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Ontogeny of skeletal ossification in *Labeo calbasu* (Hamilton, 1822) using differential fluorescence staining technique

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Abstract

The present study was carried out to study the axial skeletal development in *Labeo calbasu* by using a modified double skeletal staining technique with Alizarin red and Alcian blue. The larval samples were obtained after induced breeding of wild *L. calbasu* germplasm from the River Ganga. Samples from 1 to 20 dph (day post hatching) were preserved in 4% neutral phosphate buffered formalin solution. Alizarin red and Alcian blue were used to stain the bony and cartilage parts of the skeleton, respectively. The size of the specimens ranged from 4.6 ± 0.56 to 18.8 ± 1.3 mm. The development of skeleton was observed at very early stages. A straight notochord throughout its length and origin of caudal fin rays were seen in 3 dph. The neural spines, unbranched caudal fin rays and hypurals at ventral side of notochord were clearly visible from 4 dph. Most of the head skeletal elements and vertebral column with vertebral centrum and neural spines started appearing at 6 dph. The dorsal and caudal fins with branched rays and the opercular and jaw bones started ossifying between 10 and 20 dph. The present study gives an idea about the skeletal development process as well as detects the skeletal abnormalities in Indian major carp, *L. calbasu*.

KEYWORDS

Labeo calbasu, ontogeny, ossification, skeletal system, staining

1 | INTRODUCTION

Fish skeletal system is composed of exoskeleton and endoskeleton, where exoskeleton comprises scales and fin rays. The un-segmented spines and segmented lepidotrichial rays are the parts of the fin rays. The endoskeleton is the completely ossified part of the skeletal system that forms the framework of the body. It consists of the axial skeleton and the appendicular skeleton. The axial skeleton includes skull, vertebral column and ribs, and the appendicular skeleton includes the girdle and the supporting elements of fins. The multiplicity of the bones with their complex arrangement in fish requires a vivid study on their ontogeny for the characterization of the skeletal system as well as the differential identification of species based on the skeletal arrangement (Bose et al., 2018). In teleosts, sequence of functional changes in physiological behaviour such as feeding, respiration and swimming is accompanied with parallel sequence of morphological changes in both larvae and juveniles (Yu & Wu, 2016). Therefore, a detailed knowledge about the developmental osteology is important for phylogenetic inferences about the functional relationships among teleost taxa and the environmental performance of different developmental stages of fish (Koumoundouros et al., 1997, 1999). The skeletal abnormalities originating at the early larval and juvenile stages are mainly due to the unfavourable abiotic conditions which affect the morphology and growth of fishes. Therefore the ontogenic study of the skeletal system will not only help in taxonomy but also in the early detection of any skeletal defects in the larvae during the artificial seed production. This will help in finding the possible causes and the mitigation strategies of the skeletal abnormalities to promote effective aquaculture and resources management (Koumoundouros et al., 1997). Differential fluorescent staining technique is one of the methods to study the development of bones and cartilages; both the

