THESIS

THYROID MORPHOLOGY AND LIFE HISTORY IN UTAH TIGER SALAMANDERS

Submitted by

Debra A. Naylor

In partial fulfillment of the requirements for the
Thesis in Zoology
Weber State University
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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY DEBRA A. NAYLOR ENTITLED "THYROID MORPHOLOGY AND LIFE HISTORY IN UTAH TIGER SALAMANDERS" BE ACCEPTED AS FULFILLING PARTIAL REQUIREMENTS FOR THE THESIS IS ZOOLOGY.

Thesis Committee

Advisor

Department Chair

ABSTRACT

Some Utah Tiger Salamanders (*Ambystoma tigrinum nebulosum*) exhibit cannibalistic behavior in the field and laboratory and may display cannibal morphology as described in the literature. These animals appear refractory to metamorphosis compared to non-cannibals. I have begun a study to investigate the potential role of the thyroid gland in this phenomenon in animals from a series of high-elevation NE Utah populations. Specimens originally were fixed in the field with buffered formalin for analysis of stomach contents. Morphometric measurements were made with a caliper; thyroid glands and surrounding tissue were dissected and processed for paraffin histology. In sectioned material using an ocular micrometer, thyroid maximum length and width were measured and volume was calculated. Diameters of largest and smallest follicles were determined, follicle numbers were counted, and full and empty follicles noted. Larvae greater than 40 mm snout-vent length were qualitatively classified as cannibals or non-cannibals and were compared statistically with each other and with the entire sample. Stomach contents were removed and analyzed.

From the statistical analysis, it appears impossible to distinguish the cannibal morph from the typical animals. Results suggest the cannibals may exhibit smaller maximum follicle diameters and smaller thyroid volumes than the typical morphs, although these relationships were not significant. I speculate that the cannibal morphs may indeed have less active thyroids as caused by increased release of CRH from the hypothalamus in response to high density and stress from the environment and that this may be causing refractory metamorphosis. I also review some of the literature which suggests the cannibalistic morph may be the result of an initial cannibalistic event.

INTRODUCTION

The clouded tiger salamander, *Ambystoma tigrinum nebulosum*, inhabits temporary and permanent ponds throughout northern Utah. During their aquatic larval stage, these animals possess a tail fin, external gills, vomerine teeth and other morphological characteristics typical of

amphibians (Bogert, 1954). Their diet consists mainly of aquatic invertebrates and invertebrate eggs.

Tiger salamanders undergo metamorphosis to become terrestrial adults; during the process of transformation, the tail fin is resorbed, external gills are lost, lungs develop, the head is restructured, and skin structure is altered (Gilbert, 1994). As adults, their diet is primarily terrestrial invertebrates (Bogert, 1954). *A. t. nebulosum* larvae exhibit three distinct morphologies (Pierce et al., 1983): a large morph, a small morph, and a cannibalistic morph which appears to be refractory to metamorphosis (Stebbins, 1954; Holomuzki and Collins, 1987) and may even overwinter in ponds before transforming into the adult form. Tiger salamanders are also known to be capable of neoteny, reaching sexual maturity and breeding in the larval morphology, in cold, high-elevation ponds (Duellman and Trueb, 1986).

The primary mechanism driving metamorphosis appears to be reaching a critical minimum size (Werner, 1986). However, food availability, pond stability, temperature, iodine concentration, density of conspecifics (VanBuskirk and Smith, 1991; Crump, 1992), and photoperiod (Norris, 1997) also appear to play a role in larval transformation.

Classically, the thyroid gland has been recognized as crucial to amphibian metamorphosis (Duellman and Trueb, 1986). Several studies have underscored the importance of thyroid hormones (Wright et al., 1994). Investigators (Gilbert, 1994) demonstrated that facultative neotenes of *A. tigrinum* and the permanently neotenic species *A. mexicanum* can be made to metamorphose when supplied with exogenous thyroid hormone. Interactions among thyroid hormone and several other hormones have recently been shown to be important as well (Norris, 1997). The salamander thyroid gland is a highly vascular, follicular, paired structure

(Gorbman and Bern, 1964). It is located near the hyoid cartilages just deep to the supracoracoid muscle on either side. The cyst-like follicles are lined with secretory epithelial cells which synthesize thyroglobulin, a protein coupled to tyrosine residues which is secreted into the follicle lumen (Robbins, 1989). During this process, iodine binds to the tyrosine residues on thyroglobulin, producing the thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4). Storage as colloid in the follicle continues until secretion of hormone is stimulated. At this point, re-uptake of thyroglobulin by the follicular epithelial cells occurs. Thyroglobulin is broken down and thyroid hormones are released from the follicular cells into the bloodstream. In the target cells, T_3 is believed to be the active hormone: T_4 is often converted to T_3 in the target cells and T_3 binds more actively to membrane receptor sites (Duellman and Trueb, 1986).

