

ELECTROPHORETIC SEPARATION OF LARVAL SILVER REDHORSE (*MOXOSTOMA ANISURUM*) AND GOLDEN REDHORSE (*MOXOSTOMA ERYTHRURUM*)*

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Abstract—1. Soluble protein composition of larvae and adults of the silver redhorse (*M. anisurum*) and the golden redhorse (*M. erythrurum*) were compared with polyacrylamide gradient electrophoresis.

2. Adults and larvae of *M. anisurum* and *M. erythrurum* were readily distinguished from each other.

3. The superior resolving power of gradient vs conventional page created problems in the correlation of larval and adult forms with ontogenetic differences in proteins accounting for the lack of similarity.

INTRODUCTION

Two of the most common members of the genus *Moxostoma* in the Monongahela drainage are the silver redhorse (*M. anisurum*) and the golden redhorse (*M. erythrurum*) (Jenkins, 1970). The silver redhorse prefers freshwater streams and lakes (Scott and Crossman, 1973; Jenkins, 1980a) and is typically found from southern Virginia south to the Roanoke-Chowan drainage in Georgia (Jenkins, 1980a) along the Atlantic Slope, but its major distribution is in the Great Lakes Basin and the Ohio-Mississippi drainage as far south as the northern Ozarkian uplands. A separate population is found in the Nelson River drainage of Canada. The golden redhorse is generally sympatric with silver redhorse, although it has a more southern distribution with populations in the Mobile drainage of the Gulf Slope, the Ohio, mid and upper Mississippi, the lower Missouri basins, the Great Lakes Basin (except for Lake Superior) and the Roanoke and James River (Scott and Crossman, 1973; Jenkins, 1980b).

Both species are spring spawners (silver redhorse—initial spawning temperature 13°C, golden redhorse—15°C) (Meyer, 1962). Although both fishes have been intensively studied in order to identify the adults (Jenkins, 1970), little has been done pertaining to the identification of larval redhorses. A number of important morphological characteristics for larval identification of the two species are available (Smith, 1982). To supplement the morphological characteristics as a means for separating larval forms, we investigated the use of electrophoresis in distinguishing the two species. Electrophoresis has been used to distinguish a number of similar larval and juvenile fishes (Herzbert and Pasteur, 1975; Morgan, 1975; Brassington and Ferguson, 1976; Smith and Crossland, 1977; Sidell *et al.*, 1978; Smith *et al.*, 1980).

MATERIALS AND METHODS

Larvae were reared in the laboratory from fertilized eggs collected in the field. Adult fish were obtained by electroshocking (230 V d.c.) known spawning riffles in Buffalo Creek, a tributary stream of the Monongahela River near Fairmont, West Virginia. Eggs and milt were simultaneously hand-stripped into 203 mm dia glass preparation dishes containing a small amount of water. Eggs were allowed to water-harden for at least 1 hr, transported back to the laboratory, and incubated at 17–20°C in freshwater until hatch. Larvae were frozen at –80°C prior to electrophoresis.

Individual larvae were homogenized in distilled water (1:1, v/v) using a ground-glass tissue grinder cooled in an ice bath. The homogenate was centrifuged at 1600 g for 15 min. Dorsal white muscle from adults was treated similarly.

Precast Pharmacia acrylamide gradient gels (4–30%) were used for larval electrophoresis. A Pharmacia GE-4 II chamber and EPS 500/400 power supply were used for electrophoresis at 75 V (initial –75 mA) and 100 V (final –80 mA) for 18 hr. The buffer system was 0.09 M Tris–0.08 M borate–0.003 M Na₂EDTA, pH 8.35. Twenty microliters of homogenate were used. Twenty-four larvae and three adults of each species were analyzed. Usually, three adults and three larvae of each species were run on a gel. Two sets of replicates were run to determine gel-to-gel variation.

