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# HISTOLOGICAL EXAMINATION OF THE INTEGUMENT OF FIFTEEN SPECIES OF PLETHODONTID SALAMANDERS (AMPHIBIA: URODELA: PLETHODONTIDAE)

SUSAN H. MCMAHON
*Pennsylvania State University*
*University Park, PA 16801*

and

DENISE I. PAV
*East Tennessee State University*
*Johnson City, TN 37601*

## ABSTRACT

To test the postulate that the integumental anatomy of salamanders corresponds to the moistness of their habitats, a histological investigation of the skin of 15 species of salamanders of the family Plethodontidae was undertaken. The skin was routinely prepared for observation under a light microscope. Measurements were made of the widths of skin strata and of numbers and types of glands and of numbers of capillaries. Although each species was found to have a characteristic morphology, no correlation was found between the histology of the skin and the wetness of habitat, excepting a slight relationship in which a larger number of capillaries appear to be found in the more aquatic species than in the more terrestrial ones.

## INTRODUCTION

Species of salamanders of the family Plethodontidae are lungless; consequently, all their respiration occurs through their skin and buccal cavity. Among certain semi-aquatic plethodontids *(Desmognathus quadramaculatus, Desmognathus monticola, Gyrinophilus porphyriticus,* and *Pseudotriton ruber)* more than 85% of their oxygen is transpired through the skin (Whitford and Hutchinson, 1965). The oxygen which enters cutaneously must first be dissolved in water, making a moist habitat mandatory. However, the moisture content of habitats varies widely among plethodontids. *Leurognathus marmoratus,* for example, spends its entire life in streams, whereas species of the genus *Plethodon* prefer a terrestrial life. Mucous glands, large subcutaneous lymph spaces, and in some, a semiaquatic life help to keep the skin moist, making cutaneous respiration possible on land (Gilbert, 1965).

In one respect, plethodontids are at an advantage because their lack of air-filled lungs gives them a greater specific gravity than lunged salamanders; therefore, the likelihood of their being carried away by the stream current is lessened (Wessells, 1968). Wilder, Whipple, and Dunn (1920) suggested a correlation between the evolution of lunglessness in plethodontids and a mountain stream environment.

Since plethodontids inhabit ecological niches exhibiting a great variance in amount of moisture, a number of investigators have attempted to link physiological adaptations to the wetness of habitats. Littleford *et al.* (1947), Ray (1958), and Thorson and Svhila (1943) identified adaptations which do not necessarily retard evaporation, but do allow species living in drier environments to survive more severe desiccation than those species found in more aquatic areas. *Plethodon cinereus,* in particular, has a high "vital limit" allowing it to survive longer periods of drying than most other species (Heatwole and Lim, 1961).

In view of the correlation of the physiological factors to moistness of the habitat, the current study was undertaken to investigate the possibility of a similar link between integumental anatomy and wetness of habitat.

## METHODS

The following 15 species were chosen for study: *Desmognathus fuscus, Desmognathus ochrophaeus, Desmognathus monticola, Desmognathus quadramaculatus, Plethodon cinereus, Plethodon jordani, Plethodon richmondi, Plethodon welleri, Plethodon* *yonahlossee, Gyrinophilus porphyriticus, Pseudotriton ruber, Eurycea bislineata, Eurycea longicauda, Eurycea lucifiga* and *Leurognathus marmoratus*. All are native to the Appalachian Mountains of Northeastern Tennessee where they were collected. Three adult animals of each species were preserved in formalin. Sections of skin, measuring approximately 10 mm x 5 mm were excised from mid-dorsal and mid-ventral regions, slightly to the posterior of the mid-section of each salamander. The sections were routinely processed for histological observation and stained with either Harris hemotoxylin and aqueous eosin or periodic acid Schiff.

By microscopic examination, the following parameters were determined: width of skin layers (stratum germinativum of the epidermis, stratum spongiosum and stratum compactum of the dermis), total epidermal and dermal thicknesses, numbers and types of glands (mucous, poison, and mixed) and numbers and locations of capillaries.

