Aspects of Water Loss Physiology in Certain Plethodontid Salamanders

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Abstract

Weight reductions due to evaporative loss of water under varying conditions of temperature and relative humidity are presented for *Plethodon jordani jordani, Plethodon glutinosus,* and *Desmognathus ochrophaeus carolinensis,* the data for the latter two being somewhat less conclusive. The experimental conditions employed were those presumed to approximate conditions encountered by animals in nature.

Introduction

It is a commonplace of even casual observation that the various species of amphibians do not all live under the same environmental conditions, but that, rather, each species occurs in its own reasonably distinctive situation. These situations are, however, characterized by one relatively constant feature in that all amphibian organisms require some access to water, but even in this respect there is a diversity of habitats varying from those that are definitely aquatic to those that are almost completely terrestrial. With respect to the capacity to conserve body water and to resist, or survive, the desiccating influence of atmospheric air, the members of the Class Amphibia thus present a whole spectrum of adaptations the achievement of which must have been very crucial in the history of vertebrate evolution toward complete terrestrialism.

Among studies on the reactions of caudate amphibians to the evaporating power of air, an early one was that of Shelford (9) which afforded salamanders the opportunity to sample a humidity gradient. Both *Plethodon cinereus* and *Plethodon glutinosus* spent relatively little time in the dry and medium parts of the gradient, and spent most of the time in the moist portion. The general results enabled Shelford to state that "*P. glutinosus* is clearly more sensitive to dryness than is *P. cinereus*." Hall (3) found that *Ambystoma punctatum* could withstand evaporational loss of 11% to 47% of body weight and still survive. Littleford et al. (4) found that *P. cinereus* survived greater loss of water than two of its ecologically more aquatic plethodontid relatives, *Eurycea bislineata bislineata* and *Desmognathus fuscus fuscus.*

In a study of two plethodontids, *Aneides lugubris* and *Ensatina eschscholtzii xanthoptica* and a salamandrid, *Triturus torosus,* Cohen (1) found a differential in the time required for the specimens to be desiccated to a 20% loss of initial body weight, as follows: *Triturus* averaging 13.9 gm, a mean of 257 minutes, *Aneides* averaging 8.47 gm, a mean of 261 minutes, and *Ensatina* averaging 5.66 gm, a mean of 185 minutes. About 30% larger than *Ensatina,* the *Aneides* also required about 30% more time to lose 20% of the body weight. The *Triturus* was the largest of the three by a considerable margain, yet it lost 20% of its weight in approximately the same time as did the smaller *Aneides*. Within any one species, Cohen was unable to demonstrate a correlation between body size and rate of water loss.

In a study that encompassed eight species of Western plethodontids, Ray (8) stressed the specimens by desiccation to loss of a righting reaction, or to lethality. Ray found a definite correlation between size and rate of water loss, with the larger individuals in each species showing the slower rates of loss, a relationship which he claimed to be an expected one on the basis of a difference in surface-to-mass ratio.

The experiments reported in the present paper were undertaken to obtain information under conditions deemed to be somewhat similar to those encountered by salamanders in nature, but with the a priori understanding that any experimental procedure would necessarily be somewhat unnatural. Some of these conditions were: *1.* exposure to a degree of evaporative loss that would be essentially non-stressful; *2.* an air movement rate of a quite gentle intensity or magnitude; *3.* an experimental run of moderate length.

Materials and Methods

Routine and regular maintenance of salamanders of the type used in this study was accomplished in a constant-temperature room at 14-16°C. The animals were housed, in varying numbers, in either glass or plastic containers of various sizes, marked as to place and date of capture. Adult *Drosophila* and small or medium-sized mealworms (*Tenebrio* larvae) were fed to the animals periodically. In addition to some provision for air interchange between the room and the interior of the container, each container was supplied with sphagnum moss or wadded towel paper, partly to supply the animals with a means of concealment, but also to provide a moisture-holder that would incure a saturated air or one of quite high relative humidity within the container.

