AN ABSTRACT OF THE THESIS OF

James George Wernz for the M.S. in Zoology
(Name) (Degree) (Major)

Date thesis is presented May 11, 1966

Title PRE-HATCHING STAGES OF THE TAILED FROG, ASCAPHUS TRUEI (STEJNEGER)

Abstract approved [redacted]
(Major professor)

Description of anuran larvae with the use of staging tables is a common practice in the study of frog life-histories, but there has been no previous description or table of Ascaphus larvae. In comparing Ascaphus larvae to a simplified staging table for anurans it was found that these larvae vary considerably from those of other anurans. Five stages had to be eliminated (Fertilization, Grey Crescent, Neural Plate, Gill Circulation, and Cornea Transparent) and five new ones created (Deposition, Neural Groove, Eye Pigmentation, Body Pigmentation, and Hatching). The final result was a series of twenty-one stages, from Deposition through Hatching, through which the growth and development of the larvae could be traced and which will, it is hoped, facilitate discussion of larval Ascaphus in any further studies.

Determination of tadpole food habits through stomach and intestinal content samples was largely unsuccessful.

PRE-HATCHING STAGES OF THE TAILED FROG, ASCAPHUS TRUEI (STEJNEGER)

by

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A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1966

APPROVED:

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Date thesis is presented May 11, 1966

Typed by Gail Dailey

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PRE-HATCHING STAGES OF THE TAILED FROG, ASCAPHUS TRUEI (STEJNEGER)

INTRODUCTION

The tailed frog, Ascaphus truei (Stejneger) was first described by Stejneger in 1899. Since its description its present known range has been determined by numerous collection reports. They have been found in the coast mountains as far south as Mendocino County, California by Salt (1952), and northward throughout the coast and Cascade mountains of Oregon and Washington into the southwestern part of British Columbia (Ricker and Logier, 1935; Slipp and Carl, 1943; and Carl, 1955). It was first found east of the Cascades in Washington (Svihla and Svihla, 1933) and is now known to range into Montana (Smith, 1932; Rodgers and Jellison, 1942) as far as 200 miles north and east of the Continental Divide (Donaldson, 1934). Its range then forms the other leg of a "horseshoe" by extending southward into the Mission Mountains of Montana (Brunson and Demaree, 1951), to the Seven Devils Mountains of Idaho (Linsdale, 1933), and the Blue Mountains (Ferguson, 1954) and Wallowa Mountains of Oregon (Graf, Jewett and Gordon, 1939).

Ascaphus is endemic to this portion of the Northwest and there is only one other frog with characteristics that make it possibly related, that being Leiopelma, a frog found in New Zealand. Both are

considered under the same family but the family name has been the object of some dispute owing to the questioned nature of the "amphicoelus" vertebrae (Ritland, 1955) and priority nomenclature and misspelling (Stephenson and Stephenson, 1957). However, Van Dijk (1955) believes that the family name "Ascaphidae" has priority and should be used.

Most studies concerning Ascaphus have been on some aspect of the adult. In two studies, Ritland (1955, 1955) thoroughly covers the morphology of the vertebral column, pelvic and pectoral girdles, forearm, posterior limb, spinal nerves, and musculature of the adult. Van Dijk (1955) gives a description of the pelvic girdle of Ascaphus with emphasis on the epipubis and pubic rods, and the circulatory system of the "tail." In a later study (1959) he describes the development of the cloacal region beginning with the hind-limb anlagen. The Stephensons (1957), in New Zealand, conducted a study on Leiopelma, describing its range, habitat, feeding habits, color pattern and some of its life history. Metter (1964) describes morphological differences between two populations of Ascaphus truei and gives a little information about the ecology and life history of the tadpole, suggesting that the adults mate in the fall and the female retains the sperm until the following spring when the eggs are laid.

In spite of all the studies on Ascaphus, there is still relatively little known about the early development of the embryo. The

Stephensons (1957) note that in Leiopelma, small clusters of eggs are laid, in the spring, beneath logs or rotting stumps where moisture is conserved and that hatching takes place about six weeks later; but no description of the embryo is given during this period from egg-laying to hatching.

It was the purpose of this study to observe the development of embryos of Ascaphus during the time from egg-laying, which normally occurs in the late spring (May to July), until they hatched from their capsule and, if possible, to divide their development, during this period, into a series of developmental "stages" or periods when specific activities, morpholological changes, or differences would be noticed. An attempt would also be made to determine, more precisely, the feeding habits of the tadpoles.

The specimens were collected on streambanks and in the streams at the following locations:

1) Dutch Oven and Frissell Crossing Campgrounds on the South Fork of the McKenzie River, Lane County, Oregon.

2) Hackleman Creek at Lost Prarie Campground in the Cascade mountains of Linn County, Oregon

3) Parker Creek and Parker Falls in the vicinity of Mary's Peak Campground on Mary's Peak in Benton County, Oregon.

MATERIALS AND METHODS

Ascaphus truei were collected in 1964 and 1965 in the late spring after the snow had melted off and there had been a few days of dry weather, and again in the fall before the rain and snow returned. When there was a rainy spell it was necessary to wait a few days for the ground to dry since rain allows the frogs to travel away from the stream. They were collected at night by wading in the stream near shore using a gasoline lantern to light the bank. Once in the strong light of the lantern the frogs seemed very reluctant to move which made their capture quite easy. In most cases they were found on damp rocks, moss, or earth within two to five feet of the stream. Occasionally one could be found in the stream nestled between some rocks or in a small pool either partially or entirely submerged. They were placed in a wide-mouth plastic jar with one-half to one inch of stream water until returned to the laboratory where they were placed in Mason jars with screen tops and one inch of water and held in a controlled temperature box at 4^{o}C. If the collection trip was longer than overnight the frogs and plastic jar were placed in a Coleman ice chest with ice to keep them cool since they are intolerant of warm daytime temperatures.