## Results

The data in Tables 1 and 2 represent thicknesses of various layers of epidermis and dermis from the dorsal and ventral sections. The values given for each species are the averages of numerous measurements from three individuals. Designations as terrestrial or semiaquatic are based on criteria given by Bishop (1943). The dorsal measurements in Table 1 show very little difference between the terrestrial and semiaquatic species; indeed, the total skin widths are remarkably similar. The ventral measurements in Table 2 are not as simple to compare. In all strata, including the overall skin width, the mean thickness of the semiaquatic species is greater; however, all the differences are within the range of standard error.

Tables 3 and 4 represent the numbers and types of glands in a 1.4 mm length of skin and numbers of capillaries in a 1.8 mm length. Again, the figures reported are the averages of numerous measurements from three individuals of each species. The poison glands retained the acidic stain with both staining procedures and contained numerous granules. The mucous glands appeared basophilic and often contained web-like compartments. Mixed glands were recorded as any gland exhibiting the properties of both poison and mucous glands. The data reveal a striking similarity in the average number of each type of glands, as well as total glands, in both dorsal and ventral sections. The average number of capillaries varies only slightly in the ventral sections (Table 4), but there is a significant difference among the dorsal specimens (Table 3). The semiaquatic species are more vascularized. Indeed, when *Leurognathus marmoratus*, an aquatic species, was examined, it was found to have aneven larger number of capillaries, an average of 18.2 per 1.8 mm in both dorsal and ventral sections. This may indicate a trend toward more vascularization among the more aquatic species.

Despite the similarities of measurements given in the tables, each species was found to have a characteristic morphology. For example, *Plethodon glutinosus*, known as the "slimy salamander" for its sticky secretions, has a thick skin with many capillaries, heavy melanin deposition, and distinct, well-defined glands. The skin of *Gyrinophilus porphyriticus* is easily identified by its thick stratum germinativum, near absence of melanin, few poison glands, and unusually large mucous glands.

**TABLE 1.** *Dorsal skin strata widths. Standard errors of the mean are in parentheses.*

| Species | Stratum Germinativum | Stratum Spongiosum | Stratum Compactum | Total Dermis | Total Skin |
|---|---|---|---|---|---|
| Terrestrial Species | | | | | |
| Plethodon cinereus | 24.5 μm | 46.4 μm | 19.9 μm | 66.0 μm | 90.8 μm |
| Plethodon glutinosus | 25.2 | 204.7 | 11.7 | 216.5 | 241.6 |
| Plethodon jordani | 30.2 | 135.3 | 18.9 | 154.2 | 186.4 |
| Plethodon richmondi | 28.5 | 59.5 | 22.0 | 81.4 | 109.9 |
| Plethodon welleri | 19.4 | 51.1 | 14.9 | 66.0 | 85.4 |
| Plethodon yonahlossee | 16.1 | 154.5 | 12.0 | 166.5 | 182.6 |
| Desmognathus ochrophaeus | 28.3 | 34.4 | 10.2 | 44.6 | 72.9 |
| Mean | 24.6 (2.0) | 98.0 (25.1) | 15.7 (1.7) | 113.6 (24.6) | 138.5 (24.5) |
| Semiaquatic Species | | | | | |
| Desmognathus monticola | 31.1 | 68.5 | 18.6 | 87.2 | 119.0 |
| Desmognathus quadramaculatus | 26.0 | 76.1 | 36.2 | 112.3 | 138.3 |
| Desmognathus fuscus | 35.9 | 78.8 | 21.0 | 99.0 | 135.7 |
| Eurycea bislineata | 27.4 | 60.1 | 12.9 | 73.0 | 100.4 |
| Eurycea longicauda | 17.1 | 86.5 | 15.3 | 101.7 | 118.9 |
| Eurycea lucifuga | 15.1 | 62.0 | 15.2 | 77.2 | 92.3 |
| Pseudotriton ruber | 30.2 | 180.2 | 33.9 | 214.1 | 244.3 |
| Gyrinophilus porphyriticus | 33.2 | 79.8 | 24.6 | 104.4 | 137.6 |
| Mean | 27.0 (2.6) | 86.5 (13.8) | 22.2 (3.1) | 108.6 (15.9) | 135.8 (16.7) |

