Effects of Androgens and Estrogens on the Development Anurans

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Introduction

The Development of an Anuran

There are many different species of Anurans. Each species has a different time frame for development. However, all undergo the same basic pattern for developing from egg to froglet stage.

Males attract females by calling. Males then grasp females which stimulates the release of eggs. As eggs are released, males release their sperm coating the eggs. This process is referred to as external fertilization. The eggs, being delicate, will remain in an aqueous environment during development (Campbell 1990). There are several changes the egg cell undergoes before becoming a tadpole. The concern of this research, however, does not begin until the eggs develop into tadpoles. A detailed explanation, with photographs, of the development from the initial tadpole to the froglet stage can be found in Appendix B. The average development time from tadpole to froglet is approximately eighty-eight days (Taylor et. al 1946).

Gonadal Development

Gonads develop simultaneously from the endodermic cells. Linked germ cells flatten out and separate into two distinct strands. Gonads develop slowly for four to six weeks. The structure of the undifferentiated gonad consists of two or three germ cells. Germ cells mature with peritoneal cells

beginning to form, partially covering the sex element. The genital cavity begins to develop in the center of the gonads. The undifferentiated gonads have reached maximum development and differentiation begins (Swingle 1926).

The sex cord, forming the ovarian sacs, does not form the ovaries. Ovaries are formed by the formation of rifts in the sex cord). Rifts enlarge into a single cavity, the secondary genital cavity, and epithelium begins to condense into "egg nests". Egg nests eventually become ovaries (Swingle 1926).

The sex cord plays a major role in development of the testis. The sex cord undergoes several morphological changes forming various structures of the male reproductive system (Swingle 1926).

Metamorphosis

Anurans metamorphose from an aquatic animal, the juvenile tadpole, to a terrestrial animal, the adult frog or toad. This process is characterized by development of hind limbs, shrinking of the head, fore-limb development, and tail reabsorption. Behaviorally, among other things, anurans change from herbivores to carnivores, (Duellman 1986).

Metamorphosis is controlled by two thyroid hormones: thyroxine (T_4) and triiodothyronine (T_3), (Hill and Wyse 1989). Metamorphosis reaches maximum peak during a surge in concentrations of T_4 and T_3, (Gray and Janssens 1990). The effects of gonadal steroids on the ability to metamorphose is not certain. It is apparent, however, that "metamorphosing

tadpoles appear to have the capacity to synthesize and metabolize gonadal steroids from an early stage of development," (Gray and Janssens 1990).

Materials and Methods

Anurans were collected at the University of Notre Dame's Environmental Research Center (UNDERC). The type was *Bufo americanus* (The American Toad). The anurans collected were in the early tadpole stage (Stage 23). The anurans were divided into nineteen separate groups of 40 organisms each. They were placed in plastic dish pans labeled T1, T2, T3, T4, T5, T6, T7, C8, C9, C10, C11, C12, C13, C14, C15, E16, E17, E18, and E19. The groups with a prefix of 'T' were treated with Testosterone, a prefix of 'C' indicated Control, and a prefix of 'E' indicates a estrogen treated. The control groups numbered 8-11 were testosterone control and pans numbered 12-15 were estrogen control.

Each tank was filled with 3 L of water from the aquatic environment that the *B. americanus* originally came from. The water was monitored for algal growth and was changed for all the containers every three days. The organisms in the 'T' tanks were exposed to 1.5625mg Testosterone Propionate/300μL EtOH. The organisms in the 'E' tanks were treated with 12.5mg 17β Estradiol Benzoate/300μL EtOH. The organisms in the 'C' tanks were given 300μL EtOH. They were treated every third day for 21 days, yielding 7 treatments in all.

Before the treatments were administered, June 12, 1993, ten tadpoles from each tank were measured for snout to tail length and were staged using the standard set by Gosner (1960). Every week, thereafter, ten tadpoles were removed and measured for snout to tail length and staged. The collection dates were July 3, 1994 (1 week), July 10, 1994 (2 weeks), July 12, 1994 (Estrogen experimental and control ONLY) (2.5 weeks), and July 17, 1994 (Testosterone experimental and control ONLY) (3 weeks). The estrogen experiment and control experiments were terminated on July 12, 1994, because of cannibalism among the tadpoles in each tank.

The data was collected over a period of six weeks during June and July 1994. The data was compiled on the specified days, and statistical tests were run. One-way Analysis of Variance was run on the data within each tank to see if there were any significant differences among the animals within each separate tank. This type of test ensures that all the animals in each tank are the same and that there was no factor in a certain tank that caused a couple to grow faster or slower. Then the replicates were combined and a two-tailed T-test was run between the groups. This test was performed to test for differences between the control groups and the experimental groups of each treatment type.