For the experiments reported herein, plastic containers 6 inches in diameter were equipped in the manner just described, and each salamander selected was placed in its own individual container, appropriately marked, and thereafter not fed until after the experiments were concluded. In order that its weight might be stable, no salamander was subjected to its first experimental desiccation run until after adequate acclimation to the lack of food. For the experimental runs each salamander was transferred to and confined in a small plastic rectangular "weighing box" perforated by as many small holes as the box structure would permit. The weight of these boxes was frequently checked, and in such boxes each specimen was weighed immediately before and immediately after each desiccation run. Weighing was accomplished on a magnetically-damped chainomatic balance located in the constant-temperature room. For transport between the constant-temperature room and the environment-control chamber, the salamander in its box was placed in a small plastic bag.

The experiments were conducted in the exposure chamber of an environment-control device known as a Vapor-Temp (Model VP-200At, Blue M Manufacturing Company) which has built-in equipment giving it the capability of automatically maintaining relative humidities from 34% to 98% and dry bulb temperatures from 4°C to 77°C. Air-flow control can vary from 0 to 30 complete changes of exposure chamber volume per minute. The Vapor-Temp is furthermore provided with built-in features to record both wet-bulb and dry-bulb temperatures on a circular record sheet. This was supplemented by the installation within the exposure chamber of standard centigrade thermometers, one of which was wet-bulb, the other dry. By coating the interior of the Pyrex glass exposure chamber with an antifog solution, complete visibility within the chamber was achieved.

In preparation for an experimental run, the Vapor-Temp was turned on and regulated to the desired temperature-humidity environment within the chamber. All runs were conducted at minimal air flow. When the weighed salamander was introduced, a short wait ensued until the desired environmental conditions were re-established in the chamber, and the run was then continued for one hour. Following this sixty minutes of exposure, the animal was re-weighed, and then returned to its own individual container. Each animal was then periodically re-placed in a "weighing-box" and its weight recorded until it had re-gained its original weight. In so far as was feasible, each animal was used in all of the runs included in the program.

The animals studied in this program included 19 specimens of *Plethodon jordani jordani* and four specimens of *Desmognathus ochrophaeus carolinensis* taken at Indian Gap, Sevier County, Tennessee. Appreciation is hereby extended to the Naturalist Service of the Great Smoky Mountains National Park for cooperation with this field work. One included specimen of *Plethodon glutinosus glutinosus* was collected west and north of Dillard, Georgia, approximately 2.5 miles north of the Georgia-North Carolina border along the Betty Creek Road, in Macon County, North Carolina. All 24 specimens just mentioned were collected in a program aided by a National Science Foundation Grant administered by the Highlands Biological Station, Highlands, North Carolina, at which one of the authors was a resident investigator. The two remaining specimens of *P. glutinosus* were both Indiana specimens, one collected in the Morgan-Monroe State Forest, the other at Friends Camp in Putnam County.

Weight changes experienced by the animals during the runs are regarded as all being due to water loss: no defecation occurred during any of the runs. In reporting these changes, the raw data of weight change in grams have not been recorded; instead, the changes of weight are expressed as a proportion of the original weight.

Results

1. *Plethodon jordani*

In all cases the environmental "starting point" for the salamanders used was water-saturated air at 14-16°C. A glance at Table 1 will reveal

TABLE 1. *Weight lost by specimens of* Plethodon jordani *in one hour under various environmental conditions.*