Amphibian metamorphosis is characterized by three general stages. Premetamorphosis is the initial stage, during which growth is favored and thyroid hormone is low; the median eminence of the hypothalamus is not fully developed and high levels of prolactin (PRL) are secreted by the pituitary gland (Duellman and Trueb, 1986). Prolactin antagonizes upregulation of thyroid receptors, and negative feedback of thyroid hormone on pituitary secretion of thyroid stimulating hormone (TSH) is operating. During the prometamorphic stage, the median eminence matures and vascular connections between the hypothalamus and the adenohypophysis (anterior pituitary) are elaborated. The hypothalamus produces thyrotropin-releasing hormone (TRH) which signals the pituitary to secrete TSH, in turn causing the thyroid to release its hormones, T_3 and T_4. A positive feedback system is established and increasing levels of thyroid hormone are secreted in these animals until metamorphic climax takes place. Recent research has indicated that corticotropin-releasing factor/hormone (CRH) may cause release of TSH as well as corticotropin

(ACTH) from the anterior pituitary (Norris, 1997). Other hormonal and environmental interactions, which may include secretions from the pineal gland, are also taking place and influence the metamorphic process. During the final stage, or metamorphic climax, target cells within the external gills, tail fin, etc. undergo rapid changes in response to hormonal cues and the animal develops into an adult.

Cannibalistic *A. t. nebulosum* always possess larval morphology and have many distinctive characteristics that include larger size relative to a typical morph, relatively longer, wider, more U-shaped heads (Powers, 1907), large mouths and slit-like eyes (Pierce et al., 1983). They also have a wider interocular distance and larger dentary and vomerine teeth (Walls et al., 1993). Collins (1981) showed that the cannibal morphology is not exhibited by small larvae and is only encountered in animals greater than 40 mm snout-vent length (SVL), the size at which head differentiation in the cannibal becomes obvious. Lanoo and Bachman (1984) found all larvae greater than 50 mm SVL in their sample to be cannibals, as evidenced by presence of conspecifics in 50% of the animals' stomachs, by anecdotal evidence, and by morphological characters. Pierce et al. (1981) suggested there may be a genetic basis for the phenomenon, however, other studies (Collins and Cheek, 1983; VanBuskirk and Smith, 1991) have indicated that high density may be the cause.

I began an investigation of the role of the thyroid in development of cannibal morphology, specifically by examining the relationship between thyroid histological characteristics and morphometric measurements on animals of varying age and external appearance. I hypothesized that histological differences between the thyroid glands of cannibals and typical morphs could be found to be related to the cannibalistic life history pattern.

MATERIALS AND METHODS

I used 36 animals, 4 adults and 32 larvae, collected from four north-eastern Utah ponds. Bohman's Pond is the largest and is found on Durst mountain, near Morgan, Utah. Though it varies dramatically in size, Bohman's Pond usually retains water throughout the summer. Red Wells, Blind Spring, and Doe Pond all are found along Curtis Creek Road just east of the crest of Monte Cristo near the Utah-Wyoming border. They are approximately equal in size and much smaller than Bohman's Pond. Doe Pond is the smallest and usually dries up by mid-August; although the water levels drop significantly, Red Wells and Blind Spring usually retain some water throughout the summer. Sampling for the Bohman's Pond animals was conducted 7/12/90, the Doe Pond animals were collected 8/1/96, and the Blind Spring and Red Wells animals were collected 9/21/96.

The animals used in this study were originally collected for analysis of stomach contents; the animals were preserved in the field in buffered formalin. The following morphometric variables were measured using a hand-held caliper: snout-vent length (SVL); maximum head width (HW); interocular distance (IOD); tail length (TL); front-limb length (FLL); hind-limb length (HLL); between legs length, measured across the vent (BLL); head length (HL); head depth (HD); maximum fin height in larvae, maximum tail height in adults (FH). The area of the roof of the mouth occupied by vomerine teeth (VOMT) was also measured. Stomach contents were removed and analyzed.

A tissue block containing the paired thyroid glands was dissected from the animals, processed, and embedded in Paraplast (see Appendix A.) Each tissue sample was sectioned at 10 um using a rotary microtome and sections were mounted on microscope slides for staining with

Brookes' trichrome stain (Brookes, 1967; see Appendix B.)

Maximum thyroid length and width were measured using an ocular micrometer. Thyroid volume was calculated using two methods: as an ellipse, rotated around the long axis, and as a rectangular box. The elliptical measurement tended to underestimate the true volume, while the rectangular measurement tended to overestimate the value. Diameters of the largest and smallest follicles were measured, and the number of follicles in each section was counted. Appearance of colloid in follicles was also noted.

Statistical analysis using pairwise comparisons among data variables was carried out using the Pearson rank correlation coefficient method on SPSS-X. An analysis of variance among ponds and multiple regression analyses between morphometric variables and those variables reflective of thyroid activity were also performed.

Larvae greater than 40 mm SVL were qualitatively classified as either cannibal or typical morphs (via visual discrimination) and statistical comparisons were made between the cannibals and non-cannibals and with the group as a whole.

RESULTS

Of the 36 animals collected, 4 were from unknown localities and were not included in the statistical analysis; these included 2 adults, 1 typical larva and 1 cannibal.