Results

In the testosterone experiments, there was no significant difference within each group, for length, at any of the collection dates, except for the before treatment (Before Treat. on Table 1). This indicates that there were no internal factors within each tank that affected the development of the tadpoles. This also allows for the groups to be compared against each other with confidence that the difference in treatment versus control will be the primary factor involved if there is a difference between the experimental group and the control group. The significantly different tanks before treatment were tanks T3 and T4 and tanks T3 and T7.

The T-test was performed to see if there were any significant differences between the experimental group and the control group. The test results indicated that after 2 weeks. there was a significant difference between the sizes of the experimentals and the controls. For stages, however, there was a significant difference at every single collection date once the testosterone was administered. This indicates that the testosterone treated tadpoles developed at a faster rate than the control tadpoles.

The same statistical analysis was performed on the estrogen experimentals and controls. The only significant difference for the stage sizes within each tank was recorded before the treatment. The differences were among tanks E16 and E18 and tanks E18 and E19. However, the data, as a whole,

indicates that there is no external factor effecting the growth or development of the tadpoles within each tank.

The T-test showed that there was a significant difference between the experimental and control groups for every collection date after the introduction of the estrogen into the tanks. This indicates that the estrogen treated tadpoles developed at a faster rate than the control tadpoles.

Table 1: Mean Lengths and Standard Errors for Testosterone Treated Tadpoles

	Testosterone Experimental		Testosterone Control	
	Length (cm)	*Stage*	*Length (cm)*	*Stage*
Before Treat.	1.899 - 0.019	26.350 - 0.174	1.891 - 0.023	28.157 - 0.120
1 Week	2.071 - 0.020	28.364 - 0.160	2.104 - 0.008	32.550 - 0.118
2 Weeks	2.396 - 0.009	36.057 - 0.151	2.436 - 0.009	34.350 - 0.076
2.5 Weeks	N/A	N/A	N/A	N/A
3 Weeks	2.420 - 0.008	37.929 - 0.149	2.425 - 0.010	35.475 - 0.080

ANOVA RESULTS:
a) F=4.225; p=0.012; df=3
b) F=3.572; p=0.004; df=6

T-TEST RESULTS:
c) $p < 0.01$

Table 2: Mean Lengths and Standard Errors for Estrogen Treated Tadpoles

	Estrogen Experimental		Estrogen Control	
	Length (cm)	*Stage*	*Length (cm)*	*Stage*
Before Treat.	1.306 - 0.007	26.350 - 0.174	1.309 - 0.008	26.325 - 0.187
1 Weeks	1.608 - 0.014	26.075 - 0.110	1.451 - 0.008	27.450 - 0.080
2 Weeks	1.726 - 0.011	28.000 - 0.000	1.605 - 0.007	29.500 - 0.080
2.5 Weeks	1.773 - 0.006	28.000 - 0.000	1.648 - 0.009	30.500 - 0.080
3 Weeks	N/A	N/A	N/A	N/A

ANOVA RESULTS:
a) F=3.303; p=0.031; df=3

T-TEST RESULTS:
b) $p < 0.001$

Discussion

Effects on the Length

There is an increase in the lengths of treated versus control groups in the testosterone treated tadpoles. Goetz et. al showed that there was an increase in size of the Coho Salmon, *Oncorhynchus kisutch*, fry. (Goetz 1979) The experiments tested a wider range of steroid concentrations on the development of the salmon. Goetz found that when treated with high levels of estrogen there was an inhibition of the growth of the salmon fry. However, it was found that when the steroid treatment ceased the rate of growth for the salmon fry was greater than the control or the testosterone treated group, (Goetz 1979). Yamazaki (1976) reported that estrogen hindered growth, however, at low concentrations there was no affect. It was also found that testosterone treatments increased the growth of the salmon (Goetz 1979). The experiments were conducted at different concentrations, however, at concentration levels similar to the ones in this experiment there were no real differences between the experimentals and the control (Goetz 1979). This supports the results in this experiment that indicate that there is no notable difference between the controls and the experimentals for the testosterone treated groups.

Effects on the Stage

There were significant differences each time testosterone treated tadpoles were collected after the treatments were concluded. This has never previously been observed. However, it has been shown that the introduction of testosterone into *Rana catesbiana* tadpoles have caused the tadpole to completely differentiate it gonads to the testis, (Puckett 1940).

