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Grant No. 4092--Penrose Fund (1966), \$610.00. Evolution of tiger salamanders (Ambystoma tigrinum) on the Grand Canyon rims, Arizona.

High, mesic woodland, forest, and grassland communities of the Grand Canyon rims are isolated by the canyon physical barrier, its contained shrub desert communities, and other arid-semiarid environments surrounding Grand Canyon National Park. The north rim is approximately 1000 feet higher in elevation and correspondingly more mesic than the south rim. Tiger salamander (Ambystoma tigrinum) populations occur in the rim communities, not in the Grand Canyon. The species is broadly distributed elsewhere in both time and space, so Grand Canyon populations presumably had a common gene pool before canyon formation. Information relative to north rim vs. south rim divergence in larval (pond inhabiting) and subadult-adult (gopher burrow inhabiting) individuals was obtained in order to elucidate the evolution of tiger salamanders during the 10 million years of Grand Canyon erosion.

Between 5 July and 6 September 1966, 52 different collections of tiger salamanders and associated environmental data were made on both rims, largely in Grand Canyon National Park; 580 specimens of all age-classes were preserved. Fifty-six individuals were transported alive to Baylor University for physiological, chromosomal, and electrophoretic studies; and miscellaneous notes on still another 2,600 salamanders were gathered. A few collections of vertebrate associates (Microtus longicaudus, Thamnophis elegans), gopher burrow coinhabitants (Thomomys bottae, Scaphiopus intermontanus),

and aquatic plants from salamander ponds (Potamogeton, Sparganium, Juncus, Carex, etc.) were made.

Primary study locations, representing different seres, were the Hearst and Hull Ponds (South Rim), and Harvey, Greenland, North Boundary, Little Park, and Indian Ponds (North Rim). Bordering terrestrial habitat was considered part of a study area. Temperatures of salamanders, water stations, gopher burrows, and ground surface cover, were related to microclimatic pattern and salamander activity. Pond turbidity, water-level, and oxygen concentrations were discovered concomitantly. Relative humidity readings under ground surface cover (chiefly logs) and in gopher burrows were accumulated. Mark-recapture techniques furnished data on larval population dynamics and were facilitated by marked census plots and funnel traps in certain ponds. Observations of gulping, aggregative, defensive, migratory, metamorphic, and other behavior of larvae, subadults, and adults revealed new information related to diel and life cycles.

Critical thermal maximum (CTM) and desiccation-hydration rates were determined for subadults, adults, and larvae associated with two different primary study areas on the north rim and one on the south rim. Experimental salamanders were acclimatized at $25^{\circ} \pm 1^{\circ}\text{C.}$, and 65 ± 5 percent relative humidity, the means of the highest series of field-recorded body temperatures and humidities. Transformed individuals were assumed to modify

microenvironments under logs and in gopher burrows by their mere presence, so a microdesiccator-hydrator was designed. It was used only in desiccation experiments, since specimens were in short supply.

Blood serum electrophoresis of subadult and adult specimens from both rims was initiated, but a laboratory accident destroyed all experimental material before protein electrophoresis and karyotyping could be accomplished. Morphological data characterizing growth, transformation, and geographic variation are also incomplete at present. Thermal gradient behavior, food habits analysis, and hybridization experiments are additional aspects of the total evolutionary-ecological study, which continues through 1968. Nevertheless, some preliminary results of the Penrose Fund Research Grant are summarized below.

Field thermal studies: (1) shallow water pond temperatures were higher and more labile than deep water temperatures on both rims, but the north rim had cooler temperatures overall with less contrast between depths; (2) more larval gulping (lung-breathing) occurred at water surface temperatures of 20-21°C. and body temperatures of 17-20°C irrespective of date, time and oxygen concentration; (3) larval and subadult-adult body temperatures (means 19.2, 17.1°C., respectively) were curvilinearly related to water and substrate temperatures with evidence of more behavioral thermo-regulation at the higher environmental

temperatures; (4) all age-classes on the south rim had body temperatures $2-5^{\circ}\text{C}$. above those on the north rim; (5) gopher burrow temperatures were lower and more uniform than temperatures under surface cover, the north rim stations being significantly cooler than south rim stations; (6) salamanders were not found under cover or in burrows when temperatures exceeded 29°C . in these microenvironments.