Animals ranged from 29 mm to 54 mm SVL. Cannibals were larger on average than their typical counterparts in the larger size range (see Table 1), however, these differences were not significant. HW, IOD, VOMT, and HL were larger in the cannibals as well, although these differences diminished somewhat when values were size-adjusted (i.e., HW/SVL, IOD/SVL,

VT/SVL.) Thyroid volumes were between 0.0252 and 0.5742 mm^3; among the larger animals the range was 0.0688 and 0.5742 mm^3. The elliptical calculation did not differ significantly from the rectangular calculation and so the elliptical measurement was used. Thyroid volume (THYV), maximum follicle size (FOLMAX), and follicle numbers (FOLNUM) were greater on average among the cannibals than the larger typical larvae and among the group as a whole.

Regressions of SVL on HW, IOD, VOMT, and HL yielded a roughly linear relationship (see Fig. 1), which was significant. Follicles in any particular thyroid generally appeared to be either all empty or all full, although some follicles which might appear empty in one slide would exhibit some colloidal material a few sections later. Maximum follicle diameter varied between 0.0555 and 0.3885 mm. Minimum follicle diameter (FOLMIN) was approximately 0.0333 mm for all thyroids.

Morphometric variables were highly, significantly correlated with SVL for all groups (see Table 2), with the exception of VOMT teeth in the cannibalistic morphs. For the group as a whole, thyroid variables correlated positively with morphometrics and were statistically significant; these included VOMT with THYV, vomerine teeth to snout-vent length (VT/SVL) with THYV, VT/SVL with FOLMAX, HW with THYV, HW with FOLMAX, and IOD with FOLMAX. When qualitatively separated, correlation coefficients for thyroid variables with morphometrics in the cannibals and typical larvae greater than 40 mm SVL were no longer significant. There appeared a slight trend among the cannibal morphs toward lower values in correlation coefficients among the size-adjusted variables compared with thyroid variables. Regressions corresponding to these comparisons were performed (Fig. 2.)

Follicle number (FOLNUM) was extremely variable (range = 4 to 75 follicles per section)

and represents the maximum number of follicles that could be counted in any of the sections. In those thyroids with very few follicles (n=4), the follicles could be followed throughout the gland. However, in glands with greater follicle numbers, total number per gland could not be assessed. FOLNUM did not correlate well with any of the morphometric variables.

Analysis of variance indicated animals in all ponds were similar (see Table 3.) Appearance of colloid in follicles did separate by pond, however. Animals from Bohman's Pond (n=4) and Doe Pond (n=7) had only empty follicles. Blind Spring (n=9) had one animal with empty follicles and the remainder with full follicles. The Red Wells animals (n=10) all had full follicles. This did not correlate with any of the other variables, however. A variety of invertebrates and eggs were removed from the animals' stomachs. Not a single conspecific was found in the stomach cavities, despite field observations of several cannibalistic events.

DISCUSSION

From the statistical analysis, it appears impossible to distinguish the cannibal form from the typical morph; regressions of head morphometrics with SVL were not biphasic, but linear. When animals are separated qualitatively, the statistical analyses of each morphological type are very different. As noted in the literature (Powers, 1907; Rose and Armentrout, 1976; Pierce et al., 1983; Lanoo and Bachman, 1984; Walls et al., 1993), the cannibals displayed relatively wider heads, greater IOD, and larger vomerine teeth. Powers (1907) and Pierce et al. (1983) found that head size tended to increase less as body size increased; this appears to be reflected in my data and may help explain why it was not possible statistically to distinguish cannibals from the rest of

the group. Several of the animals in my sample displayed a transitional form (reported also in Powers (1907)) between the cannibal and typical morphology, exhibiting slightly larger heads than other animals of similar size. This continuous head enlargement among animals may also make it difficult to distinguish the different morphologies using the statistical analysis of measurements. The morphometrics for the cannibals and larger typicals are similar to other research (Pierce et al., 1983; Walls et al., 1993). However, because there are not two discreet classes of animals in my sample, qualitative assessment of cannibals versus noncannibals introduces ambiguity and possible bias.

Pierce et al. (1983) showed that regressions between head shape measurements and snout-vent length were flatter, with greater intercepts, for cannibalistic morphs than for typical morphs. This relationship is echoed in my data. For the group as a whole, as VOMT were relatively larger, THYV and FOLMAX showed corresponding increases. Similar relationships existed between IOD and HW with the thyroid variables; relatively wider heads and IOD were indicative of relatively larger thyroid morphometrics. This may simply reflect a larger thyroid in larger animals. However, in the cannibal thyroids, a trend towards flatter regressions and/or a lower correlation coefficient may indicate relatively smaller thyroid volumes with relatively smaller follicles than those found in the typical morphs. This group had a small sample size (n=6) however, and the correlations were not statistically significant. If such a relationship were to exist, it could be an indication of a less active thyroid; this would help to explain the reports of retarded metamorphosis in the cannibal morph. Several authors have reported changes in thyroid morphology which accompany transformation (Uhlenhuth and Schwartzbach, 1927; Etkin, 1968; Prahlad, 1968) and increased thyroid activity (Wright et al., 1994); these changes include

increased follicle cell proliferation, larger follicle lumen diameters and greater follicle cell height. Although I did not measure cell height or proliferation, my data implies lumen diameters (FOLMAX) may increase with increasing thyroid activity. Norman et al. (1987) found thyroid histology to be of little or no value in predicting thyroid activity, however, and instead recommended radioimmunoassay (RIA) of thyroid hormone levels in the bloodstream.