Previous work on stage development is almost unheard of. In this case there was a significant difference between the estrogen treated tadpoles and the control tadpoles in their progress towards development. The estrogen treated tadpoles arrested development at stage 28. This stage is at the point where metamorphosis begins to take shape. T_3 and T_4 are secreted by the thyroid. If T_3 productions was inhibited then the metamorphosis would not be allowed to continue. This supports the idea that 17β-estradiol (estrogen) inhibits T_3-metamorphosis. However, the estrogen did not inhibit metamorphosis to the same extent that the testosterone did, (Gray and Janssens 1990).

There is stronger evidence to support estrogen having a greater affect on the development of the tadpole. The testosterone treated tadpoles developed at an accelerated rate as compared to the control tadpoles. This evidence in a direct contradiction to the results of previous published work, (Frieden and Naile 1955). In their findings, Frieden and Naile (1955) found that testosterone inhibited metamorphosis in the *Bufo bufo japonicus* and *Rana temporaria*.

However, Norris et. al. (1973) found that there was no effect on metamorphosis by gonadal steroids.

Janssens and Gray reported on possible reasons for the blockage of metamorphosis by testosterone and estrogen. They reported on experiments performed on fish that showed that gonadal steroids prevented the release of thyroid hormones which controlled the changes in fish development, (Gray and Janssens 1990).

Conclusion

The data suggests that testosterone has no increasing effect on the growth rate of the tadpoles, but affects the development of the tadpole. The data from the estrogen experiments suggests that estrogen causes an increase in the growth rate of the tadpole, but arrests the internal development of the tadpole.

Further experiments are needed to determine the mechanisms that are involved in metamorphosis. It is important to look at the mechanisms involved in the affects of the steroids on the changes in the tadpoles.

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Appendix A

	Before Treatment Stages								6/12		
	1	2	3	4	5	6	7	8	9	10	Avg.
T1	1.85	1.70	1.60	1.80	1.90	2.05	2.10	1.95	1.90	2.05	1.89
T2	1.80	1.95	1.80	1.95	2.00	2.10	1.80	1.70	2.00	1.90	1.90
T3	1.65	1.80	2.10	2.20	1.95	1.85	2.35	2.25	2.10	2.20	2.05
T4	1.60	1.80	1.95	1.80	1.70	1.80	1.80	1.90	2.05	1.85	1.83
T5	1.70	1.85	1.80	1.85	1.80	1.90	1.90	1.90	2.10	2.00	1.88
T6	1.80	1.80	2.00	1.80	1.95	2.15	1.95	2.00	2.00	2.20	1.97
T7	1.90	1.80	1.70	1.80	1.70	1.80	1.80	1.75	1.80	1.85	1.79
C8	1.80	2.05	1.95	2.30	1.80	2.20	1.90	1.80	2.25	2.10	2.02
C9	1.85	1.70	1.80	1.90	2.00	1.75	1.70	1.90	1.90	1.80	1.83
C10	1.90	2.00	1.70	1.95	1.85	1.90	1.85	2.00	1.70	1.90	1.88
C11	1.70	1.80	1.90	1.80	1.70	1.85	1.90	1.85	2.00	1.95	1.85
C12	1.25	1.35	1.40	1.25	1.30	1.30	1.35	1.30	1.25	1.30	1.31
C13	1.30	1.30	1.30	1.35	1.25	1.35	1.25	1.30	1.40	1.25	1.31
C14	1.25	1.25	1.35	1.30	1.30	1.40	1.35	1.25	1.30	1.40	1.32
C15	1.35	1.25	1.30	1.40	1.35	1.25	1.30	1.35	1.30	1.25	1.31
E16	1.30	1.35	1.30	1.35	1.30	1.25	1.35	1.30	1.35	1.35	1.32
E17	1.25	1.25	1.35	1.30	1.35	1.25	1.25	1.35	1.30	1.35	1.30
E18	1.35	1.25	1.30	1.30	1.35	1.30	1.25	1.25	1.35	1.30	1.30
E19	1.35	1.35	1.25	1.25	1.35	1.30	1.30	1.35	1.30	1.25	1.31