Specimen No.	Initial Wt. (gm)	Percentages of initial weight lost at indicated relative humidity (in %) and temperature.				
		36% 11°C	48% 17°C	70% 15C°	85% 26°C	92.5% 29°C
A14	1.5736	13.371	10.089	11.021	6.217	2.127
A15	1.7692	5.658	11.053	10.768	3.754	2.526
A1	1.8680	3.81	24.38	10.72	9.112	——
A5	1.9740	10.088	17.509	gain	4.600	gain
A7	1.9740	14.453	9.192	3.608	4.200	1.396
A8	2.1479	13.855	13.177	10.472	5.055	3.060
A10	2.3206	15.340	9.404	8.954	3.942	1.921
A11	2.4084	11.879	11.725	8.873	2.254	5.870
A3	2.5147	10.22	8.866	6.000	4.37	0.070
A13	2.7094	12.453	10.973	9.238	3.271	4.622
A9	2.7546	11.685	7.666	6.625	3.416	1.591
A16	2.8924	13.383	13.009	9.648	3.572	2.334
A18	2.9209	9.268	9.197	6.646	2.881	3.514
A19	3.0263	13.132	17.596	2.624	4.655	0.400
A17	3.4884	8.497	8.143	7.399	4.759	3.306
A6	3.7439	9.917	11.075	9.930	4.251	7.885
A12	3.9314	7.707	9.921	9.559	2.215	2.651
A4	4.0264	7.319	8.676	4.587	3.316	3.685
A2	4.8146	36.220	——	5.198	4.22	13.10
Mean	2.7820	12.013	11.758	7.881	4.213	3.532

at once that the animals lost weight under all conditions to which they were experimentally subjected, with two exceptions. At the bottom of Table 1 the means recorded show a general trend for the animals to lose a greater proportion of body weight on exposure to progressively less humid air. With respect to this trend, a column-by-column inspection of Table 1 supports the summary which follows.

Lost more weight at 36%/11°C than at 48%/17°C = 11 specimens: A14, A7, A8, A10, A11, A3, A9, A16, A18, A17, A13;

Lost more weight at 48%/17°C than at 70%/15°C = 17 specimens: A15, A1, A5, A7, A8, A10, A11, A3, A13, A9, A16, A18, A19, A17, A6, A12, A4;

Lost more weight at 70%/15°C than at 85%/26°C = 16 specimens: A14, A15, A1, A8, A10, A11, A3, A13, A9, A16, A18, A17, A6, A12, A4, A2;

Lost more weight at 85%/26°C than at 92.5%/29°C = 11 specimens: A14, A15, A5, A7, A8, A10, A3, A9, A16, A19, A17.

Thus under all of the conditions imposed, from 11 to 17 of the 19 animals showed less water loss per hour with increasing relative humidity, or, conversely, greater water loss per hour as the relative humidity of the surrounding air decreased. In the array of experimental circumstances, perhaps the most "natural" temperatures for the salamanders were the 15° and 17° exposures; in these exposures differing by only 2°C, the relative humidity difference was of 22%, and 17 out of the 19 animals showed a difference in greater water loss in the less humid environment. Six animals showed a particularly consistent behavior under all conditions in that they lost progressively less water with increasing relative humidity, as follows: A8, A10, A3, A9, A16, A17.

The general trend of the relationships just reviewed is supportive on the thesis that *P. j. jordani* exhibits an hourly water loss rate inversely proportional to the ambient relative humidity in its environment.

In Table 1 the specimens are listed, from top to bottom, in order of increasing size as reflected by body weight, an arrangement that was deliberately adopted in view of a possibility that water loss rate might be size-related. Since evaporative water loss is skin-related, generally-accepted principles concerning surface-area-to-mass ratios might make it logical to expect that the smaller salamanders would sustain greater loss than the larger ones. If such occurred, inspection of the vertical columns in Table 1 should make it apparent. Such an inspection of Table 1, column by column, conveys strongly the idea that the salamanders were extremely variable in their response to desiccation. Within this variability, however, weak trends may be discerned at 48%/17°C, 70%/15°C, and 85%/26°C; under these conditions, the larger animals show a tendency to lose a smaller proportion of the body weight than do the smaller ones.

