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A Biological Investigation on Plethodon Larselli

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A BIOLOGICAL INVESTIGATION ON PLETHODON LARSELLI

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Chapter 1

INTRODUCTION

The Pacific Northwest with its diversified geologic, climatic, and vegetational history affords the necessary habitat for a large variety and abundance of urodeles. This multiplicity of urodeles and their environment offer an omnipresent challenge to many herpetologists.

Of particular interest to this writer is a relatively unknown species of salamander, Plethodon larselli. Although the species is not uncommon in most of its range, little is known about its life history. Burns (1954) originally collected P. larselli on the north slope of Larch Mountain, Multnomah County, Oregon, and described the specimen as a subspecies of Plethodon vandykei. Slater (1955) elaborated on the distribution of the species. The taxonomic status of P. larselli was elevated to a species by Burns (1962) in his investigation of several distinct features. Probably the most outstanding of these characters is the absence of a second phalange on the fifth digit of the hind foot, which P. vandykei exhibits. Slater (1964) was in accordance with this proposal.

It appears that Plethodon vehiculum exhibits a close ecological and distributional affinity to P. larselli. Both species are found in and near humid forests, in moist lava talus slopes, rotten wood, and under bark, but generally in drier locations than P. vandykei. Plethodon larselli is known only from the lower Columbia River Gorge

in Washington and Oregon (see Figure 1), while P. vehiculum exhibits a much wider range than larselli, spreading northward from Coos County, Oregon, through western Washington to southwestern British Columbia, including Vancouver Island (Stebbins, 1966). On the north and south sides of the lower Columbia River Gorge, where their ranges overlap, the two species occur sympatrically.

The purpose of this study is to increase the presently-lacking biological knowledge of P. larselli. A study was made, therefore, on this species relative to its geographical distribution, habitat, dehydration and rehydration rates, response to induction with varying concentrations of a gonadotropic hormone, and blood serum analysis by cellulose acetate electrophoresis. In addition, the findings of this investigation will help elucidate the relationship between P. larselli and P. vehiculum.

Chapter 2

GEOGRAPHIC AND HABITAT DISTRIBUTION

The range of P. larselli is very restricted. Burns (1962) records the known sites for the species as follows: Starvation Creek, Hood River County, Oregon; 0.5 mile south of Wyeth, Hood River County, Oregon; Ainsworth State Park, Multnomah County, Oregon; 150 feet from the summit of Larch Mountain, Multnomah County, Oregon; three miles from the summit of Larch Mountain, Multnomah County, Oregon; 0.4 mile west of Wahkeena Falls, Multnomah County, Oregon; and Archer Falls, Skamania County, Washington. In addition to these localities the author has collected larselli at Dog Creek vicinity, Skamania County, Washington; Beacon Rock State Park, Skamania County, Washington; approximately 0.5 mile west of Archer Mountain, Skamania County, Washington; Viento Creek vicinity and approximately 1.5 miles east of that area, Hood River County, Oregon; Cabin and Warren Creeks vicinities, Hood River County, Oregon; and Wahkeena Creek vicinity, Multnomah County, Oregon (see Figure 1). The east-west range of P. larselli (approximately 23 air miles) on the Oregon side of the Columbia River closely parallels the species' east-west range (approximately 22 air miles) on the Washington side of the Columbia River. The north-south range of larselli has not been explored to any extent. It seems likely, however, that in the many miles of relatively undisturbed, heavily wooded areas, such as those north and south of the present east-west range, P. larselli would find suitable habitat. However,

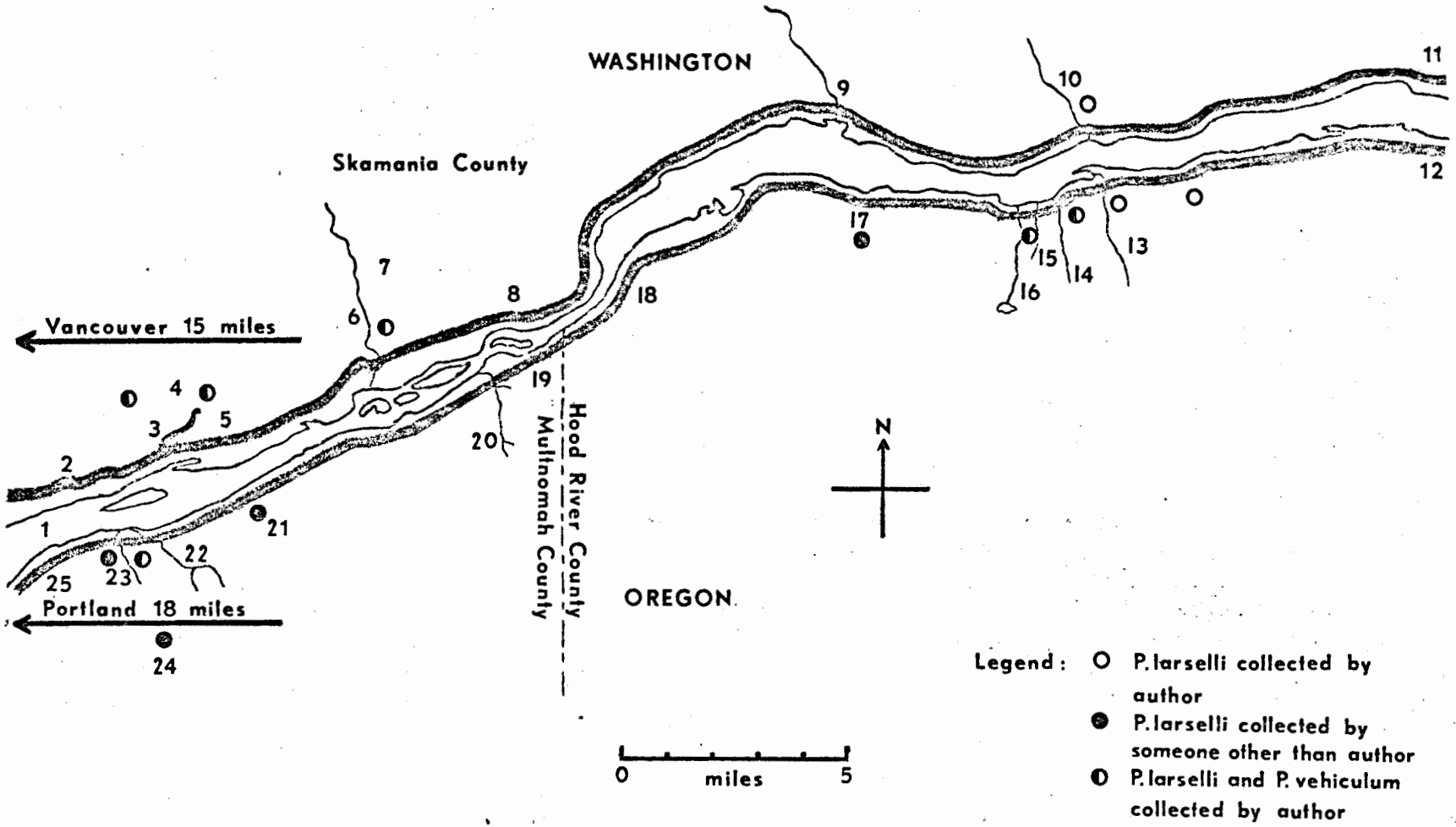


Fig. 1. Geographic Distribution of Plethodon larselli

LOCALITY INDEX FOR FIGURE 1

1. Columbia River
2. State Highway 14
3. Smith Cripe Road
4. Archer Mountain
5. Skamania
6. Hardy Creek
7. Beacon Rock State Park
8. North Bonneville
9. Wind River
10. Dog Creek
11. White Salmon
12. Hood River
13. Viento Creek
14. Starvation Creek
15. Cabin Creek
16. Warren Creek
17. Wyeth
18. Cascade Locks
19. Bonneville Dam
20. Tanner Creek
21. Ainsworth State Park
22. Multnomah Creek
23. Wahkeena Creek
24. Larch Mountain
25. Interstate Highway 80 North

the author has collected extensively in the region of Snoqualmie National Forest, which is approximately 40 air miles and almost directly north of Archer Mountain, with no success in finding larselli.

