# Update on "Raised Epiderminal Ridge Disease"

Warren F. Fox
Developmental Biology Center, University of California, Irvine, California 92717

The following short notice concerning "RER disease" appeared in the Spring, 1980 issue of the Axolotl Newsletter:

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RAISED EPIDERMAL RIDGE DISEASE

Warren F. Fox*

Symptoms:

Raised epidermal ridge 1mm high x 1mm wide with length 5mm to length of animal, colored white or grey, may branch and may start on more than one part of body. Histologically, the area showed a large number of skin glands and some dead cells, but was otherwise a fairly normal epidermis. It usually occurs on animals of 2 - 3 months of age and disappears by 6 months. Growth and feeding slows. White animals are most susceptible (no Albinos have been exposed). Death rate is not high unless secondary infection takes place.

Treatment:

No successful treatment has been found although salt treatment gives some relief. The salt causes skin to slough but ridge remains.

Causative Agent: Possibly viral.

After finding nothing ourselves in smear sections, and cultures, we had a group of California Fish and Game pathologists inspect the animals, sections and smears. They could find no causative parasite. They felt it was most likely a viral infection and felt a environmental cause was unlikely.

Secondary Infection:

Although the effects of the ridge are not too serious, the ridge seems to be a good place for bacteria and fungi to attack. Cultures at various times have indicated Mimea, Alcaligenes, Aeromonas, Vibro, and Pseudomonas, (fungial culture not done). Sensitivity test showed these bacteria resistant to the common axolotl treatments that are administered in the water (including Nitrofurazone, Tetracycline, and Penicillin). Due to the small size and large number of larve infected, injection of antibiotics was not feasible. The best treatment seems to be 500 mg. Gentamicin sulfate (Sigma G3632) and Rifampicin (Sigma R3501) to 20 liters of 50% Holtfreter's solution, but this treatment is rather expensive. Rifampicin is not very water soluble, necessitating putting it in approximately 1 liter of water on a magnetic stirrer for 30 minutes. The solution is only stable for 24 hours or less and should be used for 3 - 5 days. We have also had some luck treating with Chloramphenicol at the rate of 500 mg./20 liters, dissolved as for Rifampicin, and used for same number of days. Either anithiotic treatment must be followed by axolotl dye treatment or salt treatment due to fungal bloom after bacteria are killed.

Occurrences:

This disease has apparently been noted by Dr. D. Stocum at University of Illinois and Dr. N. Holder at King College, England.

* Center for Pathobiology
University of California, Irvine
Irvine, CA 92717

We thought we had the problem solved with resistant animals (I.U. # 4874, 4974, and 4751), but this year's progeny from those animals became infected. We contracted with Dr. Robert Busch of Rangen Research Hatchery to test the animals. That organization will do a complete survey (without T.E.M.) for $125. A list of other organizations offering similar services is attached. Preliminary results showed no causative organism. We then requested a T.E.M. analysis. The reports are attached. No indication of an infectious agent was found. Clearly, diseased of the axolotl represent a challenge for clinical personnel as well as developmental biologists!

Rangen Research
A Division of Rangen inc.
Route 1, Box 264, Hagerman, Idaho 83332
Phone (208) 837-6192

# CLINICAL DIAGNOSIS
## FINAL SUMMARY REPORT

"SICK ANIMAL"

Acc. No.: 113-81B Date Rec'd.: 4 / 7 /81 Pond Designation: Symtomatic

Station: University of California - Irvine Agent: Warren Fox

Specimen Examined: Axolotl (Ambystoma mexicanum)

(XX) External Clinical Examination
(XX) Internal Clinical Examination
(XX) Stained Tissue Smears + 4 extras
(XX) Virology: no. of pools 4
(XX) Histopathology: no. of sections 8
(XX) Primary Bacterial Isolation
(XX) Confrimed Bacterial Identification
(XX) Antibiotic Sensitivity Screening
( ) Water Quality: _____
( ) Other: _____

DIAGNOSTIC FINDINGS:

Clinical examination showed a white to grey raised epidermal lesion proliferating over the skin, eroded and necrotic gills, pale and hemorrhaged livers, and edemacious spleens. Stained tissue smears demonstrated a light incidnece of Aeromonad-like bacteria from the gills, dermal lesion, liver, and kidney.