Following electrophoresis, the gels were stained with 0.7% Buffalo Black NBR in 7% acetic acid for 3–4 hr. Destaining was accomplished using successive changes of 7% acetic acid.

Direct comparisons were made within a gel for mobility and distribution of proteins. Proteins were scored as to presence (1) or absence (0) and compared among stages and species using Jaccard's coefficient of similarity (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

Larvae and adults of *M. anisurum* and *M. erythrurum* were readily distinguished from each other using electrophoresis (Fig. 1). Within a species, all 24 larvae utilized in the study had the same complement of proteins (13 for *M. anisurum* and 15 for *M. erythrurum*) for a Jaccard's coefficient of similarity of 1.00 (Table 1). The adult forms were also very consistent (Jaccard's coefficient of 1.0).

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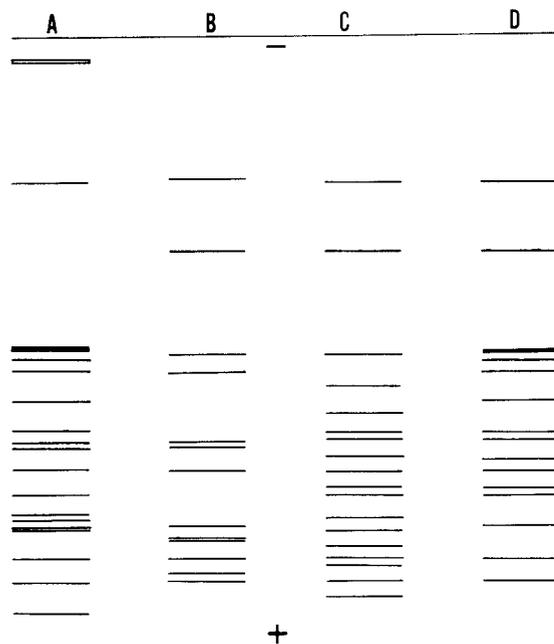


Fig. 1. Electropherograms of soluble proteins from adult (A) and larval (B) *M. anisurum* and adult (C) and larval (D) *M. erythrurum*.

The superior resolving power of gradient polyacrylamide electrophoresis created some difficulties in the interpretation of results. Part of the difficulty was related to ontogenetic proteins present in the larvae but not in the adults and conversely, the presence of some adult proteins not observed in the larval form (Fig. 1). Using Jaccard's coefficient, the adult forms had a common coefficient of 0.50 and the larval forms 0.39 (Table 1). Yet, the adult and larval *M. anisurum* had a coefficient of 0.39, whereas the adult and larval *M. erythrurum* contained a coefficient of about 0.57.

In a previous study on striped bass (*Morone saxatilis*) and white perch (*M. americana*) using column acrylamide electrophoresis, Morgan (1975) found excellent agreement between adult and larval soluble muscle proteins, although there were a few minor bands differing between larvae and adults. Agreement also was excellent within a life stage of a species (Morgan, 1975).

Excellent replication was obtained within a stage of the species for both *M. anisurum* and *M. erythrurum*. The main distinguishing character between adult and larval forms appeared to a major myogen band migrating about two-thirds of the way down the gel, as well as two denser proteins migrating anodally to this protein (Fig. 1).

Care must be taken in using this technique to compare larvae and adults due to the superior resolving power. Generally, the major differences observed between the two species centered on the major bands

Table 1. Summary of Jaccard's coefficient as derived from the proteins for the two *Moxostoma* species in Fig. 1

(n)	<i>M. anisurum</i>		<i>M. erythrurum</i>	
	Adult	Larvae	Adult	Larvae
<i>M. anisurum</i>				
Adult (3)	1.00	0.39	0.50	0.33
Larvae (24)		1.00	0.45	0.39
<i>M. erythrurum</i>				
Adult (3)			1.00	0.57
Larvae (24)				1.00

although there were also discrete differences among some of the minor bands. This technique may be particularly useful in the description of ontogenetic soluble proteins.

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