Although a number of Ascaphus were collected (male and female), only ten females were judged by external appearance to be

sufficiently gravid to merit study. These were injected over the two year period with an average of 6. 2 pituitaries each from adult female Rana pipiens in an attempt to obtain eggs.

Artificial fertilization was of no concern since the frogs mate either in the fall (Metter, 1964) or in the spring (Slater, 1931), sometime before the eggs are normally laid in May-July, and fertilization is internal.

The female Ascaphus, injected with Rana pipiens pituitaries in in Holtfreter's, was placed in a plastic holding tray (12-3/4" x 10" x 4") with approximately one inch of water and several handfuls of gravel covering the bottom of one end of the tray. She then deposited several strings of eggs, attaching them to the gravel. The eggs were separated from one another by one to two centimeters of gelatinous material. In this manner eggs were obtained, the number per female ranging from two to eighty-three.

The first eggs obtained were divided into groups of seven or eight and placed in fingerbowls containing 200 cc of 10 percent Holtfreter's solution. These fingerbowls were then placed in four different controlled temperature boxes held at $4^{o}C$, $8^{o}C$, $12^{o}C$, and $16^{o}C$ to ascertain the best temperature for the development of the embryos. By observing the mortality and growth rates of the four groups it was decided that the 12^{o} C temperature was best. There was a rapid mortality in the $4^{o}C$ group while their growth was slow.

In general mortality decreased and growth rate increased as the temperature was increased. Mortality figures for the 12°C and 16°C groups were about the same but the rate of development was more uniform for the 12°C group. subsequent groups of eggs and embryos were therefore placed in 10 percent Holtfreter's in a controlled temperature box at 12°C. This corresponds roughly to average stream temperatures at the times of collection which ranged from 9°C in late spring (near normal egg laying time) to 16°C in late summer and early fall by which time the embryos are hatched tadpoles.

In order to minimize shock to the animals due to extreme and rapid temperature changes while being studied, the fingerbowls were removed from the controlled temperature boxes, one at a time, only for the length of time needed to observe the embryos. Also, when changing the Holtfreter's in the fingerbowls, the fresh Holtfreter's was precooled to the temperature to which the embryos were acclimated.

As the embryos developed an attempt was made to stage them according to the staging table by Gosner (1960). The same stage names were employed where possible with notes and changes supplied when necessary.

RESULTS

Of the ten female Ascaphus injected with pituitaries only five responded sufficiently to lay any eggs. Collectively, these five produced a total of 178 eggs. The least number of eggs obtained from one frog was two, the most was eighty-three. These eggs were studied and treated in the manner described earlier in this paper and the results are expressed in the tables and descriptions that follow.

Test Number 1

An adult gravid female, collected at Frissell Crossing on July 3, 1964, was designated "64 T-1" and injected with a total of nine pituitaries spread out over several days in the following sequence:

Date	Number of pituitaries
7-23-64	2
7-24-64	2
7-25-64	2
7-26-64	1
7-28-64	1
7-29-64	1

No eggs were laid and the frog died on August 6, 1964.

Test Number 2

An adult gravid female, collected at Frissell Crossing on July 3, 1964, was designated "64 T-2" and injected with seven pituitaries in the following sequence:

Date	Number of pituitaries
7-23-64	2
7-24-64	2
7-25-64	2
7-26-64	1

No eggs were deposited. Frog died on August 15, 1964.

Test Number 3

An adult gravid female, collected at Frissell Crossing on July 3, 1964, was designated "64 T-3" and injected with four pituitaries in the following sequence:

Date	Number of pituitaries
7-23-64	1
7-24-64	1
7-25-64	1
7-26-64	1

No eggs were laid. Frog died on August 22, 1964.

Test Number 4

An adult gravid female, collected at Frissell Crossing on July 3, 1964, was designated "64 T-4" and injected with six pituitaries in the following sequence:

Date	Number of pituitaries
7-23-64	1
7-24-64	1
7-25-64	1
7-26-64	1
7-28-64	1
7-29-64	1

On August 6, 1964, the frog was noticed exhibiting a severe extrusion of a portion of the rectum beyond the anus. The frog died on August 8, 1964.

Test Number 5

An adult gravid female, collected at Frissell Corssing on July 3, 1964, was designated "64 T-5" and injected with six pituitaries in the following sequence:

Date	Number of pituitaries
7-30-64	3
7-31-64	1
8- 1-64	1
8- 2-64	1

The frog was placed in a screen-topped quart Mason jar in a controlled temperature box held at 12°C.

The morning of August 4, 1964, the frog was removed from the Mason jar and placed in a shallow plastic tray (12-3/4" x 10" x 4") containing pebbles and water. The water was about one inch deep and the pebbles were spread on one end of the tray up to the surface of the water. The entire ensemble was then placed in the 12°C box. That evening the female laid nine eggs. Within the next 36 hours she laid 74 more eggs for a total of 83 eggs. The eggs were entirely white, three millimeters in diameter, surrounded by several capsules and laid in a string. The outer capsular material was very sticky and adhered tightly to the pebbles. An attempt to

remove the string from the pebbles resulted in rupturing capsules of two of the eggs. The eggs were divided into groups, placed in fingerbowls of Holtfreter's solution, and then placed in controlled temperature boxes set at 4°C, 8°C, 12°C, and 16°C. Unfortunately, these temperatures varied considerably. The results of the development of these eggs are given in Table 1 in terms of hours required to reach certain stages, which are described later in the text.

Test Number 6

An adult female, showing the appearance of bearing some eggs, collected at Frissell Crossing on July 18, 1964, was designated "64 T-6" and injected with five pituitaries in the following sequence:

Date	Number of pituitaries
10-29-64	2
10-30-64	1
11- 1-64	1
11- 2-64	1

Twenty-nine eggs were laid sometime on the 8th of November, 1964. They were divided into three groups (9, 9, and 11), placed in fingerbowls of Holtfreter's solution, and held in the 12°C box. The eggs were quite a bit smaller than they would have been at the normal egg-laying time of May to July. No development followed the deposition of these eggs.

