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A NONCONVENTIONAL MORPHOMETRIC TECHNIQUE FOR MEASURING ONTOGENETIC SHAPE CHANGES IN TWO SPECIES OF CENTRARCHID FISHES

by

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ABSTRACT

A NONCONVENTIONAL MORPHOMETRIC TECHNIQUE FOR MEASURING ONTOGENETIC SHAPE CHANGES OF THE FIFTH CERATOBRANCHIAL IN TWO SPECIES OF CENTRARCHID FISHES

by

James Edward Zablotny

The shape changes of the occlusive surface of the right fifth ceratobranchial bone in pumpkinseed and bluegill sunfish were investigated across an ontogenetic series of specimens. The fifth ceratobranchials, from cleared and stained specimens, were photographed and outlines of the right occlusive surface were digitized to permit calculation of medial axes. A series of lines normal to these medial axes were drawn to the medial edge of the ceratobranchial and recorded as normal length per relative length along the medial axis. A geometric quintic equation was used to model the curve of the medial edge of the occlusive surface. Ontogenetic trajectories were calculated for the geometric coefficients across the size range of specimens studied. All geometric coefficients appear to be linearly related to standard length. The ontogenetic trajectories for ceratobranchial shape reveal that both species apparently diverge in shape at sizes greater than 35 mm standard length. For pumpkinseeds, the initiation of shape divergence from the juvenile ceratobranchial shape appears well before the switch in diet from cladocera and aquatic insects to snails.

ACKNOWLEDGMENTS

I wish to express my gratitude to the following people who have provided me with countless assistance: Donald Straney, guidance commitee chairman; Guy Bush and Robert Anstey, guidance committee members. I also thank Fred Bookstein for technical assistance with medial axis implementation; Gerald Smith, J. Alan Holman and Edward Wiley for specimens. Most of all, I thank my wife Jennifer for helping me with the completion of the manuscript.

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INTRODUCTION

In general, bony fishes utilize the external jaws for obtaining prey that is swallowed without mastication (Alexander, 1974). Highly derived euteleostean fishes (Greenwood, et. al., 1966) possess a set of pharyngeal "jaws" for mechanical manipulation of food items within the pharynx (Lauder 1983a). The euteleostean pharyngeal jaw functions as a single unit despite being derived from the posterior viscerocranium elements (Harder, 1975). The upper pharyngeal jaw consists of the second, third, and fourth pharyngobranchial bones. The lower pharyngeal jaw element is derived from the fifth ceratobranchial (Lauder, 1983). The bones of the pharyngeal jaws are usually covered with teeth on their occlusive surfaces. Extreme variability in shape of the fifth ceratobranchial and its associated dentition appear correlated with the prey type utilized by these fishes (Liem and Osse, 1975). Pharyngeal jaws are simple within the Centrarchidae and Serrannidae (the fifth ceratobranchials are separate from each other), but specialized (fifth ceratobranchials fused) in other higher euteleostean fishes such as the Cyprinodontoidei, Pomacentridae, Anabantoidea,

Girellidae, Cichlidae, Embiotocidae, Labroidei, and Odacidae (Liem and Greenwood, 1981).

Pharyngeal jaws can be used to assist in swallowing large food items. When used in pharyngeal transport, the upper and lower pharyngeal jaws are simultaneously adducted and retracted posteriorly (Lauder, 1983). By moving the upper and lower pharyngeal elements differently, the jaws will function instead as crushing surfaces masticating the food within the pharynx. For this to happen, simultaneous contraction of the pharyngeal jaw musculature adducts the upper and lower pharyngeal jaws to produce the crushing forces needed to masticate a hard food item. Based upon the taxonomic distribution and outgroup analysis, Lauder (1983) concluded that the pharyngeal jaws were primitively used for food transport. Pharyngeal trituration has arisen several times as a further, more specialized function of pharyngeal jaws. The sequences of this evolution from transport to food processing roles can be studied most conveniently within the North American centrarchid genus Lepomis. Both feeding styles are found among the 11 species of Lepomis sunfish. Two species (L. gibbosus and L. microlophus) are pharyngeal crushers, feeding extensively on snails. Lauder (1983) concluded that these two species masticate snails with muscle utilization patterns derived from the muscle patterns associated with pharyngeal transport.

Lauder (1983) investigated the functional differences in the pharyngeal jaw structure and feeding behavior in Lepomis and emphasized the structural dissimilarities between species that feed on snails and those that feed on zooplankton and aquatic arthropods. Zooplankton feeders, like bluegill sunfish (L. macrochirus), feature a narrow fifth ceratobranchial covered with filiform teeth on the occlusive surface. The two halves of the ceratobranchial are only moderately apposed to each other along the midline of the floor of the pharynx (J. Zablotny, pers. obs.). Lauder (1983) noted that the snail specializing species have a broadly widened tooth bearing occlusive surface of the fifth ceratobranchial and a fairly strong region of apposition between the right and left fifth ceratobranchials. The mollusc crushers also display hypertrophy of the muscles responsible for generating the powerful adduction of the upper and lower pharyngeal jaws.

Some Lepomis species also undergo an ontogenetic change in both food habits and pharyngeal jaw action. Although young centrarchids feed either on littoral or limnetic zooplankton (Keast, 1980), the gastropod specialists tend to shift from zooplankton to snails between 40 and 50 mm standard length. Other minor shifts in diet may occur in Lepomis due to competition based resource partitioning and niche shifting (Werner and Hall, 1976; Laughlin and Werner, 1980; Mittelbach, 1984), and predation risk (Werner, et. al., 1983a; Mittelbach,

1984), but the functional change in fifth ceratobranchial use appears only in those species which switch to feeding on snails.

The ontogenetic shift in pharyngeal jaw function in some Lepomis offers an excellent opportunity to examine the basis of morphological and functional evolution. The derived crushing morphology of L. gibbosus is achieved late enough in ontogeny (standard length of circa 45 mm, Zablotny, pers. obs.) that the morphogenetic transition can be studied in whole-mount field caught fish. This makes a comparative study of the ontogenetic basis of the evolution of pharyngeal jaw morphology feasible, since the critical ontogenetic stages are those that are most easily sampled. Because the use pattern in some species shifts in free-living individuals (and not in embryos), some questions about the process of morphological divergence become tractable. We know very little about the exact nature of the relationship between function and structure during evolution. It is in cases of ontogenetic switching in function that we can begin to assess, for example, whether changes in function precede changes in morphology (e.g. Mayr, 1958; 1960) or how important environmental factors are in precipitating morphological change (e.g. Smith-Gill, 1983).

I have examined the differences in ontogeny of the lower pharyngeal jaw in two species of Lepomis which differ in adult feeding morphology. I have focused on the lower element

(fifth ceratobranchial) because the lower pharyngeal jaws have been extensively used for taxonomic evaluation of species and evolutionary relationships among fish taxa. The species examined are phylogenetically closely related. Presently, published phylogenies on the family Centrarchidae are based on electrophoretic data (Avise and Smith, 1974; Avise, et. al., 1977), and on the morphology of the acoustico-lateralis system (Branson and Moore, 1962). Unfortunately, there are no highly resolved phylogenetic hypotheses available for evaluating the direct evolutionary relationships within the genus Lepomis (Humphries, pers. com.). Since Lauder (1983, 1983a) believes that pharyngeal transport is primitive to snail crushing, I chose to represent the primitive morphology (pharyngeal transporter) with the bluegill sunfish and the derived (snail crusher) morphology with the pumpkinseed sunfish. I am interested in determining how the primitive ontogenetic trajectory of bluegills is transformed into the derived pumpkinseed condition. This study also examines how the function of pharyngeal jaws (assessed by feeding preference) is correlated with their morphology during the transition from one feeding style to another in pumpkinseeds.

The ontogenetic shift in pharyngeal jaw function in Lepomis is a useful example of morphological change only to the extent that it can be studied quantitatively. The outline of the fifth ceratobranchial in Lepomis, for example is smoothly curved without obvious landmarks. The ontogeny of

this bone in different Lepomis species involves subtle shape differences that simple measurements of linear dimensions cannot describe well. Relatively few techniques are available for quantifying shape change in landmark-free situations (Oxnard, 1980; Bookstein, 1979, 1981a; Bookstein, et. al. 1985). In the present case, I have used the line skeleton (Blum and Nagel, 1978; Blum, 1973; Oxnard, 1980; Bookstein 1981a, 1981c; Bookstein, et. al. 1985). The medial axis is the locus of points inside an outline that are equidistant from the outline. As a nonlinear axis of symmetry of an outline, the medial axis is a useful summary of the outline shape: the outline can be recovered by associating with each medial axis point the distance from axis to outline. Because I was interested in comparing how particular regions of the fifth ceratobranchial changed with growth across species, I could not effectively use techniques such as tangent angle functions or Fourier analysis that are functions of outline arc length. Instead, I have used the medial axis of each form as a coordinate system within which to study complex shape changes.

MATERIALS AND METHODS

An ontogenetic series of 25 bluegill (Lepomis macrochirus Rafinesque) and 25 pumpkinseed (Lepomis gibbosus (Linnaeus)) sunfish, were selected from material loaned from the following sources: The University of Michigan Museum of Zoology (UMMZ); The Museum, Michigan State University; The Field Museum of Natural History (FMNH); and the University of Kansas Museum of Natural History (KU). Specimens were chosen to provide a reasonably complete coverage of standard lengths from 25 to 160 mm, spanning the size range of both species commonly found in museum collections. Additional bluegill specimens were captured by angling at Lake Ovid, Laingsburg, Michigan. Whole specimens were cleared and stained using the technique of Taylor (1967). The lower pharyngeal jaw (right and left ceratobranchial V) were dissected from the cleared and stained specimens and stored in 100% glycerine. A few grains of thymol were added to inhibit mold growth.

To standardize specimen orientation for photography, each pharyngeal lower pharyngeal jaw was positioned on a 4 mm layer of plastic sponge foam with the occlusal surface uppermost. A plastic microscope coverslip box (75x33x13 mm) served to enclose the specimen and sponge foam, which were covered by a solution of 3 parts glycerine and 7 parts 70% ethanol.

Two small sections of a transparent metric ruler were glued to the underside of a large coverslip. The coverslip was placed over the specimen with the millimeter scale of the rulers positioned along the lateral edges of the lower pharyngeal jaw. The sections of ruler provide a metric scale at or near the plane of focus during photography (Figure 1). Once placed on the microscope stage, two 100 g weights were positioned on the coverslip to level the tooth surface of the specimen to the plane of the camera lens and to minimize the central rotation of the two halves of the ceratobranchials. The resilience of the sponge foam to the force of the two weights tended to push the teeth firmly against the glass coverslip and stabilize specimens during photography.

A trinocular dissecting microscope (Wilde model M8, MPS51 camera and MPS45 Photoautomat) and Kodak Panatomic X (ISO 32) film were used for specimen photography. Only the right fifth ceratobranchial and its millimeter scale were included in the photograph. A Volpi Intralux 6000 fiber optics light source provided even illumination. Negatives were enlarged and printed on 8 1/2 x 11 in. Kodak high contrast resin coated paper.

The outline of the specimen (including the cartilaginous posterior and anterior apices) and millimeter scale were traced from the photographic prints to transparent acetate with a fine tipped permanent marking pen. Starting with the anterior apex, each outline was digitized in a clockwise

direction on a Summagraphics Bit Pad-One digitizing tablet. The number of digitized points used for shape analysis varied from 135 to 220; more points were digitized on specimens whose outline was more curved.

The medial axis (Bookstein, 1979) for each form was computed using Bookstein's (1979) program via a Tektronix T4014 graphics terminal. Hard copies of the graphics output were obtained and inspected for digitizing errors. Those forms having obvious digitizing errors were reanalyzed. The 90 degree crosshairs of a protractor were placed tangent to points along the major medial axis component and were used to draw normal lines perpendicular to the tangents of the medial axis. Based on the degree of curvature found on the specimen, 32 to 65 normal lines were drawn per specimen. Branch points on the medial axis were ignored; they were encountered only on several pumpkinseed specimens. The ends of these normal line segments at the outline boundary and at the medial axis were digitized. The length of the normals was calculated and serves as an estimate of the width function of the outline at each medial axis point. Because the medial axis is an axis of symmetry defined by the pair of "pseudonormal" lines for each point on the medial axis, I chose to analyze only the medial side of the fifth ceratobranchial. Since I used lines drawn normal to the medial axis and not to the outline, the width functions used to describe the form are an approximation of this symmetry (Blum and Nagel, 1978).

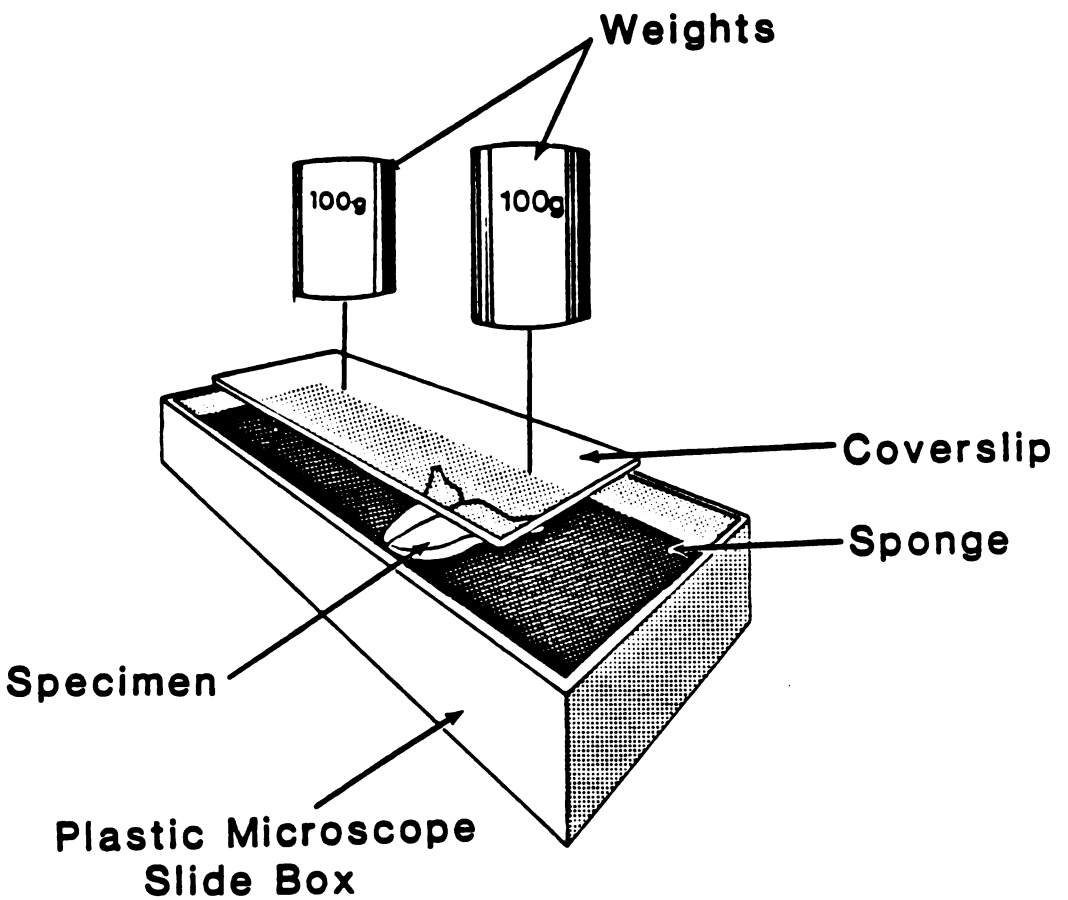


Figure 1. Alignment Apparatus for Photographing the Fifth Ceratobranchial Bones.

The medial axis and normals provide a coordinate system for analyzing differences in outline shape. I set the origin of this coordinate system at the posterior end of each ceratobranchial and standardized the arc of the medial axis to a length of one. Distance along the medial axis represents an ordinate (Figure 2). A polynomial was fitted to the plot of normal width versus relative medial axis arc length to produce an equation expressing ceratobranchial width as a function of position along the medial axis. I used a fifth order polynomial,

$$Y = a_0 + a_1x + a_2x^2 + a_3x^3 + a_4x^4 + a_5x^5$$

to model the overall curve of the medial edge of the right half of the lower pharyngeal jaw. Although the curve of the pumpkinseed specimens are more complex than for the series of bluegill specimens, comparison of the forms was simplified by the use of the quintic model for both species. All of the regression coefficients were significantly different from zero.

To make the coefficients of the regression model interpretable as shape descriptors of the estimated curve, the fifth order polynomial was reparameterized and converted algebraically to geometric form (Mortenson, 1985). This parameterization decomposes the original quintic model into six orthogonal component functions (F_0 to F_5) and a corresponding set of six regression coefficients (b_0 to b_5) of the original quintic polynomial regression model.

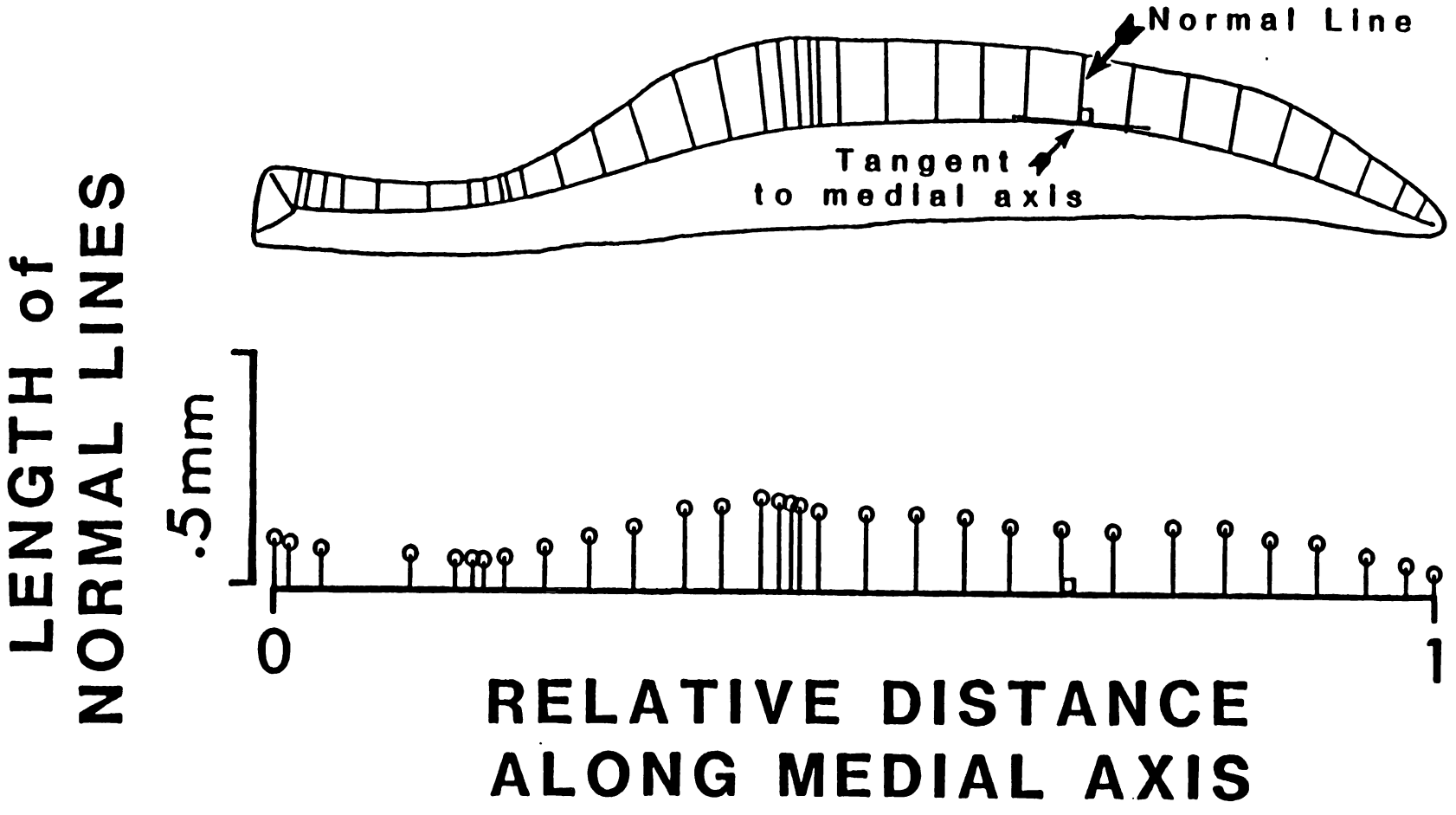


Figure 2. Measurement Protocul for Extracting Normals to the Medial Axis.