Analysis of variance indicates there are no significant differences in values of thyroid or vomerine teeth measurements for populations among different ponds. The separation of full and empty follicles according to pond is interesting and I am unsure of its significance. It is of special note, however, that ponds where field observations indicate a high incidence of aggressive interactions if not outright cannibalism, are the source of animals with empty follicles. There is anecdotal evidence to suggest that animals in these ponds are in fact, cannibals. For instance, when one Bohman's Pond sample was collected, it was noted that a large animal tried to eat a smaller one while in the collection bag filled with buffered formalin. Unfortunately, the conspecific was removed from the cannibal's buccal cavity and neither animals' identity was recorded. The Doe Pond animals (n=6) display a large amount of mutilation: 2 animals have broken tails, 1 animal is missing its left hind limb, and 1 animal is missing part of its gills on the right side. The most likely cause of these mutilations is other salamanders as there are no other predators seen in the pond that would be capable of such acts. The empty follicles in this group may indicate less thyroid activity, corresponding with the delayed metamorphosis typical of cannibals. The hypothalamic hormone, CRH, the primary vertebrate stress hormone (Denver, 1997) may be increased in aggressive animals such as those seen in this group and is believed to be involved in metamorphic transformation. Degroot (1989) showed that cortisol secretion may

play a role in controlling the thyroid, particularly in mediating environmental cues. Wright et al. (1994) also found that pituitary and adrenocortical hormones can antagonize metamorphosis in tadpoles at a specific stage. These researchers showed corticoids were inhibitory to transformation due to thyroid inhibition during the pre- and prometamorphic stages, as measured by hindlimb growth in *Rana pipiens*. In late prometamorphosis and during metamorphic climax, however, the corticoids enhanced hindlimb growth and thereby metamorphic progression. Denver (1997) found similar results in his studies of *Scaphiopus hammondii*. He also reported corticosteroids increased during late prometamorphosis and metamorphic climax and demonstrated that CRH could accelerate the rate of metamorphic change at these stages.

It is possible that the retarded transformation documented in cannibals (Rose and Armentrout, 1976; Holomuzki and Collins, 1987) may be explained, not by the thyroid, but by the pituitary gland. Recent research (Norris, 1997; Denver, 1997) indicates CRH may be the major stimulator of the thyroid axis, while TRH may release PRL. These effects are stage-specific and have a complex relationship to concentrations of thyroid receptors, thyroid hormone binding proteins, and hormone-degrading enzymes. CRH, produced in response to various environmental stimuli, is likely to be released when high densities of conspecifics occur in a pond. Density is highly correlated with occurrence of cannibalism in *A. t. tigrinum* (Crump, 1983; Lanoo and Bachman, 1984; VanBuskirk and Smith, 1991). In addition, the presence of thyroid-hormone-degrading monodeiodinase enzymes is also stage- and tissue-specific in fishes and endotherms (Denver, 1997) and is influenced by environmental effects such as photoperiod, environmental temperature and diet. In amphibians, type III deiodinase operates during premetamorphosis and lowers the concentration of T_3, slowing transformation. Type II deiodinase is active in

prometamorphosis and metamorphic climax and deiodinates T_4 to raise the concentration of T_3, thereby accelerating metamorphosis. CRH is believed to function via alteration of thyroid receptor expression or via induction of the deiodinase genes. Such interactions are likely to play a role in inducing the cannibal morph and its subsequent refractory metamorphosis.

My failure to find any salamander larvae in the stomachs of any of the animals was surprising. Rose and Armentrout (1976) found conspecifics in stomachs from all of the cannibalistic morphs they studied, while Collins and Holomuzki (1983) calculated that 84% of a cannibal morph's diet consists of other salamanders. The absence of conspecifics from the stomachs of the animals I used may be explained by the fact that they represent, for the Monte Cristo ponds, a single collection date, made late in the season when animals may be consuming less and when the ponds are generally less crowded due to metamorphosis or consumption of other larvae.

One of the possible reasons for inconclusive results in this study may be related to the transitional nature of some of the animals. Powers (1907) noted head expansion occurring 24 hrs. after a cannibalistic event. Lanoo et al. (1989), Collins and Holomuzki (1983), and Lanoo and Bachman (1984) found characters intermediate between cannibal and typical morphs, particularly in individuals between 35 and 50 mm SVL. They speculated that these animals may develop into cannibals and that cannibalism may trigger growth and induce the development of the cannibalistic morphology. Occasional cannibalism by "typical" morphs is well documented (Powers, 1907; Holomuzki, 1986; Holomuzki and Collins, 1987). It has also been shown (Walls et al., 1993) that ingestion of a critical food source in *Scaphiopus multiplicatus* leads to developmental polyphenisms and to subsequent cannibalistic behavior. Research on other species (Crump, 1992)