Table 1. Stages of development, in hours, for embryos obtained from test animal "64 T-5"

Stage No.	Time-in hours-at various temperatures			
	4°C (3.4°C - 7.7°C)	8°C (7.9°C - 9°C)	12°C (11.8°C - 19.2°C)	16°C (13.5°C - 16°C)
1	0	0	0	0
2	58	25-35		
3	70			
4	83			
5	Died			26
6				
7		61	44	
8				
9		124	102	98
10		207	128	
11		315	152	124
12			176	
13		627	209	172
14				
15		901	293	
16			317	
17		973	366	255
18				
19		1119		364
20				386
21		1404	654	

Test Number 7

An adult gravid female collected on April 23, 1965 at Parker Falls was designated "65 T-1" and injected with five pituitaries in the following sequence:

Date	Number of pituitaries
4-24-65	2
4-25-65	1
4-26-65	1
4-27-65	1

She was placed in a plastic tray with pebbles and water and held at 12°C. She laid two eggs and then died during the night of April 29th. The eggs did not develop.

Test Number 8

An adult gravid female collected on April 23, 1965, at Parker Falls was designated "65 T-2" and injected with nine pituitaries in the following sequence:

Date	Number of pituitaries
6- 2-65	2
6- 8-65	1
6-11-65	2
6-13-65	1
6-15-65	1
6-29-65	2

Twenty-three eggs were laid on the third of July, 1965, and thirteen more were produced later. They were divided up into six fingerbowls of Holtfreter's solution and placed in a 12° C. The

growth of these eggs, in terms of hours required to reach a certain stage of development, is given in Table 2.

Test Number 9

An adult gravid female captured in Hackleman Creek at Lost Prarie campground on July 4, 1965, was designated "65 T-3" and injected with eight pituitaries in the following sequence:

Date	Number of pituitaries
7-29-65	1
7-31-65	1
8- 2-65	1
8- 4-65	1
8- 7-65	2
8-10-65	2

Twenty-five eggs were laid on August 15, 1965. They were divided up into five groups and placed in Holtfreter's solution in a controlled temperature box at 12 °C. The results of the development of these eggs, in terms of hours required to attain a certain stage, are given in Table 3.

Test Number 10

An adult female, showing some signs of possessing eggs, collected at Parker Creek on November 6, 1965, was designated "65 T-4" and injected with three pituitaries in the following sequence:

Table 2. Stages of development, in hours, for embryos obtained from test animal "65 T-2"

Stage No.	Time-in hours-at 12°C (9°C-12.4°C)					
	A*	B*	C*	D*	E*	F*
1	0	0	0	0	0	0
2						
3		24		24		
4	24	43		43		
5			43			
6	43					
7						
8		72	72	89		48
9	113	89		113		111
10	137		137	137	137	135
11	163	278	161	163	161	163
12	194	299	253			183
13				209	213	
14	220	335	279	235	239	210
15						
16	246	361	331	259	265	236
17	388	480	450	379	409	365
18	458	573	543		574	457
19	554	669		568		
20		693	639		598	553
21	698		783	594	662-646	625-697

* A, B, C, D, E, and F represent fingerbowls into which the eggs were divided after being laid. They contained from 3 to 14 eggs each.

Table 3. Stages of development, in terms of hours, for embryos obtained from test animal "65 T-3"

Stage No.	Time-in hours-at 12°C (9°C-13°C)				
	A*	B*	C*	D*	E*
1	0	0	0	0	0
2					
3					
4					
5					
6					
7					
8	72	71	70	72	72
9					
10					
11					
12					
13					
14					
15			262-274		
16	264	263-281		266	264-282
17	282		284	280	
18					
19	474				
20	522	453	476	473	475
21					

* A, B, C, D, and E represent fingerbowls into which the embryos were divided. Each bowl contained five eggs.

Date	Number of pituitaries
11-10-65	1
11-12-65	1
11-13-65	1

No eggs were obtained. The animal was preserved on February 17, 1966.

An attempt was made to stage the development of the embryos according to the simplified table for anuran embryos by Gosner (1960). Where the development of Ascaphus embryos did not sufficiently fit Gosner's description, new descriptions had to be made and new stages determined. In some cases, a previously described stage was completely skipped by the Ascaphus embryos and had to be deleted from the account or replaced with a new stage. An account of the stages of pre-hatching development as finally determined for Ascaphus embryos is given on the following pages.

Description of Stages

Stage 1. Deposition (Plate I)

Stage 1 in Gosner's table is Fertilization. This can be accurately determined in other anurans in which fertilization is external; however, in Ascaphus, fertilization takes place internally before the egg is laid and the time of fertilization is not known. Rotation of the animal and vegetal poles is not visible due to the lack of pigment in the egg.

In view of these facts, Stage 1 should be called "Deposition, "

and should extend up to formation of two blastomeres. The newly laid egg is 3-4 mm in diameter, creamy-white, smooth, and surrounded by three capsular membranes: an outer, tough, sticky membrane which adheres to the substratum, a middle membrane widely separated from the egg, and an inner delicate membrane immediately surrounding the egg.

Stage 2. Two cells (Plate I)

Gosner's table indicates Stage 2 as being formation of a Gray Crescent opposite the point of sperm penetration on the egg and the expelling of the second polar body. This description is dependent upon the egg having differentially pigmented poles and some knowledge of the time of fertilization. Neither is known in Ascaphus, and the next easily observable change in the egg is the formation of two blastomeres. All early stages in Ascaphus are somewhat difficult to see due to the lack of pigment and the shallowness of the cleavage furrows.

Stage 3. Four Cells (Plate I)

In most cases the second cleavage appears at right angles to the first. The second cleavage furrow may be preceded by a "dimple" at each end.

Stage 4. Eight Cells (Plate I)

This stage is determined by counting the cells.

Stage 5. Sixteen Cells (Plate I)

Also determined by counting the cells.

Stage 6. Thirty-two Cells (Plate I)

The cells become smaller but are still distinct enough to be counted.