Mortenson (1985) noted that these functions when used together blend to produce a curve originally described by the algebraic quintic equation. The values of the coefficients b_0 and b_1 correspond respectively to the posterior and anterior widths of the medial half of the lower pharyngeal jaw. The next two coefficients, b_2 and b_3 are the slopes for the posterior and anterior ends of the form. The acceleration or change in slopes along the curve of the medial side of the fifth ceratobranchial are b_4 for the posterior and b_5 for the anterior ends. The change in magnitude and the sign of these coefficients may be used to mathematically assess the overall change in shape that is occurring during ontogeny.

The coefficients, b_i's, of the geometric quintic were calculated with an SPSS polynomial regression program. Correlations and covariance among the geometric coefficients for individual species were initially examined with a partial correlation analysis (SPSS). Since most of the coefficients were significantly correlated with standard length or size, a partial correlation analysis was used to partial out the contribution of size to the overall correlations among coefficients (Table 5).

To examine how the magnitude of the coefficients, b_i, change through ontogeny for the sunfish, each coefficient was regressed on standard length. I compared these regressions between both species with a covariance analysis to test for regression parallelism (Zar, 1984). A test for common

intercepts (Zar, 1984) was used to further examine the differences in y intercepts for regression lines having equal slopes in both species. This procedure uses a modified t-test to evaluate the regression elevations or intercepts as being the same. Only one pair of regression lines, b_0, was suited for this statistical test.

Coefficients derived from the regression equations of each coefficient versus standard length were used to calculate expected pharyngeal jaw shapes across ontogeny. The expected ceratobranchial shape was computed for 10 standard lengths from 31 to 76 mm by 5 mm increments (Table 7). The bottom of this size range was chosen at or near the standard length at which the regression lines intersected one another; the maximum size used was the maximum common to both data sets. A standard length of 111 mm was also added to the chosen size classes to represent the largest individual pumpkinseed size class in the data set. At least 50 points representing a relative arc length along the medial axis from 0 to 1 were entered in the set of geometric quintic equations with the proper coefficients for each size class. The resultant points were then plotted with a Hewlet Packard 7475A plotter (Figure 5).

To examine the sources of variation in measuring normal line lengths, a minuten insect pin was inserted on the lateral edge of one fifth ceratobranchial bone. The insect pin provided a landmark for the distance measurement used in the

test procedure. The specimen was placed in the alignment apparatus and photographed. This operation was repeated five times. Each photograph was traced twice on transparent acetate film. The distance of a normal line drawn from the edge of the form opposing the pin placement to the point of insertion of the pin was digitized five times for each tracing. A nested analysis of variance (Sokal and Rohlf, 1969) was used to estimate the magnitude of construction error due to photographic and digitizing error on the length of the normal line. F tests for among photographs and among drawings within photograph sources of variation were not significant and accounted for 41.3 percent of the total variance (Table 1.). The remaining 58.7 percent of the variance was due to errors in digitizing normal lines. The total measurement error (s^2 = .00287) and coefficient of variation, V=3.25 is actually quite small and does not contribute greatly to the estimation of the curve studied here.

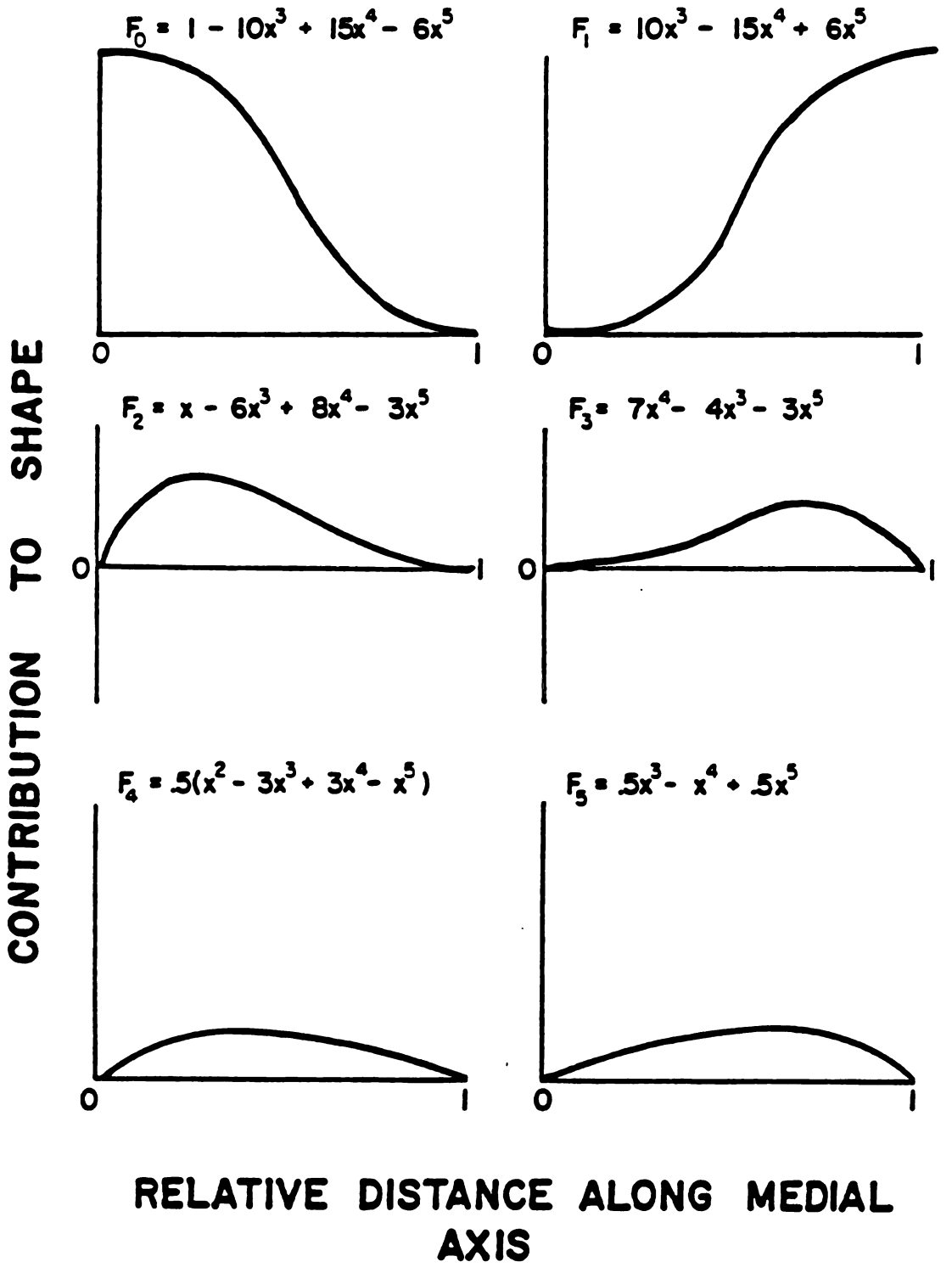


Figure 3. Blending Functions for the Quintic Polynomial.

Table 1. Two Level Nested Anova. The Sources of Variation for Measuring Normal Line Length.

Source of Variation	df	SS	MS	F_s	s^2	% total s^2
Among Photographs	4	.0485	.0121	3.87^{ns}	.0009	31.27
Among Drawings within Photographs	5	.0157	.0031	1.86^{ns}	.00029	10.0
Within Drawings	40	.0675	.0017		.0017	58.73
Total	49	.1317	.0170			

RESULTS

The medial axis constructed from the digitized outlines are depicted in Appendices A and B. The structure of the medial axis is a simple smooth curve in bluegills (Appendix A); the medial axis for pumpkinseeds (Appendix B) is more elaborate and variable than for bluegills. Many of the medial axis produced for large pumpkinseeds contain one or two triple or branch points. I defined the major medial axis components as those branches which represent the anterior-posterior axis of the ceratobranchial. Although Oxnard (1980) and Bookstein (1981a) acknowledge the usefulness of smaller branch segments for quick visual evaluation of shape differences in similar forms, I ignored them for the following reasons. Minor branches of the medial axis at 45 to 50 percent along the medial axis are consistently present for pumpkinseeds greater than 50 mm standard length. Small pumpkinseeds and bluegills did not feature any minor branches at that position along the major medial axis component. These minor branch lengths were not examined in this study. For certain cases, slight deviations in digitizing produced extraneous branches that were also excluded from the analysis.

A geometric quintic polynomial regression for normal width versus relative distance along the medial axis adequately models the curve of the right medial side of the lower pharyngeal jaw. The polynomial regression produced r^2 values for the geometric quintic equations ranging from 0.96 to 0.99 in bluegills and pumpkinseeds. The geometric quintic model fit the set of bluegill curves slightly better than pumpkinseed curves (Tables 2 and 3). Large pumpkinseeds were least likely to be fit well by a fifth degree polynomial curve. These were also the individuals with accessory branches of the medial axis near the midpoint of the outline. However, the difference in average r^2 for the quintic model fit to individuals with accessory branches (r^2=0.989, n=13) and without (r^2=0.983, n=12) suggests that no discernible imprecision was introduced by ignoring the information in accessory branches.

Although the independent "variables", F_i, of the geometric version of the quintic polynomial are formally orthogonal (Mortenson, 1985, pg. 49), the coefficients, b_i, of these functions need not be independent. With the exception of coefficient b_5, each coefficient was significantly correlated with body size in both bluegills and pumpkinseeds (Table 4). Partial correlations, removing the effect of size were significant for coefficients b_0 and b_3, b_4 and b_2, and b_5 with b_1 and b_3 in both species (Table 5).

Table 2. Coefficients of the Geometric Interpretation of the Algebraic Quintic Polynomial: Lepomis macrochirus.

Specimen	b_0	b_1	b_2	b_3	b_4	b_5	r^2	SL
LMA21	0.1012	0.0585	-1.0047	-0.6402	16.9103	-4.6099	0.993	25
LMA19	0.1169	0.0601	-1.0854	-0.5027	15.8051	-2.0660	0.988	26
LMA25	0.1150	0.0351	-1.1197	-0.8521	17.7737	-5.1853	0.995	27
LMA26	0.1204	0.0546	-1.0005	-0.5560	13.5576	0.8343	0.994	28
LMA23	0.1123	0.0537	-1.0645	-0.7114	17.9707	-4.1244	0.992	28
LMA24	0.1027	0.0445	-0.8018	-0.6629	13.7953	-3.6197	0.997	28
LMA1	0.1075	0.0416	-1.0850	-0.7893	15.8039	-5.0378	0.987	30
LMA18	0.1076	0.0428	-0.8860	-0.6667	17.0246	-4.0523	0.995	30
LMA6	0.1310	0.0705	-0.8803	-0.6995	14.9151	-1.7485	0.998	30
LMA20	0.1257	0.0776	-1.2383	-0.6036	19.2271	-2.2994	0.990	35
LMA16	0.1153	0.0842	-0.7775	-0.3102	12.3431	2.1001	0.992	35
LMA8	0.1192	0.0534	-0.8867	-1.4105	18.9814	-11.1983	0.998	37
LMA17	0.1239	0.0851	0.8082	-0.7720	15.0614	-3.8104	0.996	38
LMA2	0.1255	0.0793	-0.9951	-0.9940	21.5341	-6.4260	0.998	38
LMA10	0.1607	0.0758	-0.9839	-1.5631	20.8439	-8.8322	0.999	39
LMA9	0.1192	0.0882	-0.6132	-1.3509	16.1518	-7.7080	0.999	43
LMA7	0.1468	0.0718	-0.2999	-1.1284	12.1147	-4.4259	0.999	46
LMA12	0.3372	0.1245	-3.1775	-3.1739	57.0138	-24.1575	0.998	79
LMA3	0.3603	0.3631	-2.8435	-1.2903	44.9923	2.8059	0.997	88
LMA45	0.3918	0.2792	-3.0294	-1.1353	44.3911	0.5131	0.960	93
LMA4	0.4269	0.4208	-3.7668	-0.5098	54.0447	10.1188	0.992	100
LMA46	0.7532	0.4724	-5.1911	-2.2172	70.5409	26.8819	0.993	144
LMA47	0.3999	0.2466	-2.5277	-2.1873	45.1361	7.3176	0.998	128
LMA48	0.5968	0.3902	-4.8916	-3.2162	69.3515	-9.7602	0.996	148
LMA49	0.6758	0.3429	-5.1352	-4.7896	90.3241	-17.4446	0.999	158

Table 3. Coefficients of the Geometric Interpretation of the Algebraic Quintic Polynomial: Lepomis gibbosus.

Specimen	b_0	b_1	b_2	b_3	b_4	b_5	r^2	SL
LGI33	0.1120	0.0820	-1.1853	0.0226	14.8484	9.6943	0.961	27
LGI32a	0.1282	0.1060	-1.4321	0.3051	20.8317	3.4427	0.972	28
LGI1	0.0806	0.0425	-0.8077	-0.1366	11.8010	3.8205	0.975	28
LGI35	0.1316	0.0703	-1.6358	-0.9091	23.9717	2.5771	0.975	28
LGI30	0.0727	0.0363	-1.1448	-0.1329	15.3993	3.4809	0.964	28
LGI15	0.1311	0.0721	-1.2534	-0.6841	17.1097	2.3717	0.989	29
LGI36	0.1985	0.0779	-1.7465	-1.8874	31.3829	-11.5100	0.991	40
LGI26	0.1521	0.0910	-1.8669	-1.7382	36.0062	-6.0748	0.990	44
LGI13	0.1873	0.0939	-2.1353	-2.4917	38.2604	-11.4776	0.994	44
LGI37	0.2397	0.1403	-1.8473	-1.1571	23.8435	10.8362	0.993	46
LGI20	0.7591	0.1940	-1.0485	-0.0212	04.3855	-4.7585	0.999	50
LGI25	0.2197	0.2887	-2.3100	-0.1578	35.0971	16.8958	0.992	50
LGI14	0.3271	0.1260	-3.9572	-3.7920	63.3560	-20.3375	0.992	58
LGI39	0.2250	0.1209	-2.9282	-2.3673	46.2137	-7.7601	0.992	60
LGI27	0.2886	0.3810	-3.4050	-2.0359	62.0040	-7.7352	0.993	64
LGI41	0.3239	0.2556	-4.3052	-3.4416	69.0865	-15.4769	0.989	64
LGI28	0.2928	0.1988	-2.7373	-2.4918	47.7383	2.6453	0.993	67
LGI29	0.2971	0.2620	-3.3790	-2.2117	50.4358	-2.1333	0.991	67
LGI4	0.2201	0.1753	-2.5453	-2.5187	40.2900	-8.0946	0.990	68
LGI18	0.3736	0.1639	-5.0689	-5.7967	100.5802	-48.7783	0.977	75
LGI22	0.3206	0.5178	-2.4457	-1.0248	46.9227	27.2296	0.995	79
LGI2e	0.2472	0.2687	-3.6607	-2.6443	62.4586	-3.8982	0.988	84
LGI12	0.3929	0.3071	-4.0151	-4.2664	84.1766	-17.8465	0.980	94
LGI34	0.3999	0.4750	-2.8430	-5.1321	83.9738	-32.4243	0.994	100
LGI16	0.5448	0.7902	-6.5697	-3.5467	120.6513	9.2882	0.985	111

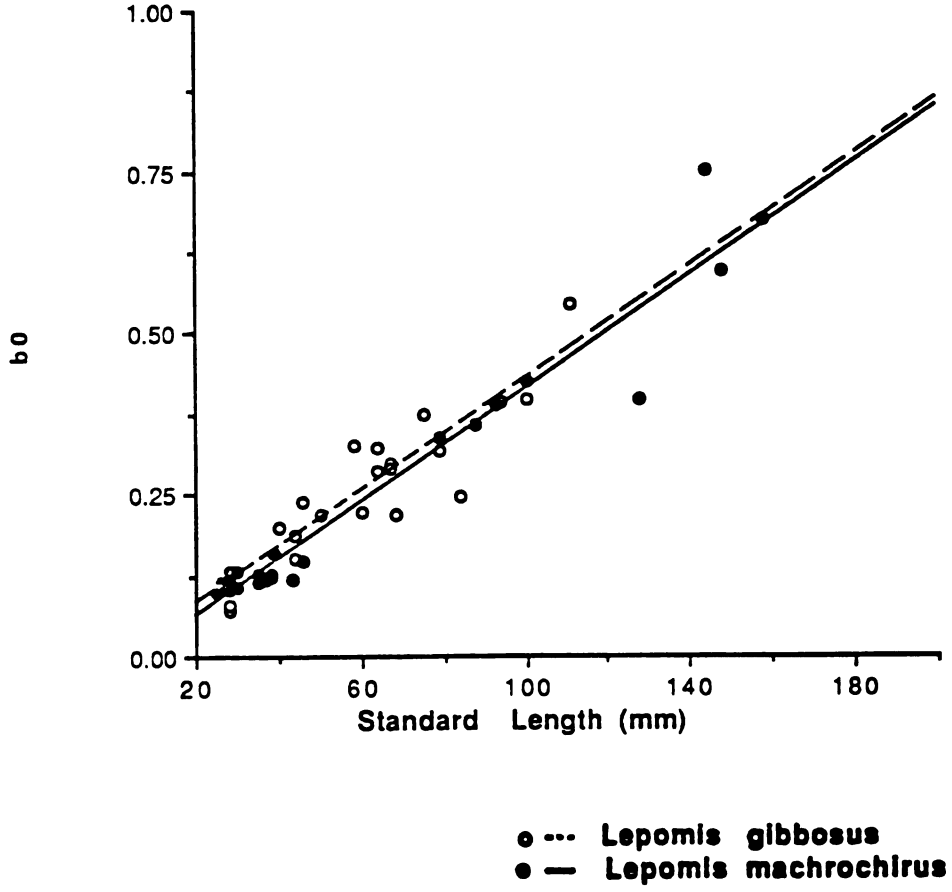


Figure 4. The Ontogenetic Trajectory of the Width of the Posterior End of the Fifth Ceratobranchial (b_0) Versus Standard Length.