**TABLE 2.** *Ventral skin strata widths. Standard errors of the mean are in parentheses.*

| Species | Stratum Germinativum | Stratum Spongiosum | Stratum Compactum | Total Dermis | Total Skin |
|---|---|---|---|---|---|
| Terrestrial Species | | | | | |
| Plethodon cinereus | 36.1 | 45.0 | 32.3 | 77.3 | 113.4 |
| Plethodon glutinosus | 35.8 | 86.1 | 23.9 | 110.0 | 145.8 |
| Plethodon jordani | 44.1 | 84.2 | 35.8 | 120.0 | 164.1 |
| Plethodon richmondi | 37.2 | 47.2 | 44.6 | 91.9 | 129.1 |
| Plethodon welleri | 22.4 | 28.6 | 19.4 | 48.1 | 70.4 |
| Plethodon yonahlossee | 22.2 | 54.0 | 23.4 | 77.4 | 99.6 |
| Desmognathus ochrophaeus | 26.4 | 21.5 | 10.9 | 32.4 | 56.9 |
| Mean | 32.0 (3.2) | 52.4 (9.5) | 27.2 (10.3) | 79.6 (11.9) | 111.3 (14.7) |
| Semiaquatic Species | | | | | |
| Desmognathus monticola | 78.7 | 81.4 | 44.2 | 125.6 | 204.3 |
| Desmognathus quadramaculatus | 22.3 | 34.1 | 22.3 | 56.3 | 78.6 |
| Desmognathus fuscus | 35.7 | 43.7 | 14.2 | 57.9 | 93.6 |
| Eurycea bislineata | 45.0 | 59.2 | 19.4 | 78.6 | 123.6 |
| Eurycea longicauda | 21.4 | 69.5 | 22.7 | 92.2 | 113.6 |
| Eurycea lucifuga | 38.0 | 62.3 | 28.0 | 90.3 | 128.4 |
| Pseudotriton ruber | 36.8 | 128.2 | 54.4 | 180.6 | 217.4 |
| Gyrinophilus porphyriticus | 49.3 | 86.3 | 33.8 | 120.1 | 169.4 |
| Mean | 40.9 (14.5) | 70.6 (10.3) | 29.9 (4.8) | 100.2 (14.6) | 141.1 (18.0) |

**TABLE 3.** *Dorsal glands and capillaries. Average number of glands in 1.4 mm and number of capillaries in 1.8 mm lenghts of integument. Standard errors of the mean are in parentheses.*

| | Glands | | | | | |
|---|---|---|---|---|---|---|
| Species | Poison | Mucous | Empty | Mixed | Total | Capillaries |
| Terrestrial Species | | | | | | |
| Plethodon cinereus | 4.0 | 4.6 | 0.0 | 0.5 | 9.1 | 11.5 |
| Plethodon glutinosus | 4.9 | 3.1 | 0.0 | 0.6 | 8.2 | 15.8 |
| Plethodon jordani | 5.8 | 6.1 | 0.2 | 0.0 | 12.1 | 10.7 |
| Plethodon richmondi | 4.1 | 5.0 | 0.1 | 0.3 | 9.5 | 12.4 |
| Plethodon welleri | 3.4 | 5.0 | 0.0 | 1.0 | 9.6 | 11.6 |
| Plethodon yonahlossee | 4.0 | 3.0 | 0.3 | 0.2 | 7.5 | 11.0 |
| Desmognathus ochrophaeus | 1.8 | 4.6 | 1.2 | 0.3 | 7.8 | 11.9 |
| Mean | 4.0 (1.2) | 4.5 (0.4) | 0.3 (0.2) | 0.4 (0.1) | 9.1 (0.6) | 12.1 (0.4) |
| Semiaquatic Species | | | | | | |
| Desmognathus monticola | 5.0 | 3.6 | 0.8 | 0.6 | 10.6 | 14.5 |
| Desmognathus quadramaculatus | 2.2 | 3.2 | 0.5 | 0.0 | 5.6 | 11.8 |
| Desmognathus fuscus | 3.4 | 4.0 | 0.9 | 2.0 | 10.4 | 15.4 |
| Eurycea bislineata | 4.5 | 4.7 | 0.1 | 1.0 | 10.2 | 16.0 |
| Eurycea longicauda | 5.7 | 2.7 | 0.2 | 1.0 | 10.2 | 14.6 |
| Eurycea lucifuga | 4.8 | 1.9 | 1.1 | 1.7 | 9.7 | 15.9 |
| Pseudotriton ruber | 4.0 | 3.8 | 0.0 | 0.5 | 7.6 | 18.2 |
| Gyrinophilus porphyriticus | 0.8 | 6.1 | 1.0 | 0.2 | 8.1 | 13.6 |
| Mean | 3.8 (0.6) | 3.8 (0.5) | 0.6 (0.2) | 0.9 (0.3) | 9.1 (0.6) | 15.0 (0.7) |