Stages 7 and 8. Mid- and Late Cleavage (Plate I)

These were distinguished by the size of the blastomeres, although with some difficulty, as in Gosner's account. However, Gosner makes an additional distinction in the Late Cleavage stage by a reduction in size of the light "hemisphere" caused by expansion of the darker area. As noted before, the Ascaphus egg possesses no pigment so that differentiation of light and dark areas is not possible.

Stage 9. Dorsal Lip (Plate I)

In this stage a small depression (dorsal lip) appears on the side of the egg where the involution of animal cells begins at the boundary between animal and yolk cells. This process is essentially the same

as described by Gosner except for the lack of pigment and the appearance of a translucent spot, 1.6-2 mm in diameter on the top of the egg. This spot is readily seen by illuminating the egg from the side and bottom.

Stage 10. Mid-gastrula (Plate I)

Involution of cells has completed one-half to three-quarters of a circle around the lower one-third of the egg. The translucent spot on the top of the egg is definite and glassy looking.

Stage 11. Late Gastrula (Plate I)

Complete encirclement of the yolk cells occurs, forming a "yolk plug." The translucent spot is, on some, as much as 2 mm in diameter. (Sectioning of two eggs in this stage showed that the translucent spot was due to a difference in the refraction potentials of light between this part and the rest of the egg, caused by the formation of a cavity under the dorsal surface of the egg.)

Stage 12. Neural Groove (Plate I)

In most anurans, this would be synonomous with the Neural Fold stage. However, in Ascaphus, there is a definite groove which appears in the surface of the egg prior to the formation of the neural folds. This groove or furrow traverses the translucent area

(which becomes white and cloudy) and possesses no elevated lateral ridges.

Stage 13. Neural Fold (Plate II)

Formation of the neural folds rapidly follows the neural groove. Two elevated lateral ridges are present, separated by the neural groove. There is no continuous raised ridge anteriorly crossing the embryo to join the anterior ends of the neural folds as indicated for other anurans in Gosner's sketch (1960). However, there is an anterior transverse ridge which is depressed in the middle at the neural groove. The head area is distinguishable as paired, widely separated areas of the neural folds.

Stage 14. Neural Tube (Plate II)

Characterized by the neural folds closing to form a tube. Closure of the folds appears to occur at the middle of the embryo first and then proceeds anteriorly and posteriorly at the same time. The head region is the last to close.

Stage 15. Rotation (Plate II)

In Gosner's tables, Rotation follows neural fold formation. In Ascaphus, it follows neural tube formation and the embryos rotate

counter-clockwise at an average rate of one revolution every three minutes.

Stage 16. Tail Bud (Plate II)

Characterized by enlargement of the tail and its extension from the surface of the egg. A pore is found on the ventral side of the tail at the point of junction between the tail and the yolk. The head becomes more swollen and has external lobes and ridges, with the eye placodes becoming visible. Somites have appeared along the back and the first indication of a tail fin occurs.

Stage 17. Muscular Response (Plate II)

In this stage the embryo exhibits periodic spontaneous flexures of its tail and body; the tail elongates and nasal pits become evident.

Stage 18. Heart Beat (Plate II)

Initial heartbeat is very weak but can be seen by rotating the embryo to view it anteriorly. The heart appears on the lower left side of the embryo just posterior to the attachment of the head to the yolk.

In other anurans and in Gosner's table the stage following Heart Beat is Gill Circulation, characterized by observing the flow of blood through the external gill filaments. In Ascaphus, however, there are

no external gill filaments formed so that this stage is not visible and therefore is eliminated.

Stage 19. Eye Pigmentation (Plate II)

This stage is represented by the cornea becoming transparent and the formation of dark pigment cells scattered in the iris of the eye. This represents the first pigment formation in the entire embryo.

Stage 20. Body Pigmentation (Plate II)

This stage is marked by the formation of pigment cells, although few and widely scattered, over the entire embryo. The pigment of the eye is noticeably heavier. Pigmentation seems to be a little heavier on the head.

Stage 21. Hatching

Activity of the embryo results in its rupturing the membranes and its emergence as a free-swimming larva. Pigmentation of the eye and body is much heavier, especially in the tail. Circulation of blood through the tail fin is noticeable.