TABLE 2. Plethodon jordani: *Mean weight loss by size groups.*

Specimens	Mean Weight (gms)	36% 11°C	48% 17°C	70% 15°C	85% 26°C	92.5% 29°C
A14, A15, A1, A5	1.7962	8.231	15.757	10.836	5.920	2.326
A7, A8, A10, A11	2.2127	13.881	10.874	7.976	3.862	3.061
A3, A13, A9, A16	2.7177	11.935	10.128	7.877	3.657	2.154
A18, A19, A17, A6	3.2948	10.203	11.502	6.649	4.136	3.776
A12, A4, A2	4.2574	17.082	9.298	6.448	3.250	6.478

For further investigation of this tendency, Table 2 has been constructed by arbitrarily dividing the 19 specimens into five groups, and presenting the loss rates as means for the resulting size groups. Such a consolidation has the virtue of affording slightly clearer evidence of a weak trend for the smaller salamanders to lose more weight at 48%/17°C, 70%/15°C, and 85%/20°C than do the larger ones.

2. Other Salamanders

Two other species of Plethodontid salamanders were studied, and these were subjected to an additional experimental run at 81% relative humidity and 16°C. The number of specimens available was small, and some of the animals failed to regain original weight and were not used in some of the experimental runs (Table 3).

TABLE 3. *Weight lost by individuals of two salamander species in one hour under various environmental conditions.*

Specimen No.	Initial Weight (gm)	Percentages of initial weight lost at indicated relative humidity (in %) and temperature.					
		36% 11°C	48% 17°C	70% 15°C	81% 16°C	85% 26°C	92.5% 29°C
A. *Desmognathus ochrophaeus carolinensis*							
E3	0.6995	21.806	21.668	16.909	------	10.769	0.516
E4	1.3226	17.753	18.937	15.359	------	5.657	0.034
E1	1.3326	12.457	11.754	12.034	9.768	0.072	2.991
E2	1.3884	18.475	-----	-----	-----	-----	-----
Means	1.1857	17.6227	17.4530	14.7673	9.7680	5.4993	1.1803
B. *Plethodon glutinosus glutinosus*							
B2	2.0423	17.808	11.663	8.517	9.1927	4.028	1.9792
B3	2.6068	14.021	19.319	9.919	10.745	-----	5.554
B1	6.5051	10.253	9.430	18.050	4.958	2.906	3.818
Means	3.7180	14.0273	13.4706	12.1620	8.2985	3.4670	3.7837

Reference to Part A of Table 3 will show that the largest specimen of *D. o. carolinensis* was still smaller in terms of body weight than the smallest specimen of *P. j. jordani* listed in Table 1. The mean percentages of weight loss of *D. o. carolinensis* shown in Table 3 were consistently greater than that of *P. j. jordani* exposed to comparable experimental conditions, a result logically to be expected on the basis of surface-mass ratio relationships. Also consistent with such a relationship was the fact that (except at 92.5%/29°C) the smallest *carolinensis* exhibited a higher hourly loss rate than did the three somewhat larger specimens, all three of which were about the same size. With respect to hourly rate of water loss in correlation with environmental condition, the mean values recorded in Part A of Table 3 show a progressive decrease as the relative humidity increased. Inspection of the separate values for each specimen indicate the same as a general trend, but with an occasional exception.

The three specimens of *P. g. glutinosus* used included two specimens near the same size plus one quite large animal (Table 3, Part B) which was in fact the largest of the total of 26 animals involved in these experiments. The two other *glutinosus* specimens were somewhat larger than the *D. o. carolinensis* and near the mean weight of the *P. j. jordani* used. This largest *glutinosus* exhibited the least weight loss in all *glutinosus* runs except those at 70%/15°C and 92.5%/29°C. For comparable environmental exposures, mean weight loss of *glutinosus* was less than of *D. o. carolinensis* in all runs except that of 92.5%/29°C, but greater than that of *P. j. jordani* in all runs except at 85%/26°C. Although not consistent for the 85%/26°C and 92.5%/29°C runs the mean percentage of weight loss in *glutinosus* generally increased progressively with reduced relative humidity (Table 3, Part B).