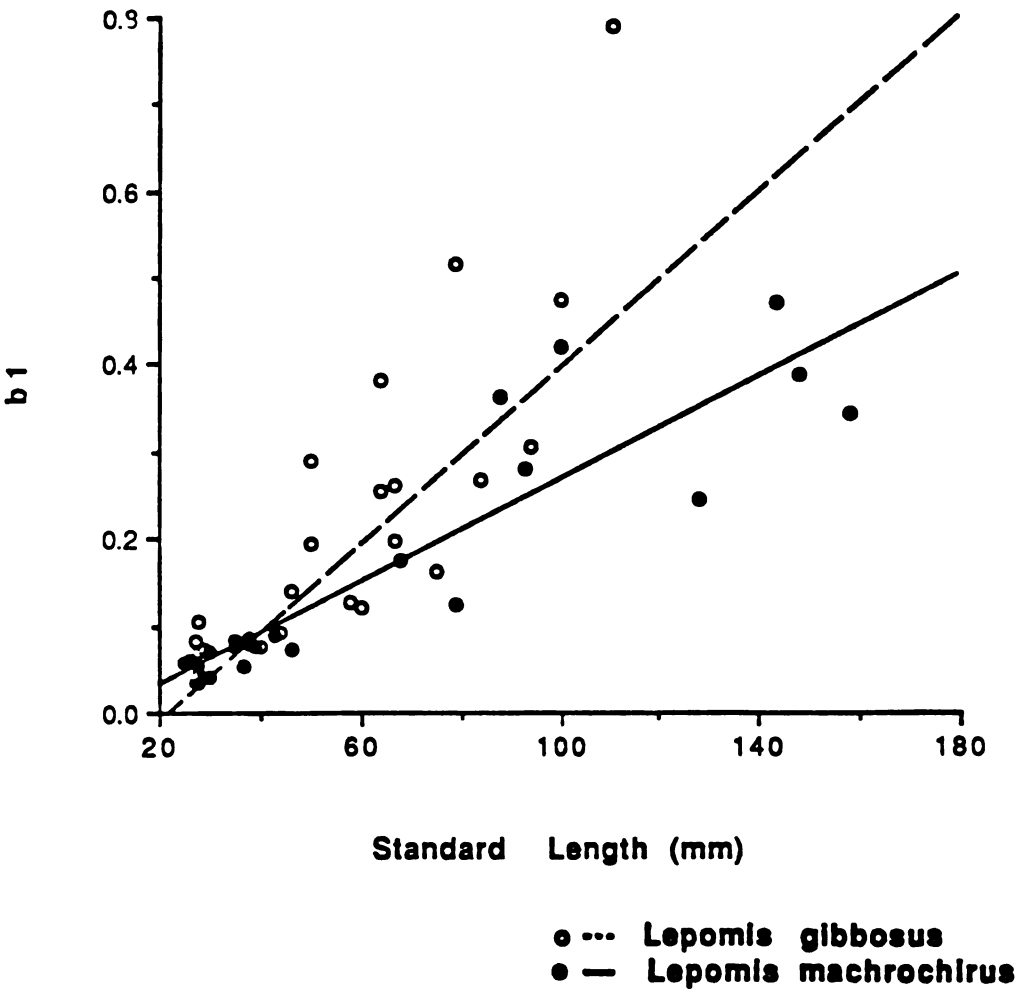


Figure 5. The Ontogenetic Trajectory of the Width of the Anterior End of the Fifth Ceratobranchial (b_1) Versus Standard Length.

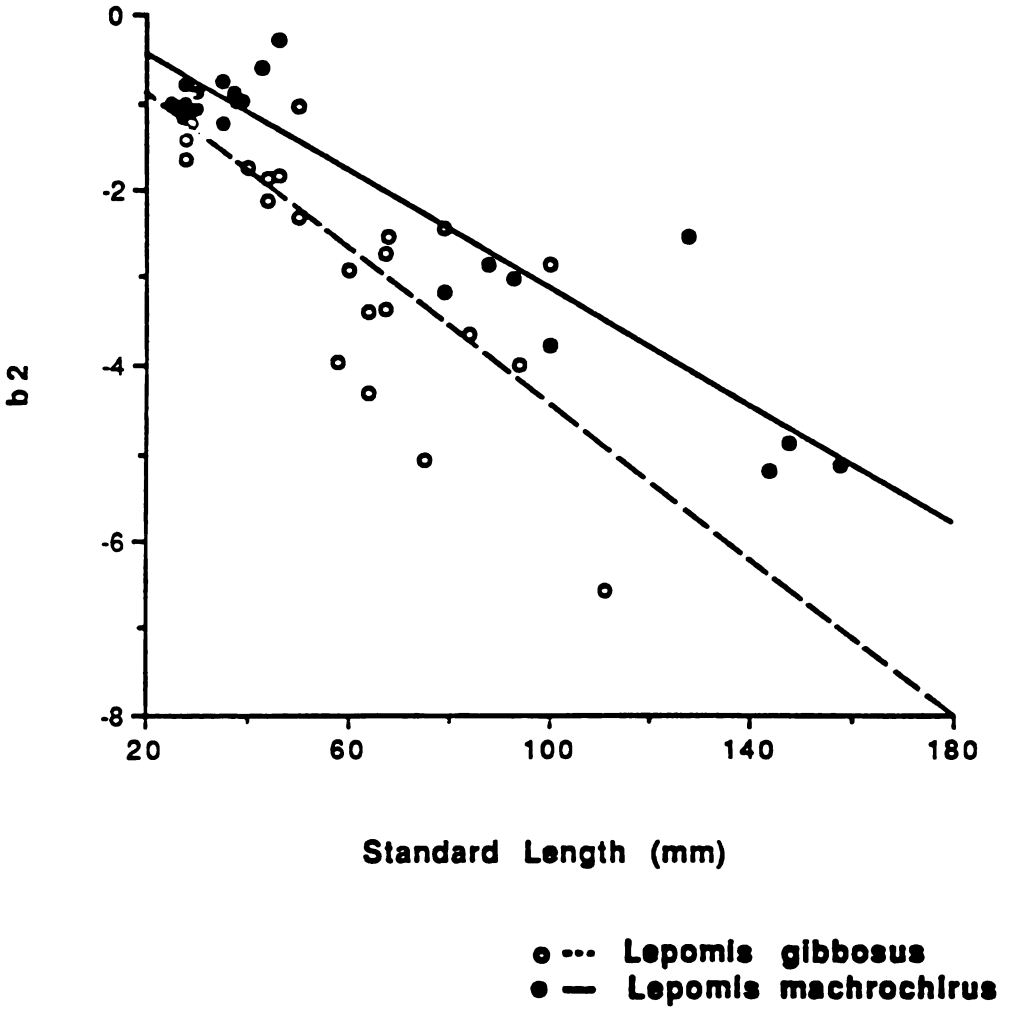


Figure 6. The Ontogenetic Trajectory of the Tangent of the Outline of the Posterior End of the Fifth Ceratobranchial (b_2) Versus Standard Length.

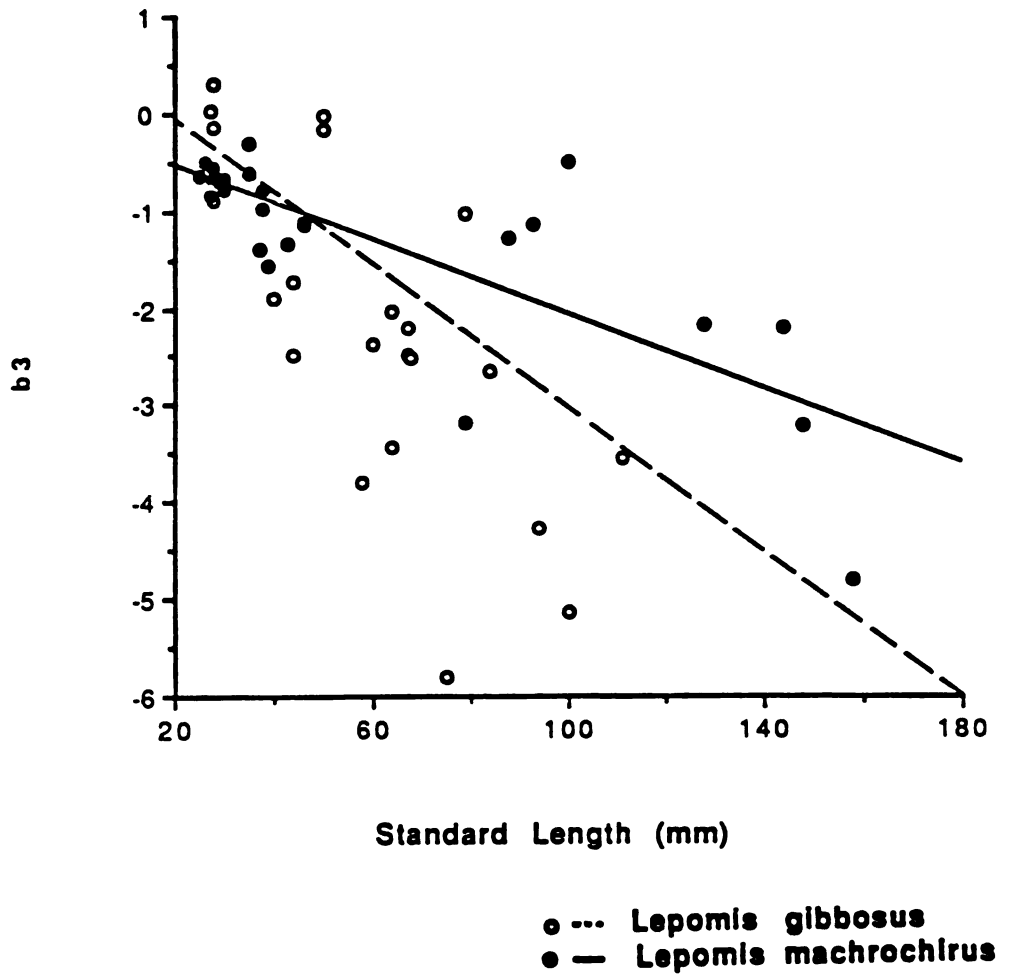


Figure 7. The Ontogenetic Trajectory of the Tangent of the Outline of the Anterior End of the Fifth Ceratobranchial (b_3) Versus Standard Length.

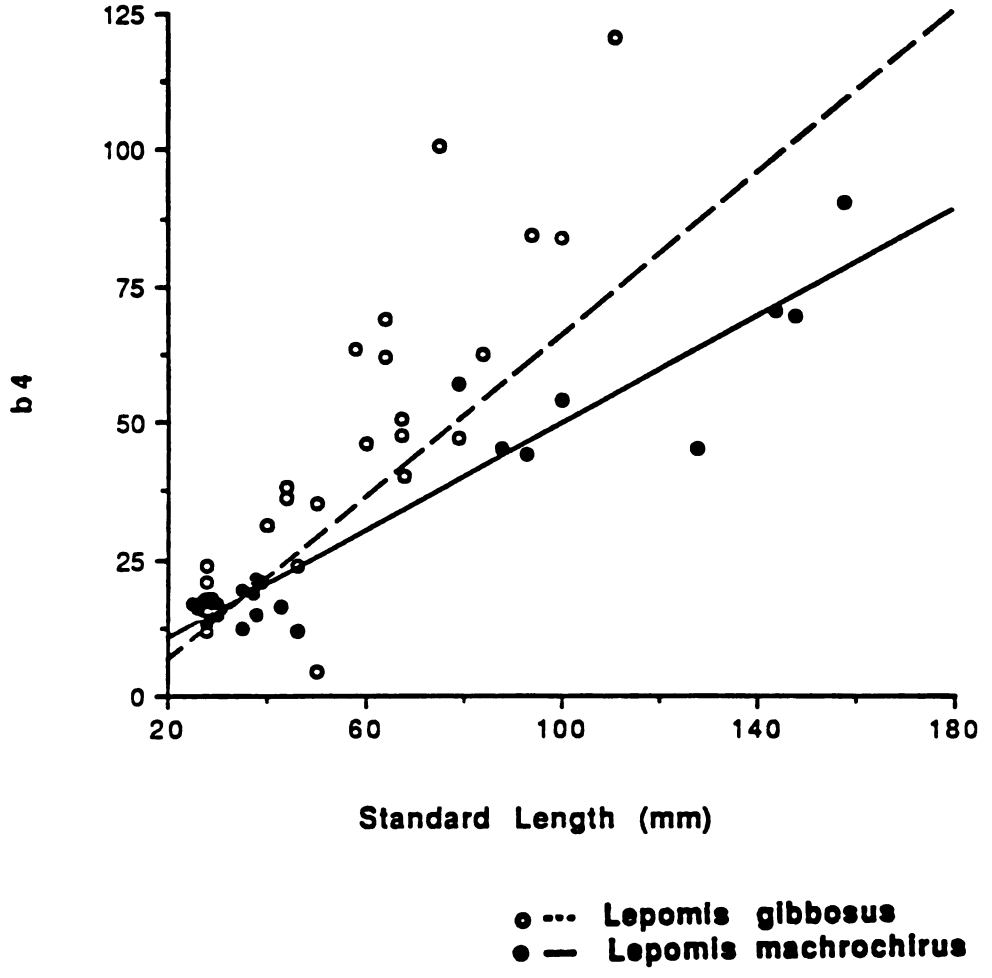


Figure 8. The Ontogenetic Trajectory of the Change in Tangent of the Outline of the Anterior End of the Fifth Ceratobranchial (b_4) Versus Standard Length.

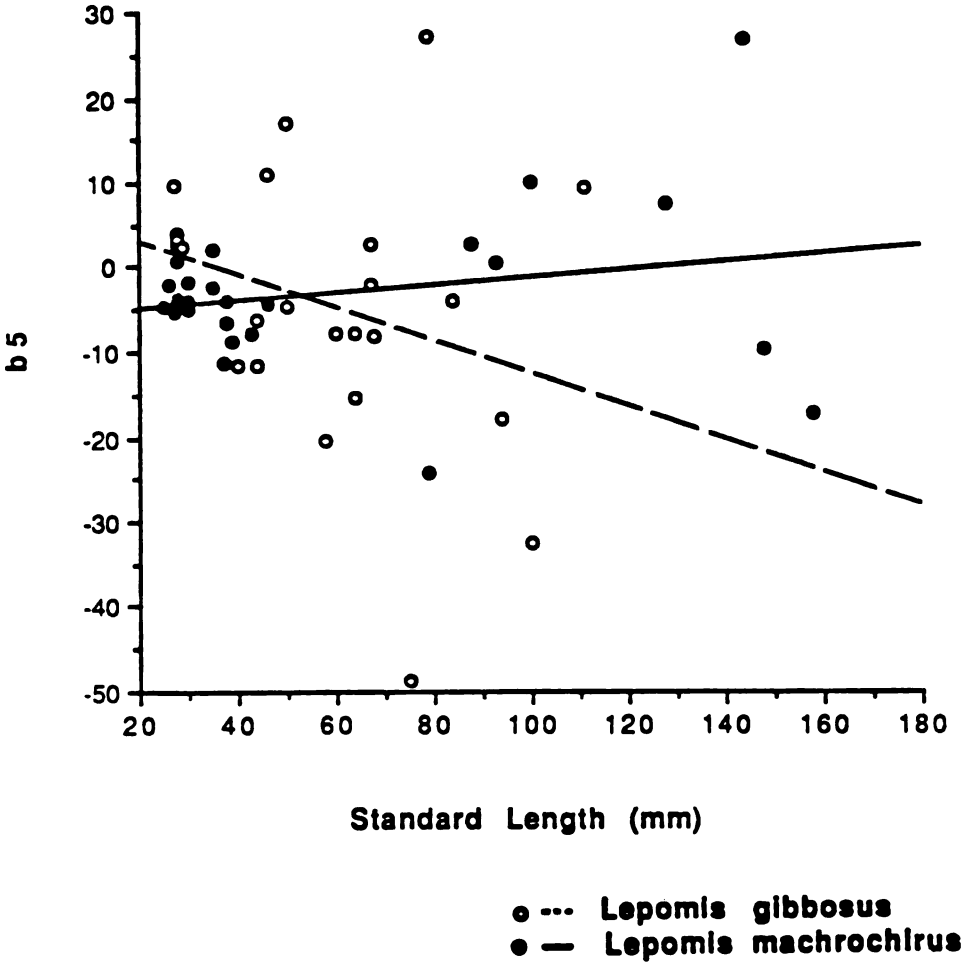


Figure 9. The Ontogenetic Trajectory of the Change in Tangent of the Outline of the Posterior End of the Fifth Ceratobranchial (b_5) Versus Standard Length.

Because the coefficients within these three groups behave differently during ontogeny, it seems that these correlations have few implications for the main purpose of this study. However, because these correlations have a complex basis (including possibly both biological and computational origins) and a difficult interpretation, further study of correlations among parameters of polynomial equations in similar situations is needed.

Ontogenetic trajectories for the coefficients of the geometric quintic are presented in Figures 4 through 9. To a good first approximation, the value of each coefficient appears to be linearly related to standard length. Over ontogeny, the coefficient b_0 is most highly correlated with standard length (Figure 4; for bluegills r^2=0.94, for pumpkinseeds r^2=0.85). The quality of the fit of coefficients b_1 through b_4 varies from good to fair. Coefficient b_1 correlated well with standard length (Figure 5; for bluegills r^2=0.83, for pumpkinseeds r^2=0.69). Coefficient b_2 also correlated reasonably well with standard length (Figure 6; r^2=0.88 for bluegills, r^2=0.58 for pumpkinseeds). For coefficient b_3 (Figure 7), both species show fair correlations with size (r^2=0.61 for pumpkinseeds, r^2=0.58 for bluegills). Coefficient b_4 (Figure 8) correlated well with standard length for bluegills (r^2=0.90) and pumpkinseeds (r^2=0.77). Coefficient b_5 does not appear to correlate with body size for the two species (Figure 9; bluegills r^2=0.04, pumpkinseeds

r^2=0.18). The variance inherent to b_5 tends to increase with fish body size. Because a log transformation can frequently improve the fit in situations where variances increase with size, I also regressed the log transforms of b_5 and standard length. This transformation resulted in a worse fit of the model (Figure 10; bluegills, r^2=0.0063; pumpkinseeds, r^2=0.0856). I conclude that there is no clear association between b_5 value and standard length.

Coefficients b_0 describes the width of the posterior end of the ceratobranchial (the point of insertion of the fifth branchial adductor). The slope of the ontogenetic change of b_0 with size appeared to differ significantly between bluegills and pumpkinseeds. Inspection of residuals, though, indicated that one pumpkinseed, labeled LGI20, has an unusually wide posterior end for its size (Figure 4). Examination of the photograph of this ceratobranchial revealed that its posterior region was slightly occluded by overlying soft tissue and the thickness of the posterior edge was overestimated. Therefore, this specimen was excluded from analysis of b_0. Digitization of the remainder of the form appeared reasonable and the rest of the coefficients for specimen LGI20 were not removed from the analysis. The slopes of the ontogenetic trajectories of b_0 in fact do not differ significantly between species (Table 6; standard length by species interaction is not significant). Likewise, there is statistically no detectable difference between intercepts for

Table 4. Linear Regression of the Geometric Coefficients versus Standard Length.

Lepomis macrochirus Rafinesque

Coefficient	n	r^2	Y = mx + b
b_0	25	0.94	Y = .0044x - .02435
b_1	25	0.83	Y = .00295x - .02847
b_2	25	0.88	Y = -.03273x + .12152
b_3	25	0.61	Y = -.01926x - .15357
b_4	25	0.90	Y = .48884x + .87434
b_5	25	0.04	Y = .04471x - 5.7218

Lepomis gibbosus (Linnaeus)

Coefficient	n	r^2	Y = mx + b
b_0	25	0.41	Y = .0041x + .03397
b_0	24	0.85	Y = .0044x - .00425*
b_1	25	0.69	Y = .0061x - .13597
b_2	25	0.58	Y = -.0403x - .26624
b_3	25	0.58	Y = -.0523x + .98594
b_4	25	0.77	Y = 1.0646x - 14.9917
b_5	25	0.18	Y = -.2312x + 5.9002

* Specimen LGI20 was excluded from this regression.

Table 5. Correlation Analysis for the Interaction between Geometric Coefficients with Size Partialed Out.

