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THE EFFECT OF PRESERVATION ON UROGENITAL PAPILLA LENGTH IN THE LEAST BROOK LAMPREY, *LAMPETRA AEPYPTERA*

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INTRODUCTION

In male lampreys, the urogenital papilla becomes elongate as the spawning season approaches. Kott et al. (1988) reported that the length of the urogenital papilla, expressed as a proportion of branchial length, is taxonomically useful in distinguishing among male lampreys of nonparasitic species. Subsequently, Sneen and Cochran (1990) reported a significant difference in relative papilla length of *Ichthyomyzon gagei* from the southeastern U.S. and *I.* cf. *gagei* from Wisconsin.

Fixing and preservation may significantly alter morphological measurements of lampreys (e.g., Beamish, 1982; Cochran, 1987; Cochran and Pettinelli, 1988; Morkert and Bergstedt, 1990; Docker and Beamish, 1991). Typically, these processes result in shrinkage, which may be relatively more severe in lampreys than in most other fishes because of their reduced skeletal investment. The purpose of our study was to examine the effect of fixing and preservation on the ratio of urogenital papilla length to branchial length in the least brook lamprey, *Lampetra aepyptera*. Such an effect, if unaccounted for, might confound the use of this measure for taxonomic purposes. We expected that urogenital papilla length would be reduced in preserved specimens, but we were unsure if the ratio of papilla length to branchial length would be similarly affected.

METHODS

We collected *Lampetra aepyptera* on 12 March 1991, at several sites in Marshall and Itawamba counties, Mississippi (Table 1). Eight adult male lampreys, hereafter referred to as Group A, were held alive and returned to the laboratory for measurement. After the lampreys were anesthetized with tricaine methanesulfonate (MS 222), total length of each specimen was measured to the nearest millimeter, and branchial and urogenital papilla lengths, as defined by Kott et al. (1988), were measured to the nearest 0.01 mm with a digital micrometer. The lampreys were fixed for two days in 10% formalin, soaked in water for two days, and preserved in 70% ethanol. During this process, they were held in individual vials so that their identities could be maintained. After 33 days in ethanol, each lamprey was remeasured for total length, branchial length, and urogenital papilla length. Paired t-tests were used to assess statistically the changes in these measures.

Table 1. Sites in Mississippi at which *Lampetra aepyptera* were collected on 12 March 1991. The sample size indicated is the number of adult males included in later analyses. Those in Group A were measured both prior and subsequent to preservation, whereas those in Group B were measured only after preservation.

County	Stream	Sample Size A	Sample Size B
Marshall (T4S,R1W,S6)	Tributary to Chewalla Creek	1	0
Marshall (T4S,R1W,S5)	Tributary to Chewalla Creek	7	21
Itawamba (T11S,R9E,S14)	Tributary to Bull Mountain Creek	0	6

In addition to lampreys in Group A, a set of 27 adult male *L. aepyptera* (Table 1) were fixed in the field in 10% formalin after anesthesia with MS 222. After transfer to 70% ethanol in the laboratory, they were held until July 8, 1991, before being measured. Hereafter, these lampreys will be referred to as Group B.

RESULTS AND DISCUSSION

Branchial length and total length were significantly correlated in both living and preserved samples, but urogenital papilla length was not correlated with either variable (Table 2). Kott et al. (1988) found mean branchial length and mean total length to be highly correlated among lamprey species. They also found mean total length and mean urogenital papilla length to be weakly correlated among species, but only when parasitic lampreys were included in the analysis. When parasitic species were excluded, the two variables were uncorrelated.

Fixing and preservation effected significant reductions in total length, urogenital papilla length, and the ratio of urogenital papilla length to branchial length (Table 3). Although branchial length was reduced in the preserved specimens, the decline was not significant, and the mean

Table 2. Correlation among total length (TL), branchial length (BL), and urogenital papilla length (PL) in samples of adult male *Lampetra aepyptera*. Sample sizes (n) for each group are indicated.

Sample	n	TL vs BL	BL vs PL	TL vs PL
Group A				
live	8	0.893**	-0.128	-0.098
preserved	8	0.962**	-0.265	-0.256
Group B				
preserved	27	0.893**	0.040	0.016

**p < 0.01

percentage reduction (2.2% of the mean for living specimens) was not as great as for total length (4.8%) or urogenital papilla length (13.5%). The stability of branchial length apparently reflects the relatively greater investment of skeletal tissue in the branchial region. The mean percentage decline in the ratio of urogenital papilla length to branchial length (11.4%) obviously resulted primarily from the change in urogenital papilla length.

The mean ratios of urogenital papilla length to branchial length we observed in this study (Table 3) are well below the mean of 0.408 reported for *L. aepyptera* by Kott et al. (1988), although we did record individual values as high as 0.519 among the preserved specimens in Group B. We probably observed lower ratios because we collected our specimens in advance of the spawning season. Kott et al. (1988) reported a change in the mean ratio at one collection site from 0.226 on 6 February to 0.388 on 18 March.

