A NEUROANATOMICAL ATLAS OF ASIAN SEABASS, *Lates calcarifer* LARVAL DEVELOPMENT AND METAMORPHOSIS

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Introduction

Introduction: Lates calcarifer (Asian seabass) is a major aquaculture species in Singapore, but larval development and metamorphic progression remain poorly characterised under local farming conditions.

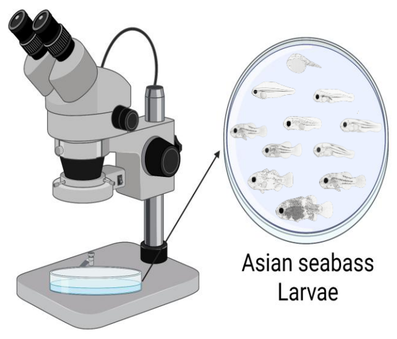
Background: Early larval stages are highly sensitive to environmental and genetic variation, leading to mortality that compromises production and survival. Brain anatomy and developmental processes also remain poorly understood despite the importance of neurohormonal regulation in growth, metamorphosis, and homeostatic behaviours.

To address these, we established a morphological staging atlas to support standardised developmental profiling across farms and rearing conditions, and are developing a neuroanatomical atlas to characterise brain development and enable future studies of growth, behaviour, and stress regulation.

Methods

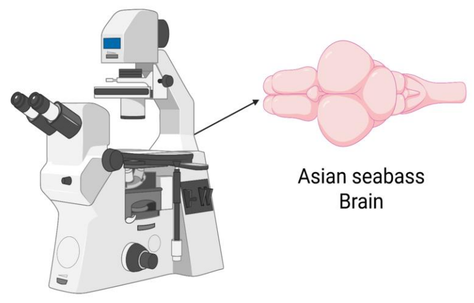
Larvae of different age were sampled from Singapore Aquaculture Technologies farms over Sept to Nov 2025.

Morphological images were captured using stereo microscope, from 0 days post hatching (dph) to 15 dph. Key morphological features were recorded and quantified to ascertain staging.



The measurement of the larvae were taken as follows:

1.Eye length
2.Head height
3.Standard Length
4.Flexion angle



Further, neuroanatomical profiles are being collected with immunostaining and a newly developed RAM-FISH in-situ hybridization for gene expression analysis.