It seems apparent that the Dog Creek and Viento Creek vicinities are at or near the most eastern extent of the species' range, and the Archer Mountain and Wahkeena Creek vicinities are at or near the most western extent of the species' range. Moisture seems to be playing a vital role in limiting the dispersal of larselli to the immediately adjacent eastern and western areas as indicated by average annual rainfall at several selected weather stations in the Columbia River Gorge (see Table 1). Moisture content is high in the center of the species' range and decreases rapidly to the east and west. Hood River has an average rainfall of 1.17 inches during July, indicating that there has been no threat of drought to the eastern extremity of the species' range during the drier summer months. The major vegetational formations gradually change from western hemlock, western red cedar, and douglas fir, which provide an excellent canopy, in the Viento Creek and Dog Creek vicinities, to yellow pine, oak woodland, and grassland in the Hood River and White Salmon vicinities, thus restricting the species' dispersal to the east. Talus slopes, which serve as excellent protective microhabitats for larselli during the drier months, are absent in the Portland and Vancouver vicinities and, therefore, apparently restrict dispersal of the species to the immediate west.

Plethodon vehiculum has been found sympatrically with P. larselli at the following sites: Beacon Rock State Park, Skamania

TABLE 1. AVERAGE SEASONAL AND ANNUAL RAINFALL IN LOWER COLUMBIA RIVER GORGE*

Locality	J	F	M	A	M	J	J	A	S	O	N	D	Annual
Portland	6.02	4.63	4.04	2.68	1.93	1.46	0.50	0.64	1.85	2.85	6.15	6.68	39.43
Cascade Locks	11.73	8.67	8.46	5.21	3.51	2.24	0.56	1.01	3.05	5.84	12.21	12.70	75.19
Hood River	4.78	3.65	3.11	1.56	0.95	0.72	1.17	0.30	1.04	1.98	5.35	5.45	30.06
Vancouver	5.41	4.30	3.56	2.54	1.96	1.53	0.53	0.73	1.90	2.71	5.88	6.11	37.16
Wind River	14.82	9.48	10.07	5.83	3.64	2.13	0.47	0.98	3.57	6.76	13.38	15.71	86.84
White Salmon	5.58	3.61	3.19	1.52	1.09	0.76	0.25	0.31	1.19	2.08	5.04	5.87	30.49

* U.S.D.A., 1941

County, Washington; Archer Falls, Skamania County, Washington; approximately 0.5 mile west of Archer Mountain, Skamania County, Washington; Starvation Creek vicinity, Hood River County, Oregon; Cabin and Warren Creeks vicinities, Hood River County, Oregon; and Wahkeena Creek vicinity, Multnomah County, Oregon (see Figure 1). While vehiculum exhibits a much more extensive north, south, and west range than larselli, it apparently does not occur as far east as larselli. It has already been mentioned that in moving from west to east, in the lower Columbia River Gorge, several ecological factors are altered, namely, a decrease in annual rainfall and a change in the major vegetational formations. It also seems that the interesting exclusions and overlaps in the distribution of larselli and vehiculum suggest some type of competition. It is, however, difficult to understand why larselli has not expanded its range into the apparently suitable, northern regions, especially when it sympatrically exists in abundance with vehiculum along the lower Columbia River Gorge.

Plethodon larselli has been collected in abundance during its active season (September, October, and November) at all the sites indicated in Figure 1. However, during winter months this species withdraws from its customary habitat, in talus slopes that are usually north-facing, under stones and forest debris, becoming difficult to obtain, apparently due to its inhabiting the depths of rocky fragmented slopes. Egg deposition probably occurs during late September and October, with young hatching or emerging early in the spring.

Chapter 3

DEHYDRATION AND REHYDRATION RATES

As with all members of this genus, P. larselli is a lungless, thin-skinned, terrestrial salamander and is dependent on the skin remaining moist for cutaneous gaseous exchange, a vital respiratory component. While existing under terrestrial conditions, larselli must contend with the threat of dessication which may have its greatest impact through reduction of gaseous exchange. Any resistance to dessication exhibited by this species would consequently increase its survival capacity when subjected to unfavorable dry situations. Therefore, a study was made on its dehydration and rehydration rates.

The dessication chamber consisted of a glass tube approximately one inch in diameter and seven inches in length. The two ends of the dessicator were sealed by number four rubber stoppers with size B Quick Disconnects in the center of each plug. Rubber tubing with size B Quick Disconnects was used to couple one end of the dessicator to a Manostat Flowmeter, and the other end to a Drierite cylinder. The experimental animal was enclosed in a preweighed fiberglass screen so that a relative constancy of exposed surface area could be maintained during dehydration. The salamander and jacket were then placed in the preweighed dessication chamber and weighed. Air from a Dyna-Vac Pump, dehydrated by blowing through a Drierite cylinder (anhydrous calcium sulfate) and regulated by an adjustable clamp, was passed through the dessication chamber. The calibrated flowmeter assured a constant air

stream (approximately 1.06 l/min). The specimen and dessicator were reweighed at 10 minute intervals for 40 minutes. The total dehydration time for immature salamanders was 30 minutes. The experimental animal was then removed from the fiberglass screen envelop and placed in a preweighed petri dish and weighed, then transferred to saturated filter paper in a second petri dish. Another saturated filter paper was placed over the individual. The rehydrating animals were moved to a dry, preweighed petri dish and weighed at 20 minute intervals for one hour. The dehydrating and rehydrating experiments were done at room temperature (21 - 22°C). All weighings were made on a Mettler balance (precision ±.1 mg). The salamanders were grouped into the following average snout-vent lengths: P. larselli, 46.4 mm (11 individuals); P. larselli, 31.2 mm (9); P. vehiculum, 45.6 mm (8); P. vehiculum, 28.5 mm (5). All animals were measured from the tip of the snout to the anterior angle of the vent. Surface areas were obtained by skinning the salamanders and transferring the traced outline to graph paper (20 squares to the inch).

The results of dessication and rehydration are presented in Table 2 and 3. Factorial analyses and Duncan's multiple range tests, where necessary, of the raw data show that there were significant differences among the adults and juveniles of P. larselli and P. vehiculum in the rates of dehydration and rehydration (Tables 4 - 15). Before statistical treatment, the raw data were converted to mg of weight lost per square inch of surface area per 10 mm snout-vent length, thus minimizing any effect from the surface-volume relationship. The statistical analyses were set up for the following

TABLE 2. AVERAGE AND STANDARD DEVIATION (IN PARENTHESES) OF RATE OF DEHYDRATION IN *larselli* AND *vehiculum* ADULTS AND JUVENILES (in mg body weight).

	Time (min)			
	10	20	30	40
larselli adults (8)*	8.54 (1.24)	7.20 (1.17)	6.97 (0.97)	7.00 (1.21)
larselli juveniles (9)	15.64 (3.21)	12.31 (2.20)	11.82 (2.13)	----
vehiculum adults (8)	7.14 (1.22)	5.48 (0.77)	5.14 (0.70)	4.93 (0.69)
vehiculum juveniles (4)	14.66 (5.56)	12.77 (3.13)	----	----

* Number of specimens

TABLE 3. AVERAGE AND STANDARD DEVIATION (IN PARENTHESES) OF RATE OF REHYDRATION IN *larselli* AND *vehiculum* ADULTS AND JUVENILES (in mg body weight).