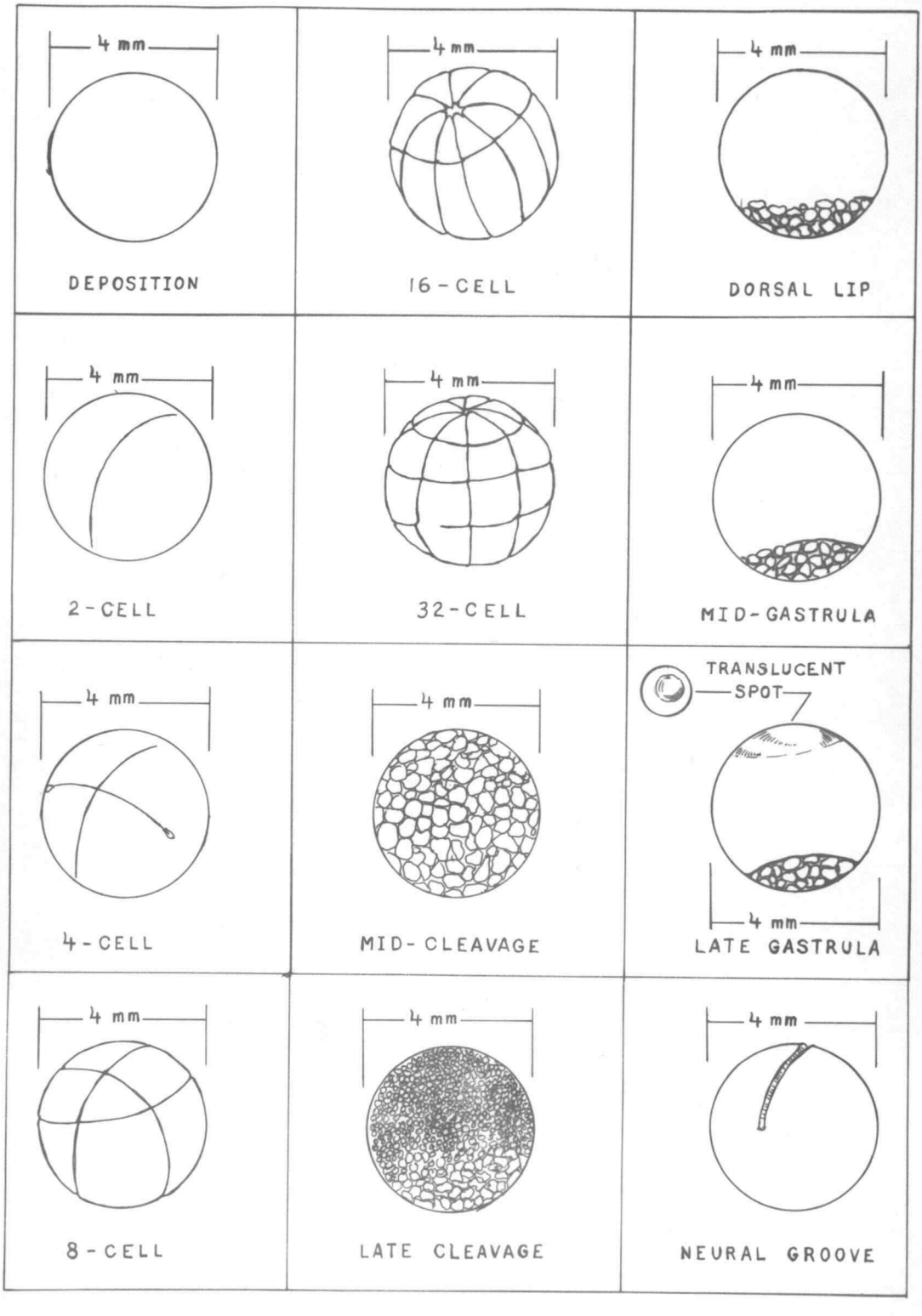


Plate I. Developmental stages 1 - 12

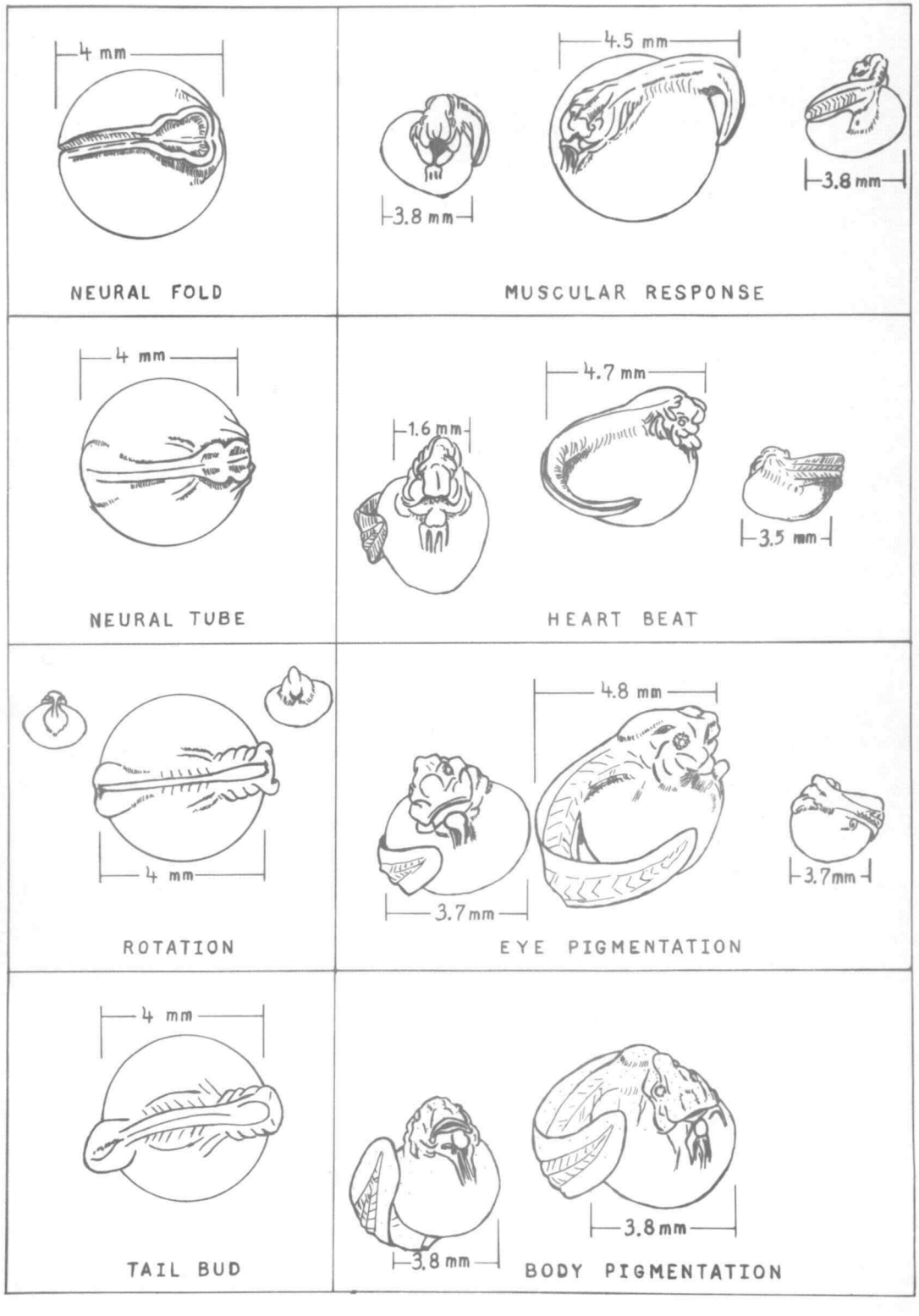
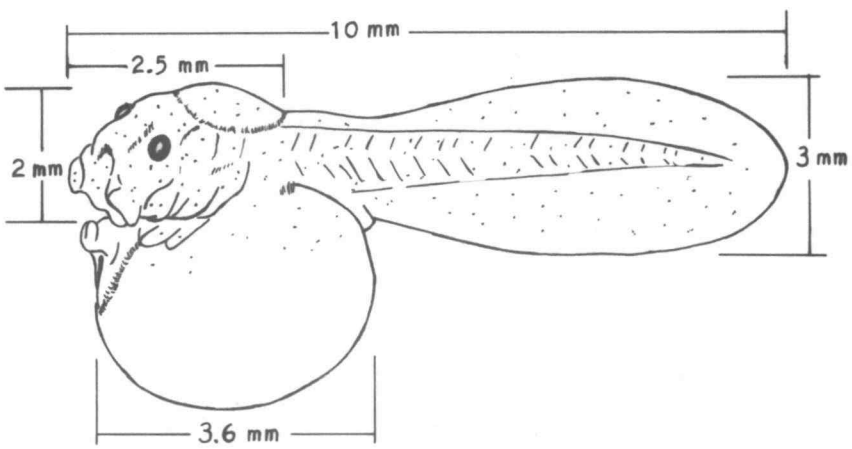


