AN ATLAS ILLUSTRATING BONE DEVELOPMENT OF LARVAL ASIAN SEABASS (*LATES CALCARIFER*)

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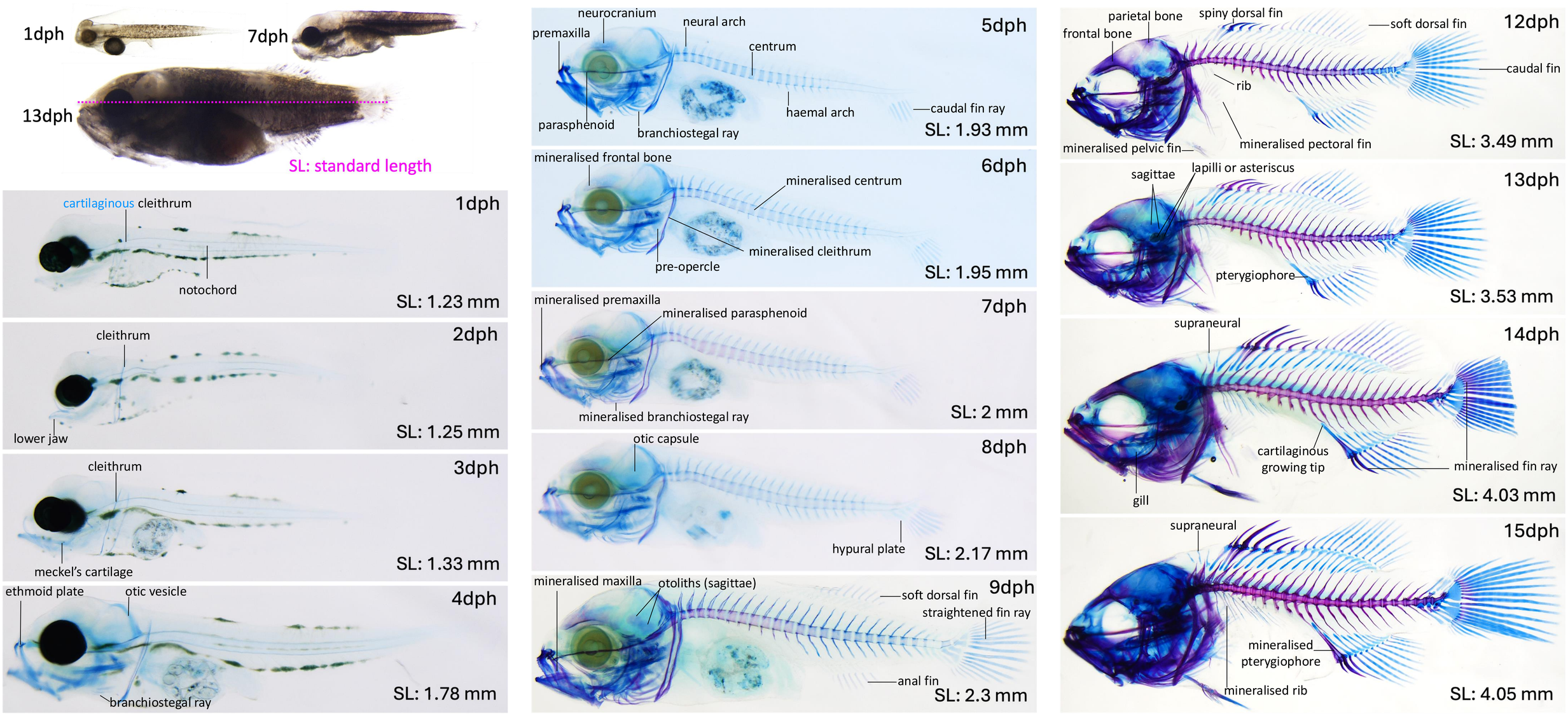
Introduction

This atlas presents skeletal development and early expression of osteogenic genes in Asian seabass larvae from 1 to 15 days post-hatching (dph). We employed Alcian Blue and Alizarin Red double staining to differentiate cartilaginous and mineralized skeletal structures. In-situ Hybridization Chain Reaction (HCR) was used on whole larvae to detect the expression of genes essential for bone formation.