	Before Treatment Stages								6/12		
	1	2	3	4	5	6	7	8	9	10	Avg.
T1	28	29	30	27	28	27	29	28	30	28	28
T2	28	30	28	28	29	27	28	27	29	27	28
T3	30	27	28	27	29	28	29	28	27	27	28
T4	29	30	28	28	27	30	27	28	28	30	29
T5	27	27	28	28	30	28	28	30	28	27	28
T6	28	28	28	28	27	28	30	27	29	30	28
T7	28	28	27	27	28	28	28	28	28	27	28
C8	30	28	30	28	29	30	28	30	27	30	29
C9	28	30	28	29	28	30	28	27	28	28	28
C10	28	29	27	28	27	28	28	28	30	27	28
C11	27	28	30	27	30	28	27	29	28	27	28
C12	25	25	26	25	26	26	28	27	27	25	26
C13	27	28	25	25	26	27	26	26	28	28	27
C14	25	27	27	26	25	25	26	25	27	25	26
C15	27	25	25	28	28	27	28	28	25	28	27
E16	25	26	26	26	26	26	28	26	26	26	26
E17	26	28	26	28	25	25	27	28	26	28	27
E18	26	25	28	26	27	26	25	25	27	26	26
E19	25	28	28	26	28	25	26	26	28	25	27

After Treatment Stages 7/3

	1	2	3	4	5	6	7	8	9	10	Avg.
T1	2.15	2.05	2.15	2.10	2.20	2.00	2.25	1.95	1.95	1.90	2.07
T2	2.15	2.25	2.20	2.00	2.20	2.20	2.00	2.10	2.15	2.00	2.13
T3	1.90	2.10	1.90	2.15	1.95	2.10	2.15	1.95	2.10	2.20	2.05
T4	2.15	2.00	2.10	2.20	2.15	2.00	1.90	2.05	1.95	2.05	2.06
T5	2.05	2.25	1.90	2.05	1.90	2.20	2.25	2.05	2.20	2.15	2.10
T6	2.20	2.05	2.15	2.20	2.10	2.05	1.95	2.25	2.10	2.20	2.13
T7	1.90	2.20	2.10	1.95	2.25	2.15	2.15	1.90	2.15	1.95	2.07
C8	2.05	2.10	2.05	2.15	2.05	2.15	2.10	2.05	2.15	2.10	2.10
C9	2.10	2.05	2.10	2.05	2.10	2.05	2.20	2.15	2.15	2.05	2.10
C10	2.10	2.05	2.05	2.20	2.20	2.15	2.05	2.05	2.05	2.15	2.11
C11	2.05	2.15	2.15	2.15	2.05	2.05	2.20	2.15	2.10	2.10	2.12
C12	1.40	1.55	1.50	1.40	1.45	1.55	1.50	1.40	1.45	1.40	1.46
C13	1.45	1.50	1.45	1.40	1.45	1.50	1.40	1.45	1.50	1.40	1.45
C14	1.50	1.40	1.45	1.40	1.50	1.40	1.50	1.40	1.40	1.40	1.44
C15	1.40	1.50	1.45	1.50	1.40	1.50	1.45	1.40	1.50	1.50	1.46
E16	1.50	1.55	1.55	1.50	1.55	1.60	1.50	1.70	1.60	1.70	1.58
E17	1.60	1.50	1.50	1.70	1.75	1.55	1.65	1.55	1.75	1.50	1.61
E18	1.50	1.55	1.70	1.55	1.60	1.50	1.70	1.50	1.75	1.70	1.61
E19	1.75	1.70	1.50	1.70	1.55	1.75	1.60	1.70	1.60	1.60	1.65

After Treatment Stages 7/3

	1	2	3	4	5	6	7	8	9	10	Avg.
T1	32	32	33	35	33	34	32	35	33	33	33
T2	34	34	32	35	35	33	34	40	34	32	34
T3	33	31	33	35	32	33	31	33	32	35	33
T4	34	34	42	33	31	34	32	35	34	33	34
T5	32	32	34	31	33	33	34	33	31	35	33
T6	33	35	33	35	32	31	35	32	35	32	33
T7	34	31	35	32	33	35	33	34	33	32	33
C8	33	33	33	33	33	33	33	32	33	33	33
C9	32	31	33	32	33	33	31	33	33	31	32
C10	33	33	33	33	32	33	33	31	33	33	33
C11	31	31	33	33	33	32	33	33	32	33	32
C12	27	28	27	28	27	28	27	27	28	27	27
C13	28	27	28	27	27	28	28	28	27	28	28
C14	27	27	27	27	28	27	27	27	28	27	27
C15	28	28	27	28	27	28	27	28	27	28	28
E16	27	26	25	26	26	26	27	26	27	26	26
E17	25	27	26	27	27	25	26	26	25	27	26
E18	26	25	26	25	26	26	26	27	26	26	26
E19	27	26	27	26	25	27	26	26	25	26	26