Discussion

One of the most striking features of the results just presented is the marked amount of variation observed. One specimen of *P. jordani* lost as much as 13% of body weight in relatively humid air at 92.5% relative humidity, while one other specimen lost a rather insignificant 0.07% of body weight; these represent the extremes of responses to conditions that evoked an average loss of 3.53% of body weight. In the 36%/11°C runs there occurred the largest "spread" between greatest and least weight loss, a difference of 32.41% of body weight under conditions where the mean weight loss was 12.0%; yet in this "driest" air of these 36%/11°C runs, one animal lost only 3.81% of body weight. The smallest spread in response was seen in the runs at 85%/26°C where the difference was 6.9% under conditions where the average loss was 4.2%. The more scant data on the other salamanders, *D. o. carolinensis* and *P. glutinosus*, exhibited differences more intermediate in magnitude, but nevertheless show variation.

In spite of the very considerable variation manifested, salamanders of all three taxa exhibited a trend, as has been indicated in the previous section, to lose body weight in direct proportion to the dryness of the surrounding air. This response of the animals to the evaporative power of air is in thorough accord with logical expectation.

In view of the generally-held view that small organisms have a higher ratio of surface area to mass than do larger organisms, another logical expectation was that the smaller salamanders would lose more weight in percentage of initial weight, during the 60-minute runs than would the larger salamanders. This expectation was borne out only to the extent that the data presented above give evidence of a weak trend. Inspection of the Tables will show several deviations from this expectation, and one gross violation of it was exhibited by *P. jordani* No. A2 which was the largest of the *jordani* series, yet it sustained the greatest weight loss under two sets of conditions, 36%/11°C and 92.5%/29°C. These experiments thus fail to confirm unequivocally a definite correlation between size and rate of water loss found by Ray (8) yet indicate more of a trend in this direction than was found by Cohen (1), who found no correlation.

MacMahon (5) studied water loss rate in *P. jordani*, *P. glutinosus*, and also *Plethodon yonahlosse* at 20°C, air flow of 2000 cc/minute, and 0% relative humidity, and concluded that the living salamander was unable to exert any effective physiological control over loss of water but suffered a rate of loss determined entirely by body composition and body proportion (surface-volume ratio). MacMahon paralleled his studies of living salamanders by the use of inanimate "model salamanders" made up of 80% water and 20% agar by weight, and cast in molds that were exact replicas of typical individuals of the three species, and found that these lost weight at the same rate as did their living counterparts. In another report (MacMahon, 6) based on 573 specimens of the same three species, air flow rates of 0, 1000, 2000, and 3000 cc/minute were used, and runs were made a 0% and 100% relative humidity, all at 20°C. That water loss was least in *P. jordani* and greatest in *P. yonahlosse* was ascribed by MacMahon to the fact that the former had the greatest, the latter the least, surface-volume ratio. Hourly weight checks during the experimental runs enabled MacMahon to determine a critical activity point (CAP) at which the salamander concerned failed to accomplish a righting reaction. Although all three species were variable, in general both *P. jordani* and *P. yonahlosse* had lesser CAP values than *P. glutinosus*. In addition *P. glutinosus* from montane areas had smaller CAP values than specimens from non-montane localities. These latter considerations constrained MacMahon to propose a genetically-controlled CAP in these salamanders.

In the present report, in all runs except that in the most humid air, the greatest mean water loss rates were experienced by specimens of *D. o. carolinensis*, the smallest organism used, a result in conformity with MacMahon's (5) emphasis on the role of the surface-volume ratio. It may also be pertinent, however, to point out that *D. o. carolinensis* lives in natural situations that are typically somewhat more moist than those harboring either *P. jordani* or *P. glutinosus*, so the faster rate of water loss could be due to intrinsic morphologic as well as physiologic factors that are genetically determined and which are part and parcel of the total adaptive make-up of the species.