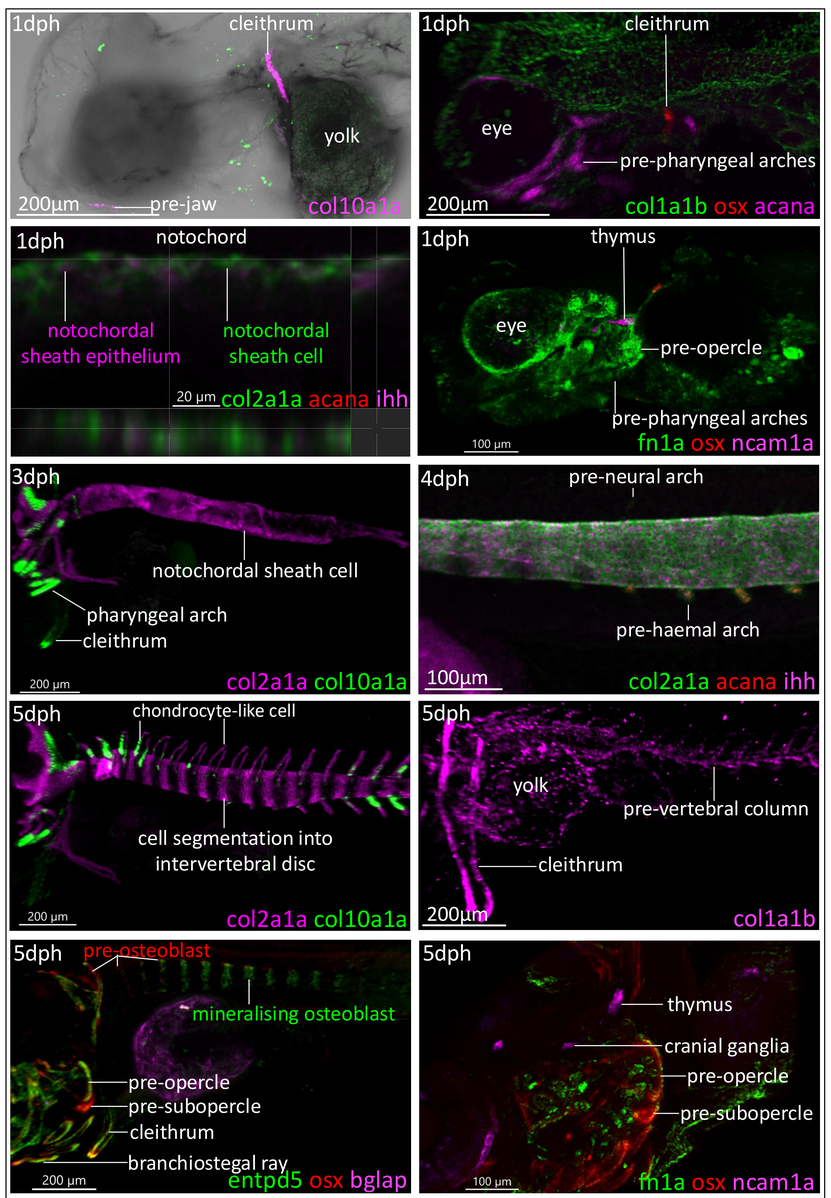
Conclusion

Our findings illustrate the transition from a Col2a1a-containing cartilaginous scaffold in young larvae to the gradual appearance of mineralized bone accompanied by increased expression of osteogenic genes including *col1a1b, col2a1a, col10a1a, acana, ihh, runx2b, fn1a, ncam1a, entpd5, osx, bglap* and *ctsk* as development progresses.