	After Treatment Stages								7/10		
	1	2	3	4	5	6	7	8	9	10	Avg.
T1	2.30	2.35	2.45	2.35	2.40	2.30	2.35	2.35	2.45	2.40	2.37
T2	2.40	2.45	2.40	2.45	2.30	2.40	2.30	2.45	2.35	2.30	2.38
T3	2.30	2.35	2.45	2.30	2.35	2.40	2.35	2.30	2.50	2.35	2.37
T4	2.35	2.50	2.20	2.35	2.30	2.45	2.40	2.50	2.45	2.30	2.38
T5	2.50	2.30	2.40	2.45	2.35	2.60	2.50	2.35	2.50	2.30	2.43
T6	2.50	2.50	2.30	2.35	2.50	2.50	2.35	2.40	2.45	2.50	2.44
T7	2.45	2.40	2.45	2.35	2.40	2.40	2.45	2.30	2.45	2.40	2.41
C8	2.45	2.40	2.35	2.45	2.50	2.45	2.50	2.40	2.35	2.45	2.43
C9	2.40	2.50	2.35	2.40	2.50	2.45	2.40	2.45	2.35	2.50	2.43
C10	2.45	2.50	2.45	2.50	2.35	2.50	2.50	2.35	2.50	2.35	2.45
C11	2.35	2.50	2.40	2.45	2.50	2.40	2.50	2.50	2.40	2.45	2.45
C12	1.55	1.60	1.55	1.60	1.65	1.55	1.60	1.60	1.55	1.60	1.59
C13	1.60	1.65	1.65	1.65	1.60	1.65	1.55	1.65	1.65	1.60	1.63
C14	1.65	1.65	1.60	1.60	1.55	1.65	1.60	1.60	1.55	1.65	1.61
C15	1.55	1.65	1.65	1.55	1.65	1.60	1.60	1.65	1.65	1.60	1.62
E16	1.70	1.65	1.80	1.75	1.70	1.65	1.75	1.80	1.70	1.65	1.72
E17	1.65	1.75	1.70	1.65	1.75	1.70	1.80	1.75	1.85	1.75	1.74
E18	1.75	1.80	1.80	1.80	1.70	1.85	1.65	1.60	1.70	1.80	1.75
E19	1.65	1.75	1.75	1.65	1.75	1.70	1.60	1.80	1.60	1.85	1.71

	After Treatment Stages								7/10		
	1	2	3	4	5	6	7	8	9	10	Avg.
T1	35	36	35	35	34	37	36	38	36	37	36
T2	34	37	38	36	35	36	36	35	34	35	36
T3	37	34	36	34	37	37	36	38	37	37	36
T4	35	37	36	38	36	35	34	37	36	35	36
T5	36	38	35	37	37	36	37	36	37	38	37
T6	38	36	38	34	36	37	36	35	37	36	36
T7	34	38	35	36	35	34	38	37	36	34	36
C8	34	35	34	34	34	35	35	34	34	35	34
C9	35	34	34	34	35	34	34	35	35	34	34
C10	34	35	35	34	34	34	34	34	34	34	34
C11	35	34	34	35	34	35	34	34	34	35	34
C12	30	30	29	30	30	29	30	29	30	29	30
C13	29	29	30	29	29	30	29	30	29	30	29
C14	30	29	30	29	30	29	30	30	29	29	30
C15	29	30	29	30	29	30	29	29	30	30	30
E16	28	28	28	28	28	28	28	28	28	28	28
E17	28	28	28	28	28	28	28	28	28	28	28
E18	28	28	28	28	28	28	28	28	28	28	28
E19	28	28	28	28	28	28	28	28	28	28	28