Lepomis gibbosus (Linnaeus)

	b_0	b_1	b_2	b_3	b_4	b_5
b_0	-	ns	ns	ns	ns	ns
b_1		-	ns	0.66^{***}	ns	0.68^{***}
b_2			-	-0.46^{*}	-0.85^{***}	ns
b_3				-	-0.68^{***}	0.88^{***}
b_4					-	-0.49^{*}
b_5						-

Lepomis macrochirus Rafinesque

	b_0	b_1	b_2	b_3	b_4	b_5
b_0	-	0.46^{*}	-0.77^{*}	ns	ns	ns
b_1		-	-0.43^{*}	0.75^{***}	ns	0.63^{***}
b_2			-	ns	-0.81^{***}	ns
b_3				-	-0.46^{*}	0.86^{***}
b_4					-	-0.46^{*}
b_5						-

* $p < 0.5$, ** $p < 0.05$, *** $p < 0.005$

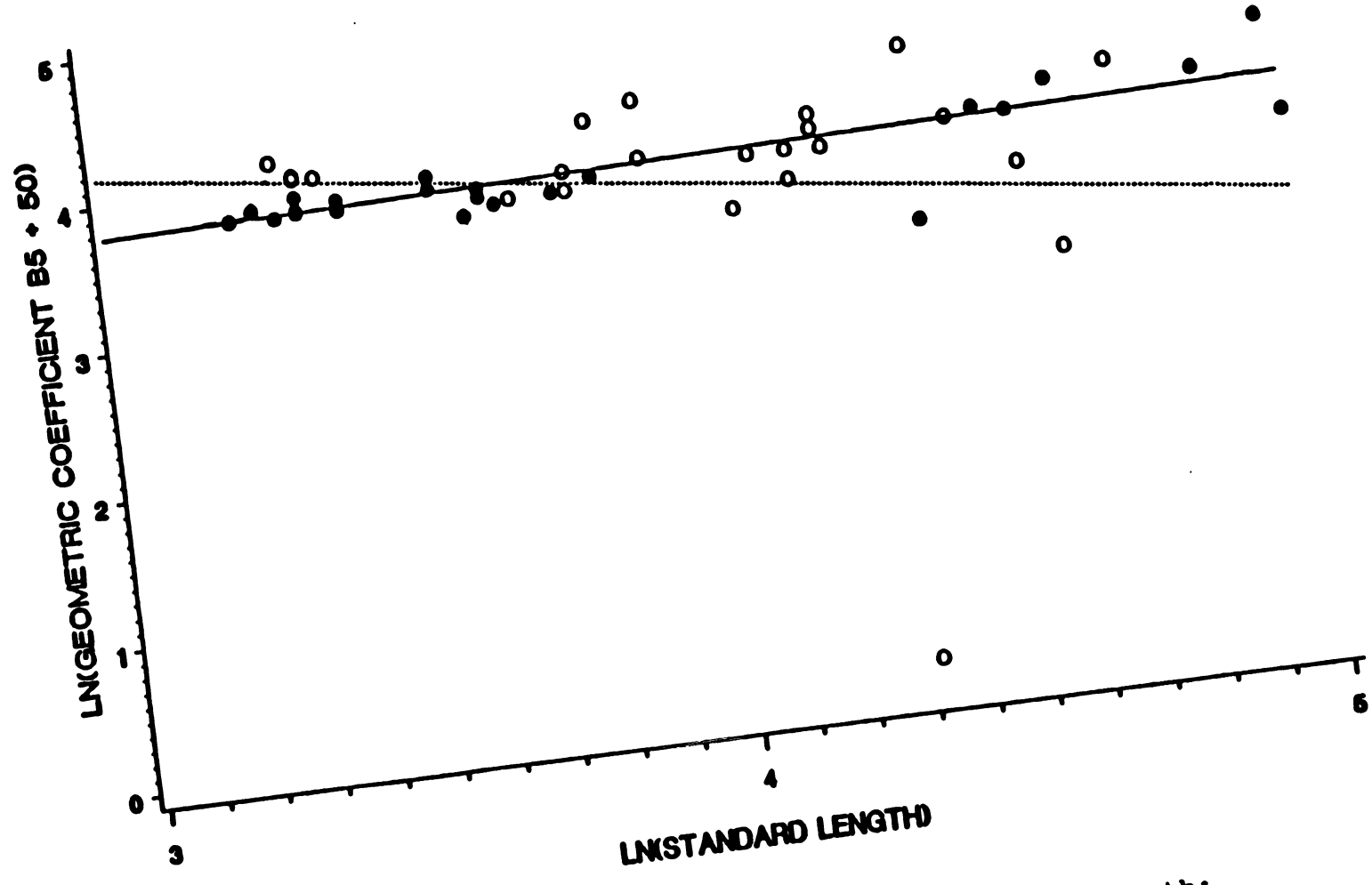


Figure 10. Regression for b_5 versus Standard Length: Natural Log-Natural Log Transformation.

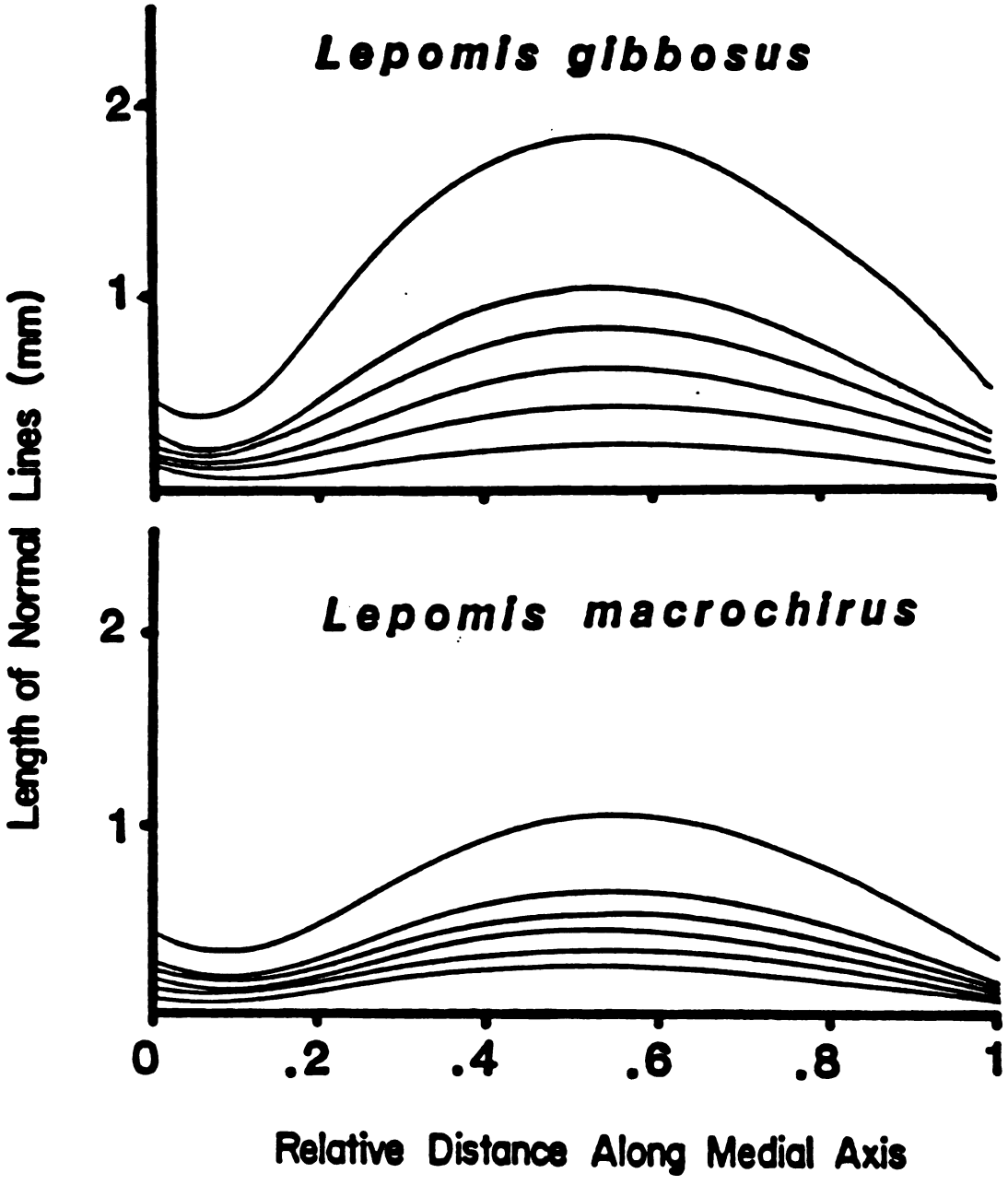


Figure 11. Expected Curve Shape of the Medial Side of the Fifth Ceratobranchial of Pumpkinseed and Bluegill Sunfish. Calculated Curves Constructed for Specimens Having Standard Lengths of 31, 41, 51, 61, 71, and 111 mm.

these two trajectories (Table 7). Although the posterior end of the ceratobranchial in pumpkinseeds averages slightly wider than in bluegills (Figure 4), the difference in regression intercepts is well within the range of variation seen within species. Both species appear to share a common pattern of growth of the posterior width of the fifth ceratobranchial, best described by the equation $y=0.0044x-0.0143$.

Coefficient b_1 describes the width of the anterior end of the ceratobranchial. The anterior portion of the lower pharyngeal jaws of adult pumpkinseeds is usually broader than in adult bluegills (Figure 5). The slope of the regression of coefficient b_1 on standard length is significantly greater (Table 6, $p<0.001$) in pumpkinseeds than in bluegills (Table 4: bluegills, slope= 0.00295; pumpkinseeds slope= 0.0061). The two regression lines intersect at a standard length of 34 mm and the data points at smaller sizes do not differ appreciably between species. The two species seem to diverge in anterior ceratobranchial width relatively early in ontogeny. Faster growth in this feature may provide pumpkinseeds with greater surface area for stronger articulation with the anterior pharyngeal elements to better support the large forces generated in crushing snails.

Divergence between species of the trajectory of b_2 is less marked than for b_1, b_3, or b_4. Coefficient b_2 is the tangent at the posterior end of the outline curve. The values of b_2 influences the shape of the geometric regression on the

posterior third of the curve (Figure 6). Slopes of the trajectories of b_2 are marginally distinguishable (p=.033; Table 6). Values of b_2 are consistently smaller in pumpkinseeds than in bluegills, even in the range of sizes (<40 mm) where the two species become indistinguishable in coefficients b_1, b_3, and b_4. Because the slopes of the trajectories differ between species, the trajectories must intersect. Whether they do at some ontogenetic stage before the earliest ones included here, or whether the ceratobranchials of the two species begin development with different values of b_2, can not be resolved with the data at hand. Within the ontogenetic range studied here, the contribution of b_2 to the shape of the posterior arm of the ceratobranchial decreases with size. The decrease is slightly faster in pumpkinseeds. Overall, the aspects of shape indexed by b_2 seems of minor importance in determining differences between bluegills and pumpkinseeds.

Coefficient b_3 is the tangent of the outline of the curve at the anterior end and influences the shape of the regression in the anterior third of the curve (Figure 7). As with coefficient b_2, coefficient b_3 decreases as body size increases (Figure 7). The decrease is faster in pumpkinseeds (Table 4; slope=-0.052) than in bluegills (Table 4; slope=-0.019). The two ontogenetic trajectories for coefficient b_3 intersect at 34 mm standard length (Figure 7) as do the trajectories for b_1. The ontogenetic trajectories for coefficient b_3 appear

to be negatively related to the ontogenetic trajectories of b_1, the width function of the anterior end of the ceratobranchial (Figure 5 and 7). However, the high degree of association between these two variables is accountable to high correlations with size (Table 5).

Coefficient b_4 represents how rapidly the tangent of the outline changes at the posterior end of the curve. Its value affects curve shape most in the posterior part of the middle third of the curve (Figure 8). Pumpkinseeds change significantly faster in this coefficient with size than do bluegills (Table 4: Bluegills slope=0.489; pumpkinseeds slope=1.065; Table 6: $p<0.001$). The ontogenetic trajectories of coefficient b_4 diverge earlier during ontogeny (at 28 mm standard length) than for the other coefficients (Figure 8). How the tangent of the outline changes near the posterior end of the ceratobranchial strongly influences the shape for the edge where the fifth ceratobranchials articulate. The more rapid growth of the tooth covered occlusive surface in pumpkinseeds produces the flared posterior edge of the fifth ceratobranchial (Figure 12). Examination of the ontogenetic trajectory of coefficient b_4 for other euteleost fishes may be useful in understanding the evolution of a fused fifth ceratobranchial in these pharyngognathous fishes.

The slopes of the ontogenetic trajectories for bluegills and pumpkinseeds were statistically indistinguishable in coefficients b_5 (Figure 9). This coefficient is the analog

of b_4 (Figure 8). Bluegill ceratobranchials change relatively little in coefficient b_5 over ontogeny (slope=0.045) but pumpkinseeds show a general decrease with size (slope=-0.23). However, the variance within species is considerable, preventing the test of parallelism of slopes from detecting differences between species. Unlike other coefficients, data for b_5 suggest that within species variation in this coefficient may increase with size. The variation in shape indexed by b_5 can not be explained with a size-based model.

The shapes of the fifth ceratobranchial in both sunfishes was adequately modeled by the geometric quintic regression. Figure 11 displays the expected medial outlines of this bone in pumpkinseeds and bluegills at different sizes (along a "straightened" medial axis abscissa). This figure summarizes visually the growth trajectories illustrated numerically in Figures 4 through 9. The posterior end of the ceratobranchial developed similarly in the two species. Although similar at young stages, the width of the anterior tip of the ceratobranchial grows faster in pumpkinseeds than in bluegills yielding an adult pumpkinseed ceratobranchial with a wider anterior end. The slopes or tangents of the posterior and anterior regions of the ceratobranchial are negative and become more steeply

Table 6. Statistical Tests for Regression Line Parallelism for Inter-Species Comparisons of the Geometric Coefficients versus Standard Length Regressions.

Coefficient b_0

Source of Variation	Sum of Squares	df	Mean Square	F	Sig. of F
Within+Residual	.09285	45	.00206		
SL	1.14551	1	1.14551	555.148	0
Species	.00354	1	.00354	1.715	.197
SL by Species	.00003	1	.00003	.015	.904ns
(model)	1.14908	3	.38303	185.626	0
(total)	1.24194	48	.02587		

ns = not significant

Coefficient b_1

Source of Variation	Sum of Squares	df	Mean Square	F	Sig. of F
Within+Residual	.30503	46	.00663		
SL	.79497	1	.79497	119.888	0
Species	.06994	1	.06994	10.548	.00218
SL by Species	.10610	1	.10610	16.000	.00023***
(model)	.97101	3	.32367	48.812	0
(total)	1.27604	49	.02604		

*** p <.001

Table 6 (cont'd.).

Coefficient b_2

Source of Variation	Sum of Squares	df	Mean Square	F	Sig. of F
Within+Residual	21.86753	46	.47538		
SL	76.02410	1	76.02410	159.922	0
Species	10.23439	1	10.23439	21.529	.00003
SL by Species	2.28600	1	2.28600	4.809	.03341*
(model)	88.54449	3	29.51483	62.087	0
(total)	110.41202	49	2.25331		

* p <.05

Coefficient b_3

Source of Variation	Sum of Squares	df	Mean Square	F	Sig. of F
Within+Residual	38.77362	46	.84290		
SL	42.18086	1	42.18086	50.042	0
Species	7.47786	1	7.47786	8.872	.00461
SL by Species	11.68191	1	11.68191	13.859	.00054***
(model)	61.34064	3	20.44688	24.258	0
(total)	100.11426	49	2.04315		

*** p <.001

Table 6 (cont'd.).

Coefficient b_4

Source of Variation	Sum of Squares	df	Mean Square	F	Sig. of F
Within+Residual	5851.50169	46	127.20656		
SL	22544.35223	1	22544.35223	177.226	0
Species	3824.36815	1	3824.36815	30.064	1.71E-6
SL by Species	3552.78130	1	3552.78130	27.929	3.36E-6***
(model)	29921.50168	3	9973.83389	78.407	0
(total)	35773.00337	49	730.06129		

*** p <.001

Coefficient b_5

Source of Variation	Sum of Squares	df	Mean Square	F	Sig. of F
Within+Residual	7360.20445	46	160.00444		
SL	6.62088	1	6.62088	.041	.83970
Species	19.00690	1	19.00690	.119	.73192
SL by Species	611.66709	1	611.66709	3.822	.05665ns
(model)	637.29488	3	212.43163	1.328	.27690
(total)	7997.49932	49	163.21427		

* p <.05

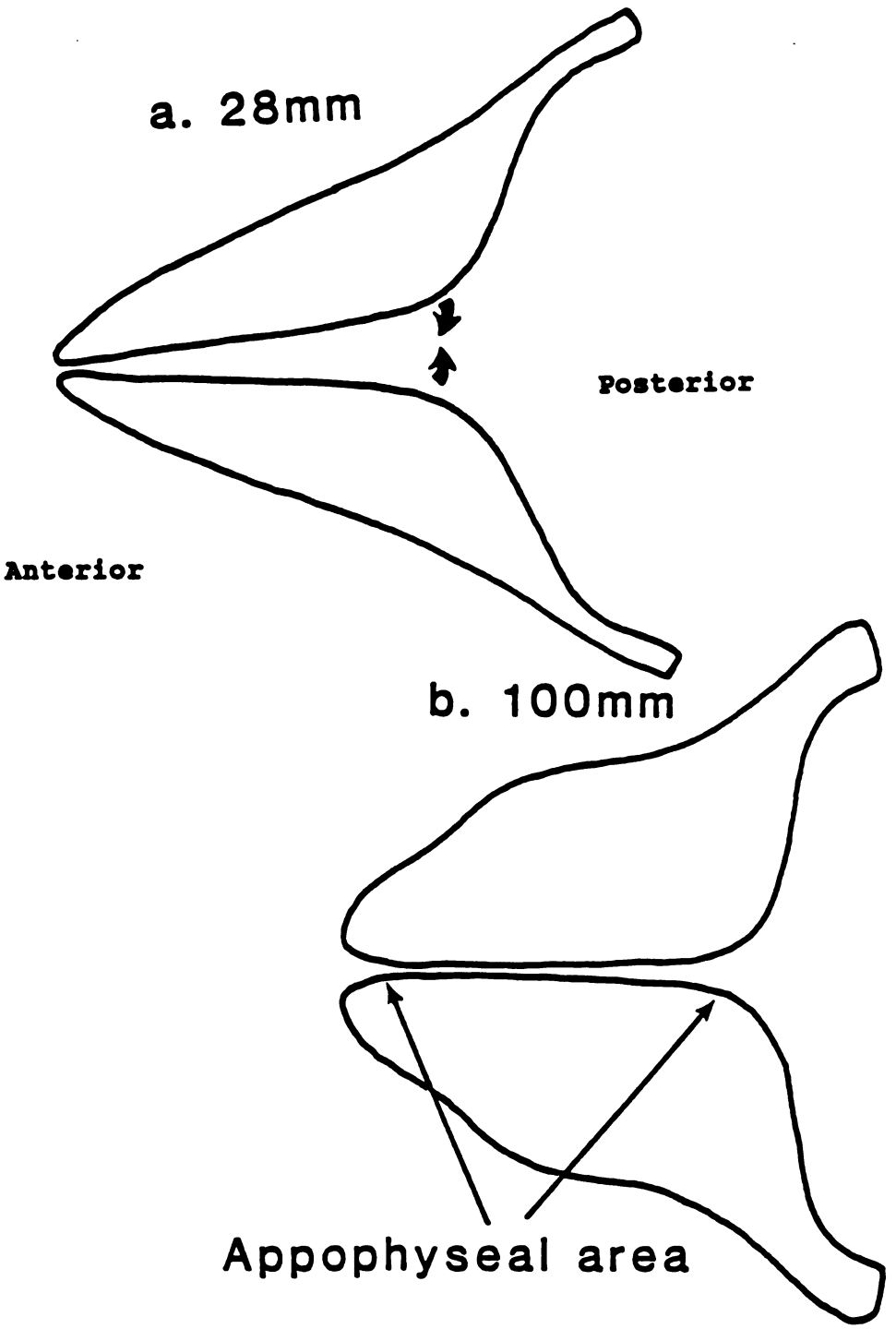


Figure 12. Growth Pattern of the Appophyseal Edge in the Fifth Ceratobranchial of Pumpkinseed Sunfish.

negative with increased body size. The slope of the outline at each end was always more steeply negative in pumpkinseeds than in bluegills. The slope of the outline at the posterior end changes faster in pumpkinseeds than in bluegills. This influences the shape of the tooth covered occlusive shelf of pumpkinseeds which forms the edge along which the two ceratobranchials articulate. The ontogenetic trajectory for the influence of anterior curve acceleration was quite variable among individuals and this aspect of ceratobranchial shape is probably explained better by non-ontogenetic variables.

