Fax:* *E-mail: vargasleiton@gmail.com* *Dirección postal:*
Fabiola Quesada ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 88480103* *Fax:* *E-mail: fabiquesada07@yahoo.com* *Dirección postal:*	***Carolina Rodríguez*** ***UNA- Escuela de Medicina Veterinaria*** *Teléfono: 88221707* *Fax:* *E-mail: carolina_guarin@yahoo.com* *Dirección postal:* 1564-1200 Pavas
Andrea Chaves ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 25624508* *Fax:* *E-mail: chavesr_a@yahoo.com* *Dirección postal:*	***Michelle Brown*** ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 88439294* *Fax:* *E-mail: mcbrown08@gmail.com* *Dirección postal:*
Angie Quesada ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 88734465* *Fax:* *E-mail: lamirrisa@gmail.com* *Dirección postal:* Jardines de Moravia, del Salón comunal 100 mts. Este y 150 mts. Sur	***Manuel Ureña*** ***SENASA-MAG*** *Teléfono: 22609049* *Fax: 22609049* *E-mail: furena@senasa.go.cr* *Dirección postal: 3-3006 Cenada, Heredia*
Luis Mariano Arroyo ***SENASA-MAG*** *Teléfono: 22608300 Ext.2110* *Fax:22609046* *E-mail: marroyo@senasa.go.cr* *Dirección postal: 3-3006 Cenada, Heredia*	***Edwin García*** ***SENASA-MAG*** *Teléfono: 22608648* *Fax: 22608648* *E-mail: agracia@senasa.go.cr* *Dirección postal: 3-3006 Cenada, Heredia*
Alexander Durán ***SENASA-MAG*** *Teléfono: 27583514* *Fax: 27585639* *E-mail: aduran@senasa.go.cr* *Dirección postal: 3-3006 Cenada, Heredia*	***Marco Pizarro*** ***SENASA-MAG*** *Teléfono: 88690646* *Fax: 27585639* *E-mail: apizarro@senasa.go.cr* *Dirección postal: 3-3006 Cenada, Heredia.*

Isabel Hagnaver ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 83774513* *Fax:* *E-mail: isaghv@yahoo.es* *Dirección postal:*	***María Pía Martín*** ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 83838778* *Fax:* *E-mail: piamartin21@yahoo.com* *Dirección postal:*
Karolina Solís ***UNA-Escuela de Medicina Veterinaria*** *Teléfono: 83601237* *Fax: 24946435* *E-mail: ksolisa@gmail.com* *Dirección postal:* 213-4100	***Andrew A. Cunningham*** ***Institute of Zoology. The Zoological Society of London*** *Teléfono: +44 (0)20 7449 6674* *Fax: +44 (0)20 7483 2237* *E-mail: A.Cunningham@ioz.ac.uk* *Dirección Postal: Regent's Park, London, England NW1 4RY*