	After Treatment Stages								7/12		
	1	2	3	4	5	6	7	8	9	10	Avg.
T1	-	-	-	-	-	-	-	-	-	-	0.00
T2	-	-	-	-	-	-	-	-	-	-	0.00
T3	-	-	-	-	-	-	-	-	-	-	0.00
T4	-	-	-	-	-	-	-	-	-	-	0.00
T5	-	-	-	-	-	-	-	-	-	-	0.00
T6	-	-	-	-	-	-	-	-	-	-	0.00
T7	-	-	-	-	-	-	-	-	-	-	0.00
C8	-	-	-	-	-	-	-	-	-	-	0.00
C9	-	-	-	-	-	-	-	-	-	-	0.00
C10	-	-	-	-	-	-	-	-	-	-	0.00
C11	-	-	-	-	-	-	-	-	-	-	0.00
C12	1.60	1.70	1.65	1.75	1.60	1.60	1.70	1.70	1.65	1.70	1.67
C13	1.70	1.60	1.65	1.70	1.65	1.55	1.70	1.60	1.70	1.60	1.65
C14	1.60	1.65	1.70	1.65	1.60	1.70	1.60	1.70	1.55	1.70	1.65
C15	1.55	1.70	1.60	1.65	1.65	1.70	1.55	1.65	1.70	1.60	1.64
E16	1.75	1.80	1.75	1.80	1.75	1.80	1.80	1.80	1.70	1.80	1.78
E17	1.80	1.80	1.80	1.70	1.80	1.70	1.80	1.80	1.80	1.70	1.77
E18	1.70	1.80	1.80	1.75	1.80	1.75	1.80	1.75	1.80	1.80	1.78
E19	1.80	1.80	1.75	1.70	1.75	1.75	1.80	1.80	1.80	1.75	1.77

	After Treatment Stages								7/12		
	1	2	3	4	5	6	7	8	9	10	Avg.
T1	-	-	-	-	-	-	-	-	-	-	0
T2	-	-	-	-	-	-	-	-	-	-	0
T3	-	-	-	-	-	-	-	-	-	-	0
T4	-	-	-	-	-	-	-	-	-	-	0
T5	-	-	-	-	-	-	-	-	-	-	0
T6	-	-	-	-	-	-	-	-	-	-	0
T7	-	-	-	-	-	-	-	-	-	-	0
C8	-	-	-	-	-	-	-	-	-	-	0
C9	-	-	-	-	-	-	-	-	-	-	0
C10	-	-	-	-	-	-	-	-	-	-	0
C11	-	-	-	-	-	-	-	-	-	-	0
C12	30	31	30	31	30	30	31	30	30	30	30
C13	31	30	31	31	31	31	30	31	30	31	31
C14	30	31	30	30	31	30	31	30	31	30	30
C15	31	31	30	31	30	31	30	31	30	31	31
E16	28	28	28	28	28	28	28	28	28	28	28
E17	28	28	28	28	28	28	28	28	28	28	28
E18	28	28	28	28	28	28	28	28	28	28	28
E19	28	28	28	28	28	28	28	28	28	28	28

	After Treatment Stages								7/17		
	1	2	3	4	5	6	7	8	9	10	Avg.
T1	2.30	2.45	2.50	2.40	2.50	2.30	2.35	2.35	2.45	2.40	2.40
T2	2.40	2.45	2.40	2.45	2.30	2.40	2.45	2.50	2.40	2.50	2.43
T3	2.45	2.50	2.40	2.50	2.35	2.40	2.35	2.30	2.50	2.35	2.41
T4	2.35	2.50	2.50	2.35	2.40	2.50	2.45	2.40	2.45	2.30	2.42
T5	2.50	2.30	2.45	2.50	2.40	2.50	2.50	2.35	2.50	2.30	2.43
T6	2.50	2.50	2.30	2.35	2.50	2.50	2.35	2.40	2.45	2.50	2.44
T7	2.45	2.40	2.45	2.35	2.30	2.35	2.35	2.45	2.40	2.50	2.40
C8	2.45	2.40	2.35	2.45	2.50	2.45	2.50	2.40	2.35	2.45	2.43
C9	2.30	2.35	2.35	2.45	2.40	2.30	2.35	2.35	2.45	2.50	2.38
C10	2.45	2.50	2.45	2.50	2.35	2.50	2.50	2.35	2.50	2.35	2.45
C11	2.35	2.50	2.40	2.45	2.50	2.40	2.50	2.50	2.40	2.45	2.45
C12	-	-	-	-	-	-	-	-	-	-	0.00
C13	-	-	-	-	-	-	-	-	-	-	0.00
C14	-	-	-	-	-	-	-	-	-	-	0.00
C15	-	-	-	-	-	-	-	-	-	-	0.00
E16	-	-	-	-	-	-	-	-	-	-	0.00
E17	-	-	-	-	-	-	-	-	-	-	0.00
E18	-	-	-	-	-	-	-	-	-	-	0.00
E19	-	-	-	-	-	-	-	-	-	-	0.00