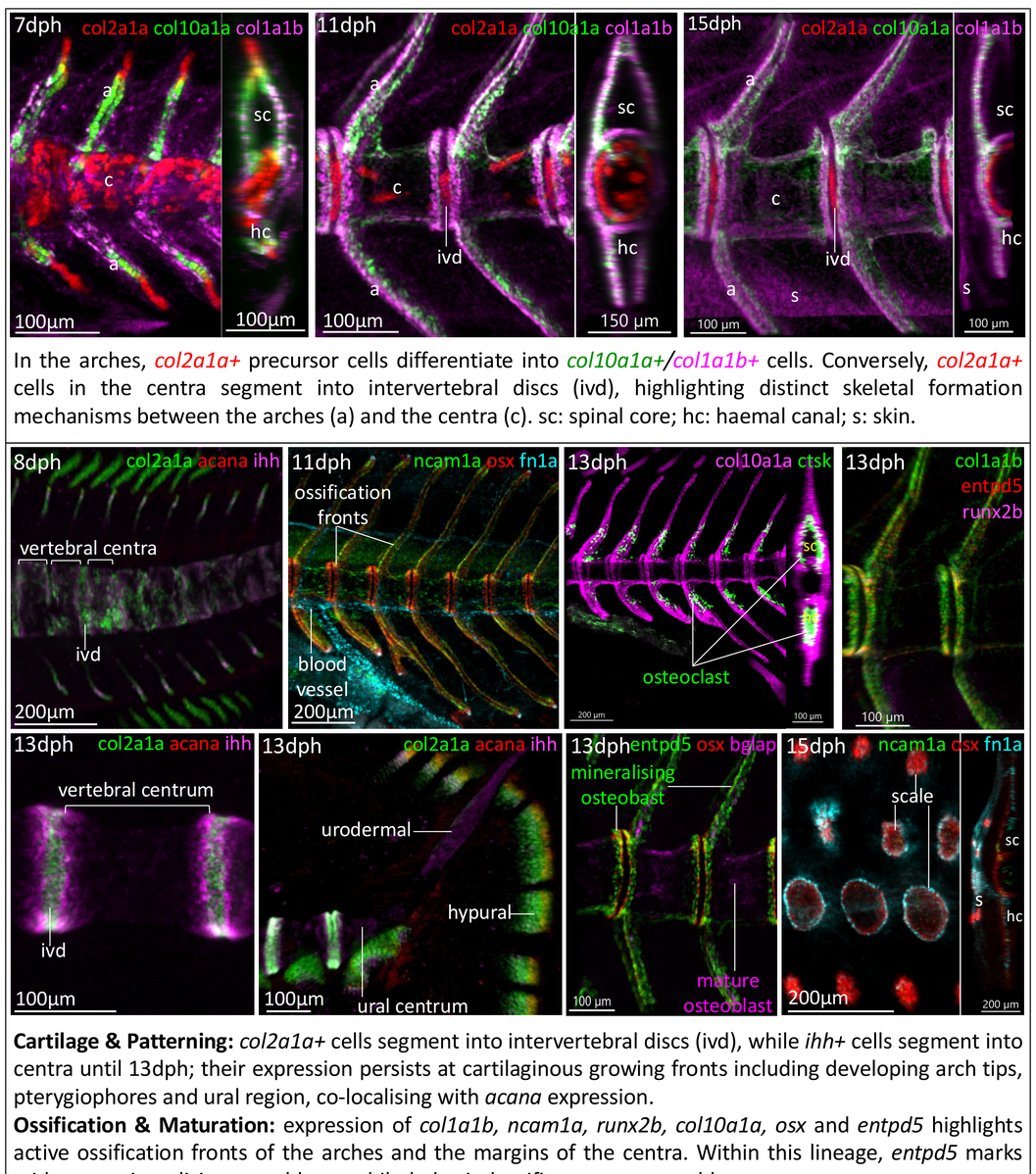
Cartilage and bone formation in Asian seabass larvae



Spatiotemporal expression pattern of osteogenic genes in Asian seabass larvae revealed by HCR