With respect to *P. glutinosus*, the mean values in Table 3 indicate a slower rate of water loss than was characteristic of *D. o. carolinensis*, a result consonant with the surface-volume relationship since *glutinosus* is a larger animal. However, *glutinosus* also lost more water per hour than did *P. jordani* in all runs except that at 85%/26°C. The mean weight in Table 3 for *P. glutinosus* is skewed upward because of one quite large animal. To make an additional comparison, the two most similar *glutinosus* (B2, B3) and the four most comparable *jordani* (A8, A10, A11, A3) were singled out and separate sub-means calculated for each group; the mean initial weight for both groups was slightly greater than 2.3 grams. In terms of these sub-means, *P. glutinosus* still lost more water in an hour than did *P. jordani*, and under all of the experimental conditions. These *P. glutinosus* vs. *P. jordani* comparative responses to evaporative water loss are not in strict accord with the thesis that surface-volume relationships control water loss. The possibility exists that the capacity of *P. glutinosus* to lose water at a slightly faster rate could be one facet of its organization making possible the higher CAP value found by MacMahon (6). Certain it is that *P. glutinosus* as a total population must be so constituted as to be reasonably well adapted to quite a variety of conditions since it has a very extensive range as compared to either *P. jordani* or *D. o. carolinensis*, a range that includes much more non-montane territory than the high-rainfall southern Appalachian mountainous area where it overlaps both of the other two forms (2). MacMahon (6) pointed out that because of higher temperature and lower rainfall animals in non-montane areas may have had to withstand greater moisture-loss stress, and may have been the objects of selective action resulting in the higher CAP.

Throughout this paper attention has been focused on the relative humidity of the atmosphere surrounding the experimental animals, and no analysis or special study has been made of temperature relationships. In general, and with one exception, higher experimental humidities have been accompanied by higher temperatures where evaporation rates could logically be expected to be greater. Furthermore, full recognition is given to the small sample sizes, particularly as applied to *D. o. carolinensis* and *P. glutinosus*, and to the consequent fact that the results do not support any high degree of conclusiveness. This investigation was undertaken in the spirit of an exploratory study utilizing a basic experimental design that differed from several other studies of a like nature chiefly in that less severe physiological stresses were imposed on the experimental animals. It was felt desirable that the actual results, as set forth in the Tables, be made a matter of record, along with the logical inferences that may be derived from them in terms of indicated trends.

The material presented in this paper formed a portion of a somewhat broader program used in another connection (7). This broader program took note of the recovery of the experimental animals following evaporative water loss. The time required for the recovery of 99% to 100% of initial weight was variable, but occurred as follows:

P. jordani—3 hrs. 35 min. to 38 hrs. 35 min.
P. glutinosus—2 hrs. 20 min. to 23 hrs. 11 min.
D. o. carolinensis—4 hrs. 30 min. to 22 hrs. 56 min.

Summary

In this preliminary study nineteen specimens of *Plethodon jordani* were subjected to relative humidity and temperature conditions as follows: 36%/11°C, 48%/17°C, 70%/15°C, 85%/26°C, and 92.5%/29°C. To

supplement this, four specimens of *Desmognathus ochrophaeus carolinensis* and three specimens of *Plethodon glutinosus* were exposed to the same conditions plus an additional experimental run at 81%/16°C. All experimental runs were one hour in length, and weight losses were all considered to be due to evaporative loss of water. Weight losses were presented in tabular form as percentages of initial weight. Weight loss was quite variable, the extremes extending from as little as 0.07% to as much as 36.22% of initial body weight. The most distinct trend evinced by the results was weight loss, or loss of water, in direct proportion to atmospheric dryness. The data gave weak support to the thesis that water loss is very largely controlled by the ratio of surface-to-mass (or volume). Other inferences from the data suggested a taxonmic, genetically controlled, difference in response to desiccation. After a desiccation run, the animals recovered initial weight in periods varying generally from 3 to 23 hours.

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