Table 7. Calculated Geometric Coeffients for Expected Ceratobranchial Shapes Across Ontogeny.

Lepomis gibbosus (Linnaeus)

Standard Length	b_0	b_1	b_2	b_3	b_4	b_5
31	0.13215	0.05313	-1.5155	-0.6354	18.0109	-1.267
36	0.15415	0.08363	-1.7170	-0.8969	23.3339	-2.423
41	0.17615	0.11413	-1.9185	-1.1584	28.6569	-3.579
46	0.19815	0.14463	-2.1200	-1.4199	33.9799	-4.735
51	0.22015	0.17513	-2.3215	-1.6814	39.3029	-5.891
56	0.24215	0.20563	-2.5230	-1.9429	44.6259	-7.047
61	0.26415	0.23613	-2.7245	-2.2044	49.9489	-8.203
66	0.28615	0.26663	-2.9260	-2.4659	55.2719	-9.359
71	0.30815	0.29713	-3.1258	-2.7274	60.5949	-10.515
76	0.33015	0.32763	-3.3290	-2.9889	65.9179	-11.671
111	0.48415	0.54113	-4.7395	-4.8194	103.1789	-19.763

Table 7 (cont'd.).

Lepomis machrochirus Rafinesque

Standard Length	b_0	b_1	b_2	b_3	b_4	b_5
31	0.11205	0.06298	-0.8931	-.75063	16.0284	-4.3358
36	0.13405	0.07773	-1.0568	-.84693	18.4726	-4.1122
41	0.15605	0.09248	-1.2204	-.94323	20.9168	-3.8887
46	0.17805	0.10723	-1.3841	-1.0395	23.3610	-3.6651
51	0.20005	0.12198	-1.5477	-1.1358	25.8052	-3.4416
56	0.22205	0.13673	-1.7114	-1.2321	28.2494	-3.2180
61	0.24405	0.15148	-1.8750	-1.3284	30.6936	-2.9945
66	0.26605	0.16623	-2.0387	-1.4247	33.1378	-2.7709
71	0.28805	0.18098	-2.2023	-1.5210	35.5820	-2.5474
76	0.31005	0.19573	-2.3660	-1.6173	38.2618	-2.3238
111	0.46405	0.29898	-3.5115	-2.2914	55.1314	-0.7590

DISCUSSION

The morphometric technique presented here uses the medial axis in a nonconventional way to describe the ontogenetic shape changes of the fifth ceratobranchial in pumpkinseed and bluegill sunfish. Original applications of the medial axis were used to visually represent complex shapes (Oxnard, 1980) or to provide landmarks for measuring lengths and angles of medial axis arcs (Bookstein et. al., 1985). The technique used for this study is nonconventional to these other medial axis applications in that the medial axis is used as a landmark for setting a coordinate system for numerically analyzing the curvature of the ceratobranchial bone. Numerical approaches are easier than purely visual descriptive methods for discerning subtle changes in shape and provide the means for statistical evaluation of shape changes. For centrarchid ceratobranchials, the medial axis is ideal for testing whether this technique can provide a means to mathematically model curvature of this simple landmark free form. Limitations of the technique are few and may be particular to this data set. Most difficulties encountered usually were involved with digitizing the ceratobranchial outline. These situations were easily avoided by tracing the outline of the form on clear acetate.

The medial axis for centrarchid fifth ceratobranchial bones features a long arc which represents the anterior-posterior axis. This major axis of the ceratobranchial was always present in both bluegill and pumpkinseed sunfishes. The major axis of the fifth ceratobranchial is the "operational homology" used for comparative purposes in this study. Branch points were usually found only at the endpoints of the medial axis in bluegill ceratobranchials and were present there as well as elsewhere along the medial axis in pumpkinseed ceratobranchials. Because of the noise in digitizing the posterior end of the ceratobranchial (J.E. Zablotny, pers. obs.), branch lengths at the posterior end of the fifth ceratobranchial were not included within the sample of normal lines. The side branches at 45 to 58% of the distance along the major medial axis were only found in large individual pumpkinseeds and in only two of the larger bluegill specimens.

The individual ontogenetic trajectories of the shape descriptors of the fifth ceratobranchials in bluegill and pumpkinseed sunfish are linearly related to size. This is a nontrivial result; inspection of an age series of ceratobranchials would not easily suggest a linear shape change over ontogeny because of the curvature of the bone. This seems contradictory to the expected since shape change is usually modeled as a nonlinear ontogenetic process (e.g. Alberch et. al., 1979). The curvilinear relationship of shape

with time should asymptote to a target adult morphology. Perhaps, the strictly linear change of shape descriptors with size found here is due to bias in sampling fishes within 25 and 155 mm standard length. This size range is only a subset of the possible size range (both species can exceed 350 mm standard length; Lee et. al. 1980) and especially excludes large male bluegills. However, very large specimens of bluegill and pumpkinseed sunfish are uncommon in nature and none greater than 150 mm were available for this study. The bias towards small individuals reflects the availability of specimens from museums. It is likely that large specimens greater than 150 mm may display different ontogenetic trajectories for shape descriptors of the fifth ceratobranchial than the values reported here.

Size also has some drawbacks as an index of age in these centrarchids. Growth rates in fishes are directly related to available resources and overcrowding tends to reduce resource availability and to decrease growth rates. Dominey (1980) has shown that size related alternate breeding strategies for centrarchids occur in naturural populations as well. Dominey (1980) discovered subtle phenotypic differences between "sneaker" and territorial breeding male bluegill. Rapid sexual development in "sneaker" males may indirectly affect the development of other structures. The sexual condition of the specimens used in this study was not ascertained and this phenomena may have contributed to the variance observed in the

shape descriptors that were correlated with size. However, the relatively tight associations between shape descriptors and size suggests that these possible drawbacks pose little problem for this study; if anything, identification of these effects would probably increase the goodness of fit of the linear relationships.

The specializations in the shape of the fifth ceratobranchial in adult pumpkinseeds are presumably derived from an ancestral shape within Lepomis. Phylogenetic understanding of the genus Lepomis is limited at this time. The phylogenetic arguments for the evolution of trophic specialization in Lepomis presented here are generalizations based on current knowledge of the group. Humphries (pers. com.) suspects that the warmouth sunfish, Chaenobryttus gulosus, is the outgroup for Lepomis. Initial changes in the shape of the fifth ceratobranchial probably were derived from an ancestral sunfish possessing similar pharyngeal jaw morphology to the pharyngeal jaw type found in Chaenobryttus. As adults, species of Lepomis have lower pharyngeal jaws featuring various degrees of apposition between right and left fifth ceratobranchial bones (see Trautman, 1981, p. 585, Fig.141, #11; p.589, Fig. 142, #9; p.580, Fig. 140, #9; and p. 599, Fig. 145, #10). By visual inspection, bluegill fifth ceratobranchials are intermediate in the extent of apposition and I base the changes in morphology of pumpkinseed ceratobranchials in terms of deviation from bluegill

pharyngeal jaw shape. Small individuals of species of Lepomis appear to have apposition sites similar to the apposition site in warmouth sunfish lower pharyngeal jaws.

The primitive ceratobranchial shape features a rather narrow occlusive shelf filled with fine, filiform teeth. Additionally, primitive fifth ceratobranchials possess a narrow apophysis or articulating surface located anteriorly between the right and left fifth ceratobranchials elements (Figure 8). The ceratobranchial shapes mostly associated with snail crushing include: a broad crushing surface studded with molariform teeth, a broad region apposition of the left and right halves of the lower jaw and an increase in the lateral depth of the ceratobranchial. Ideally, the processes of morphological specialization in the fifth ceratobranchial need be addressed to understand the type and roles of mechanisms which have occurred in the evolution of the genus Lepomis.

The process for morphological differentiation in trophic structures of the Centrarchidae have not been addressed. Fink (1982) mentions that in nature "changes in developmental timing and their epigenetic consequences are suspected of being instrumental in acquisition of evolutionary novelties, including those often major changes associated with large scale cladal diversity." The gastropod crushing type of pharyngeal jaw develops from a precursor shape common to bluegill sunfish. All of the shape descriptors within this study are similar between bluegills and pumpkinseeds at some

point in development, and some descriptors have identical trajectories in both species. This means that both species have common shapes at some time in development (Figure 9). For body sizes less than 35 mm standard length, pumpkinseeds and bluegills have lower pharyngeal jaws of very similar shape. The ceratobranchial elements of the viscerocranium are endochondral (Harder, 1975) and do not begin to ossify until fish exceed 20 mm standard length. The data from this study do not address species differences in growth or shape of the cartilaginous model of the fifth ceratobranchial. However, the data strongly suggest that divergence in shape occurs at sizes greater than 34 mm in standard length. Since the cartilaginous model is nearly (but not completely) ossified in fishes of about 28 mm standard length (J. E. Zablotny, pers. obs.), the ontogeny of the bony fifth ceratobranchial begins at a very similar shape in both species.

The shape descriptors exhibiting wide differences in ontogenetic slopes between pumpkinseeds and bluegills always have greater absolute values of slopes in pumpkinseeds. The morphological differences in the two species of centrarchid lower pharyngeal jaws may be easily explained in terms of a common hetrochronic change. Increased ossification rates of the occlusive tooth bearing surface of the pharyngeal jaw in pumpkinseeds accelerate the development of width in pumpkinseed pharyngeal jaws as compared to pharyngeal jaw development in bluegills.

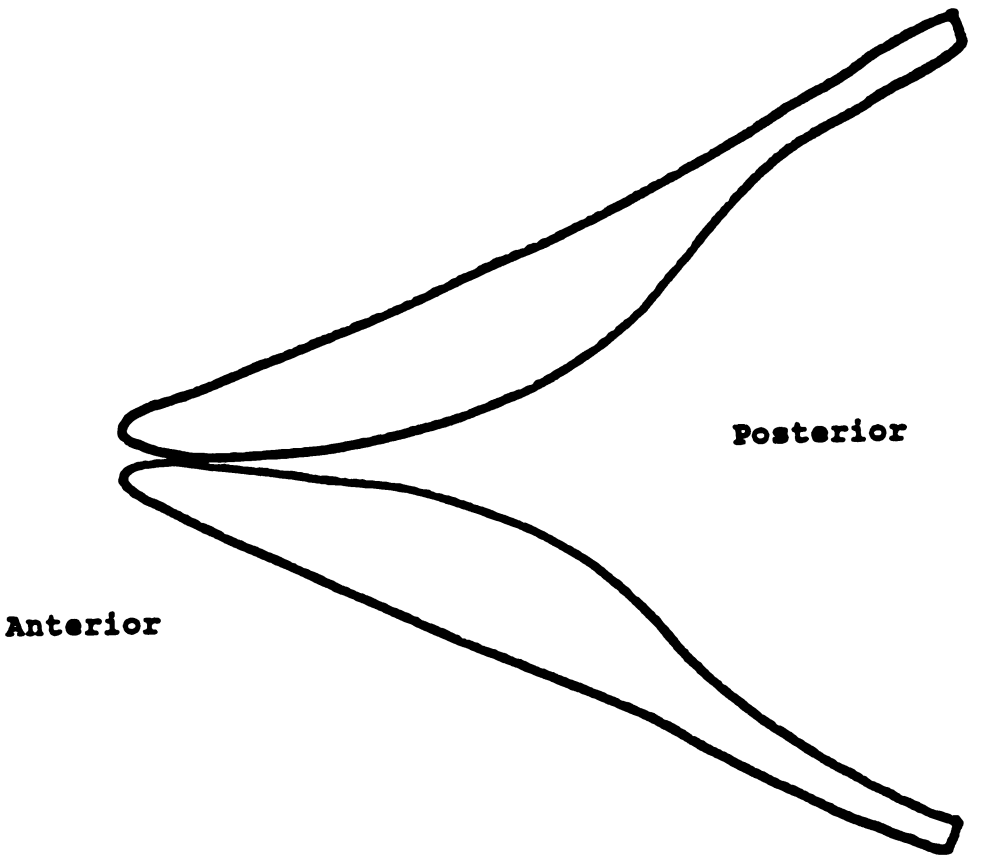


Figure 13. Primitive Fifth Ceratobranchial Shape: Chaenobryttus gulosus

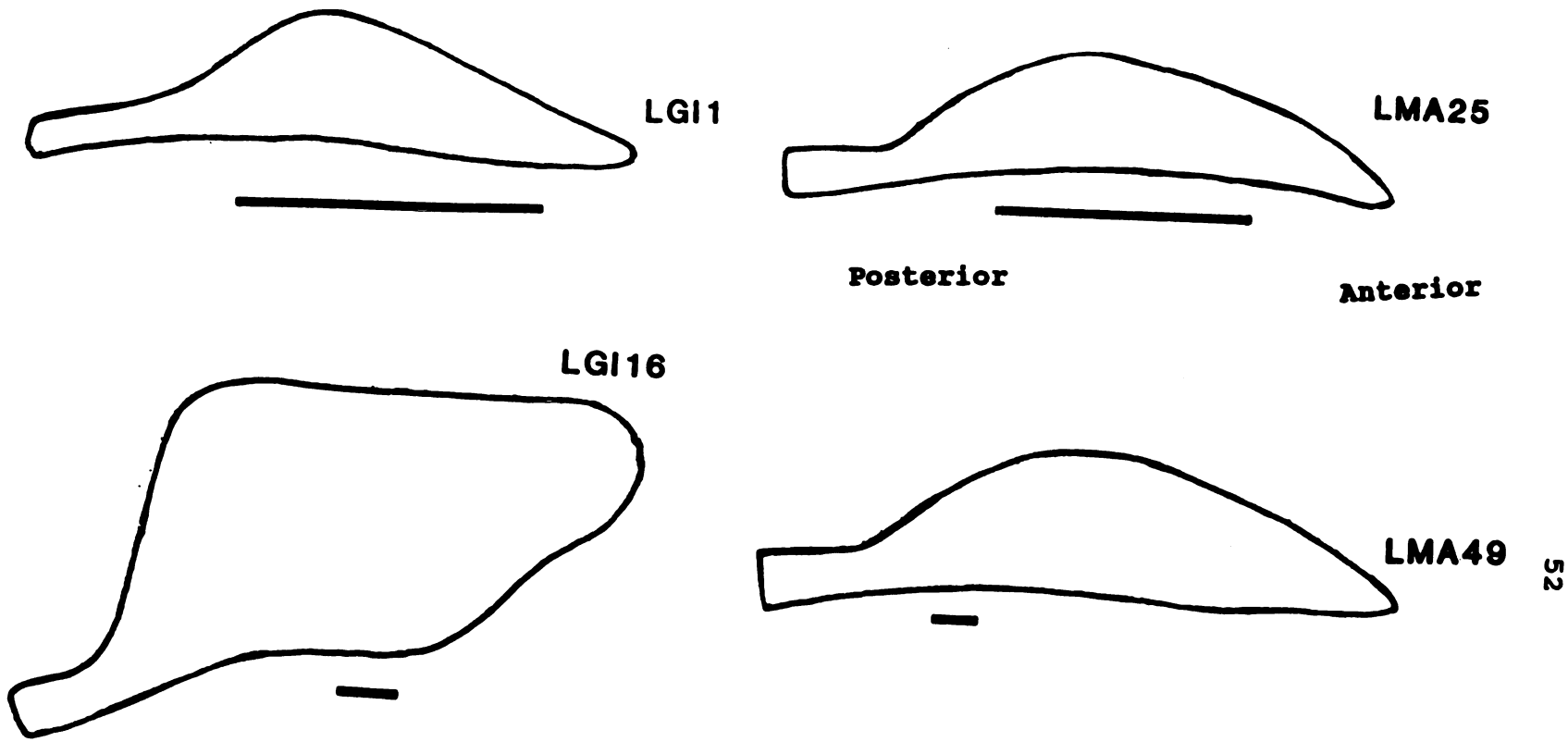


Figure 14. Similarity in Fifth Ceratobranchial Shape Between Small (<28 mm standard length) Pumpkinseeds and Bluegills.

DEVELOPMENTAL RELATIONSHIP BETWEEN MORPHOLOGY AND BEHAVIOR

Mayr (1960, 1976) proposed that behavior always precedes morphological change during evolution. Although Bock (1976) mentions that the study of structures by themselves may not be adequate to evaluate adaptation, the shape analysis techniques employed in this study, and published ecological data on sunfish feeding, provide the means for examining ontogenetic shape change as a set of continuous values and permit hypotheses construction in terms of ontogenetic change and ecological correlation. The appearance of new behaviors may invoke changes in the selection of structures that are used in the behavior pattern (Mayr, 1960).

The data from this study reveals that pumpkinseed fifth ceratobranchial shape is well differentiated from bluegill ceratobranchial shape at approximately 50 mm standard length. The evidence I gathered suggests that the initial shaping of the fifth ceratobranchial appears approximately from 28 to 34 mm standard length, well before the trophic switch to snails by pumpkinseeds. However, Mittlebach (1984) notes small pumpkinseeds do include a small portion of pond snails in the diet whenever small hatchling snails are present. Young pumpkinseeds may be severely constrained by gape size which prevents them from harvesting larger snails. Smaller pumpkinseeds are also known to crush small snails during times of high availability (Mittlebach, 1984). However, these snails make up only a small percentage of the total diet and

do not reflect a major change in diet for small pumpkinseeds (Mittlebach, 1984). This may reflect the lack of morphological specialization in small pumpkinseeds which is necessary for the fifth ceratobranchial to structurally accommodate the compressive forces produced by pumpkinseeds during bouts of snail crushing.

Morphological constraints may negatively affect the energetic reward for switching to other prey types in response to interspecific competition. Behavioral plasticity in switching behavior appears to occur in nature. For localities in South Michigan, Mittlebach (1984) noted that pumpkinseeds begin switching to snails from 45 mm to 50 mm in standard length. Pumpkinseeds greater than 70 mm standard length readily take snails as the major dietary component. Ontogenetic changes in the shape of the fifth ceratobranchial with body size in pumpkinseeds may be associated with a decrease in handling times for processing pond snails as body size increases in pumpkinseeds (Mittlebach, 1983). Osenberg and Mittlebach (1988) note that large pumpkinseeds have a greater probability of crushing a snail shell than small pumpkinseeds. Although small pumpkinseeds are able to crush snails, these individuals require longer handling times and tend to reject more snails that are unsuitable for crushing (Osenberg, 1988). The behavioral switch to snails in pumpkinseeds may be consistent for regions where other sunfishes are in sympatry. Ontogenetic shape constraints for

pumpkinseeds may not provide competitive parity with other centrarchid species within the community.