The percentage reduction in total length of preserved adult *L. aepyptera* in this study was comparable to that observed in other studies. Cochran and Pettinelli (1988) reported a mean reduction of 7.6% in total length of adult *Ichthyomyzon* cf. *gagei* fixed in 10% formalin and preserved in 70% ethanol. *Ichthyomyzon unicuspis* treated in a similar fashion shrank by an average of 6.6% (Cochran and Marks, in press). Beamish (1982) and Docker and Beamish (1991) presented regressions for converting between live and preserved total lengths of adult *I. gagei* and large larval *L. aepyptera* fixed and preserved in 5% formalin. For individuals 113.4 mm in length, the mean total length of living lampreys in Group A (Table 3), their equations predict shrinkage by 2.9% and 3.1%, respectively. Average shrinkage of 100-mm larval sea lampreys (*Petromyzon marinus*) was 3.8% when fixed and preserved in 5% formalin and 4.3% in 10% formalin (Morkert and Bergstedt, 1990).

We do not know how long it takes for morphological measurements of lampreys preserved in 70% ethanol to stabilize. Docker and Beamish (1991) stated that large ammocoetes of *L. aepyptera* preserved in 5% formalin achieved constant length and weight in 7 days. Morkert and Bergstedt (1990) reported that most shrinkage of larval sea lampreys fixed and preserved in 5 or 10% formalin occurred within the first 2 hours. In the present study, the measurements for lampreys in Group B, which were stored for nearly 4 months in ethanol, were not substantially different from those for Group A, which were preserved for only one month (Table 3). However, to adequately assess the effect of duration in preservative on morphological measurements would require paired comparisons of measurements for individual lampreys.

Table 3. Mean total length (TL), branchial length (BL), urogenital papilla length (PL), and ratio of urogenital papilla length to branchial length (PL/BL) for samples of adult male *Lampetra aepyptera*. Standard errors are in parentheses, and sample sizes (n) for each group are indicated. For group A, the results of paired comparisons between measurements for live and preserved specimens are indicated in the appropriate columns.

Sample	n	TL (mm)	BL (mm)	PL (mm)	PL/BL
Group A	8				
live		113.4 (4.3)	11.87 (0.72)	3.52 (0.24)	0.306 (0.031)
preserved		108.0 (4.2)	11.50 (0.53)	3.09 (0.31)	0.275 (0.033)
paired t value		(22.97***)	(0.24)	(3.74**)	(3.01*)
Group B	27				
preserved		107.9 (2.0)	11.37 (0.18)	3.37 (0.27)	0.298 (0.024)

*p < 0.05
**p < 0.01
***p < 0.001

We have identified an effect of fixing and preservation on the ratio of urogenital papilla length to branchial length. If this ratio is to be used effectively in comparisons among lamprey taxa or among populations from different geographic locations, then not only should all lampreys be at a comparable reproductive state (Kott et al., 1988), but all should be treated identically with respect to fixing and preservation. Differences among individuals or groups with respect to types or concentrations of preservatives, or with respect to schedules of fixing and preservation, might contribute to variability that obscures taxonomic differences, or worse, to biases that are confounded with taxonomic effects.

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MUSEUM NOTES

The Auburn University Museum Fish Collection

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HISTORY

The Auburn University Museum fish collection (standard symbolic code - AUM, Leviton et al., 1985; Leviton and Gibbs, 1988: 281 [address correction]) was established in 1930 by entomologist, Frank E. Guyton. Guyton collected fishes while sampling streams of east-central Alabama with students from the Alabama Polytechnic Institute (A.P.I.), the forerunner of Auburn University. Specimens from these early collections formed the basis of descriptions and systematic treatments of taxa such as the redeye bass *Micropterus coosae* (holotype and paratypes; Hubbs and Bailey, 1940), Alabama spotted bass *M. punctulatus henshalli* (paratypes; Hubbs and Bailey, 1940), bronze darter *Percina palmaris* (paratypes; Bailey, 1940), and eastern redfin darter *Etheostoma whipplii artesia* (specimens used in color description; Hubbs and Black, 1941). In fact, A.P.I. specimens and Guyton's contributions are acknowledged, in all of the above works. In most instances, A.P.I. specimens were donated to Michigan and are now cataloged in the Museum of Zoology. However, in a few cases (notably *Micropterus coosae* and *M. p. henshalli*) paratypes were retained at A.P.I., and are now part of the AUM fishes type collection.

The AUM fish collection built up gradually through the efforts of Guyton, and fisheries biologists such as H.S. Swingle, John D. Black, and Jack S. Dendy. William Smith-Vaniz, a student of Dendy's, contributed numerous collections and used the Auburn Fish Collection as the basis for a Master of Science thesis, which culminated with the publication of