	Time (min)		
	20	40	60
larselli adults (8)*	3.89 (0.60)	3.25 (1.04)	3.45 (0.56)
larselli juveniles (8)	4.01 (2.01)	4.24 (1.60)	3.83 (1.17)
vehiculum adults (7)	1.97 (0.87)	1.44 (0.82)	1.78 (0.69)
vehiculum juveniles (4)	1.41 (1.55)	2.17 (0.65)	2.34 (1.57)

* Number of specimens

TABLE 4. ANALYSIS OF DEHYDRATION DATA ON *larselli* AND *vehiculum* ADULTS (5% level of significance). Significant differences are denoted with a /.

Factorial Analysis			
Variables	Degrees of Freedom	F	Remarks
Species (Factor A)	1	58.53 with 1.& 3 d.f.	significant
Time (Factor B)	3	14.59 with 3 & 49 d.f.	significant
Interaction (Factor AB)	3	0.36 with 3 & 49 d.f.	not significant
Error	49		
Total	63		

Multiple Range Test of Factor B

N=16 s^2=0.84 with 49 d.f.

v_2=49 $S\bar{y}=\sqrt{s^2/N}$ =0.23

g	2	3	4	
SSR	0.65	0.69	0.89	
	B_4	B_3	B_2	B_1
$\bar{x}$	5.97	6.06	6.35 /	7.84

TABLE 5. ANALYSIS OF REHYDRATION DATA ON *larselli* AND *vehiculum* ADULTS (5% level of significance).

Factorial Analysis			
Variables	Degrees of Freedom	F	Remarks
Species (Factor A)	1	50.86 with 1 & 30 d.f.	significant
Time (Factor B)	2	1.76 with 2 & 30 d.f.	not significant
Interaction (Factor AB)	2	0.12 with 2 & 30 d.f.	not significant
Error	30		
Total	41		

TABLE 6. ANALYSIS OF DEHYDRATION DATA ON *larselli* ADULTS AND JUVENILES (5% level of significance). Significant differences are denoted with a /.

Factorial Analysis

Variables	Degrees of Freedom	F	Remarks
Species (Factor A)	1	160.07 with 1 & 2 d.f.	significant
Time (Factor B)	2	13.44 with 2 & 40 d.f.	significant
Interaction (Factor AB)	2	2.57 with 2 & 40 d.f.	not significant
Error	40		
Total	53		

Multiple Range Test of Factor B

N=18 s^2=0.28 with 40 d.f.

v_2=40 $S\bar{y}=\sqrt{s^2/N}$ =0.12

g	2	3	
SSR	0.36	0.37	
	B_3	B_2	B_1
$\bar{x}$	9.37	9.73 /	12.03

TABLE 7. ANALYSIS OF REHYDRATION DATA ON *larselli* ADULTS AND JUVENILES (5% level of significance).

Variables	Degrees of Freedom	Factorial Analysis F	Remarks
Species (Factor A)	1	1.93 with 1 & 35 d.f.	not significant
Time (Factor B)	2	0.27 with 2 & 35 d.f.	not significant
Interaction (Factor AB)	2	0.52 with 2 & 35 d.f.	not significant
Error	35		
Total	47		

TABLE 8. ANALYSIS OF DEHYDRATION DATA ON larselli ADULTS AND vehiculum JUVENILES (5% level of significance).

Factorial Analysis			
Variables	Degrees of Freedom	F	Remarks
Species (Factor A)	1	15.29 with 1 & 9 d.f.	significant
Time (Factor B)	1	1.33 with 1 & 9 d.f.	not significant
Interaction (Factor AB)	1	0.01 with 1 & 9 d.f.	not significant
Error	9		
Total	15		

TABLE 9. ANALYSIS OF REHYDRATION DATA ON *larselli* ADULTS AND *vehiculum* JUVENILES (5% level of significance).

Factorial Analysis			
Variables	Degrees of Freedom	F	Remarks
Species (Factor A)	1	7.53 with 1 & 15 d.f.	significant
Time (Factor B)	2	0.01 with 2 & 15 d.f.	not significant
Interaction (Factor AB)	2	0.82 with 2 & 15 d.f.	not significant
Error	15		
Total	23		

TABLE 10. ANALYSIS OF DEHYDRATION DATA ON *larselli* JUVENILES AND *vehiculum* ADULTS (5% level of significance). Significant differences are denoted with a /.

Factorial Analysis

Variables	Degrees of Freedom	F	Remarks
Species (Factor A)	1	847.04 with 1 & 2 d.f.	significant
Time (Factor B)	2	57.24 with 2 & 2 d.f.	significant
Interaction (Factor AB)	2	4.16 with 2 & 35 d.f.	significant
Error	35		
Total	47		

Multiple Range Test of Factor B

N=16 s^2=0.60 with 35 d.f.

v_2=35 $S\bar{y}=\sqrt{s^2/N}$ =0.19

g	2	3
SSR	0.55	0.58

	B_3	B_2	B_1
$\bar{x}$	8.14	8.54 /	10.85

Multiple Range Test of Factor AB

N=8 s^2=0.60 with 35 d.f.

v_2=35 $S\bar{y}=\sqrt{s^2/N}$ =0.27

g	2	3	4	5	6
SSR	0.79	0.83	0.85	0.87	0.89

	A_2B_3	A_2B_2	A_2B_1	A_1B_3	A_1B_2	A_1B_1
$\bar{x}$	5.14	5.48 /	7.14 /	11.14	11.59 /	14.56

TABLE 11. ANALYSIS OF REHYDRATION DATA ON *larselli* JUVENILES AND *vehiculum* ADULTS (5% level of significance).

Factorial Analysis			
Variables	Degrees of Freedom	F	Remarks
Species (Factor A)	1	46.81 with 1 & 30 d.f.	significant
Time (Factor B)	2	0.55 with 2 & 30 d.f.	not significant
Interaction (Factor AB)	2	0.17 with 2 & 30 d.f.	not significant
Error	30		
Total	41		

TABLE 12. ANALYSIS OF DEHYDRATION DATA ON *larselli* AND *vehiculum* JUVENILES (5% level of significance).

Factorial Analysis			
Variables	Degrees of Freedom	F	Remarks
Species (Factor A)	1	0.33 with 1 & 9 d.f.	not significant
Time (Factor B)	1	2.01 with 1 & 9 d.f.	not significant
Interaction (Factor AB)	1	0.14 with 1 & 9 d.f.	not significant
Error	9		
Total	15		

TABLE 13. ANALYSIS OF REHYDRATION DATA ON *larselli* AND *vehiculum* JUVENILES (5% level of significance).

Factorial Analysis			
Variables	Degrees of Freedom	F	Remarks
Species (Factor A)	1	14.17 with 1 & 15 d.f.	significant
Time (Factor B)	2	0.58 with 2 & 15 d.f.	not significant
Interaction (Factor AB)	2	0.56 with 2 & 15 d.f.	not significant
Error	15		
Total	23		

TABLE 14. ANALYSIS OF DEHYDRATION DATA ON *vehiculum* ADULTS AND JUVENILES (5% level of significance).

Factorial Analysis			
Variables	Degrees of Freedom	F	Remarks
Species (Factor A)	1	24.16 with 1 & 9 d.f.	significant
Time (Tactor B)	1	1.07 with 1 & 9 d.f.	not significant
Interaction (Factor AB)	1	0.02 with 1 & 9 d.f.	not significant
Error	9		
Total	15		

TABLE 15. ANALYSIS OF REHYDRATION DATA ON *vehiculum* ADULTS AND JUVENILES (5% level of significance).

