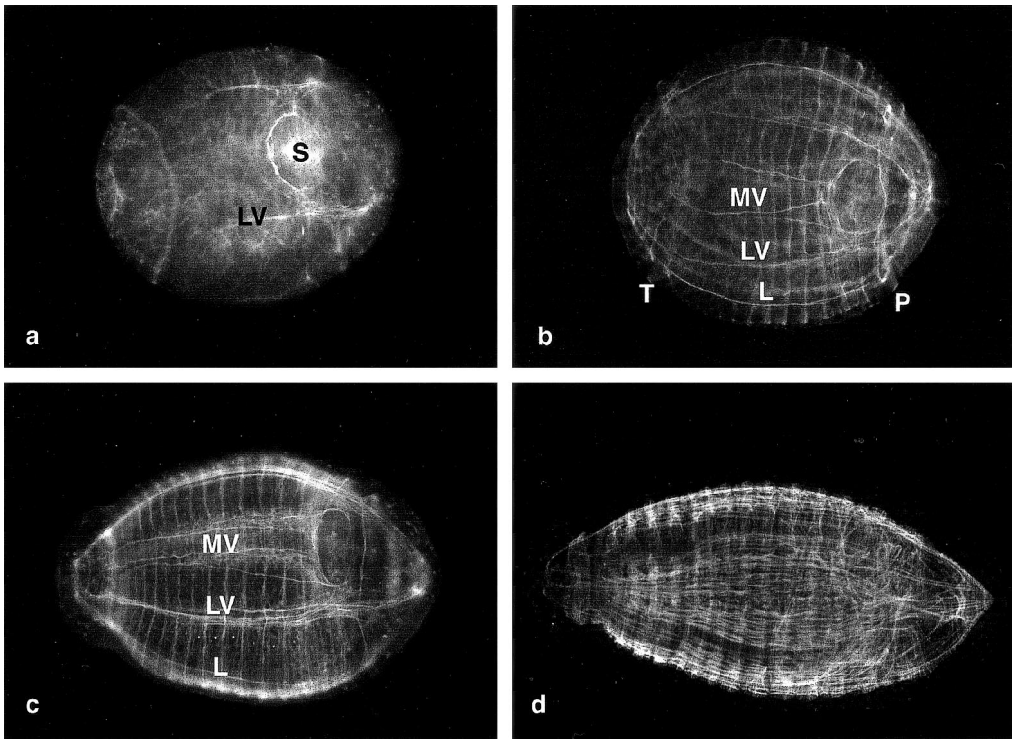

Figure 1. *(a) Ventral view of an early embryo (240 × 175 μm) showing the stomodeum (S) with phalloidin labeling of the lower lip and lateroventral muscles (LV). (b) Ventral view showing the prototroch (P), telotroch (T), paired midventral muscles (MV), lateroventral muscles (LV), and lateral muscles (L). Circular musculature formation is incomplete, with a gap between the most posterior circular muscle band and the telotroch. (c) Ventrolateral view showing thickened midventral (MV) and lateroventral (LV) muscles. Circular muscle bands are complete to the telotroch. L = lateral muscle. (d) Metatrochophore showing greatly increased complexity of the larval musculature.*

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Differentiation of Pharyngeal Muscles on the Basis of Enzyme Activities in the Cichlid *Tramitichromis intermedius*

Aaron N. Rice, David S. Portnoy, Ingrid M. Kaatz, and Phillip S. Lobel (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts 02543)

One of the key morphological features of cichlid fishes is their highly developed pharyngeal jaw complex used in feeding (1). Although many studies focused on the anatomy (2) and function (1) of pharyngeal muscles, the potential physiological differences between them have not been examined in detail. The purpose of this paper is to investigate the capacity for anaerobic activity of the muscles in the pharyngeal jaw complex, and to assess whether they are all the same functional type. Finding different types would suggest that various muscles in the complex may have functions other than mastication.