has shown ingestion of conspecifics early in development to trigger accelerated growth in *Lepidobatrachus laevis* and *L. llanensis* tadpoles. Research on *A. tigrinum* (Lanoo et al., 1989) documented accelerated growth following a single cannibalistic event. Their study showed early metamorphosis in such animals, however, and speculated that such animals were an occurrence of a new morphology, previously uncharacterized in the literature. Such conflicting reports of retarded metamorphosis (see also Crump, 1992) are confusing and as yet unexplained. It seems reasonable, in light of the literature, that many of the intermediate forms may be on their way to acquiring the cannibal morphology; this may explain the linear nature of the regressions and suggests there may be a window of time during which a cannibalistic event may trigger development of the cannibal morphology via maximum digestive efficiency or higher levels of hormone following ingestion of a conspecific. More research is needed on the populations I studied, however, to determine whether in fact, transitional forms are developing into cannibals and whether this is triggered by ingestion of other salamanders. The length of the larval period is largely a result of genetics which, in part, controls the rate at which an individual grows and reaches critical minimum size for transformation. Cannibalism is likely a polygenic trait (Collins and Cheek, 1983); genetically-programmed individuals exceeding the threshold for developmental polyphenism may become cannibalistic in response to environmental triggers mediated by varying levels of hormones such as CRH and thyroid hormone, both of which act by altering gene expression. More research is needed on these subjects to discover exactly what mechanisms might lead to the cannibalistic morphology, to explain the variable observations on refractory metamorphosis, and to understand the relationship of the phenomenon of cannibalism to environmental factors.

TABLE 1

MEAN MORPHOMETRIC AND THYROID MEASUREMENTS IN TIGER SALAMANDERS

	cannibals			large typicals					all animals		
	mean	std dev	n	mean	std dev	n	t	p	mean	std dev	n
SVL	48.33	3.615	6	40.82	12.53	11	-1.41	0.179	41.71	7.752	35
HW	15.17	1.602	6	13.55	1.695	11	-1.92	0.074	12.57	2.279	35
HW/SVL	0.314	0.021	6	0.300	0.025	11	-1.12	0.278	0.303	0.027	35
IOD	10.83	0.753	6	10.00	1.095	11	-1.65	0.119	9.371	1.352	35
IOD/ SVL	0.224	0.007	6	0.222	0.014	11	-0.42	0.683	0.227	0.020	35
VOMT	9.67	1.506	6	6.455	3.415	11	-1.55	0.141	6.152	3.073	33
VT/SVL	0.201	0.034	6	0.161	0.057	11	-1.53	0.147	0.143	0.052	33
HL	16.50	2.345	6	15.09	1.868	11	-1.36	0.194	14.00	2.733	35
THYV	0.351	0.193	6	0.198	0.109	10	-2.05	0.060	0.192	0.153	34
FOLMAX	0.240	0.093	6	0.178	0.058	10	-1.66	0.119	0.169	0.083	34
FOLNUM	43.17	9.988	6	34.89	13.26	9	-1.30	0.217	32.73	17.47	30

Cannibals and large typicals all greater than 40 mm SVL.
SVL = snout-vent length in mm; HW = head width at its widest point in mm; IOD = inter-ocular distance in mm; VOMT = area of palate occupied by vomerine teeth in mm^2; VT/SVL = vomerine teeth/snout-vent length; HL = head length in mm; THYV = thyroid volume in mm^3; FOLMAX = diameter of largest follicle per thyroid in mm; FOLNUM = maximum number of follicles per section.

TABLE 2

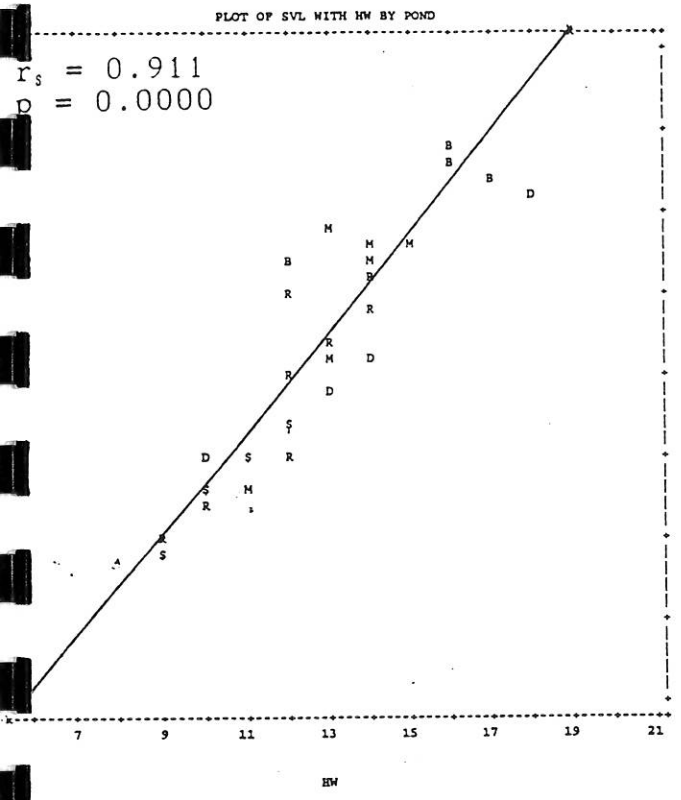
CORRELATION COEFFICIENTS IN TIGER SALAMANDERS