Factorial Analysis			
Variables	Degrees of Freedom	F	Remarks
Species (Factor A)	1	0.05 with 1 & 15 d.f.	not significant
Time (Factor B)	2	0.25 with 2 & 15 d.f.	not significant
Interaction (Factor AB)	2	0.52 with 2 & 15 d.f.	not significant
Error	15		
Total	23		

comparisons of species and maturity: adult P. larselli versus adult P. vehiculum; adult P. larselli versus juvenile P. larselli; adult P. larselli versus juvenile P. vehiculum; juvenile P. larselli versus adult P. vehiculum; juvenile P. larselli versus juvenile P. vehiculum; adult P. vehiculum versus juvenile P. vehiculum. The adults of P. larselli lost water at a significantly higher rate than the adults of P. vehiculum, while losing water at a significantly lower rate than the juveniles of P. larselli and P. vehiculum. The juveniles of P. larselli had a significantly higher rate of water loss than P. vehiculum adults, while showing no significant difference when compared to P. vehiculum juveniles. The adults of P. vehiculum lost water at a significantly lower rate than the juveniles of P. vehiculum. In all the species and maturity comparisons except those including P. vehiculum juveniles, the rate of water loss was most rapid during the first period. After this 10 minute interval, the rate of water loss decreased significantly during the remaining dehydration intervals and remained unchanged through the termination of the experiments. This suggests that some type of behavioral and/or physiological mechanism exists in P. larselli adults and juveniles and in P. vehiculum adults which retards the rate of water loss after the initial period of dehydration. It also indicates that P. larselli exhibits this mechanism at an earlier developmental stage than P. vehiculum. This is further illustrated in the significant variation that was found in the A-B interaction between P. larselli juveniles and P. vehiculum adults, that is, the interaction due to the maturity of the two species and the dehydrating time intervals, while no such significant variation

resulted from the A-B interaction between P. vehiculum juveniles and P. larselli adults. While no significant differences were noted for rates of rehydration at any given time interval, there were significant results for several species-maturity comparisons of total rehydration rates. The adults and juveniles of P. larselli rehydrated at significantly higher rates than P. vehiculum adults and juveniles. Even though P. larselli dehydrates more rapidly than P. vehiculum, P. larselli rehydrates significantly faster than P. vehiculum.

The relationship between these data and the distribution of the two species clearly indicates that P. larselli exhibits a greater capacity to survive in relatively drier areas than P. vehiculum. This is further emphasized by the fact that only one P. larselli juvenile died after dehydration, while two adult and four juvenile P. vehiculum died after dehydration. This strongly suggests that P. larselli can tolerate a greater total loss of water than P. vehiculum, as well as regain any water loss in a shorter period of time, thus enabling the species to endure the drier eastern areas of its range. A similar difference in habitat tolerance was shown by Dumas (1956) for P. vehiculum and Plethodon dunni. Whether P. larselli is competitively displaced into drier habitats, as P. dunni apparently forces P. vehiculum to select lower humidities when both species are together, is not presently known.

Chapter 4

INDUCED OVIPOSITION

Although I have found no reference in the literature to eggs of Plethodon larselli having been induced, several investigators have successfully stimulated oviposition in plethodontids. Anderson (1958) induced oviposition in Batrachoseps attenuatus with implants of whole pituitary glands and with gonadogen injections, while Highton and Savage (1961) obtained egg deposition in Plethodon cinereus with injections of the same hormone, and Plethodon shenandoah was induced to deposit eggs by injections of ovine luteinizing hormone (Highton and Worthington, 1967).

On November 23, 1968, eight gravid females, four P. larselli and four P. vehiculum, were collected at Starvation Creek, Hood River County, Oregon, and kept at 16°C for two months, with no oviposition occuring in the laboratory. A concentrated solution (6 mg/ml) of Ovine Pituitary Luteinizing Hormone (Nutritional Biochemicals Corporation) was prepared in amphibian Ringer's solution along with two dilutions (dilution factors: 1:6,666 and 1:266,666). Three specimens of both species each received .05 cc of a different concentration of the gonadotropic hormone, and one specimen of each species received .05 cc of amphibian Ringer's solution. The experimental animals were anesthetized with MS 222 (Tricaine Methanesulfonate, from Kent Chemicals Limited) and then given intraperitoneal injections at the posterior of the abdomen with a one cc Tuberculin syringe and a number

27 hypodermic needle. A total of three injections were given at five day intervals, with no additional injections given after egg deposition. Following injections, the individuals were separated into plastic containers, approximately eight inches in diameter, containing moistened paper towels and a few pebbles, and these were placed in a refrigerator which was maintained at 16°C. The females remained with any deposited eggs and both were kept at the previously mentioned temperature. The females were fed vestigial-winged Drosophila melanogaster flies at two day intervals. All egg measurements were done under a dissecting scope by calipers.

Oviposition occurred only in P. larselli receiving the most concentrated gonadotropic hormone. Four eggs were deposited 84 hours after the initial injection, and two additional eggs were laid five hours later. All the eggs were deposited separately, no further than one inch apart, with the exception of two ova which were present together and wedged between two pebbles. The occurrence of single eggs is probably the result of unnatural laboratory conditions, for Dumas (1955) reported a grape-like cluster of Plethodon dunni eggs in nature, and Stebbins (1966) states that Plethodon vehiculum eggs occur in grape-like clusters in nature. The deposited eggs were creamy white with a cloudy transparent outer jelly capsule. The diameters (in mm) of the eggs, including the outer jelly capsule, were, 5.0, 5.0, 5.5, 5.5, 5.5, and 5.2. The mean egg diameter was 5.28 mm.

On March 4 a slight growth of mold was noticed at a junction between the moist paper towel and one of the eggs wedged between two pebbles. The following day the egg was missing and, upon examination

of the female's abdominal wall, was clearly seen in her stomach. Oophagy has been reported by Highton and Savage (1961) for P. cinereus and Noble (1954:413) mentioned cryptobranchid salamanders devouring their own eggs, as well as observations by the author of egg-eating by Plethodon vandykei. It is interesting to note that the mold and the egg were eliminated before the mold spread to the other egg which was closely associated in position to the contaminated egg. Furthermore, vestigial-winged fruit flies were in abundance at the time of inspection, and prior observations indicate that this food type is taken readily by the salamanders. The remaining eggs, all separate in position, eventually became covered with mold and were removed; two eggs on March 26, and three eggs on April 6. The last three eggs to become contaminated plainly exhibited embryos.

Brooding behavior has been reported by Highton and Savage (1961) for P. cinereus, by Gordon (1952) for Aneides aeneus, and by Noble (1954:413-415) for several species of salamanders. While no continuous brooding behavior was noticed, the female was found on infrequent occasions to be in contact with the eggs. Furthermore, it was observed the day after egg diameters were taken, that an egg had been moved approximately 18 cm from a position clear of any pebbles to a position against a pebble. No such observations were further detected even though container disturbances continued for feeding purposes.

Chapter 5

ELECTROPHORETIC ANALYSES

Dessauer and Fox (1956) suggested that it is possible to differentiate between taxa on the basis of paper electrophoretic analysis of plasma protein fraction characteristics. Bertini and Rathe (1962) investigated the hemoglobin of several species of anurans by electrophoretic analysis, and indicated that the results may serve as a valuable index to the biochemical specificity of individuals and to interspecific affinity. Dumas (1966) was able to show a number of differences between various species of ranids by paper electrophoretic separation of blood serum proteins. Therefore, an attempt was made to determine the mobility and heterogeneity of the plasma protein fractions in P. larselli and P. vehiculum.