Likewise, pumpkinseeds are usually the only representative Lepomis and mollusc crushing fish species in the northern part of its range. At the time of Pleistocene glaciation, Bailey and Smith (1981) noted that L. gibbosus was the only Lepomis to occupy the Atlantic drainage refugia. Scarola (1979) mentioned that pumpkinseeds are the most common sunfish in the Northeast. The feeding habits for allopatric populations of pumpkinseeds differ from those found in sympatric with other centrarchids. Confer and Blades (1975) remarked that pumpkinseeds from New England ponds utilize a stereotypical behavior when capturing suspended zooplankton, a preferred prey item found in open water habitat. Apparently pumpkinseeds can be encountered in littoral zone habitat in some of these Northeastern lakes (C. Folt, pers. com.). In New Brunswick, Reid (1930) stated that dragonfly nymphs were the preferred prey item taken by pumpkinseeds. The causal agents for this behavior pattern and the associated pharyngeal jaw morphology of these populations have yet to be studied. The appearance of crushing behavior in pumpkinseeds may be initiated by the presence of superior competitors that prey on zooplankton.

Pumpkinseeds from different lake communities may be under different selection pressures for rates of development in the pharyngeal jaws. I hypothesize that pumpkinseeds from high

competitive environments should express greater developmental acceleration for producing the crushing type morphology than those from noncompetitive lake systems. Behavioral plasticity in switching behavior may indirectly provide the natural selection for increased rates of growth and shape modification of the pharyngeal jaws in pumpkinseeds in competitive environments.

Resource partitioning appears to be a major mechanism for alleviating competitive effects in centrarchid communities (Werner and Hall, 1976) and may factor heavily in the natural selection of trophic morphology. Keast (1980) remarks that resource partitioning is commonly used by larval and post larval fish communities at peak periods of utilization while these young fishes are relatively undifferentiated regarding trophic structures. Similarly, there are no significant morphological differences between bluegills and pumpkinseeds less than 34 mm standard length that might serve to alleviate interspecific competition. For specimens less than 35 mm standard length, t-tests show no significant differences between bluegills (n=9) and pumpkinseeds (n=6) for the shape descriptors b_0, b_1, b_4, low significance for b_2, and b_3 and high significance for b_5. However, this high significant difference between pumpkinseeds and bluegills for coefficient b_5 is suspect due to the low correlation with standard length.

Resource partitioning in larger centrarchids has also been noticed for competition based centrarchid communities.

Werner and Hall (1976) demonstrate decreased growth rates in addition to niche shifting for coexisting pumpkinseed, bluegill, and green sunfishes. Werner and Hall (1976) believe that the niche shifting into suboptimal diets are results of interspecific competition in centrarchid fish communities. High predation risk by largemouth bass also forces small bluegills to switch to vegetation based prey from open water prey (Werner et. al 1983). Unlike pumpkinseeds, the switch in habitat type and prey is not correlated with morphological changes in the pharyngeal jaw of bluegills. The shape analysis of the fifth ceratobranchial hints that a morphological bottleneck may prevent young pumpkinseeds from effectively using feeding refuges distinct from the diets of its competitors while at small body sizes. Examination of the plots of the expected shapes (Figure 5) indicates strong similarity in shape of pumpkinseed ceratobranchials to bluegill ceratobranchials for the smaller size classes.

MORPHOLOGICAL PLASTICITY

Epigenetic modification (Waddington 1953, 1956a, 1956b) or morphological plasticity (Smith-Gill, 1983) occurs in the trophic structures of fishes. Meyer (1987), Sage and Selander (1975), and Kornfield and Taylor (1983) all noted the presence of phenotypic plasticity in trophic morphology of certain species of cichlid fishes. Meyer (1987) strongly suggests different feeding behaviors as the principal influence on trophic morphology in Cichlasoma managuense. Rubin and Lanyon

(1984) mention that minor alterations in loading on bone can drastically remodel bone.

Within the Centrarchidae, Ehlinger and Wilson (1987) found intraspecific morphometric differences between bluegills occupying open water and vegetation habitat types. The pumpkinseed sunfish may be the best species for testing hypotheses of environmental influences on the shaping of trophic morphology. The wealth of ecological and morphological data on pumpkinseeds provide adequate background for understanding the sources of morphological plasticity observed in nature. Unlike redear sunfish, pumpkinseeds switch feeding modes between crushing and pharyngeal transport when presented hard and soft food types (Lauder, 1983). Pumpkinseeds may experience force loading during snail crushing that vastly differ from the forces produced by pharyngeal transport which may cause new bone remodeling patterns in the ceratobranchial. Detailed captive breeding studies may also help to separate any epigenetic effects of behavioral plasticity that may confound hypotheses concerned with the genetic basis off structural shapes. I suggest further studies be undertaken to evaluate the plasticity of diet switching with regards to community structure in pumpkinseeds and to correlate the impact of switching behavior to the ontogenetic trajectory of the morphogenesis of the fifth ceratobranchial bone.

INFLUENCES OF BEHAVIOR ON MORPHOLOGICAL DIVERSIFICATION

Mayr (1976) noted that behavioral changes and the change in selection pressure usually cause rampant structural reorganization in the organism. In the centrarchids, no major repatterning of muscle homologies are observed in the pharyngeal region. Lauder (1983) tested for differences in muscle activity and morphology between snail crushing and zooplankton feeding species of centrarchids. According to Lauder (1983) the bluegill and pumpkinseed share the derived condition ("thickened dorsal intermuscular aponeurosis, left and right obliquus dorsalis 2 and anterior transervsus dorsalis do not form a continuous sheet of muscle at the midline") over the primitive condition ("obliquus dorsalis 4 passes anteromedially beneath the posterior transverse dorsalis anterior fibers") found in Micropterus and the green sunfish Lepomis cyanellus. This structural repatterning found in the upper pharyngeal jaw structure is not correlated with the behavior shift in pharyngeal jaw manipulation and appears to have arisen independently of the behavior modification in the centrarchids. Greater cross sectional areas have been found in the fifth branchial adductor, levatores externi 3 and 4, pharyngocleithralis externus and internus, and pharyngohyoideus muscles for pumpkinseeds as compared with bluegill pharyngeal musculature (Lauder 1983).

Lauder (1983) noted most changes in the musculature of the pharyngeal jaws involve an increase in area of the physiological cross section of pharyngeal musculature between

zooplankton feeders and gastropod crushers. Major differences in function result from changes in muscle firing patterns with modification in fifth ceratobranchial shape rather than through the evolution of new muscle groups. For Lepomis species feeding on zooplankton or soft bodied prey, pharyngeal transport involves an overlapping activity pattern of the pharyngocleithralis internus with the retractor dorsalis. Both the upper and lower pharyngeal jaws retract simultaneously, moving the food items into the esophagus (Lauder, 1983). Coactivation of the pharyngeal muscles adduct the upper and lower pharyngeal jaws during stereotypical crushing mode (Lauder, 1983). The redear, pumpkinseed, and green sunfish depend on this behavioral pattern for crushing snail shells (Lauder, 1983).

Changes in the shape of the lower pharyngeal jaw correlate well with the appearance of ecological and species diversification in fishes. Unlike the more derived pharyngognath teleost fishes like the Cichlidae, Embiotocidae, Labridae, Odacidae, and Scaridae (Liem and Greenwood, 1981), the centrarchidae all share the primitive condition of possessing an unfused lower pharyngeal jaw. The medial sides of the fifth ceratobranchials closely appose each other in pumpkinseed specimens larger than 75 mm standard length (J. E. Zablotny, pers. obs.). I hypothesize that the broadly apposing medial edges of the paired fifth ceratobranchial in large pumpkinseeds serves to reduce the degrees of freedom of

movement of the lower pharyngeal jaw while undergoing snail crushing. To promote crushing efficiency, this may prevent the halves of the ceratobranchials from yielding and folding around a gastropod shell during adduction of the pharyngeal jaws. The nonspecialized centrarchidae feature a narrow region of close apposition in the lower pharyngeal jaw as opposed to the broad zone of contact found in pharyngognath gastropod crushers like the pumpkinseed and redear and pumpkinseed sunfish.

The results of this study reveal that a geometric quintic is reliable for modeling the ontogenetic shape changes in the fifth ceratobranchial bone in pumpkinseed and bluegill sunfish. Pumpkinseeds appear to diverge in ceratobranchial shape from bluegills approximately 35 mm standard length, well before the ontogenetic diet shift to snails. It is suggested that the plasticity in switching behavior may influence selection for more rapidly developing crushing morphology in pumpkinseeds from highly competitive habitats than those from less competitive habitats. Epigenetic influences of switching behavior on fifth ceratobranchial morphology may also occur. Comparisons of populations of pumpkinseeds allopatric and sympatric with other centrarchid species may be of use in testing for epigenetic and evolutionary changes in morphological shape of the fifth ceratobranchial. Finally, the centrarchids of the genus Lepomis may provide insight into understanding the evolution of more ecologically complex,

speciose assemblages of teleost fishes.

APPENDICES

APPENDIX A

DIGITIZED OUTLINES AND MEDIAL AXES

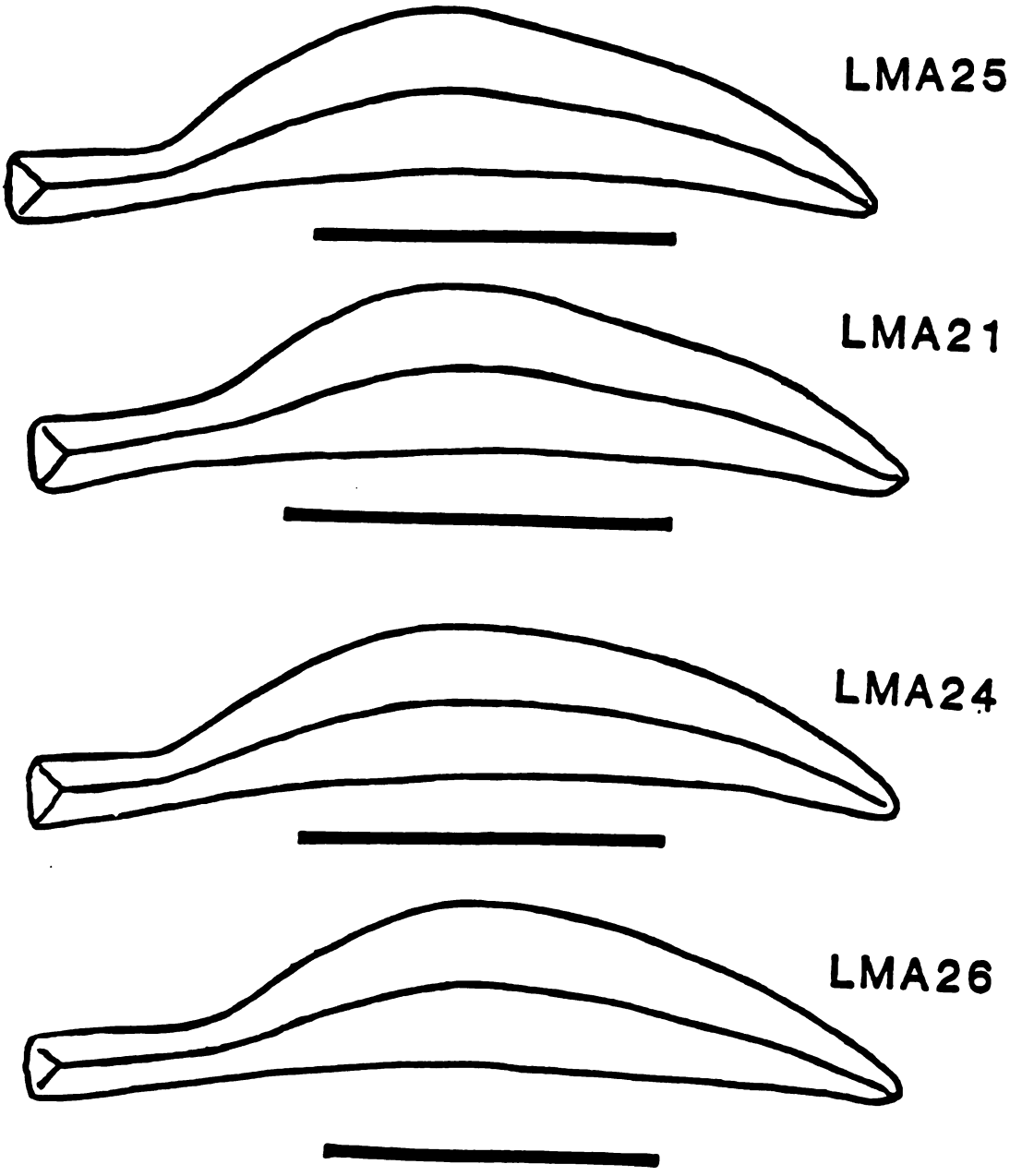


Figure 15. Digitized Outlines and Medial Axes of Bluegill Sunfish. Specimens are Ranked by Size from Small to Large. Scale Distance is Equal to One Millimeter.

Figure 15. (cont'd.).

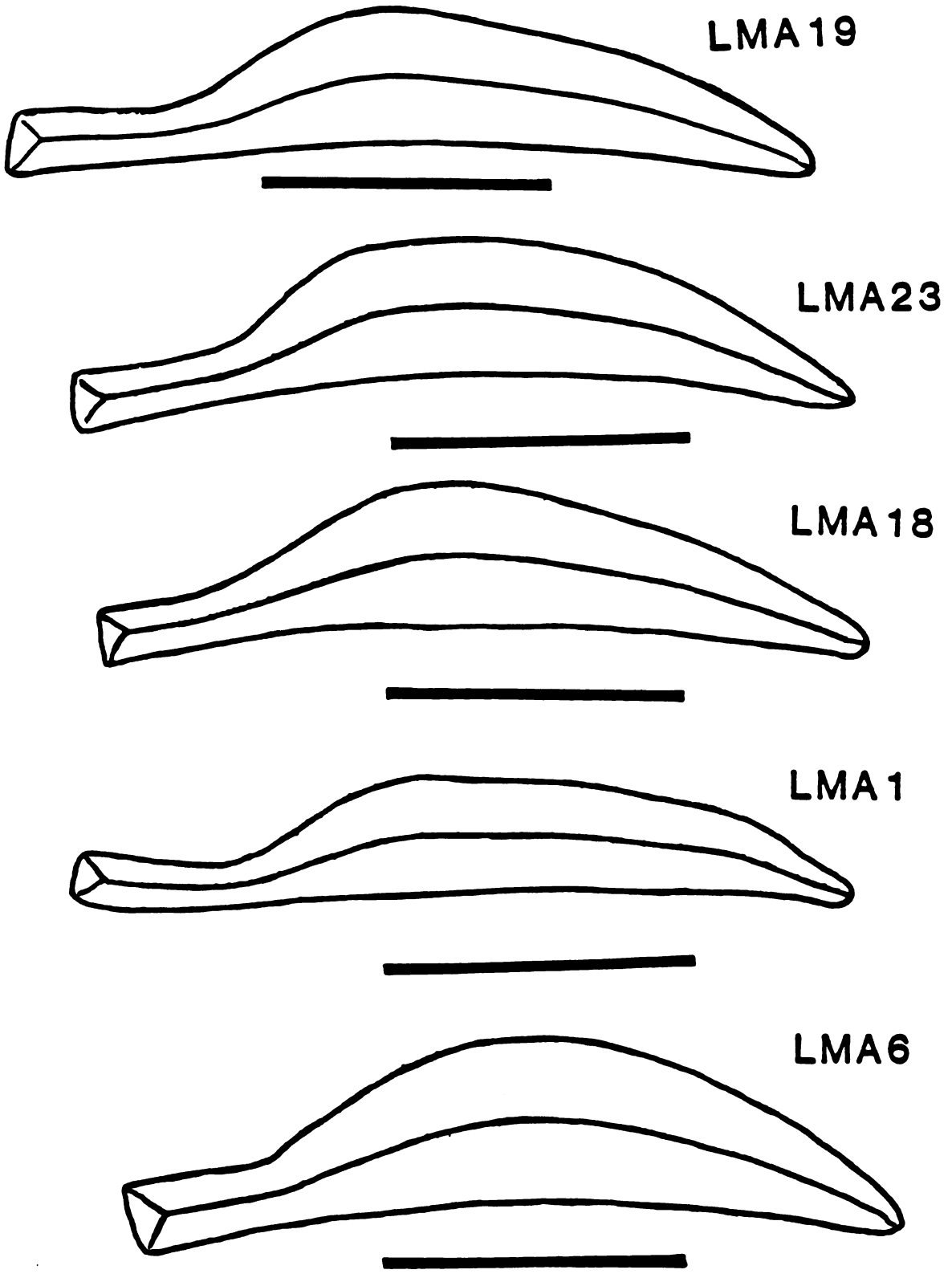
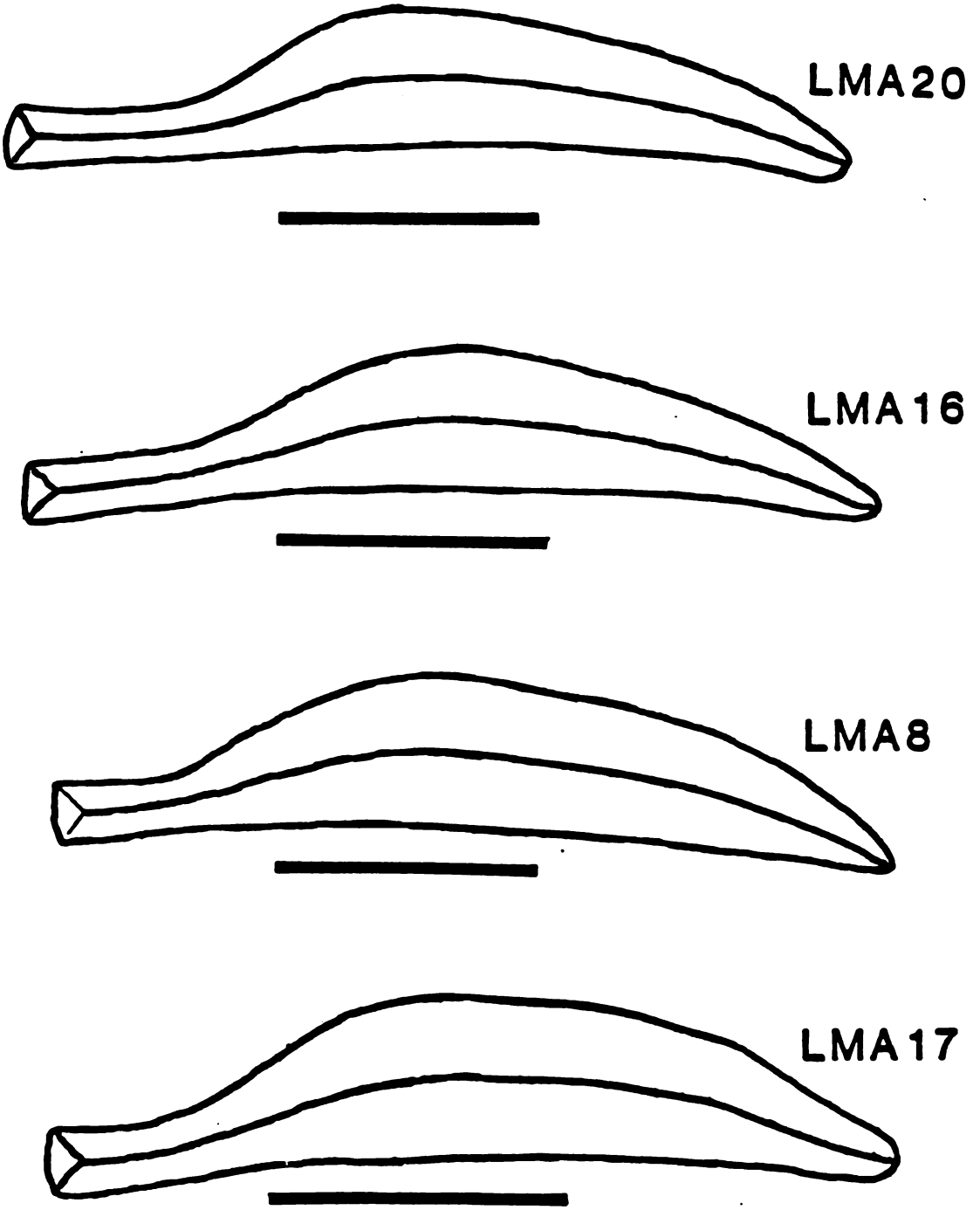


Figure 15. (cont'd.).