Results

Figure 1. Early developmental atlas Asian seabass under standard farm conditions

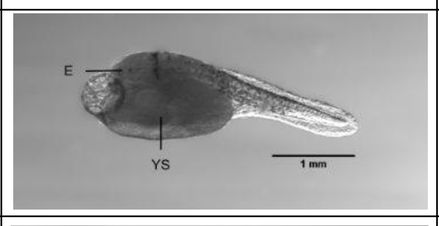
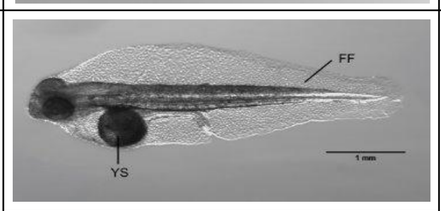
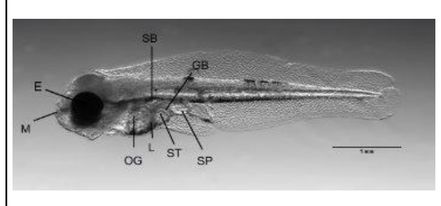
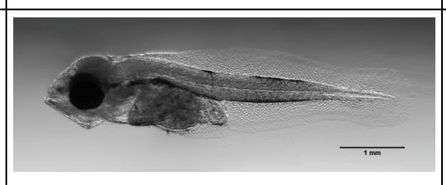
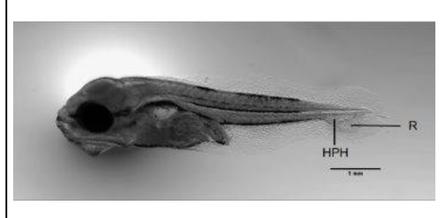
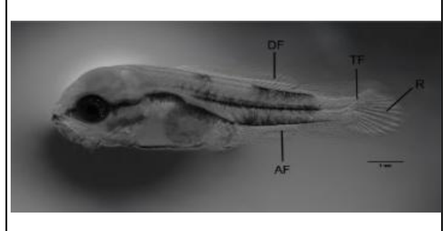
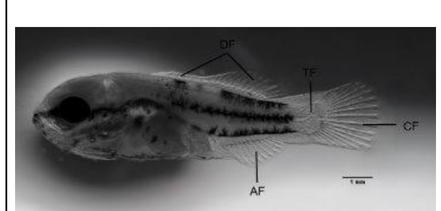
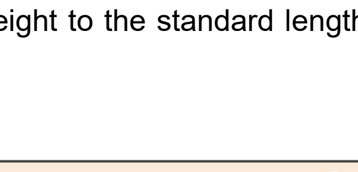
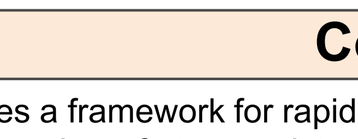
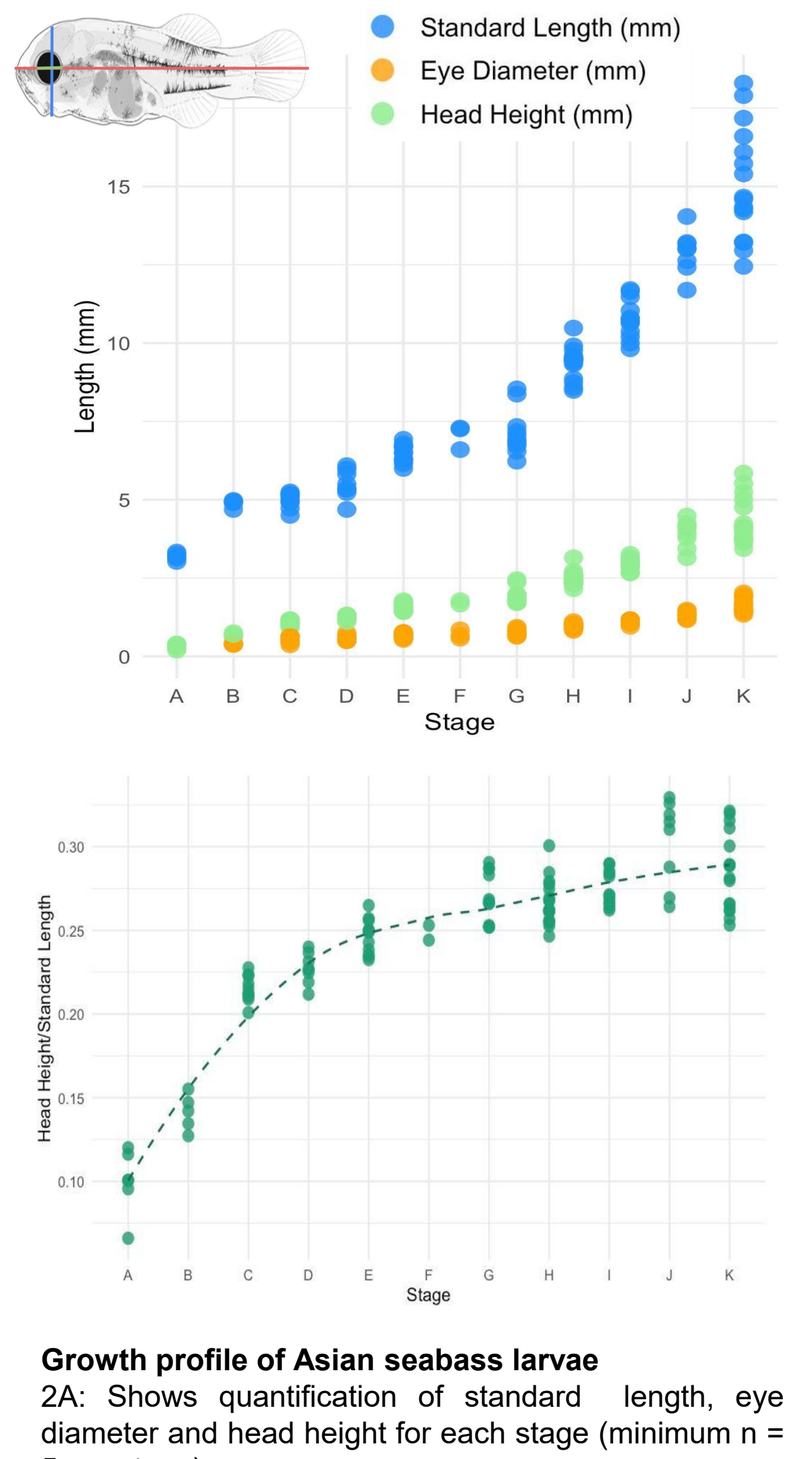
Stage	Description	Images	Day(s) Observed
A	• Yolk sac is the biggest (YS) • Unpigmented eye (E)		0
B	• Fin fold (FF) surrounds the body • Yolk sac is absorbed		1
C	• Mouth (M) opens • Eye fully pigmented • Organs and Swim bladder formation • Pectoral fins developed		2
D	• Oil globule disappears • Jaws fully formed		4
E	• Hypural, Parhypural, and haemal spine (HPH) formation • Rays (R) formed • Metamorphosis starts		5
F	• Tail flexion (TF) • Starting roughly flexed at 10 to 25° • Metamorphosis ongoing		7
G	• Ossification of HPH spines (more rays formation) • Posterior dorsal fin (DF) and anal fin (AF) starts forming • Tail flexion 28 to 45°		8
H	• Caudal (CF) and posterior dorsal fin are more differentiated • Anterior dorsal fin starts to form • Tail flexion 50° to 70°		9/10
I	• Raying of anterior dorsal fins • Fins/Rays are more defined		11
J	• Ventral fin (VF) observed • HPH spines are nearly invisible		12
K	• Complete absence of the finfold		14