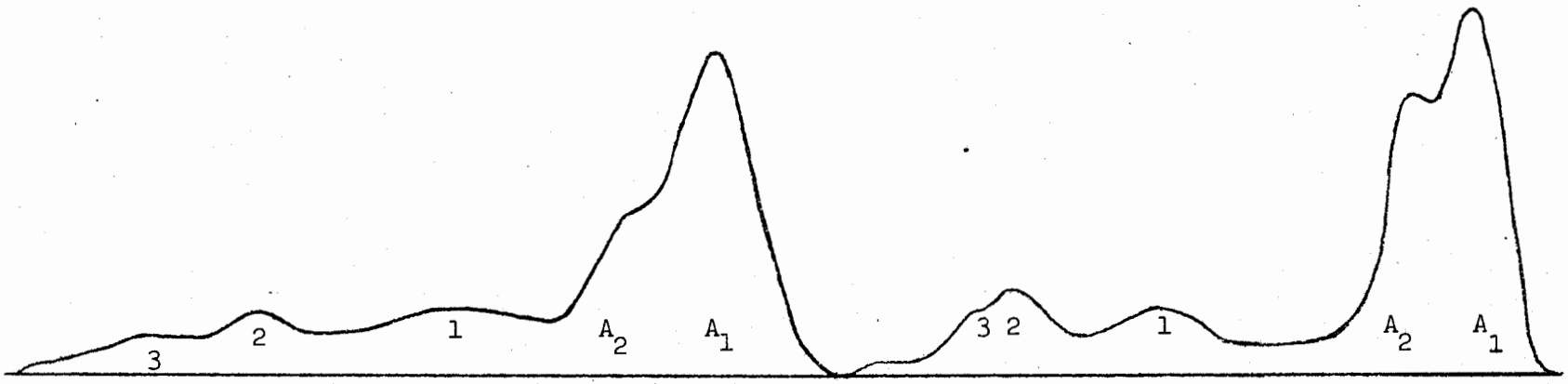
The plasma proteins were studied by means of a Beckman/Spinco Model R-110 Microzone Electrophoresis apparatus and a Model R-110 Microzone Densitometer. The blood samples were obtained from the heart, which was exposed immediately after killing the animal in a saturated chloretone solution. The blood was drawn into capillary tubes, allowed to clot, and the serum separated by centrifugation. The serum was then subjected to electrophoretic analysis following the procedure outlined in the Beckman Instruction Manual RM-IM-3 on pages 23 to 41. For each analysis 0.25 μl of serum was used. The samples were applied on the Beckman Electrophoresis Membranes (number 324330) after obtaining liquid-vapor equilibrium in the cell with barbital

buffer at pH 8.6 and ionic strength of 0.075. Electrophoresis was run at a 250 volt, 5ma current for 20 minutes. Staining was accomplished with Beckman Fixative Dye number 324340 (Ponceau-S stain). Several electrophoretic analyses were made of each serum sample. The cellulose acetate membranes were dried at 90°C for 20 minutes, and then placed in a clean plastic envelop. The plasma separations were scanned with the densitometer.

Figures 2,3, and 4 are tracings of the separation patterns which most closely represented the average for each species. Table 16 compares the results of the electrophoretic separations in which the no delay group represents those specimens analyzed within one day after collection, and the delay group indicates the specimens kept under laboratory conditions (16°C) for no more than four months before electrophoresis. The relative concentrations of each protein fraction are expressed as average percentages of the total relative concentrations of all the protein fractions, and were determined as outlined in the Densitometer Instruction Manual RM-TB-005 on pages 29 and 30. The migration of each fraction is expressed in average mm. Figures 5 and 6 represent the ranges, means, and one standard deviation on either side of the means for relative percent concentrations and distance moved for each protein fraction. The standard deviations extend beyond the sample ranges because of the small N (number of observations) involved in computing the statistic.

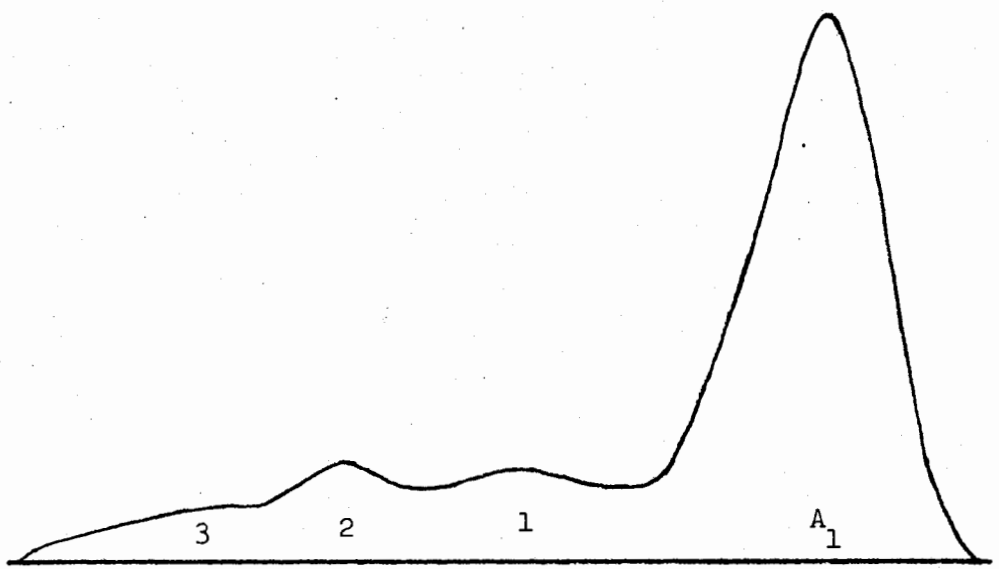
The results obtained from the electrophoretic study indicate significant differences for the interactions of the two groups (no delay and delay) between and within the two species. While the raw

Fig. 2. Typical electrophoretic patterns for larselli.



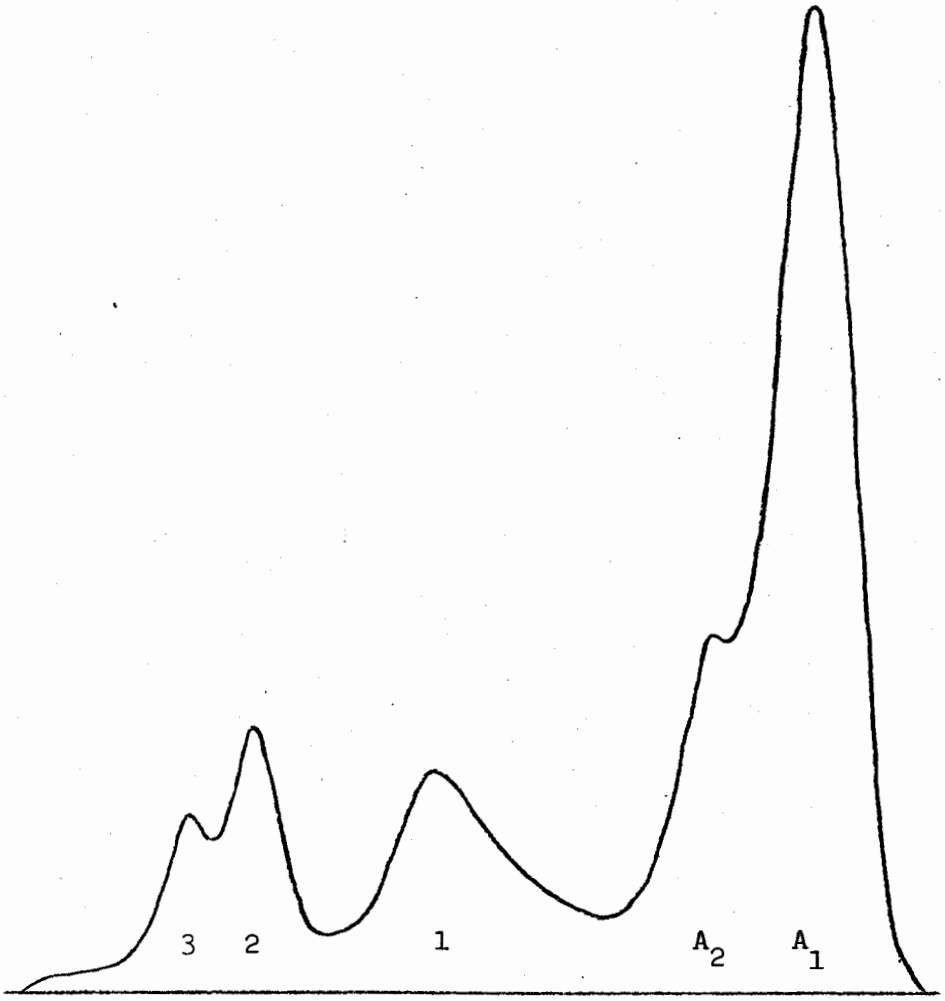
Plethodon larselli (delay)