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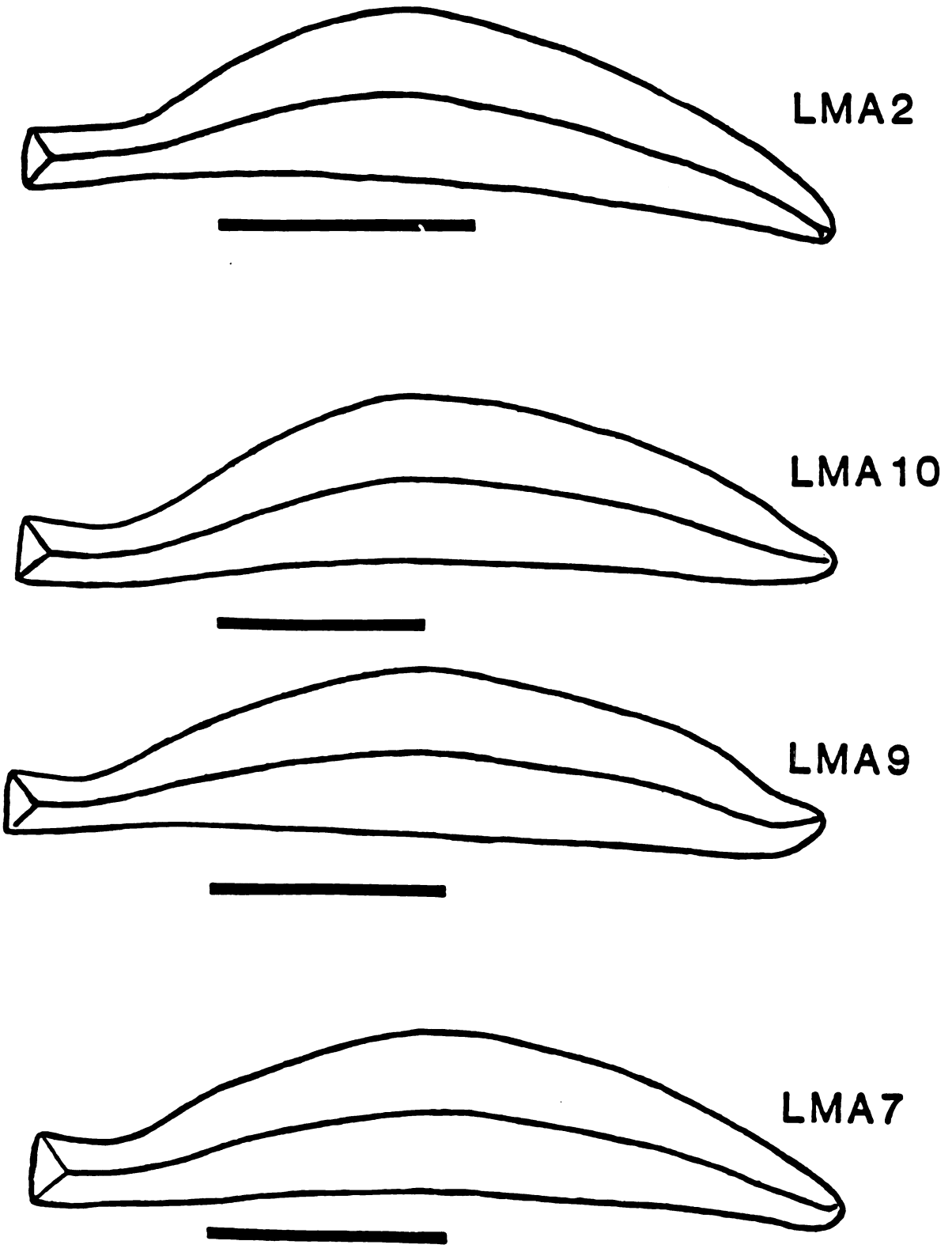


Figure 15. (cont'd.).

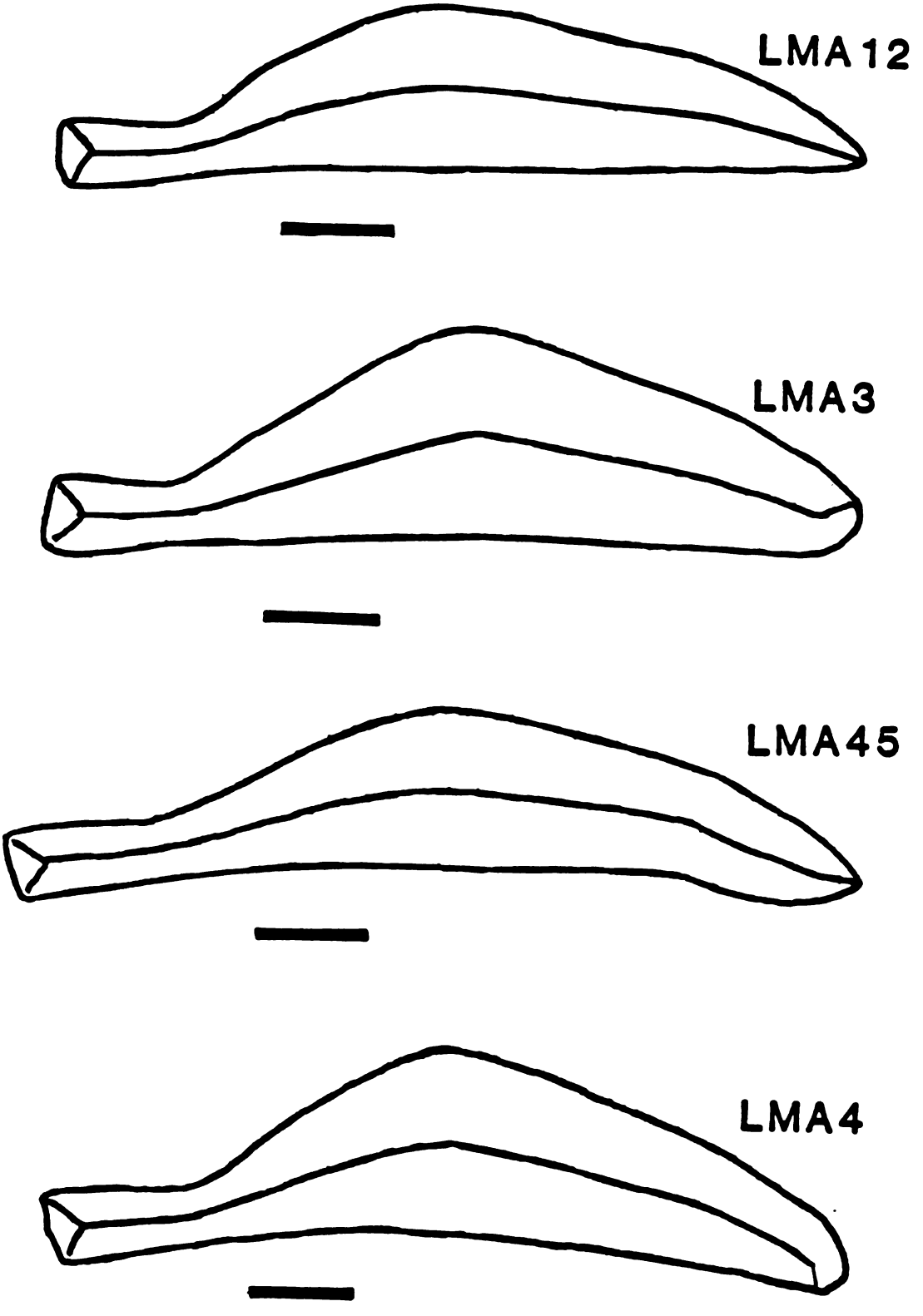
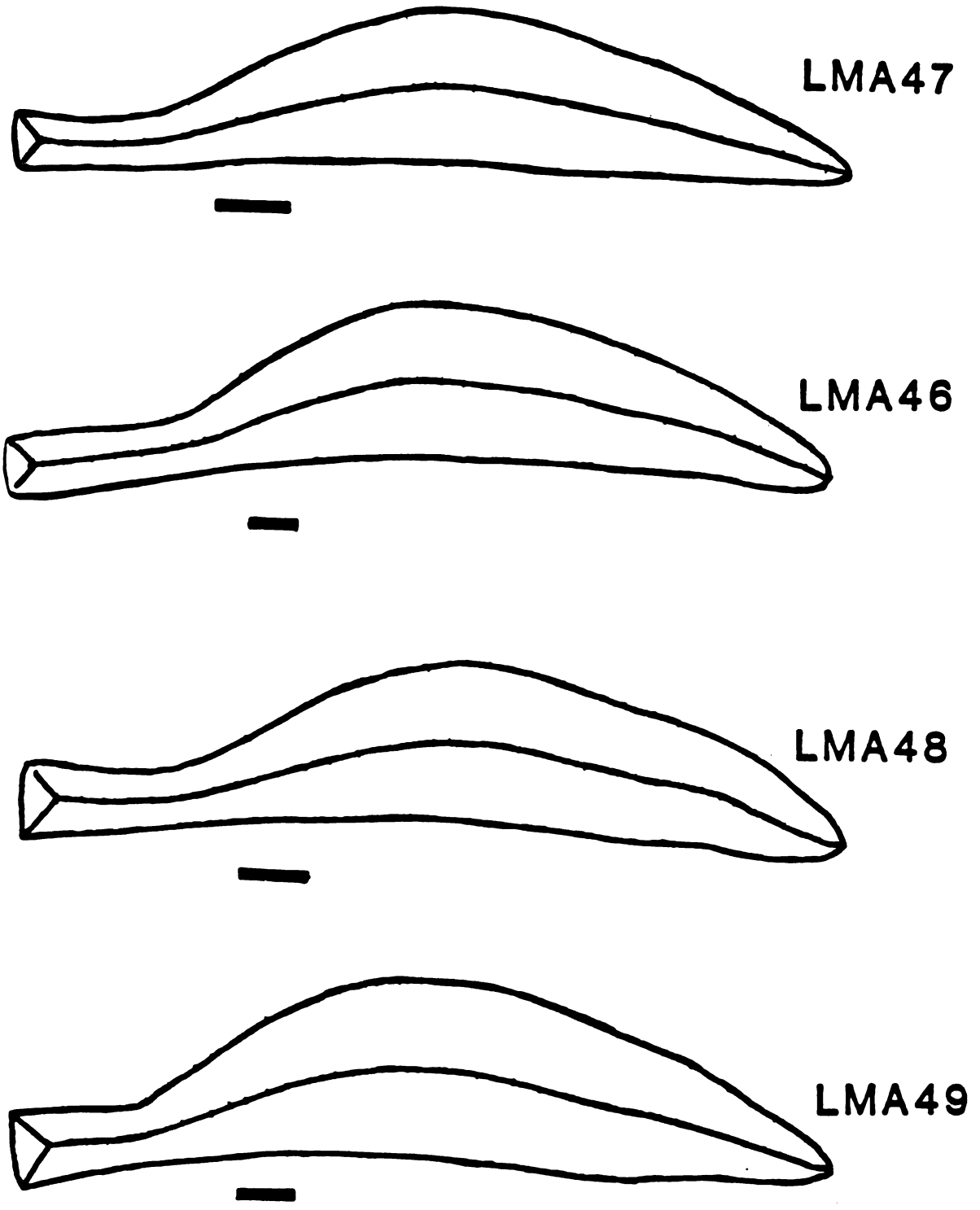


Figure 15. (cont'd.).



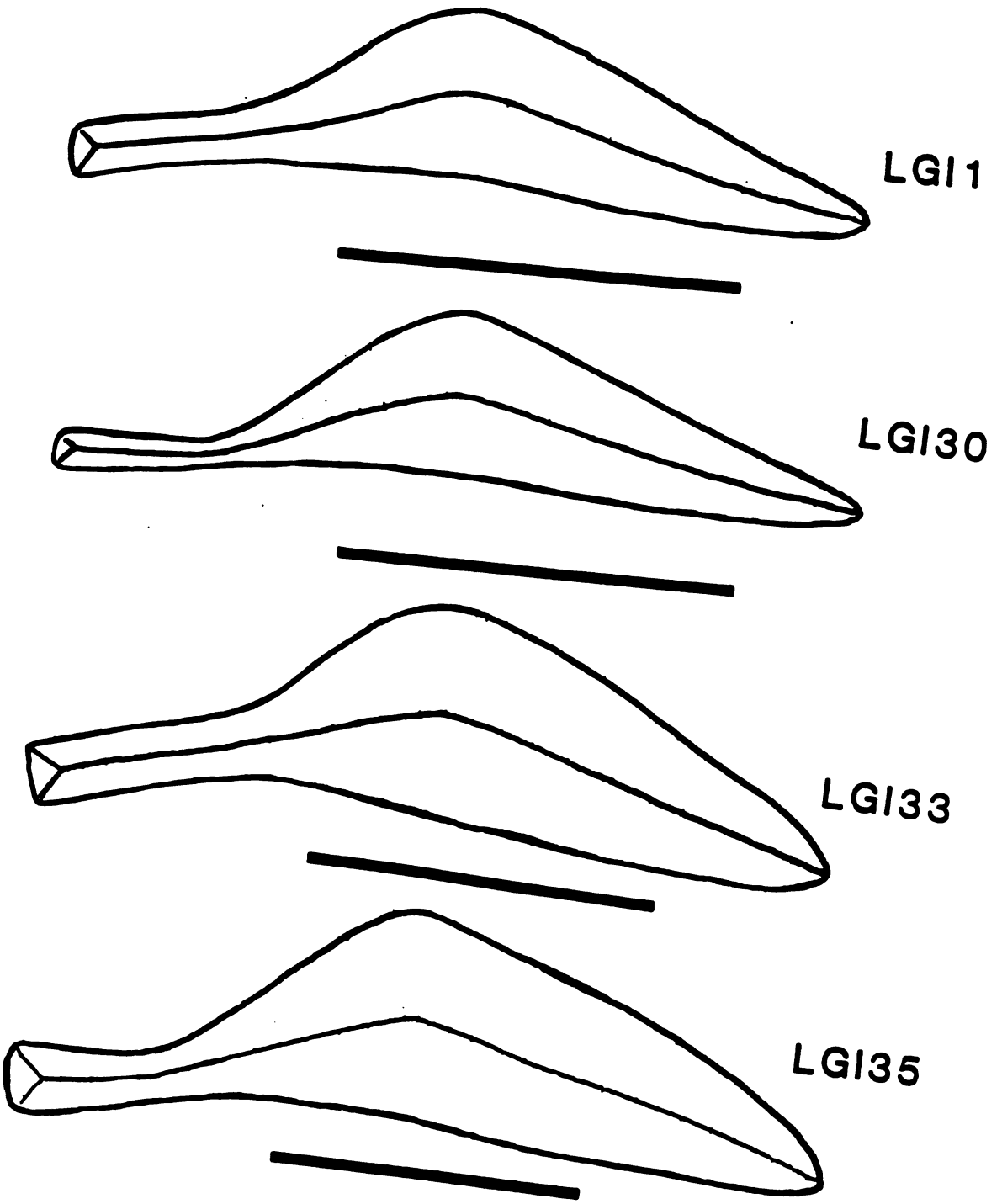
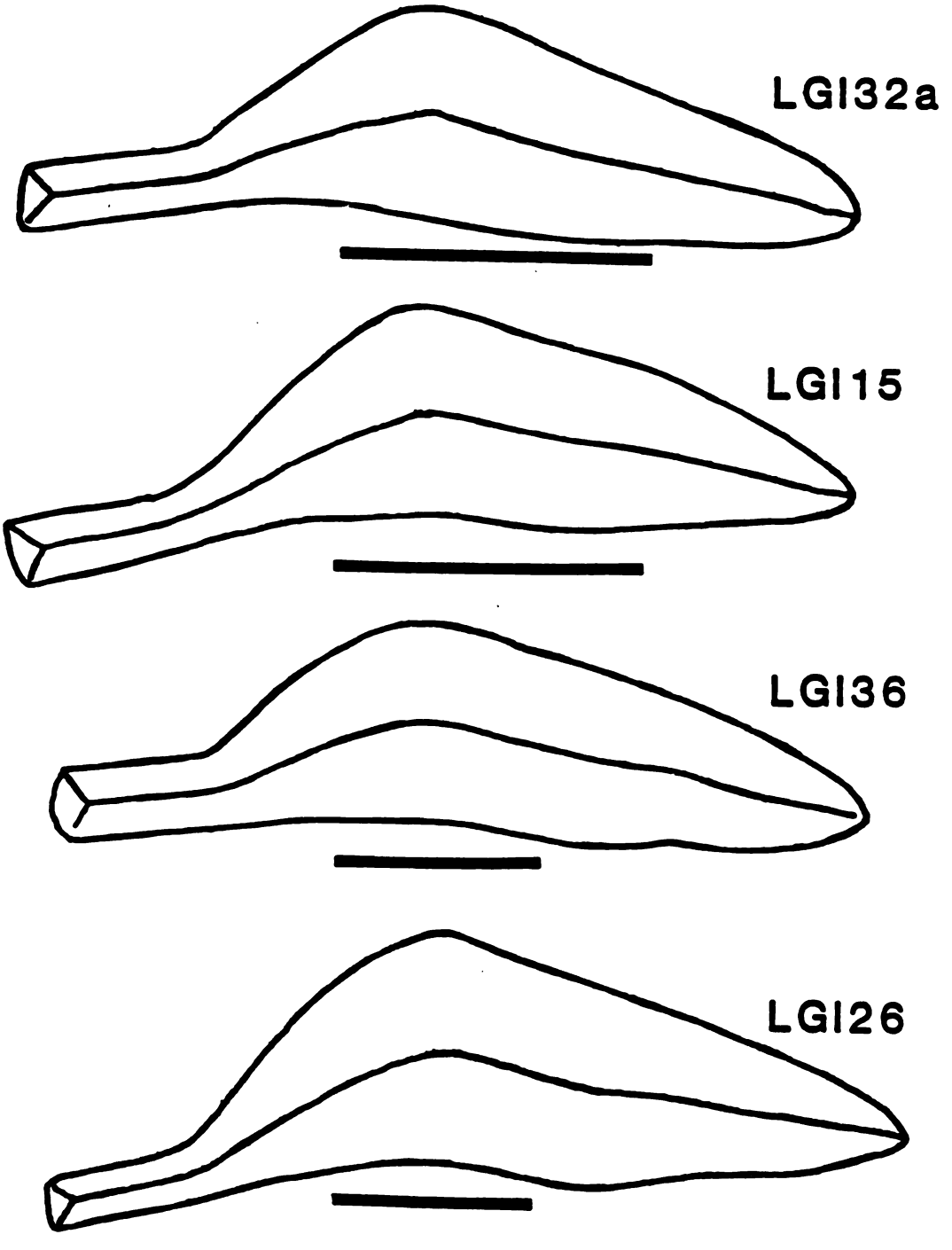


Figure 16. Digitized Outlines and Medial Axes of Pumpkinseed Sunfish. Specimens are Ranked by Size from Small to Large. Scale Distance is Equal to One Millimeter.

Figure 16. (cont'd.).