	CANNIBALS			LARGE TYPICALS			ALL ANIMALS		
	corr	n	sig	corr	n	sig	corr	n	sig
SVL with HW	0.7483	6	0.087	0.7565	11	0.007	0.9110	32	0.000
SVL with IOD	0.9065	6	0.013	0.8264	11	0.002	0.9287	32	0.000
SVL with VOMT	0.0980	6	0.853	0.8161	10	0.004	0.8588	30	0.000
SVL with HL	0.8493	6	0.032	0.8092	11	0.003	0.7855	32	0.000
VOMT with THYV	-0.0364	6	0.945	0.1950	10	0.589	0.4581	30	0.011
VOMT with FOLMAX	0.1806	6	0.732	0.3942	10	0.260	0.5286	30	0.003
VT/SVL with THYV	-0.0527	6	0.921	0.2239	10	0.534	0.4528	30	0.012
VT/SVL with FOLMAX	0.2690	6	0.606	0.4267	10	0.219	0.5435	30	0.002
HW with THYV	0.3372	6	0.513	0.0046	10	0.990	0.4703	31	0.008
HW with FOLMAX	-0.2946	6	0.571	0.2769	10	0.439	0.4401	31	0.013
HW/SVL with THYV	0.4420	6	0.380	-0.1145	10	0.753	0.0023	31	0.990
HW/SVL with FOLMAX	-0.2423	6	0.644	0.2469	10	0.492	-0.0870	31	0.641
IOD with THYV	0.1327	6	0.802	0.2364	10	0.511	0.5097	31	0.003
IOD with FOLMAX	0.0213	6	0.968	0.3701	10	0.293	0.5454	31	0.002
IODSVL with THYV	0.1035	6	0.845	0.3675	10	0.296	-0.1047	31	0.575
IODSVL with FOLMAX	0.4280	6	0.397	0.5173	10	0.126	-0.1097	31	0.557

corr = correlation coefficient; n = number of animals per sample; sig = statistical significance; SVL = snout-vent length in mm; IOD = inter-ocular distance in mm; VOMT = area of palate occupied by vomerine teeth in mm²; HL = head length in mm; THYV = thyroid volume in mm³; FOLMAX = diameter of largest follicle per thyroid in mm; VT/SVL = vomerine teeth/snout-vent length; HW = head width at its widest point; IODSVL = inter-ocular distance/snout-vent length.

TABLE 3		
ANALYSIS OF VARIANCE		
	F	P
VOMT by POND	1.5667	0.2213
FOLMAX by POND	0.3077	0.8196
FOLNUM by POND	0.7745	0.5184
THYV by POND	0.4421	0.7248
VT/SVL by POND	1.2239	0.3202

VOMT = vomerine teeth; FOLMAX = maximum follicle size; FOLNUM = maximum number of follicles per section; THYV = thyroid volume; VT/SVL = vomerine teeth/snout-vent length.



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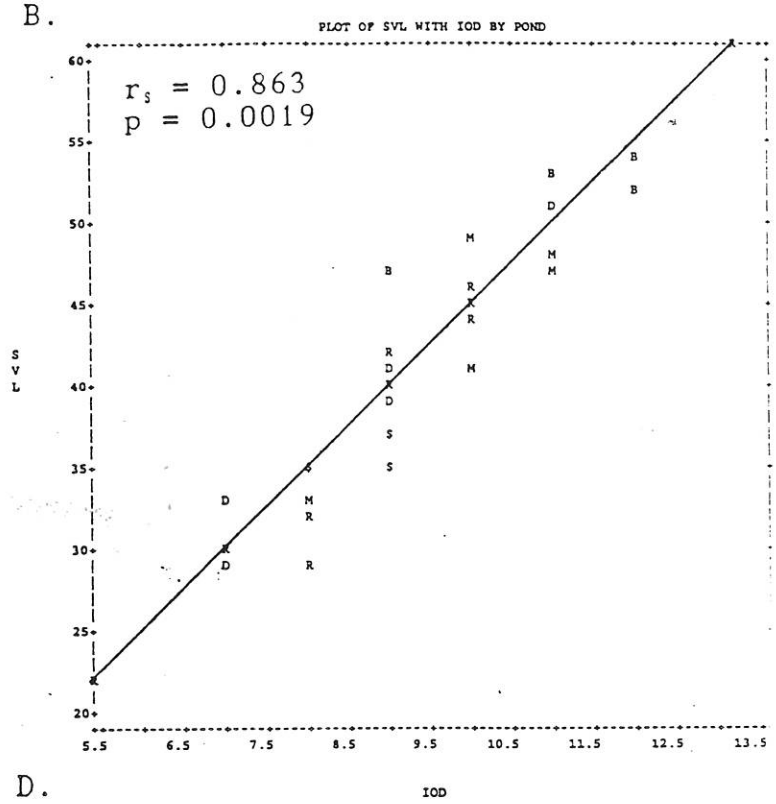