Plethodon larselli (no delay)



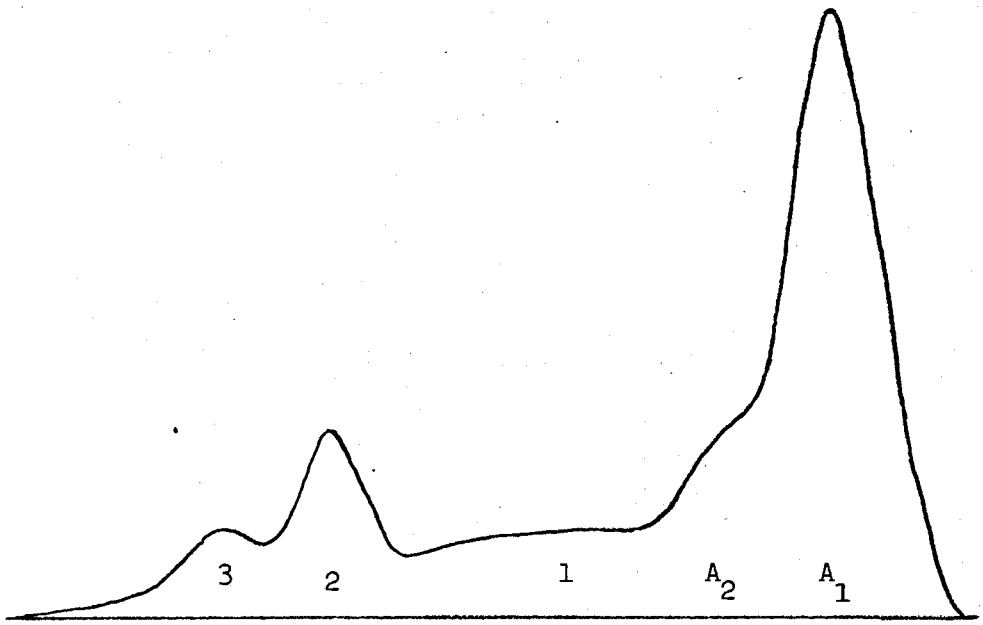
Plethodon larselli (delay)

Fig. 3. Typical electrophoretic pattern for vehiculum.

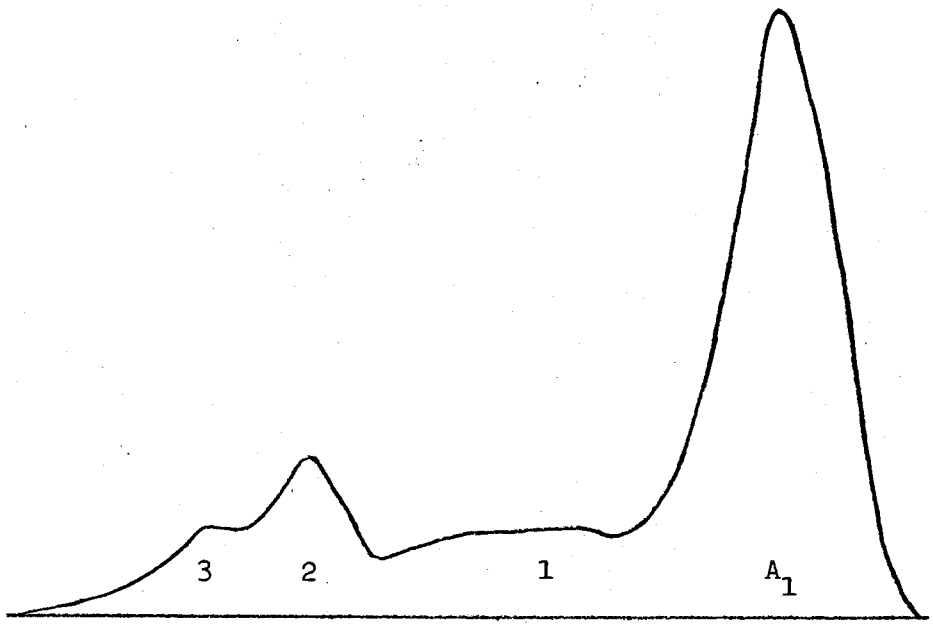


Plethodon vehiculum (no delay)

Fig. 4. Typical electrophoretic patterns for vehiculum.



Plethodon vehiculum (delay)



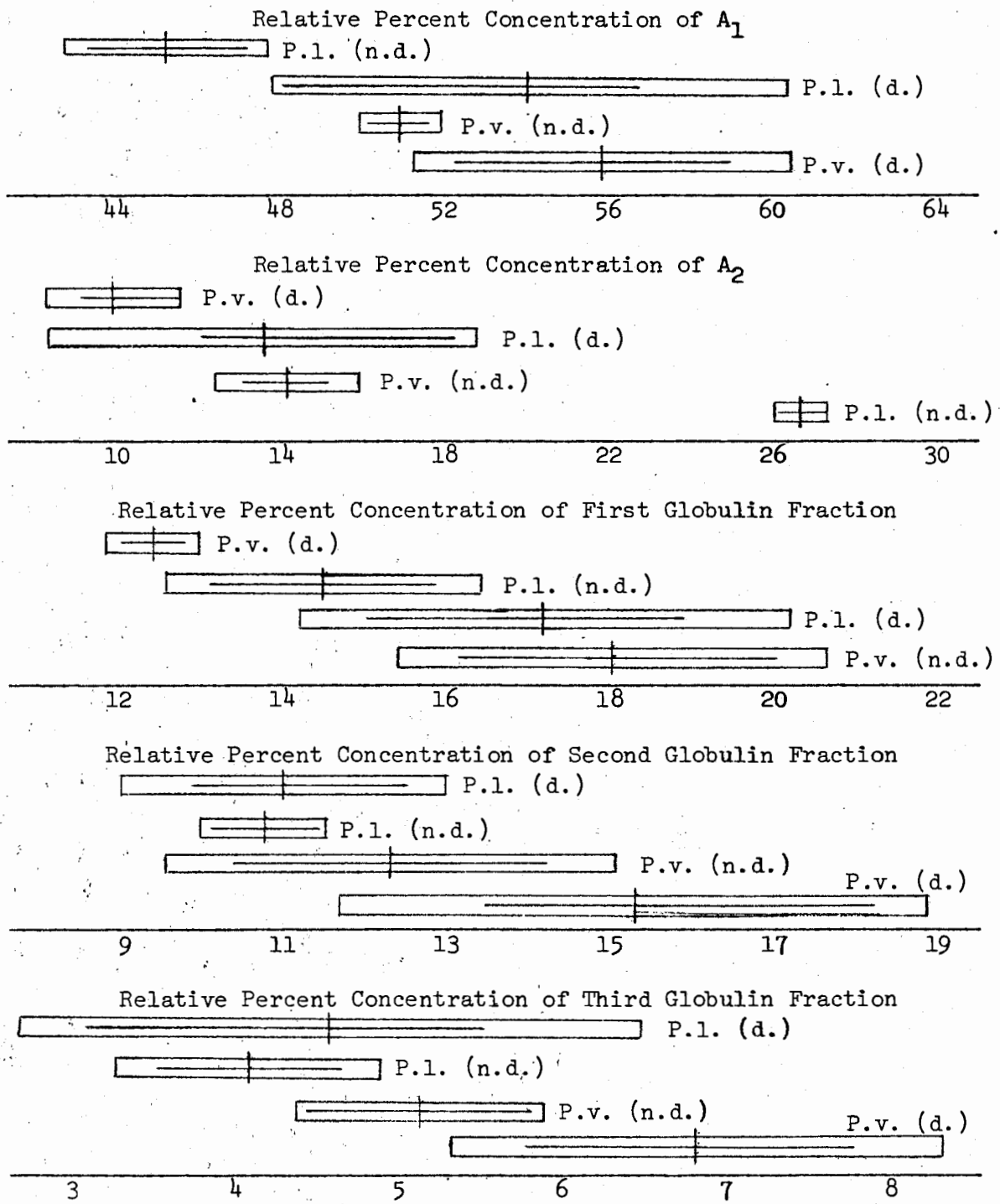
Plethodon vehiculum (delay)