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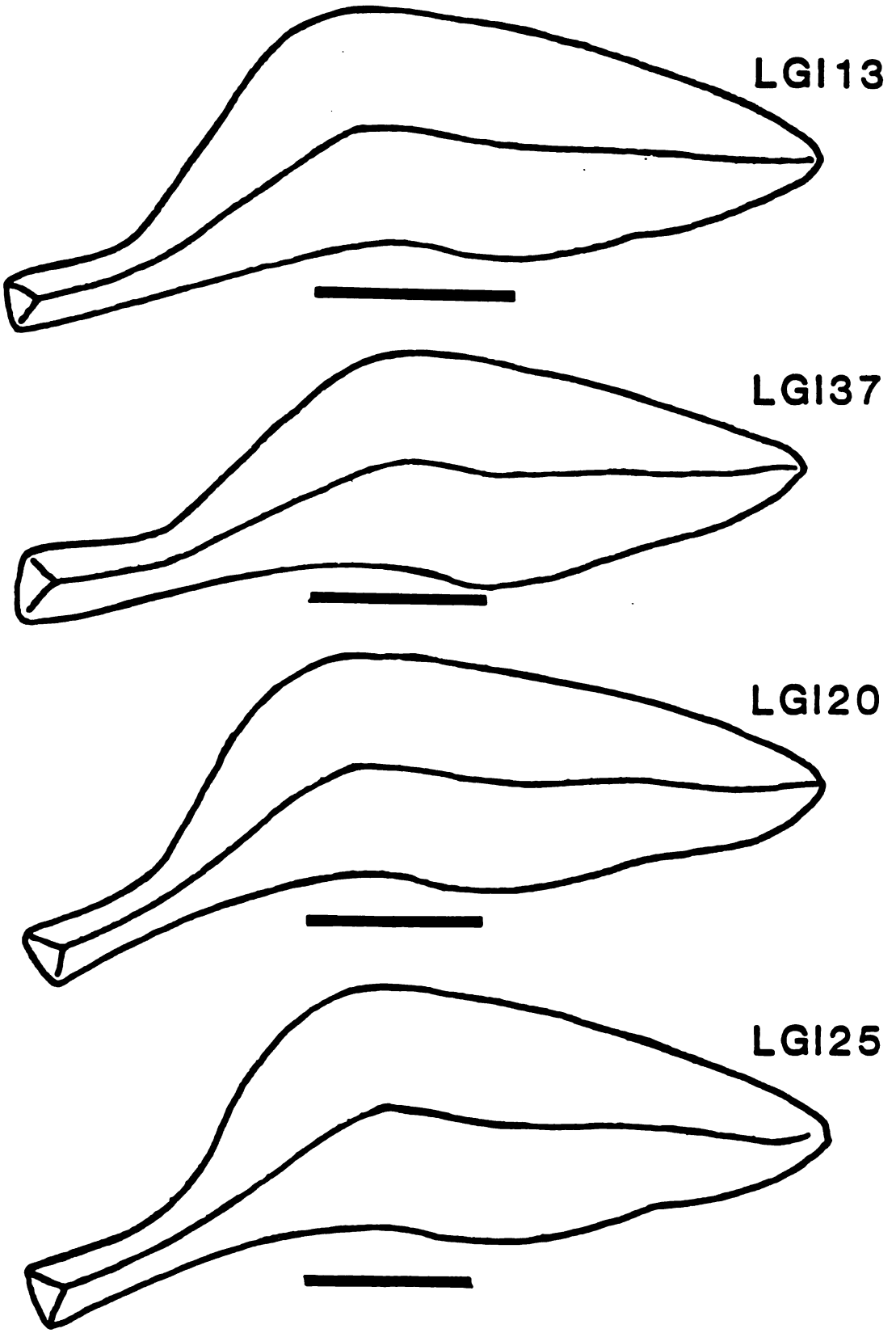

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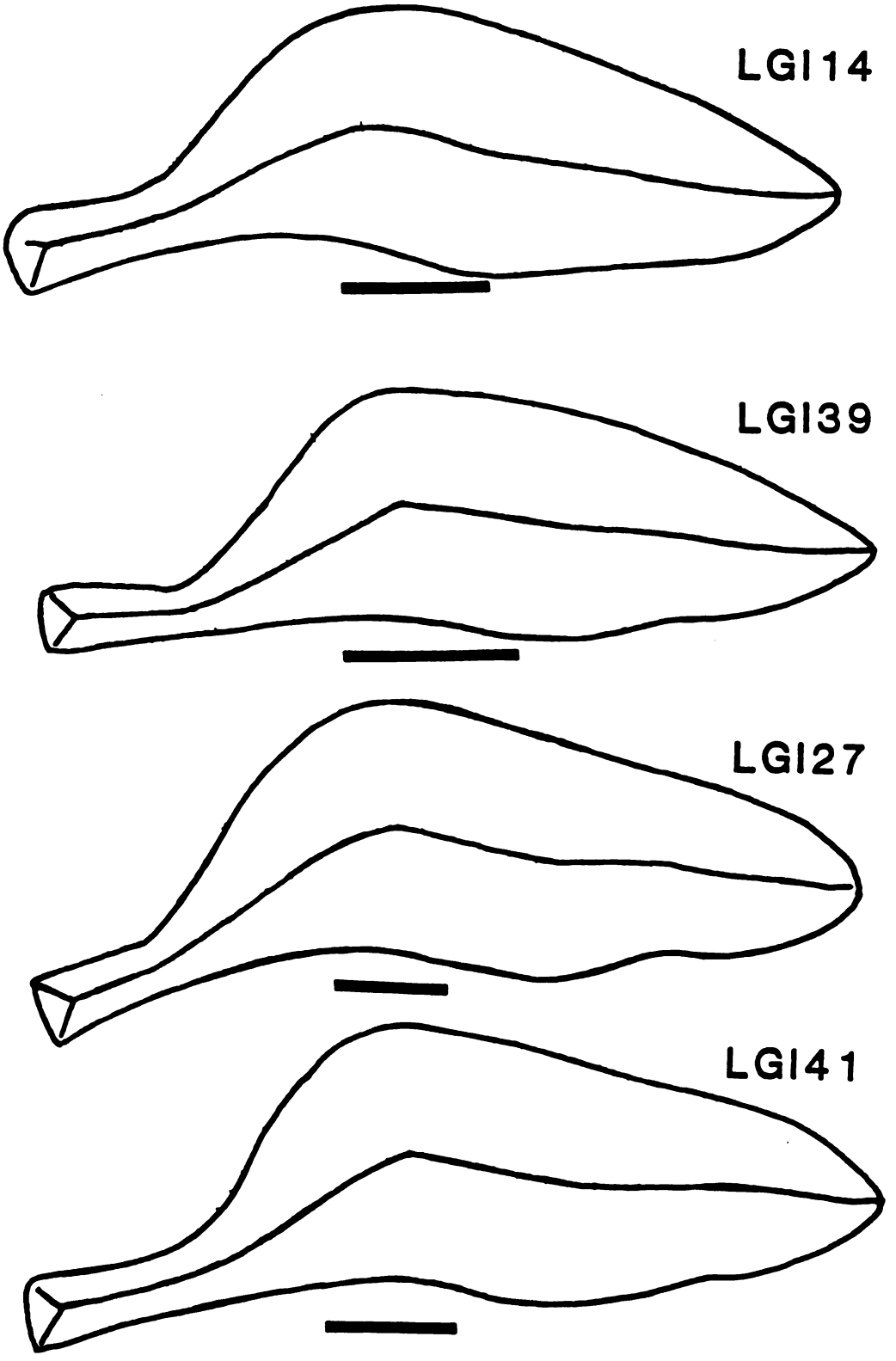

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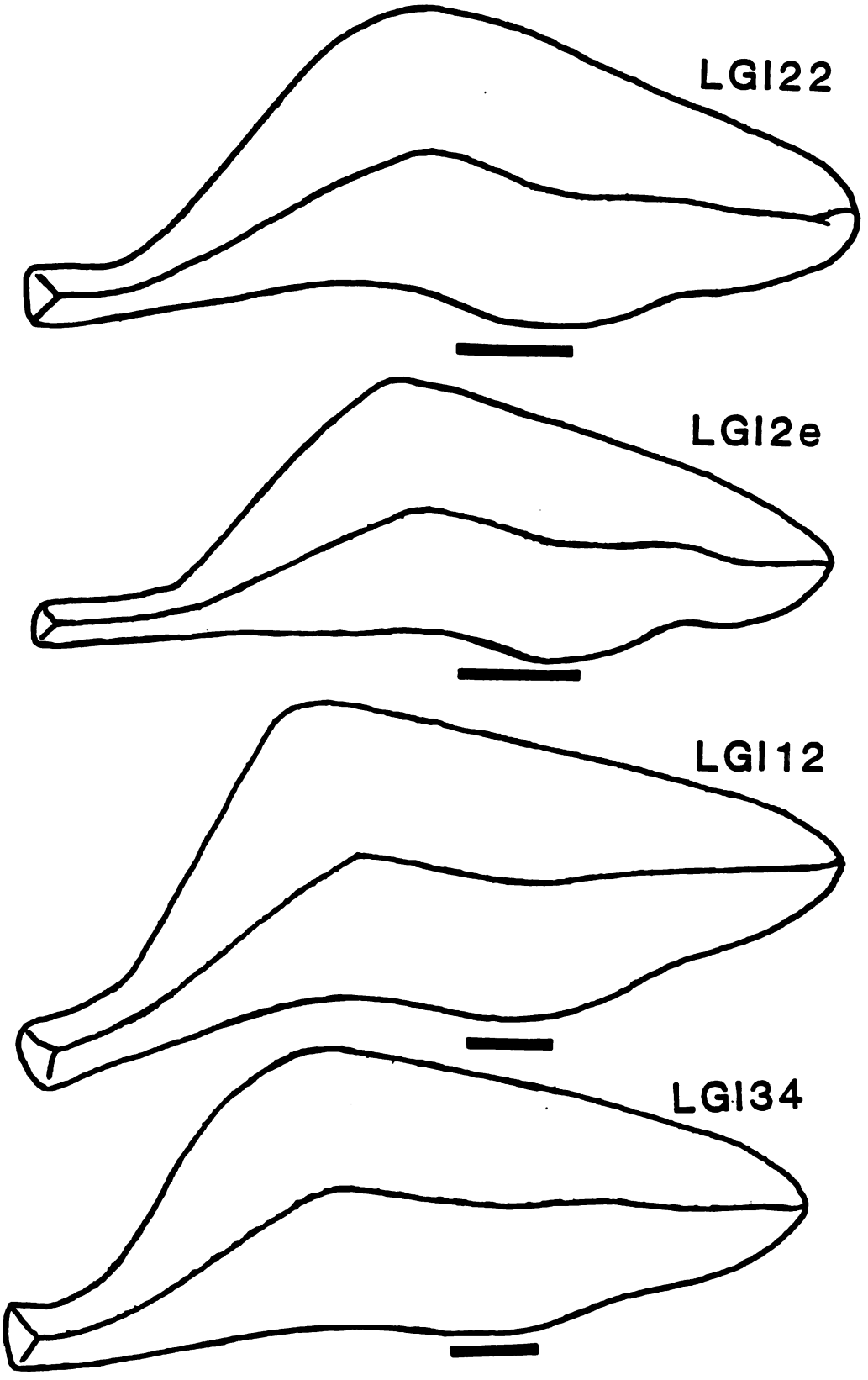

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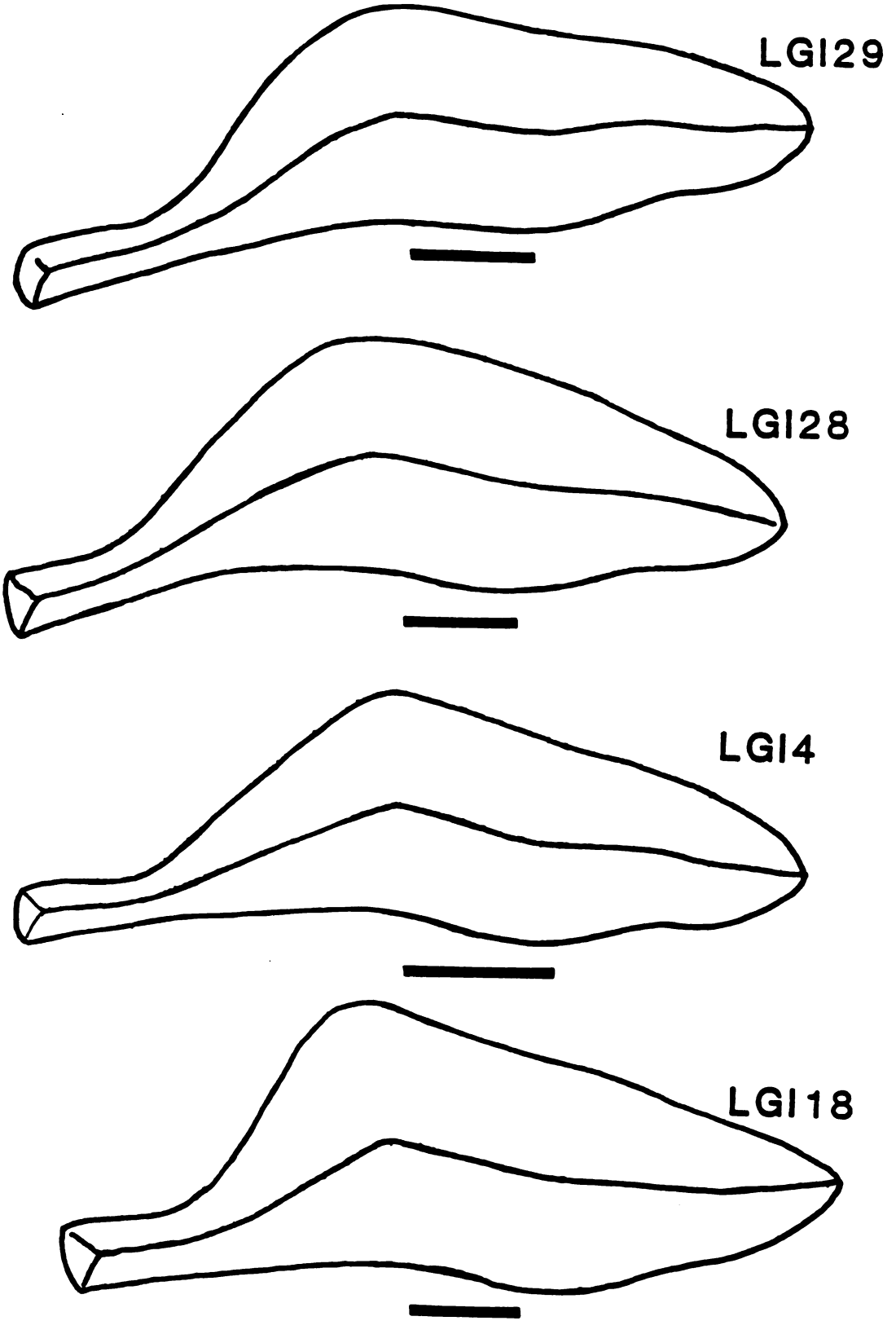

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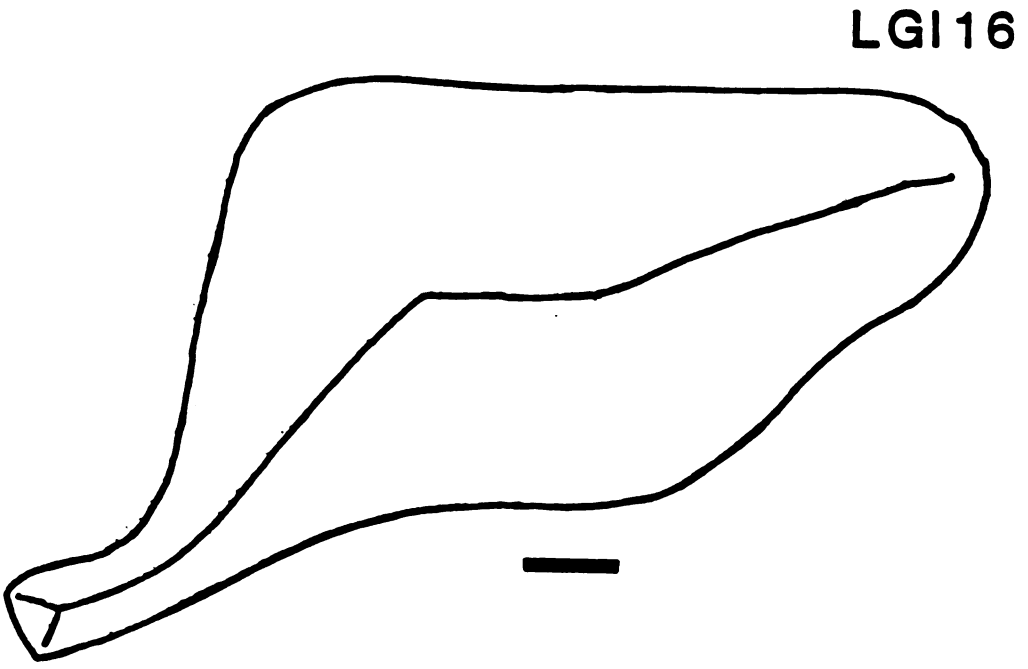

APPENDIX B

SOURCES AND LOCALITY DATA FOR SPECIMENS

Lepomis gibbosus:

University of Michigan Museum of Zoology, UMMZ
- 103258- MI., Macomb Co., Missmoris Gravel Pit, 24 April 1935, G. P. Cooper: LGI13 44mm; LGI14 58mm; LGI20 50mm; LGI15 29mm; LGI25 58mm; LGI26 64mm; LGI32A 28mm; LGI33 27mm; LGI35 28mm;
- 103263- MI., Macomb Co., Huron River, 24 April 1935, G. P. Cooper: LGI2E 84mm; LGI4 68mm; LGI28 67mm; LGI29 67mm;
- 196846- MI., Huron Co., Pigeon River, 27 July 1908, A. S. Seathers: LGI1 28mm; LGI30 28mm;
- 2103258- LGI26 44mm;

Field Museum of Natural History, FMNH
NY., North Rose, Sodus Creek, 21 October 1925, A. C. Weed:
- 13426- LGI34 100mm;
- 13431- LGI16 111mm;
- 13435- LGI12 94mm;
- 13443- LGI18 75mm; LGI22 79mm;

- 42342- ILL., Skokie Lagoon, 30 July 1939, L. P. Woods LGI39 60mm; LGI41 64mm;
- 43300- LGI36 40mm; LGI37 46mm;

Lepomis macrochirus:

University of Michigan Museum of Zoology, UMMZ
- 71380- MI., Newaygo Co., Long Lake, 9 July 1926, Langloise and Moody: LMA2 38mm; LMA7 46mm; LMA8 37mm; LMA10 39mm;
- 107943- MI., Jackson Co., Watkins Lake, 29 September 1934, I.F.R. Staff: LMA3 88mm; LMA4 100mm; LMA6 30mm;
- 113172- MI., Mason Co., Gooseneck Lake, 4 September 1936, E. R. Kuhne: LMA20 25mm; LMA23 28mm; LMA24 28mm; LMA25 27mm; LMA26 28mm;
- 210030- FLA., Lake Co., N. Shore Wildcat Lake, R. M. and S. Bailey: LMA9 43mm;

Field Museum of Natural History, FMNH
4332- LMA20 35mm;
43327- LMA1 30mm; LMA16 35mm; LMA17 38mm; LMA18 30mm; LMA19 26mm;
43998- LMA12 79mm;

Personal Collection- J. E. Zablotny
MI., Eaton Co., Lake Ovid
October 1986:
-LMA45 93mm; LMA46 144mm; LMA47 128mm; LMA48 148mm; LMA49 158mm

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BIBLIOGRAPHY

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