Bacteriological examination detected a heavy amount of Aeromonad-like bacteria from the gills and a moderate to heavy incidence of Aeromonas spp. from the liver, spleen, kidney, and dermal lesion. An antibiotic sensitivity test showed this Aeromonas spp. to be sensitive in decreasing order to Gentamicin, Nalidixic Acid, and Neomycin Sulfate.

Virological assay using standard methods and materials on CHSE-214, RTG-2, FHM, and FT cell lines detected no virus.

Histopathological examination observed lymphoid accumulation and edema in the gills. The liver exhibited fatty degeneration with proteinacious material evident throughout. Heavy lymphoid accumulation was located in the liver periphery and bacterial involvement and/or nutritional problems were indicated. Widespread tubular necrosis and general edema were evident in the kidney. The epidermis within the dermal lesion was hyperplastic, ragged in appearance and exhibited necrosis and edema throughout. Many of the nuclei were pycnotic and karyolytic. Areas of disorganization were exhibited in the dermis and the muscle appeared normal. Periodic Acid Schiff (PAS), Gimenez, and Hoechst stains did not demonstrate fungal involvement, Chlamydia, or mycoplasmas, respectively, in the dermal lesion.

Gimenez and Hoechst stains of the FT cells after viral screen did not demonstrate Chlamydia or mycoplasmas, respectively.

Station: University of California - Irvine Agent: Warren Fox

The E.M. photographs did not show any viral involvement.

NOTIFICATION OF DIAGNOSTIC RESULTS:
Person Contacted: Warren Fox Date: 4 /7 /81

RESOLUTION:

The epidermal lesion pathology suggested viral involvement, although none was detected in the virological assay. Bacterial involvement appears to be secondary and it might be suggested to treat with antibiotics either by food or injection along with water treatments

*This report does not constitute a fish health certification and cannot be used for that purpose.*

CLINICIAN: Ronald E. Kinnunen Date: 7 /23/81

"HEALTHY ANIMALS"

Rangen Research
A Division of Rangen inc.
Route 1, Box 264, Hagerman, Idaho 83332
Phone (208) 837-6192

# CLINICAL DIAGNOSIS
## FINAL SUMMARY REPORT

Acc. No.: 113-81A Date Rec'd.: 4 / 7 / 81 Pond Designation: Asymtomatic

Station: University of California - Irvine Agent: Warren Fox

Specimen Examined: Axolotl (Ambystoma mexicanum)

(X) External Clinical Examination
(X) Internal Clinical Examination
(X) Stained Tissue Smears + 3 extras
(X) Virology: no. of pools 4
(X) Histopathology: no. of sections 8
(X) Primary Bacterial Isolation
(X) Confirmed Bacterial Identification
(X) Antibiotic Sensitivity Screening
( ) Water Quality:
( ) Other:

DIAGNOSTIC FINDINGS:

Clinical examination showed pale livers and spleens. Stained tissue smears demonstrated a moderate amount of Aeromonad-like bacteria from the gills and a light incidence in the liver, spleen, and kidney.

Bacteriological examination detected a moderate amount of Aeromonad-like bacteria from the gills and a light incidence of Aeromonas spp. from the liver, kidney, and integument. An antibiotic sensitivity test showed this Aeromonas spp. to be sensitive in decreasing order to Gentamicin, Nalidixic Acid, Neomycin Sulfate, and Streptomycin.

Virological assay using standard methods and materials on CHSE-214, RTG-2, FHM, and FT cell lines detected no virus.

Histopathological examination observed a light to moderate amount of lymphoid elements in the gills. The liver exhibited fatty degeneration (possible nutritional problems) with heavy lymphoid accumilation in the liver periphery. General edema and widespread tubular necrosis were noted in the kidney. The epidermis, dermis, and muscle appeared normal. Periodic Acid Schiff (PAS), Gimenez, and Hoechst stains did not demonstrate fungal involvement, Chlamydia, or mycoplasmas, respectively.

The E.M. photographs did not show any viral envolvement.