Plate II. Developmental stages 13 - 20



HATCHING

Plate III. Developmental stage 21 - Hatching

DISCUSSION

Although the embryology of numerous anurans has been studied and staging tables have been composed for many of them, there has previously been no study of larval Ascaphus. During the course of this study it was noticed that the stage names as utilized in other general tables applied fairly well to Ascaphus larvae, since the names are of a very basic nature. Even though these names are reasonably applicable there are definite morphological differences which need to be studied in further detail. Some of the obstacles and problems encountered are presently being attacked by the writer but many more remain to be solved.

Copulation of male and female Ascaphus in the laboratory has been observed during the spring (1966) and, although this does not solve the problem of the exact time of fertilization of the eggs, it does eliminate the necessity of the female retaining the sperm in a viable condition over winter, after a fall mating, as suggested by Metter (1964).

Of the five females which did not produce eggs, four (64 T-1, 64 T-2, 64 T-3, 64 T-4) died shortly after injection of the pituitaries, and one did not die and was preserved later. The four deaths could be attributed to several causes. In three of these the death could have been due to a too rapid build-up of hormones caused by the successive

injections of pituitaries; in the fourth, however, only four pituitaries were injected, and although they were injected on successive days, this dosage is not felt to be particularly damaging since the Rana pipiens being used as a source of pituitaries were small. Another factor could have been the container in which these animals were placed, which was a quart Mason jar with a screen top. As demonstrated by later successful animals, the females seem to prefer to lay their eggs on rocks, pebbles, or rough surfaces rather than in a small smooth jar. If the pituitary injections were sufficient to instigate deposition, but the stimuli in the environment were not, the resulting conflict could possibly have been fatal. A third possible factor could be that the females were not fertilized, and the subsequent injection of pituitaries resulted in a physiological upset.

The one female (64 T-5) which did not produce eggs and which did not die was collected in November, 1965, and showed only small eggs within her abdomen. In this case it was considered late for a gravid female to be laying eggs. The eggs she was carrying could have been the following year's, in which case they would not have been fertilized. On the other hand, it is possible that they were eggs that should have been deposited during the past spring. If this is the case, then why were they not laid? Is it possible for a non-fertilized female to retain her eggs for a whole year and lay them the next

spring or does she reabsorb them and not become gravid again until the second spring thereafter?

In two other cases (64 T-6, 65 T-1) the females laid eggs which did not develop. One of these females (65 T-1) died after laying only two eggs. This female was collected in the early spring and it is quite possible that she was not fertilized. In the other instance (64 T-6), however, the female was collected in July which gave her ample opportunity to have bred before she was captured, but it is still possible that she had not. This frog deposited twenty-nine eggs, none of which developed. Again the question arises as to what happens to the eggs of a female which has not mated.

The three remaining females did lay eggs which were fertile and which developed as normally as could be expected of embryos in an artificially manufactured laboratory environment.

Most anurans produce eggs which have a dark, almost black, "animal pole" where the animal cells are located, and a "vegetal pole" composed of light-colored yolk cells. Rotation of these eggs shortly after fertilization, which is external, brings the dark animal cells to the top of the egg and assures the observer that fertilization has occurred. The dark cells can then absorb some of the sun's rays and "incubate" the eggs. In addition, the cleavage furrows are rather deep and easily observed. In Ascaphus, however, there is no pigment in the egg, and this fact does not permit distinguishing between animal

and vegetal cells; the egg is fertilized internally so that rotation is not observable; cleavage furrows are very shallow and the inner membrane is smooth and very close to the egg. These factors make it difficult to observe the early divisions and are partially responsible for the lack of early observations as noticed in Tables 1, 2, and 3. This problem was partly solved by removing the capsular layers and illuminating the egg with a soft light from the side.

By the time Late Cleavage to Late Gastrula (Plate I) were reached, observations were easier due to the development of a translucent spot on the dorsal part of the egg and the dorsal lip of the blastopore on the ventral. As pointed out in the description of the stages the "spot" is due to a difference in the refraction of light through a cavity developing beneath the surface of the egg. Other anurans develop a similar cavity but its presence is masked by the dark pigmented cells above and it is not seen unless the egg is sectioned.

In other frogs there is developed a neural or medullary plate prior to the formation of the neural folds and neural groove. A structure resembling this was observed, in Ascaphus, only on two occasions which was not considered sufficient to merit marking this as a stage.

Development of a Neural Groove (Plate I) prior to Neural Fold (Plate II) formation is also slightly irregular as compared to other frogs. In other anurans the neural groove does not appear until the

neural folds are formed, and is generally considered merely as the area between the two elevated folds, not a depression into the surface of the egg. In Ascaphus, a depression forms in the surface of the egg before the two lateral neural folds appear. The neural folds (Plate II) themselves are different from most anuran embryos in that the anterior transverse neural fold, in Ascaphus, is divided into two halves while that of other frogs is complete.