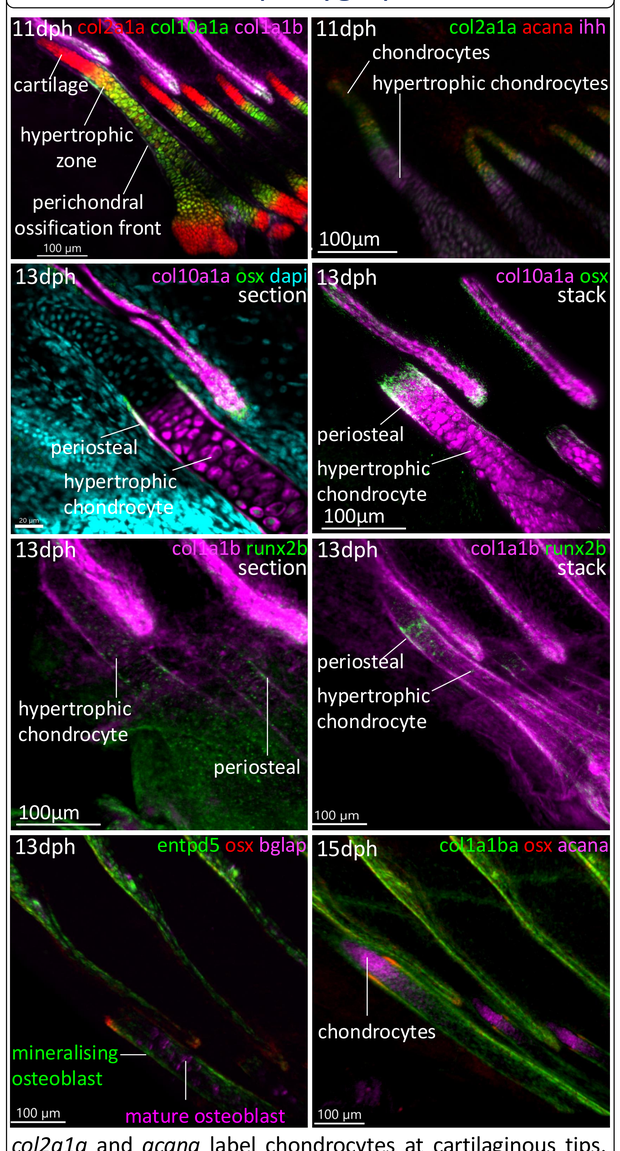
Osteogenic genes including *col1a1b, col2a1a, col10a1a, acana, ihh, osx, fn1a* and *ncam1a* are spatiotemporally expressed at 1dph. At 5dph, *col2a1a+* and *col10a1a+* cells at neural/haemal arches form cartilaginous scaffolds. While active osteoblast markers *osx* and *entpd5* highlight ossification of cleithrum, opercle, branchiostegal ray and vertebral centra.



In the arches, *col2a1a+* precursor cells differentiate into *col10a1a+*/*col1a1b+* cells. Conversely, *col2a1a+* cells in the centra segment into intervertebral discs (ivd), highlighting distinct skeletal formation mechanisms between the arches (a) and the centra (c). sc: spinal core; hc: haemal canal; s: skin.

Cartilage & Patterning: *col2a1a+* cells segment into intervertebral discs (ivd), while *ihh+* cells segment into centra until 13dph; their expression persists at cartilaginous growing fronts including developing arch tips, pterygiophores and ural region, co-localising with *acana* expression.
Ossification & Maturation: expression of *col1a1b, ncam1a, runx2b, col10a1a, osx* and *entpd5* highlights active ossification fronts of the arches and the margins of the centra. Within this lineage, *entpd5* marks mid-stage mineralising osteoblasts, while *bglap* indentifies mature osteoblasts.
Matrix & Scale Development: *fn1a* is present at the tips of ossified arches and the periphery of newly forming scales on the skin (s) alongside *ncam1a* and *osx*. *fn1a* is also expressed in blood vessels.
Remodeling: *ctsk+* osteoclasts are restricted within the neural and haemal canal, driving localised bone resorption to accommodate the spinal cord (sc) and the blood vessel within haemal canal (hc).

Anal fin pterygiophores



col2a1a and *acana* label chondrocytes at cartilaginous tips, while *ihh* marks hypertrophic chondrocytes within pterygiophores. *osx* is restricted to the periosteal layer and the transdifferentiation zone. *runx2b* is expressed in both the periosteal layer and hypertrophic chondrocytes, while a small internal cell population expresses *bglap*.

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