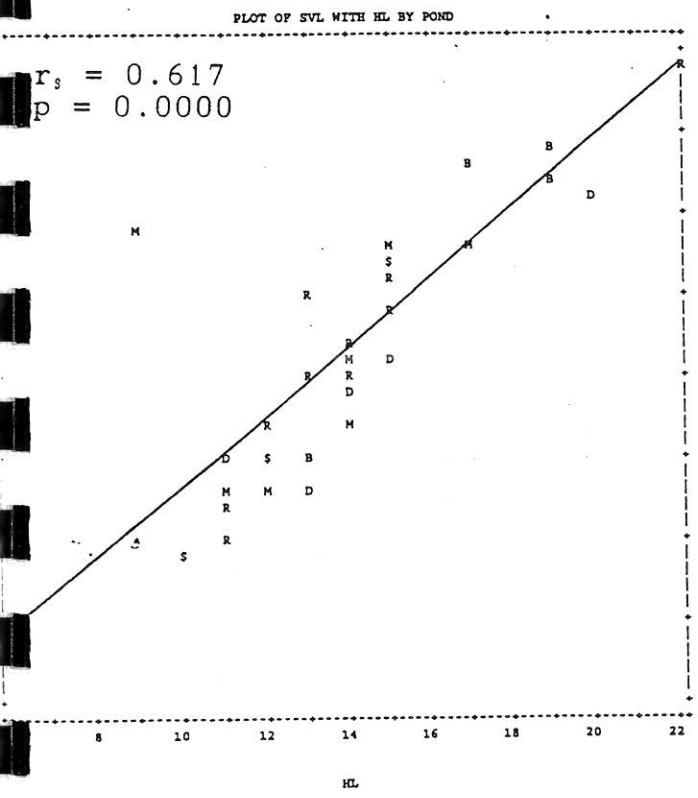

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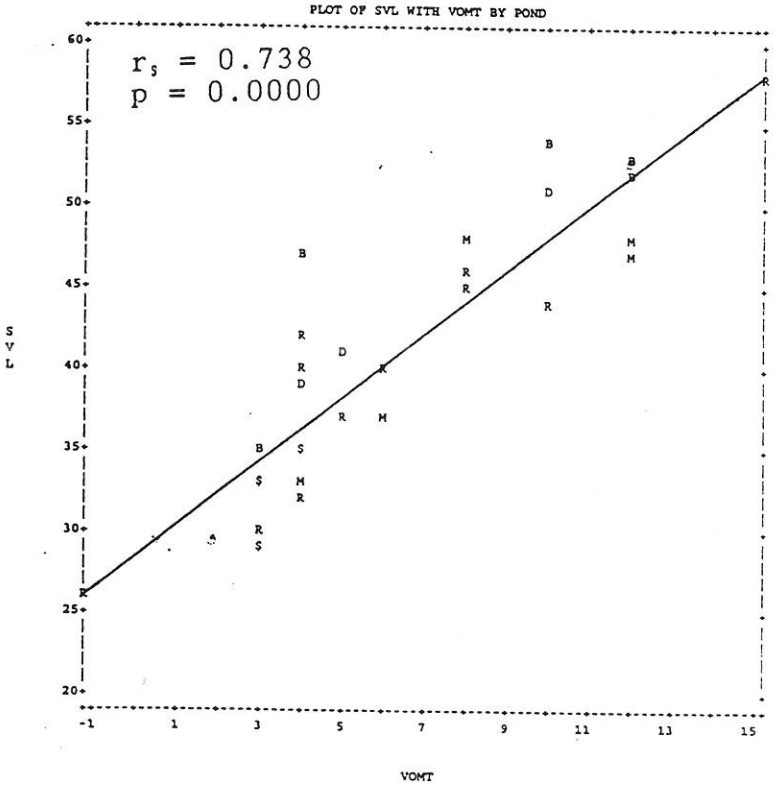

Figure 1. Regressions of SVL with head morphometrics in all animals.
A. SVL with HW.
B. SVL with IOD.
C. SVL with HL.
D. SVL with VOMT.
N = no location; R = Red Wells; B = Bohman's Pond; M = Blind Spring; D = Doe Pond; $ = multiple occurrence.