TABLE 16. CHARACTERISTICS OF larselli AND vehiculum SERUM FRACTIONS.
Figures are in average percentages and mm with standard deviations in parentheses.

	Albumen 1		Albumen 2		Globulin 1		Globulin 2		Globulin 3	
	Concentration	Moved	Concentration	Moved	Concentration	Moved	Concentration	Moved	Concentration	Moved
larselli* (2)**	44.4 (2.6)	18.8 (3.2)	26.4 (0.6)	21.8 (1.8)	14.4 (1.9)	29.8 (1.1)	10.7 (0.8)	15.0 (4.2)	4.1 (0.8)	28.5 (0.7)
vehiculum* (2)	50.7 (1.0)	25.8 (0.4)	14.0 (1.7)	17.3 (1.1)	18.0 (2.7)	35.0 (1.4)	12.3 (2.8)	16.0 (1.4)	5.1 (0.8)	22.0 (1.4)
larselli*** (4)	54.0 (6.9)	28.5 (4.7)	13.4 (5.3)	13.9 (1.8)	17.1 (3.0)	29.9 (4.0)	11.0 (2.1)	22.9 (4.8)	4.6 (1.9)	26.3 (7.5)
vehiculum*** (3)	55.8 (4.7)	34.2 (5.2)	9.7 (1.7)	14.5 (0.7)	12.4 (0.6)	24.7 (2.9)	15.3 (3.6)	21.0 (1.4)	6.8 (1.5)	32.2 (2.3)

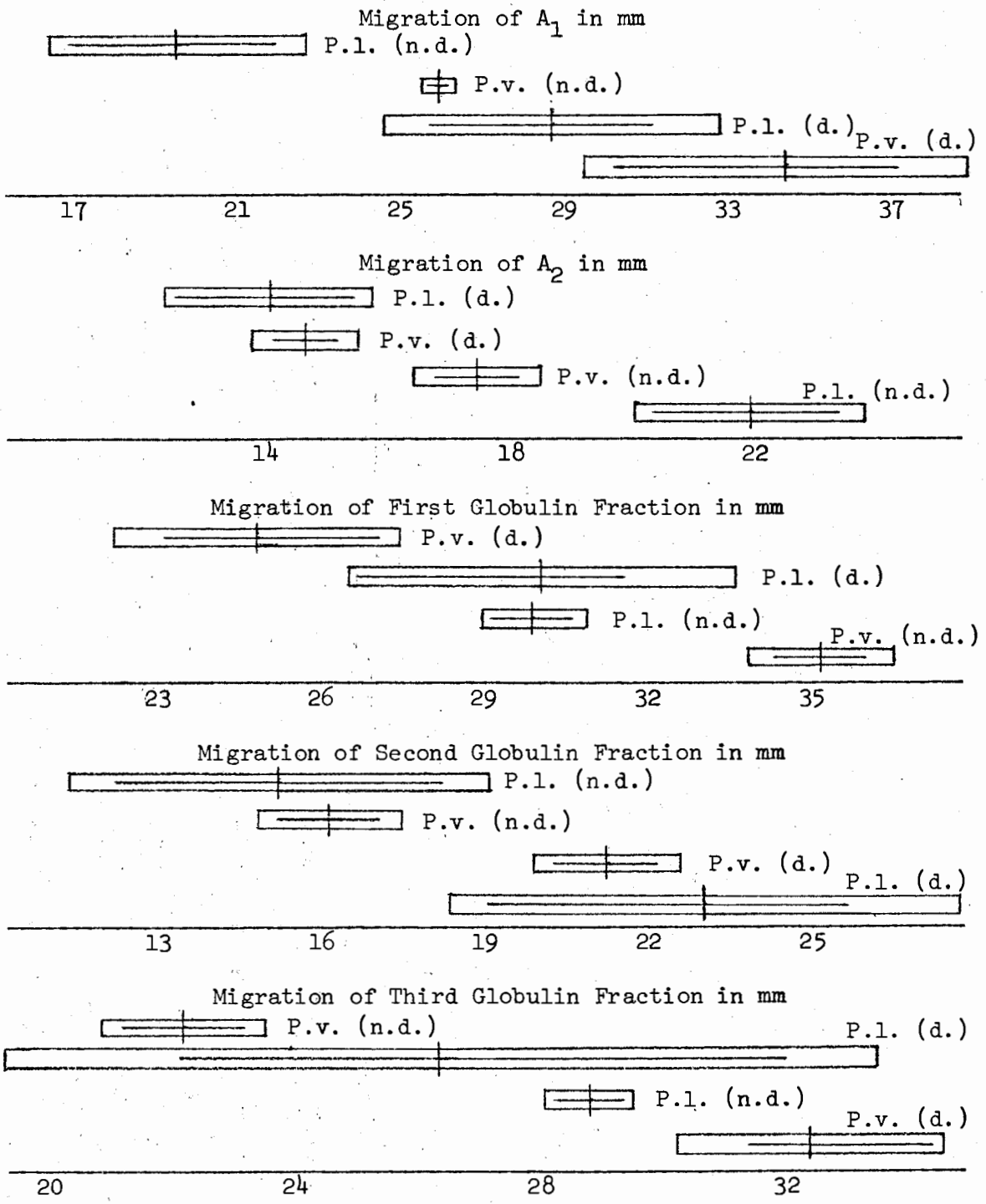
* No delay specimens
** Number of specimens
*** Delay specimens

Fig. 5. Comparison of relative percent concentrations of blood protein fractions for larselli and vehiculum. Horizontal lines represent observed ranges; vertical lines represent means; rectangles denote one standard deviation on either side of means.



Legend: P.l. = Plethodon larselli, P.v. = Plethodon vehiculum, n.d. = no delay, d. = delay, A = albumen

Fig. 6. Comparison of migrations of blood protein fractions for larselli and vehiculum. Horizontal lines represent observed ranges; vertical lines represent means; rectangles denote one standard deviation on either side of means.