	After Treatment Stages								7/17		
	1	2	3	4	5	6	7	8	9	10	Avg.
T1	38	39	38	39	40	39	38	39	40	38	39
T2	39	40	38	39	38	39	37	38	39	40	39
T3	39	38	37	38	37	38	37	39	37	36	38
T4	39	38	37	37	39	37	38	36	40	37	38
T5	36	37	39	37	36	39	37	38	40	40	38
T6	37	36	39	36	37	39	37	36	39	37	37
T7	36	39	37	36	39	37	38	39	36	37	37
C8	35	35	35	35	36	35	36	35	36	36	35
C9	36	35	35	36	36	36	35	36	35	36	36
C10	35	35	36	36	35	35	36	35	36	35	35
C11	35	36	36	35	36	35	36	35	36	35	36
C12	-	-	-	-	-	-	-	-	-	-	0
C13	-	-	-	-	-	-	-	-	-	-	0
C14	-	-	-	-	-	-	-	-	-	-	0
C15	-	-	-	-	-	-	-	-	-	-	0
E16	-	-	-	-	-	-	-	-	-	-	0
E17	-	-	-	-	-	-	-	-	-	-	0
E18	-	-	-	-	-	-	-	-	-	-	0
E19	-	-	-	-	-	-	-	-	-	-	0

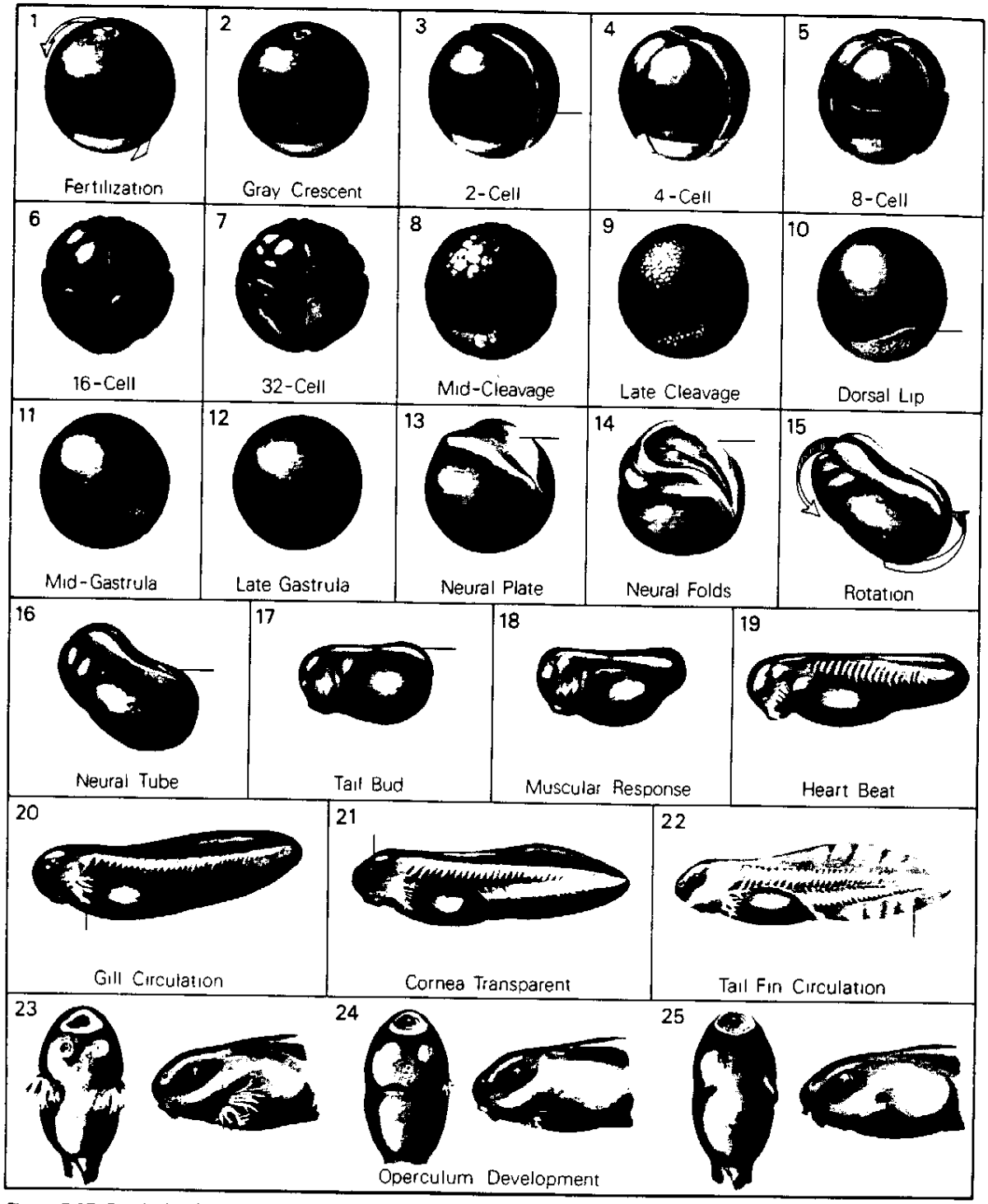

Figure 5-17. Standard early stages of development of anurans. Stages are according to Gosner (1960). Guidelines indicate major features mentioned in text.