**TABLE 4.** *Ventral glands and capillaries. Average number of glands in 1.4 mm and number of capillaries in 1.8 mm lengths of integument. Standard errors of the mean are in parentheses.*

| | Glands | | | | | |
|---|---|---|---|---|---|---|
| Species | Poison | Mucous | Empty | Mixed | Total | Capillaries |
| Terrestrial Species | | | | | | |
| *Plethodon cinereus* | 3.2 | 6.2 | 0.0 | 0.0 | 9.3 | 12.2 |
| *Plethodon glutinosus* | 1.5 | 7.1 | 0.0 | 0.9 | 9.5 | 13.2 |
| *Plethodon jordani* | 2.0 | 7.2 | 0.2 | 0.5 | 9.8 | 10.1 |
| *Plethodon richmondi* | 3.6 | 4.8 | 0.0 | 0.0 | 8.7 | 12.1 |
| *Plethodon welleri* | 3.2 | 5.2 | 0.0 | 0.0 | 8.3 | 13.7 |
| *Plethodon yonahlossee* | 0.5 | 8.3 | 0.0 | 0.0 | 8.8 | 12.1 |
| *Desmognathus ochrophaeus* | 1.3 | 8.2 | 0.0 | 0.0 | 9.5 | 12.5 |
| Mean | 2.2 (0.4) | 6.7 (0.5) | 0.03 (0.03) | 0.2 (0.1) | 9.1 (0.2) | 12.1 (0.3) |
| Semiaquatic Species | | | | | | |
| *Desmognathus monticola* | 5.0 | 6.2 | 0.0 | 0.0 | 11.2 | 9.9 |
| *Desmognathus quadramaculatus* | 0.7 | 4.7 | 0.3 | 0.0 | 5.7 | 13.0 |
| *Desmognathus fuscus* | 2.2 | 7.2 | 0.0 | 0.4 | 9.8 | 12.9 |
| *Eurycea bislineata* | 2.5 | 7.5 | 0.0 | 0.8 | 10.8 | 15.2 |
| *Eurycea longicauda* | 5.8 | 3.0 | 0.0 | 1.5 | 11.3 | 11.2 |
| *Eurycea lucifuga* | 1.8 | 4.3 | 5.3 | 0.0 | 11.5 | 12.4 |
| *Pseudotriton ruber* | 2.3 | 4.2 | 0.0 | 1.1 | 7.6 | 15.8 |
| *Gyrinophilus porphyriticus* | 0.2 | 7.7 | 0.2 | 0.2 | 8.2 | 13.4 |
| Mean | 2.6 (0.7) | 5.6 (0.6) | 0.7 (0.7) | 0.5 (0.2) | 9.5 (0.8) | 13.0 (0.7) |

## Discussion

Since the physiology of water loss is related to the moisture content of their habitats (Littleford *et al.*, 1947; Ray, 1958), it was thought that perhaps their integumental microanatomy might also correlate to the wetness of habitat. Indeed, a possible trend was seen in the number of capillaries: the more aquatic species had a slightly greater number of capillaries on their dorsal sides than the more terrestrial species. One would expect aquatic species to exhibit more vascularization since the oxygen content of water is lower than that of air. However, among the 15 species studied, no link was discovered between the thickness of any of the layers of skin or of the total width of the skin and habitat moistness. Nor was the number or types of glands shown to be related to wetness of habitat.

This study supports the observation by Littleford *et al.* (1947) that, although there are integumentary differences among species, they are not significant in water conservation. They believe that the skin alone is not a major contributing factor toward raising the vital limit of salamanders. McCourt (1954) also expresses the idea that, although differences among individual species do exist, they are probably not significant in the facilitation of respiration.

It appears, then, based on dorsal and ventral integumental sections, that anatomy alone is not sufficient to account for differences in survival rates during times of dryness. Physiological factors are important and should be further studied to determine which are important and to what degree each plays a role in salamander survival and habitat selection.


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