Legend: P.l. = Plethodon larselli, P.v. = Plethodon vehiculum, n.d. = no delay, d. = delay, A = albumen

data were taken from those patterns showing two albumen fractions, other patterns were obtained from the delay groups of both species which exhibited no second albumen fraction (see Figures 2 and 4). This indicates that laboratory conditions inspired some physiological factors which influenced plasma protein composition. Even when the second albumen fraction was exhibited by the delayed specimens of both species, it was present in lower concentrations and with a decreased mobility. Although such factors as geographic variation, season, and starvation are known to affect electrophoretic patterns, they do not apply in this study; all specimens were collected in an area approximately 22 miles in east-west extent and six miles in north-south extent with no major physical barriers, as well as the season being uniform throughout collection, and all individuals fed well on vestigial-winged Drosophila melanogaster flies. It does seem likely, however, that moisture is an important factor since specimens were kept in a somewhat more damp environment than their normal habitat. Furthermore, the albumen fractions of the blood are related to osmoregulatory mechanisms, thus a situation could have been set up that provoked a diluting effect on the albumen-two fractions. While a significant difference in the concentrations and migrations of the globulin fractions were also exhibited by the no delay and delay groups, an explanation for these differences is not presently known; however, the answers may rest within physiological and genetical considerations.

The larselli (no delay) and vehiculum (no delay) patterns reveal several significant differences. The albumen-one component in larselli displays significantly less concentration and mobility than

that in vehiculum, while the albumen-two component for larselli reveals a significantly higher concentration and mobility than that for vehiculum. The first globulin fraction of vehiculum exhibits a significantly greater mobility than that of larselli, while the third globulin component migrates less than that for larselli. The larselli no delay group and the larselli delay group reveal differences only in the two albumen fractions. The albumen-one fraction of the delay specimens is greater in both concentration and mobility, while the albumen-two fraction is less in both concentration and mobility than that for the no delay specimens. The no delay group for larselli and the delay group for vehiculum show several significant differences. The concentrations and mobilities of the first albumen fraction and the second and third globulin fractions for larselli are significantly less than those for vehiculum, while the second albumen component is greater in concentration and mobility than that for vehiculum. The concentrations of the plasma protein molecules for larselli (delay) and vehiculum (no delay) reveal no significant differences; however, the first and second globulin, and albumen-two fractions are significantly different in mobility. The first globulin and albumen-two molecules are less mobile for larselli than for vehiculum, while the second globulin molecules are more mobile for larselli than for vehiculum. The no delay group for vehiculum shows significant differences from its delay group for both concentration and migration of several protein fractions. While the concentration differences for the second and third globulin, and albumen-one fractions for the no delay and delay specimens are not significant, the mobilities for

these fractions are significantly higher in the delay specimens than in the no delay specimens. The first globulin and albumen-two fractions of the no delay specimens are greater in concentration and migration than those for the delay specimens. The only significant difference found between larselli delay and vehiculum delay was for the concentration of the first globulin fraction in which larselli exhibited a greater concentration than vehiculum.

The plasma proteins revealed both concentration and migration characteristics that can serve as a means of elucidating basic similarities and differences between larselli and vehiculum. It is possible to recognize the patterns of the no delay groups of larselli and vehiculum based on concentration and mobility differences in their first and second albumen, and first and third globulin fractions. It is, however, necessary to recognize that various physiological variations will cause differences in the patterns and, therefore, certain allowances must be made before such separations may be used for diagnostic characteristics.

Chapter 6

SUMMARY

The eastern limits of the range for P. larselli were probably more extensive during the colder and more moist periods of the post-Pleistocene, while the western boundaries presumably remained relatively unchanged from the present. The existing extensiveness of the north-south range is open to investigation. The present restriction of the species' range may be due to several factors. If larselli became genetically separated from its basic stock a relatively short time ago, then it would seem that the species has not been in existence long enough to allow invasion of adjacent, suitable habitat, especially since the dispersal rate of terrestrial salamanders is extremely slow. The possibility also exists that larselli lacks the reproductive potential necessary to extend its range, or that a relatively high mortality rate, among the young and/or adults, keeps the population sufficiently low so that range extension is impossible. Also, if competition exists with its sympatrically abundant relative, P. vehiculum, the dispersal ability of larselli may be severely repressed. It is evident that the distribution of larselli presents several interesting zoogeographical problems which are worthy of further investigation.

An interesting sympatric and allopatric situation between larselli, vehiculum, and dunni occurs in the lower Columbia River Gorge. On the south side of the river all three species occur

sympatrically in the areas of greater moisture, while an allopatric gradient is present in the less damp regions. Plethodon vehiculum exists in the less damp areas with the exclusion of dunni and the overlap of larselli, while larselli exists in the least damp areas with the exclusion of both vehiculum and dunni. On the north side of the Columbia River larselli and vehiculum occur sympatrically without dunni in high moisture areas, while larselli exists in the less damp regions with the exclusion of vehiculum.

The rates of dehydration and rehydration show significant differences between the two species. The juveniles of both species dehydrated at significantly higher rates than the adults of either species. While there was no significant difference between the rates of rehydration for adults and juveniles of larselli, both exhibited significantly greater rehydration rates than either adults or juveniles of vehiculum, indicating a definite survival advantage for larselli under adversely dry conditions. Furthermore, even though larselli adults dehydrate significantly faster than vehiculum adults, they are able to tolerate a greater total water loss, thus, it appears that some type of mechanism is present in larselli that enables it to withstand a greater percentage of dessication. Consequently, if the two species were faced with drying conditions, larselli would have a longer period of time with which to find a suitable habitat, and upon contact with a moist surface would regain water significantly faster than vehiculum. A mechanism also exists in both species which retards the rate of water loss after the initial period of dehydration. This mechanism is apparently present in larselli at an earlier developmental

stage than in vehiculum.

Oviposition was successfully induced in a gravid female of larselli with an injection of Ovine Pituitary Luteinizing Hormone. Observations of the spent female with her eggs indicate that brooding behavior studies may be worthy of investigation.

Electrophoretic separations of the blood serum proteins showed a number of significant differences. It was possible to distinguish between the two species by concentration and migration of the first and second albumen fractions when the analysis was made immediately after collecting. This was indicated by significant concentration differences for albumen-one and two fractions and by significant mobility differences for albumen-one and two and the first and third globulin fractions. If, however, the specimens are kept under laboratory conditions for any length of time significant changes occur in all the blood protein fractions in either concentration or migration characteristics, with the exception of the third globulin fraction of larselli. Therefore, to obtain accurate comparisons the specimens should be analyzed shortly after collection. It should be noted that due to difficulty in determining an accurate end point for the third globulin fraction of either no delay or delay specimens, the value of this fraction as a diagnostic measure is dubious.

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APPENDIX

AUTHOR'S COLLECTING SITES FOR PLETHODON LARSELLI

Hood River County (Oregon)

Approximately 1.5 miles east of Viento Creek; T.3N,R.9E,S.36.

Viento Creek vicinity; T.3N,R.9E,S.34-35.

Cabin Creek and Warren Creek vicinities; T.2N,R.9E,S.4.

Starvation Creek vicinity; T.2N,R.9E,S.3-4.

Multnomah County (Oregon)

Wahkeena Creek vicinity; R.1N,R.6E,S.18.

Skamania County (Washington)

Dog Creek vicinity; T.3N,R.9E,S.27-28.

Beacon Rock State Park vicinity; T.2N,R.6E,S.24-25.

Archer Falls vicinity; T.2N,R.6E,S.32.

Approximately 0.5 miles west of Archer Mountain; T.2N,R.6E,S.31.