Bass *et al.* (3) demonstrated that fundamentally different types of muscles can be distinguished by the activity level of energetic enzymes. Assaying these enzymes in muscles tissues can indicate whether a muscle functions primarily through aerobic or anaerobic

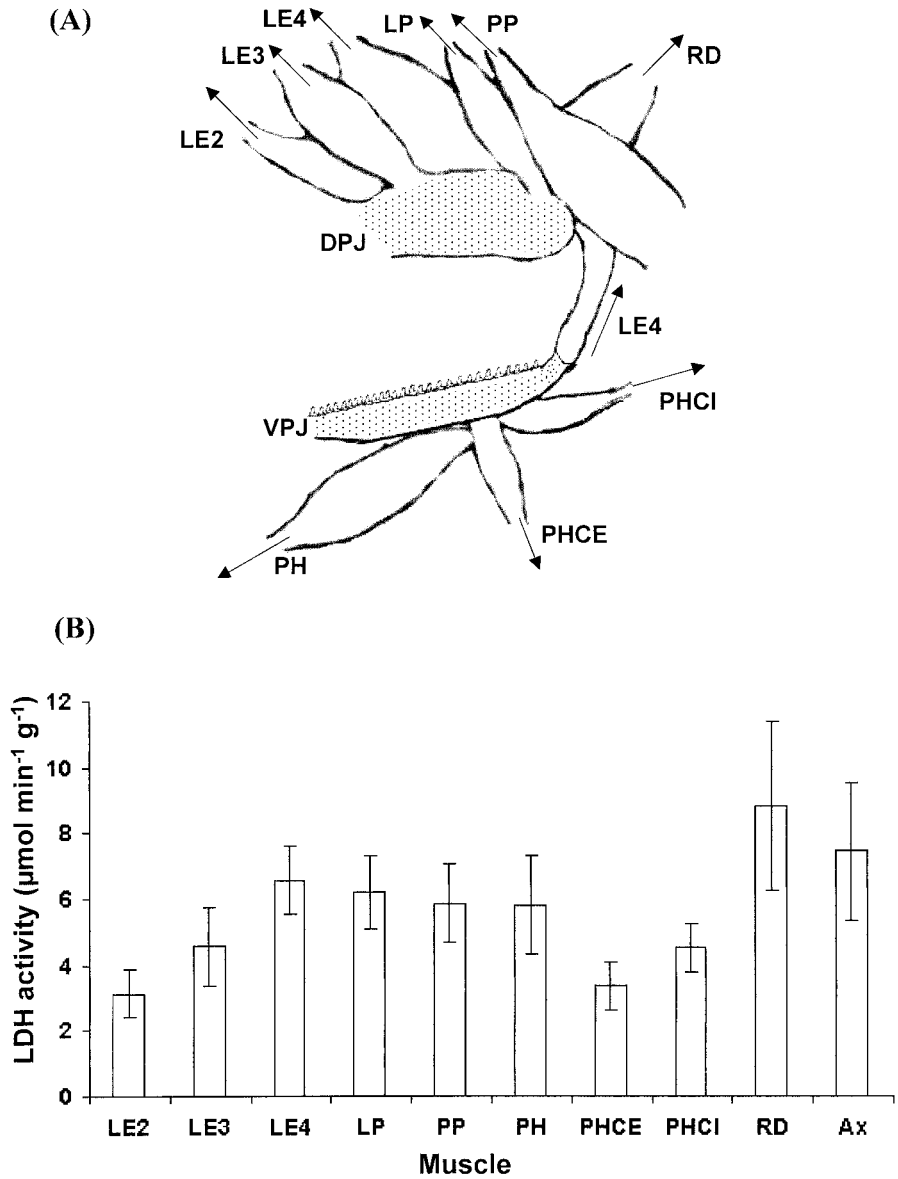

Figure 1. *(A) The* Tramitichromis intermedius *pharyngeal muscles. Arrows indicate the direction of movement of the pharyngeal jaws due to muscular contraction. Abbreviations are as follows: LE2, levator externus 2; LE3, levator externus 3; LE4, levator externus 4; LP, levator posterior; PP, protractor pectoralis; PH, pharyngohyoideus; PHCE, pharyngocleithralis externus; PHCI, pharyngocleithralis internus; RD, retractor dorsalis; DPJ, dorsal pharyngeal jaw; VPJ, ventral pharyngeal jaw. (B) Enzymatic activities of* L*-lactate dehydrogenase from different* T. intermedius *pharyngeal muscles.*