Laboratory thermal studies: (1) the CTM of subadults was $0.3-0.7^{\circ}\text{C}$. above that of adults; (2) north rim subadults had a significantly lower CTM than south rim subadults, but the north rim vs. south rim difference in CTM of subadults was not significant; (3) the mean CTM of all north rim transformed specimens (39.4°C .) was significantly lower than the 40.1°C . mean for the south rim; (4) larvae had a significantly lower CTM (mean 37.8°C .) than transformed specimens, the south rim vs. north rim difference in larval CTM being significant; (5) two different north rim pond populations had insignificantly different CTM values.

Humidity and desiccation-hydration analysis: (1) logs and gopher burrows with 38-60% relative humidities did not contain transformed salamanders, while those with 42-94% relative humidities did; (2) north and south rim humidity values overlapped broadly, but the north rim had a significantly higher mean value than the south rim (72.4 vs. 62.1%); (3) subadults

lost and gained water more rapidly than adults; (4) north rim specimens had significantly higher rates of desiccation (mean, 12.7 mg. H_2O loss/hr./gm.wt.) and hydration (mean, 67.5 mg. H_2O gain/hr./gm.wt.) than south rim specimens (10.8, 52.2, respective means); (5) two different north rim pond populations had significantly different desiccation and hydration rates.

Population features: (1) later seres contained fewer, smaller larvae than early seres; (2) twice as many larvae were collected in the 40-60 mm. snout-vent length, size-class (mean of class, 52.2 mm., s-v.) than in any other size-class on the north rim, whereas south rim ponds had as many larvae in this class as in all others from 20 to 90mm. (mean of 40-60 mm. size-class, 50.1 mm., s-v.); (3) one south rim pond supported 134 more larvae per 625 square foot plot than a north rim pond of comparable seral stage and size; (4) number of larvae per study plot declined 0.5 individuals per day in the first three weeks of study, 1.5 individuals per day during the last three weeks with differences between rims due to season; (5) percent of larval sample transforming increased 0-60 during the first three weeks on the south rim and 51-90 during the last three weeks on the north rim; (6) marked larvae moved laterally an average of 3.8 feet per day, the inter-rim difference not being significant; (7) marked larvae were recovered in post-transformation, over-land migration up to 250 yards from their home ponds.

Behavioral observations: (1) diel vertical migrations of larvae apparently were related to light-temperature regimes; (2) larval feeding aggregations resembled those of Scaphiopus tadpoles; (3) non-breeding migrations of subadults-adults and adult breeding migrations were correlated with rainfall of 0.14 inches or more; (4) subadults and adults entered and left Thomomys burrows and often used Microtus runways on rainy nights; (5) tight spiral coiling in subadults was associated with low relative humidities under surface cover; (6) tail wagging, caudal secretion and an erect body posture constituted defensive behavior in response to physical contact.

Morphological data: (1) larval color-pattern varied with seral stage, turbidity, diel cycle, and degree of transformation, inter-rim differences not being apparent; (2) north rim larvae had significantly more gill rakers (mean, 20.3) on the anterior face of the third arch than south rim larvae (mean, 19.2), and number of gill rakers increased ontogenetically until transformation; (3) newly transformed specimens averaged 53.3 mm. snout-vent length on the north rim, 65.4 mm. s-v. on the south rim, but considerable differences between pond populations obtained; (4) north rim individuals lost their dorsal yellow marking and became olive with black spots and reticulations before reaching adult size (mean, 93.5 mm., s-v.), while the larger south rim adults (mean, 97.1 mm., s-v.) usually remained yellow-spotted; (5) north rim subadults had 33-66 (mean, 46.8)

yellow body spots on the dorsum and sides between extremes of limb insertion, an insignificantly different number compared to south rim subadult[†]s with 34-64 (48.7) spots; (6) south rim adults had 15-45 (32.6) spots; (7) south rim adults had a brown-bronze iris, and north rim adults had a bronze-gold iris.

This preliminary data suggests considerable evolutionary divergence of tiger salamanders on the Grand Canyon rims, although Ambystoma tigrinum nebulosum Hallowell is applicable to both rim populations.¹ Evolutionary direction is closely tied to inter-rim environmental differences, and larval and subadult-adult adaptations are correlated with pond and gopher burrow niches that vary accordingly. Natural gene flow, if any, between the Grand Canyon rims may never be ascertained, for the grantee has observed that current ecological modifications of the Colorado River by Glen Canyon Dam and Lake Powell are altering Grand Canyon National Park as a natural laboratory of evolution. Certainly the proposed Marble and Bridge Canyon dams would eliminate this unique "laboratory" aspect of the Grand Canyon.

¹Frederick R. Gehlbach, "Ambystoma tigrinum" In Catalogue of American Amphibians and Reptiles, Amer. Soc. Ichthyol. and Herpetol., N.Y., 1967, (in press).