Development of the neural tube proceeds in the same general manner as in other frogs except that it precedes Rotation stage (Plate II), in Ascaphus, whereas it follows rotation in others. Rotation is indicated by the embryo turning counterclockwise within its capsules, not necessarily by any great morphological change. This action apparently sets in soon after neural tube formation after which the embryo rapidly enters the next stage, which is Tail Bud (Plate II). It is this apparent rapid transition which caused the many blanks at Stage 15 (Rotation) in Tables 1, 2, and 3. More frequent observations, perhaps every six to ten hours instead of every eighteen to twenty-four, may help make a clearer distinction between the times that these stages occur.

The formation of the tail bud is not greatly different from that in other frogs except in the head region. The nasal pits are not evident and the head has a flattened anterior appearance to it with the beginning of a depression in the center which will eventually become

the sucker and mouth. (In other anurans, the head is rounded anteriorly with very little indication of mouth or sucker parts.) There is also a pore developed under the tail bud which eventually becomes the anus, and the first indication of a tail-fin appears.

Muscular Response (Plate II) is initiated by spasmodic contraction of the embryo, causing it to twitch. Since an action on the part of the embryo is the criterion responsible for denoting this stage, the stage name does not vary considerably from one anuran to another but other differences are evident. The eye placodes become visible and the anterior edge of the head, in side view, takes on a rounded appearance but still retains the sucker and mouth depression. In addition, there develops, below the mouth, a small "plateau" of tissue which is supported anteriorly by three ridges of tissue and posteriorly by being attached to the embryo and yolk. This is apparently peculiar to Ascaphus and, as far as this writer knows, is encountered in no other anuran. Its function is still unknown.

Heart Beat stage (Plate II) is characterized by the rhythmic contraction of the heart which is found on the left side of the embryo ventral to the head and posterior to the mouth area. Its action can best be seen by illuminating the embryo from the opposite side. The tail has grown considerably by this time. Ridges have been noticed, for some time now, in the area where gills and visceral arches would normally be expected in anurans. However, no external gills ever

develop in Ascaphus, so that externally visible Gill Circulation (Gosner, 1960) never occurs. On the other hand, dissection of one-year-old tadpoles has shown that they do possess internal gills, but there is no evidence of an external spiracle to allow passage of water over the gills. The exact time of development of these gills and how they function, if they do at all, are questions yet to be answered. Living, as these tadpoles do, in rapidly flowing streams with a high oxygen content it is possible that their integument may suffice for their respiratory needs so that they have no need of gills.

Stage 19, Eye Pigmentation (Plate II), corresponds to Gosner's Cornea Transparent stage but since this is the first appearance of pigment in the embryo, even though it occurs at the same time the cornea becomes transparent, it was felt that it should be recognized more fully.

The appearance of pigment in the body (Stage 20, Plate II) is a phenomenon which is quite noticeable in animals having pigmentless, or at least light-colored, eggs. Since Ascaphus does have a pigmentless egg the appearance of dark pigment cells is a noteworthy event. The dark cells seem to be slightly more concentrated in the head region than over the rest of the body. There are also two shelf-like structures developed, one on each ventro-lateral margin of the head where the embryo joins the yolk. The function of this shelf, as in the case of the "plateau," which is still present, is unknown.

Hatching (Plate III) finally occurs by strong repeated muscular movements of the embryo which result in the rupturing of its confining capsules and its release into its environment. At this point, the Ascaphus embryo still has a large yolk sac whereas other anuran embryos are relatively yolk free at hatching. Other anuran larvae also possess small paired ventral suckers just behind and to the side of the mouth region, which they use to hold themselves to vegetation and rocks while eating. "Tailed frog" larvae possess no such paired structures but have a large sucker (with a centrally located mouth) which does not really become functional until some hours after hatching. This would seem to create a problem for the larvae. How do they keep from being swept downstream until their sucker-mouth becomes functional? Also, what kind of food do the free-swimming tadpoles eat? In the laboratory their large yolk sac enables them to survive for quite some time without eating. Does this also serve them in nature until their suckers develop? The writer has never found newly hatched tadpoles in nature although older tadpoles can be readily found clinging to rocks in the swiftly running water. Attempted analysis of stomach contents of one- and two-year-old tadpoles in 1965 showed a few diatoms and a great deal of algal material that was too severely masticated to be identified readily. The most that can be said at this point is that their main food may be algae that forms a thin covering over the rocks and boulders in the streams and that the

larvae scrapes this material off the surface of the rocks as it inches its way along with its sucker.

This study just opens the door of natural studies of Ascaphus and is far from comprehensive. More embryos should be reared and observed. It would be a definite help if serial sections could be made of the embryos in their various stages of development. The eggs should be stained after deposition (presently being done) to provide an accurate check on the early development. Especially important would be a more accurate temperature regulating device on the controlled temperature boxes, and a study of the odd and unusual structures developed by the larvae.

SUMMARY AND CONCLUSIONS

Its endemic nature, uniqueness, and the lack of previous work on this frog were three major factors considered in the selection of a problem concerning Ascaphus truei. A general review of the literature and a study of the larvae were undertaken in the spring and summer of 1964 but the decision to narrow the study to the pre-hatching stages was not made at the beginning of the study. Not until this decision was made was the seasonal nature of the gravid females considered a real obstacle to the problem.

Adult "tailed frogs" were collected over a two year period (1964-1965) at four different locations in Oregon by wading, at night, along stream edges with a gasoline lantern. The gravid females collected were injected with pituitaries and an attempt was made to stage the eggs obtained according to the table by Gosner (1960). A summary of the females collected and the eggs laid is found in Table 4. Some of the eggs obtained never developed. Observance of mating frogs in the spring of 1966, during the writing of this paper, indicate the real possibility that at least two of the females tested (those that laid a total of 31 eggs which did not develop) probably had not been fertilized. Five other females which laid no eggs at all may have been in the same state. The remaining three females, whose eggs and embryos were successfully staged, laid a total of 147 eggs.