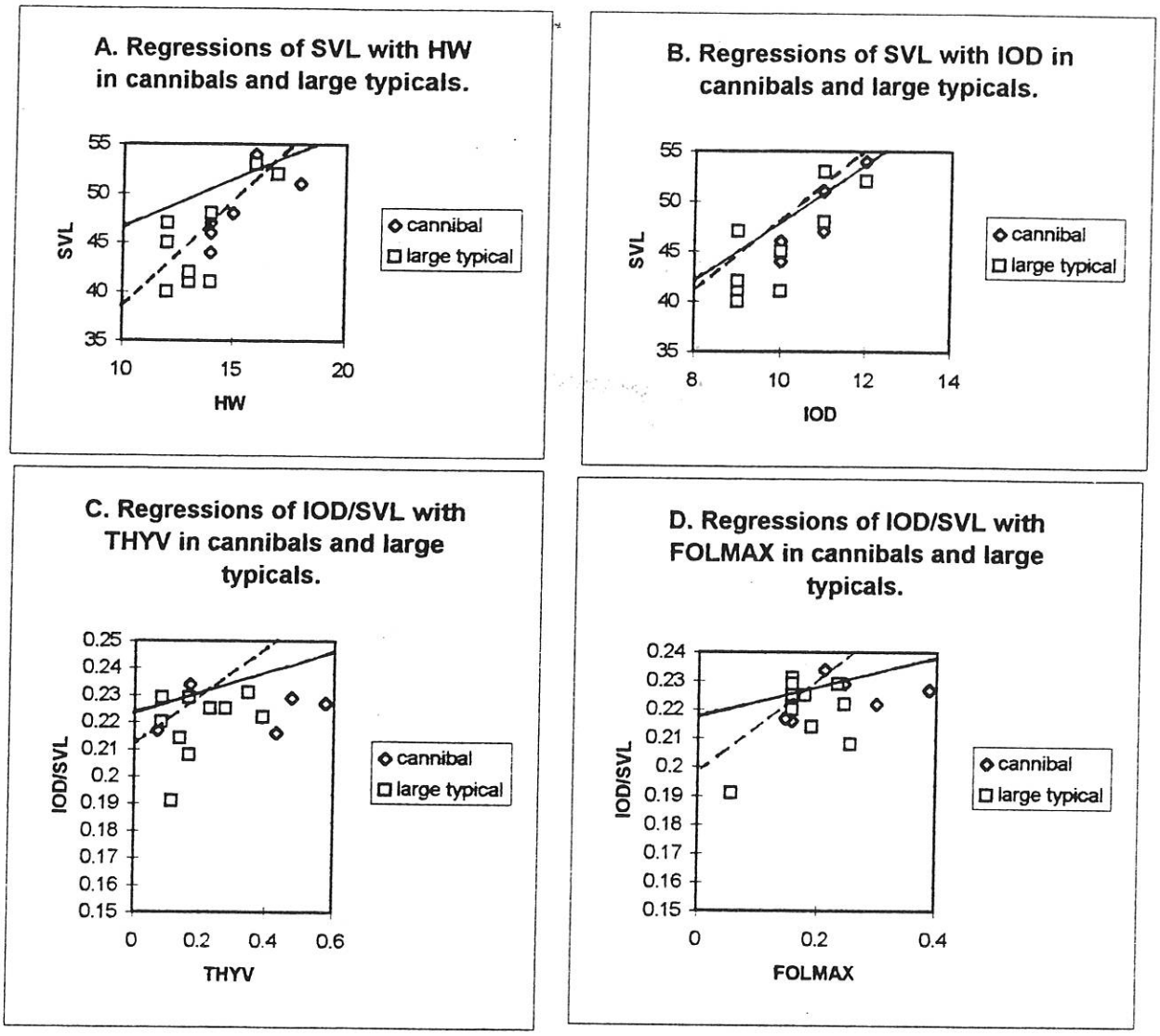

Figure 2. Regressions of SVL on head morphometrics for cannibals and large typicals. Solid line = cannibals; dashed line = large typicals. A. Regressions of SVL with HW in cannibals and large typicals. Cannibals: correlation = 0.74826; intercept = 46.3; significance = 0.0070. Large typicals: correlation = 0.75654; intercept = 38.8; significance = 0.0871. B. Regressions of SVL with IOD in cannibals and large typicals. Cannibals: correlation = 0.90649; intercept = 42.0; significance = 0.0127. Large typicals: correlation = 0.82637; intercept = 40.9; significance = 0.0017. C. Cannibals: correlation = 0.10348; intercept = 0.2225; significance = 0.8453; Large typicals: correlation = 0.36748; intercept = 0.2140; significance = 0.2962. D. Cannibals: correlation = 0.42801; intercept = 0.219; significance = 0.3972. Large typicals: correlation = 0.51735; intercept = 0.200; significance = 0.1257.

APPENDIX A

Tissue processing schedule

70% EtOH, overnight; 80% EtOH, 1 hr.; 95% EtOH, 1 hr.; 100% EtOH I, 1 hr.; 100% EtOH II, 1 hr.; xylene I, 1 hr.; xylene II, 1 hr.; Paraplast I, 1 hr.; Paraplast II, 1 hr.; Paraplast III, 1 hr.; embed.

APPENDIX B

Brookes' stain schedule

Xylene I, 5 min.; xylene II, 5 min.; 100% EtOH I, 15 dips; 100% EtOH II, 15 dips; 95% EtOH, 15 dips; 70% EtOH, 15 dips; distilled H_2O, 15 dips; 70% EtOH, 15 dips; 1% Orange G (Gurr) in 95% EtOH, 10 min.; 70% EtOH, 15 dips; distilled H_2O, 15 dips; 1% HOAc, 15 dips; 0.5% Wool Green (Lissamine Green) in 0.5% HOAc, 1 min.; running water until green clears rinse; 70% EtOH, 15 dips; 95% EtOH I, 15 dips; 95% EtOH II, 15 dips; 100% EtOH I, 15 dips; 100% EtOH II, 15 dips; xylene I, 5 min.; xylene II, 5 min.; coverslip.

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