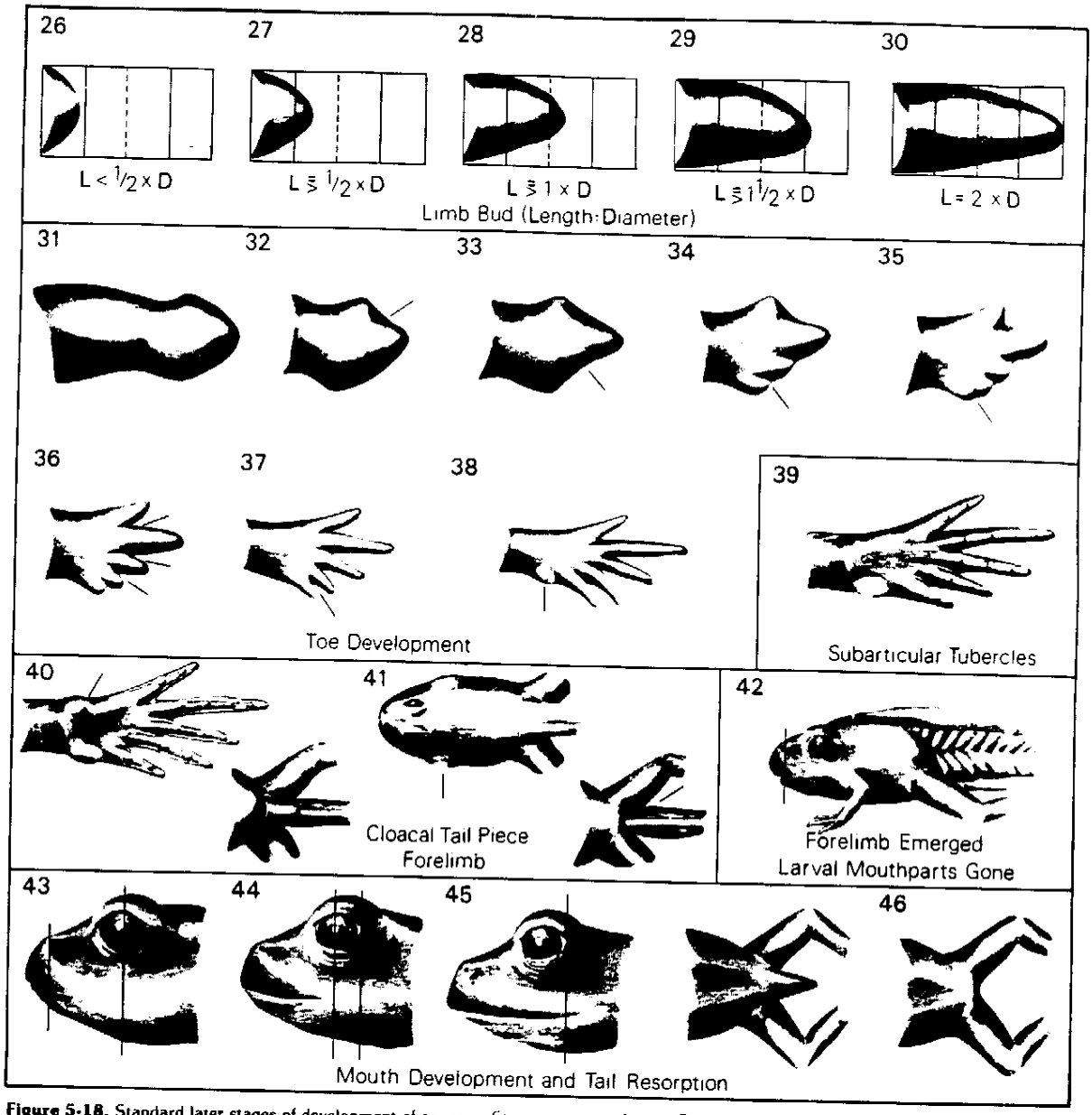

Figure 5-18. Standard later stages of development of anurans. Stages are according to Gosner (1960). Pigmentation is not shown. Guidelines indicate major features mentioned in text.