pathways. Comparative analysis between muscles can further elucidate the degree of functional specialization that these tissues have undergone relative to other muscles in the body. This technique has been employed in a variety of different taxa—for example, fishes (4), frogs (5), and bats (6)—to demonstrate functional differences between muscle types.

Captive-bred *Tramitichromis intermedius* (born in July 2000 from wild-caught parents from Lake Malawi, Africa) were kept in 75-gallon aquaria. Fish were euthanized with MS-222, and the opercles were removed. Muscles involved in movement of the dorsal and ventral pharyngeal jaws were removed and weighed: levator externi 2, 3, 4 (LE2, LE3, LE4), levator posterior (LP), protractor pectoralis (PP), pharyngohyoideus (PH), pharyngocleithralis externus (PHCE), pharnygocleithralis internus (PHCI), and retractor dorsalis (RD) (Fig. 1A). Axial muscle (Ax) from the tail was also removed and served as a comparison for fast-twitch muscle. Muscle nomenclature follows Liem (1). Tissues were homogenized in 1 ml of buffer (7.5 m*M* Tris and 1 m*M* EGTA, pH 7.6), and analyzed for activities of L-lactate dehydrogenase (LDH; E.C. 1.1.1.27), in order to indicate capacity for anaerobic respiration. Using a Perkin-Elmer Lambda 3B UV/Vis spectrophotometer, enzymes were assayed using 50 m*M* TEA, 5 m*M* EGTA, 0.15 m*M* NADH, 0.24 m*M* pyruvate, pH 7.6, at 340 nm. Enzyme activities were calculated as micromoles of product per minute per gram of tissue (4), and differences between muscle groups were analyzed using a one-way ANOVA.

The mean (±SE) LDH activities of the muscles were as follows: LE2: 3.15 ± 0.71, LE3: 4.56 ± 1.19, LE4: 6.57 ± 1.02, LP: 6.20 ± 1.12, PP: 5.87 ± 1.18, PH: 5.82 ± 1.48, PHCE: 3.38 ± 0.73, PHCI: 4.52 ± 0.72, RD: 8.83 ± 2.57, Ax: 7.44 ± 2.09 (Fig. 1B). These results show that the pharyngeal muscles examined differ significantly in levels of LDH activity ($n = 8$, $P = 0.0152$). A post-hoc Fisher's Protected Least Significant Difference test revealed that significant differences existed between Ax and PHCE ($P = 0.0469$), LE2 and RD ($P = 0.0143$), LE3 and RD ($P = 0.0374$), PHCE and RD ($P = 0.0355$).

The differences in LDH activity in this muscle complex shows that several muscles examined have different capacities for anaerobic activity. Functional muscle types can be differentiated on the basis of enzyme activities by comparing ratios between activities of aerobic and anaerobic enzymes. Without determining aerobic activity, we cannot conclusively demonstrate that the muscles examined are different functional types. These preliminary data suggest that more than one muscle type may be present, but analysis of the aerobic capacity is necessary.

The presence of different muscle types would suggest that the pharyngeal complex may be performing a dual function. In addition to mastication, the pharyngeal jaws have also been hypothesized to function in sound production (7). Spectrograms of sounds produced by cichlids (8) suggest that this behavior involves very rapid muscle contraction and occlusion of the pharyngeal jaws mediated by rapid muscle contraction (7). These muscles would need to be capable of powerful burst activity, as opposed to more slow-twitch muscles involved in mastication. Observable differences in enzymatic properties of pharyngeal muscles are further representative of the complexity of this structure, and perhaps the result of its dual function.

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