Table 4. Summary of animals tested and numbers of eggs laid

Animal No.	Number of pituitaries injected	Number of eggs produced	Development of eggs (+ or -)
64 T-1	9	0	-
64 T-2	7	0	-
64 T-3	4	0	-
64 T-4	6	0	-
64 T-5	6	83	+
64 T-6	5	29	-
65 T-1	5	2	-
65 T-2	9	38	+
65 T-3	8	25	+
65 T-4	3	0	-
Total eggs produced		178	

While observing the Ascaphus embryos it was found that internal fertilization of the frogs and the lack of pigment in the eggs necessitated the omitting of the first two of Gosner's stages (Rotation of the egg and Gray Crescent formation) which were replaced with one stage, Deposition. It was later noticed that some other of Gosner's stages also had to be eliminated and replaced by descriptions and stages that would fit the larval development of Ascaphus. A Neural Groove stage had to be inserted to accommodate formation of a neural groove prior to neural fold formation, and Neural Plate stage (Gosner, 1960) had to be deleted since it does not appear. Rotation and Tail Bud stages were reversed from Gosner's table, and his Gill Circulation stage was eliminated since external gills are not developed in these larvae. The cornea does become transparent in Ascaphus but this was replaced by Eye Pigmentation which occurs at the same time and marks the first formation of pigment in the embryo. This is followed by Body Pigmentation and Hatching. These descriptions mark the successful completion of staging the larvae of Ascaphus before they become free-swimming tadpoles. It is possible, however, that further, more detailed observations may make it necessary to further revise these stages.

Attempts to determine food habits of the tadpoles have, at this time, been relatively unsuccessful but future studies might prove more fruitful.

Much information was obtained from this study and new problems were revealed. Many more remain to be solved. Of particular interest might be more careful study of the development of unusual embryonic structures (especially the "plateau"), their function, and their ultimate fate.

BIBLIOGRAPHY

Brunson, Royal B. and Herold A. Demaree, Jr. 1951. The herpetology of the Mission Mountains, Montana. Copeia No. 4:306-308.

Carl, G. Clifford. 1949. Extension of known ranges of some amphibians in British Columbia. Herpetologica 5(6):139-140.

Carl, G. Clifford. 1955. Northward extension of the known range of the tailed frog, Ascaphus truei Stejneger. Herpetologica 11(3):202.

Carl, G. Clifford and Ian McTaggart Cowan. 1945. Notes on some frogs and toads of British Columbia. Copeia No. 1:52-53.

Donaldson, Lauren R. 1934. The occurrence of Ascaphus truei east of the continental divide. Copeia No. 4:184

Ferguson, Denzel E. 1954. An annotated list of the amphibians and reptiles of Union County, Oregon. Herpetologica 10(3):149-152.

Gosner, Kenneth L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16 (3):183-190.

Graf, William, Stanley G. Jewett and Kenneth L. Gordon. 1939. Records of amphibians and reptiles from Oregon. Copeia No. 2:101-104.

Linsdale, Jean M. 1933. Records of Ascaphus truei in Idaho. Copeia No. 4:223.

Metter, Dean E. 1964. A morphological and ecological comparison of two populations of the tailed frog, Ascaphus truei Stejneger. Copeia No. 1:181-195.

Noble, G. K. and P. G. Putnam. 1931. Observations on the life history of Ascaphus truei Stejneger. Copeia No. 3:97-101.

Ricker, William E. and E. B. S. Logier. 1935. Notes on the occurrence of the ribbed toad (Ascaphus truei Stejneger) in Canada. Copeia No. 1:46.

Ritland, Richard M. 1955. Studies on the post-cranial morphology of Ascaphus truei. I. Skeleton and spinal nerves. Journal of Morphology 97(1):119-178.

Ritland, Richard M. 1955. Studies on the post-cranial morphology of Ascaphus truei. II. Myology. Journal of Morphology 97(2):215-282.

Rodgers, Thomas L. and William L. Jellison. 1942. A collection of amphibians and reptiles from western Montana. Copeia No. 1:10-13.

Rugh, Roberts. 1948. Experimental embryology. A manual of techniques and procedures. Minneapolis, Burgess. 480 p.

Salt, George W. 1952. The belltoad, Ascaphus truei, in Mendocino County, California. Copeia No. 3:193-194.

Slater, James R. 1934. Notes on northwestern amphibians. Copeia No. 3:140-141.

Slater, James R. 1931. The mating of Ascaphus truei Stejneger. Copeia No. 2:62-63.

Slipp, John W. and G. Clifford Carl. 1943. Northward extensions of the range of Ascaphus. Copeia No. 2:127.

Smith, Hobart M. 1932. Ascaphus truei Stejneger in Montana. Copeia No. 2:100.

Stejneger, L. 1899. Description of a new genus and species of discoglossid toad from North America. Proceedings of the United States National Museum 21(1178):899-901.

Stephenson, N.G. 1951. Observations on the development of the amphicoelus frogs, Leiopelma and Ascaphus. Journal of the Linnaean Society 42:18-28.

Stephenson, N.G. and Elsie M. Stephenson. 1957. Field observations on the New Zealand frog, Leiopelma Fitzinger. Transactions of the Royal Society of New Zealand 84(4):867-882.

Svihla, Arthur and R. D. Svihla. 1933. Notes on Ascaphus truei in Kittitas County, Washington. Copeia No. 1:37-38.

Van Dijk, D.E. 1959. On the cloacal region of anura in particular of Ascaphus. Annals of the University of Stellenbosch 35, Sect. A, No. 4:169-249.

Van Dijk, D.E. 1955. The "tail" of Ascaphus: a historical resume and new histological-anatomical details. Annals of the University of Stellenbosch 31, Section A, No. 1:1-71.