Figure 2: Growth as a function of developmental stage

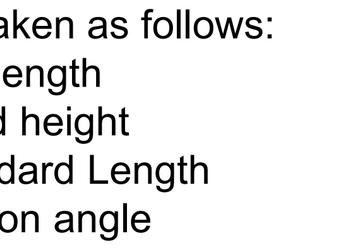


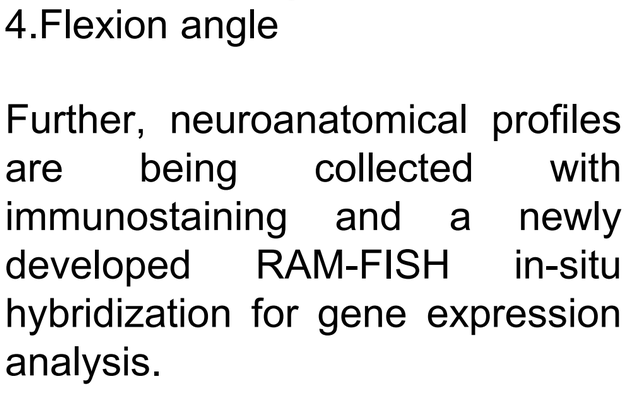
Growth profile of Asian seabass larvae

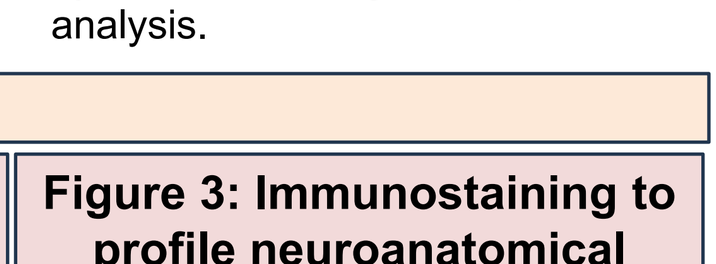
2A: Shows quantification of standard length, eye diameter and head height for each stage (minimum n = 5 per stage).

2B: Ratio of head height to the standard length across the stages

Figure 3: Immunostaining to profile neuroanatomical development at each stage







Stage C Stage E Stage F

Immunostaining with neurotransmitters, neural and metabolic function markers.

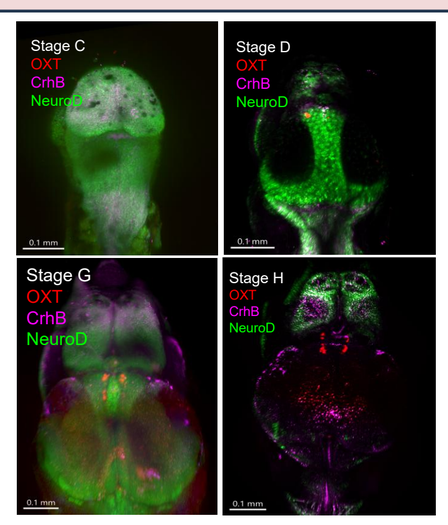
3A: Stained with anti-Tyrosine Hydroxylase (TH; green), anti-Extracellular signal-Regulated Kinase (ERK; purple) and DAPI nucleus stain (cyan).

3B: Stained with anti-serotonin from rabbit (5-HT; purple), and DAPI nucleus stain (cyan).

3C-E: anti-TH (green) across stages C, E and F, shows rapid progression of differentiated TH neurons.

Scale bar: 100µm.

Figure 4: Neuroanatomical gene expression profile using *In-situ Hybridisation Chain Reaction (HCR)*



Spatial expression patterns of oxytocin (*OXT*), corticotropin releasing hormone B (*crhB*), and *NeuroD* during brain development across stages (Stages C, E, G, and I shown). *OXT* expression was first detected at Stage E in the telencephalon. In contrast, *crhB* and *NeuroD* expression were detectable from the earliest stages in the telencephalon, optic tectum, and trunk regions. Scale bar: 0.1mm

Conclusion

- The atlas provides a framework for rapid developmental characterisation.
- It will support mapping of stress-related gene and protein expression across key tissues, brain regions, and larval stages.
- It will inform hypotheses for genetic and nutritional interventions in early Asian seabass development.

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2. Shadrina, A. M., & Pavlov, D. S. (2015). Embryonic and larval development of the Asian seabass *Lates calcarifer* (Pisces: Perciformes: Latidae) under thermostatically controlled

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