NOTIFICATION OF DIAGNOSTIC RESULTS:
Person Contacted: Warren Fox Date: 4 / 7 / 81

RESOLUTION:

*This report does not constitute a fish health certification and cannot be used for that purpose.*

TECHNICIAN: Ronald E. Kinnunen Date: 7 123181
(Clinical Lab Supervisor)

# Diagnostic Services Available

As the demand for exotic fishes increases, so too does the demand for quality fish disease diagnostic services. Although some governmental institutions occasionally accepted tropical fish specimens in the past, they have become increasingly reluctant to handle diseased specimens from individual aquarists. Each month we receive numerous inquiries from hobbyists who wish to submit diseased specimens to fish pathologists for a diagnosis. In an attempt to satisfy the needs of the hobbyist, we sought the names of fish health specialists who would accept specimens from hobbyists, dealers, importers and commercial breeders of tropical fishes. To date we have the names and locations of seven such laboratories. As additional qualified persons respond to our solicitations, we will publish their names in the *Hobbyist*.

We suggest you contact the particular laboratory you have chosen regarding cost of analysis and their preferred methods for handling and preserving tissues. For histopathological examination, most laboratories find 10% buffered neutral formalin (B.N.F.) to be a satisfactory perservative. For bacteriological examination, the tissues should be submitted in the fresh or frozen state. If you are confused about how to obtain and prepare 10% B.N.F. or how to package tissues for mailing, we suggest the following: try to obtain some concentrated formaldehyde from your local mortician. Dilute this with 9 parts distilled water and add a pinch of borax as a buffer. Unless the formalin is neutralized in some way, formic acid crystals may form which interfere with the pathologist's diagnosis. This solution should be stored in a safe place, preferably in a dark bottle at room temperature. Tissues (or the entire fish with incised abdomen) should be soaked in the 10% B.N.F. for 24 hours, wrapped in formalin-soaked gauze and sealed in an air-tight plastic bag for shipping. A comprehensive case history must accompany the specimen. For more details, read the February fish health column entitled "Post-Mortem Examination."

John F. Kuhns, Research Director, Aquascience Research Group, 512 East 12th Avenue, North Kansas City, Missouri 64116, (816) 842-2590.

Instructions: $5.00 minimum charge to hobbyists, $10.00 minimum charge to businesses. Freeze tissues for bacteriological or virus analysis. For histopath. use 10% B.N.F. Taxonomic ID, water analysis and consulting services also available.

Species Accepted: No limitations on fish, inquire about invertebrates.

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Dr. Robert A. Busch, Director of Research, Rangen Research Hatchery, Route 1, Hagerman, Idaho 83332, (208) 837-~~4402~~. 6192

Instructions: Some diagnostic and consultative services may be available at no charge. Contact Rangen for policy before submitting specimens. Prefer fishes be submitted live or freshly refrigerated. All phases of laboratory diagnostic services are available excluding water analysis.

Species Accepted: All cold-blooded aquatic animals are accepted from fish culturists, breeders and importers—not from individual hobbyists.

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Charles Dale Meryman, Director, Fish Doctor Laboratory, Inc., 9225 Bay Plaza Blvd., Suite #408, Tampa, Florida 33619, (812) 626-1805.

Instructions: All phases of laboratory diagnostic services are available including pond-side consulting in Florida. Also conducts pollutant bioassays, taxonomic ID and surgical procedures. Contact laboratory for fee schedule, preservation and shipping instructions.

Species Accepted: No limitations.

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Dr. Donald F. Amend, Tavolek Laboratories, 2779 152 Ave. N.E., Redmond, Washington 98052, (206) 883-2150.

Instructions: 10% B.N.F.

Species Accepted: Tissues accepted from major importers or breeders only.

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Dr. Raymond A. Bendele, Texas Veterinary Medical Diagnostic Laboratory, P.O. BOX 3040, College Station, Texas 77840, (713) 845-3414.

Instructions: $5.00 minimum charge per fish. 10% B.N.F. for histopath. exam. Freeze tissues for bacteriological and viral analysis.

Species Accepted: No limitations.

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Dr. G.W. Klontz, Dept. of Fishery Resources, College of Forestry, Wildlife and Range Sciences, University of Idaho, Moscow, Idaho 83843, (208) 885-6336.

Instructions: 10% B.N.F.

Species Accepted: Preference given to unusual disease conditions.

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Dr. R.E. Wolke, Marine Pathology Laboratory, Dept. of Animal Pathology, University of Rhode Island, Kingston, Rhode Island 02881, (401) 792-2334.

Instructions: 10% B.N.F. or Bouin's fixative.

Species Accepted: Exotic marine fishes preferred. Preference given to importers, breeders, or public aquariums.