

## Short Note

# Notes on the Spawning Activity of the Tiny Scale Barb (*Thynnichthys thynnoides*, Bleeker 1852) in Relation to its Gonadal Development in Perak River, Malaysia

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The tiny scale barb (*Thynnichthys thynnoides*, Bleeker 1852) is a freshwater species of the Cyprinidae family. The species is widely distributed in Southeast Asia, from the lower Mekong basin to Thailand and Malaysia, including the Sumatra and Borneo<sup>1</sup>. The tiny scale barb is also a potamodromous fish that naturally migrates through streams and floodplains to spawn<sup>2, 3</sup>. The species inhabits the streams and lakes and synonymous with the Perak River, Malaysia, which is one of the important habitats for the fish that continuously attracts the local human population to harvest the fish during their migration. The importance of the Rui River, especially from Kuala Rui to Kampung Kerunai as a spawning ground for this fish was recently reported, but the exact location of the spawning activity has yet to be highlighted<sup>4</sup>. Such information is crucial as most of the harvesting of these fish takes place along the Rui River during their mass migration from October to December every year. Thus, there is a need to identify the exact location of the spawning activity to better conserve future populations. Hence, this study was undertaken to address this

issue, and was performed by studying the gonadal development of the fish during their migration season.

The study was conducted at monthly interval from October to December 2013 at two different stations (Fig. 1) along the Rui River, one at the Kuala Rui (5°27.54' N, 101°10.53' E) and the other one at Kampung Kerunai (5°28.74' N, 101°9.99' E), some ~12 km apart. This area is known to be one of the important migration routes for the tiny scale barb population between October and December every year<sup>5, 6</sup>. Although the migration may start from much lower in the Perak River, the Rui River is considered as the upper most destinations for the migrating population<sup>5, 6</sup>. At each site the fish were caught using a hand net, sexed and kept on ice during transport to the laboratory where the total length (cm) and body weight (g) of the fish were recorded. The fish were then dissected, before their gonads were later prepared for histological examination. The gonadal tissues were fixed in 10% (v/v) neutral buffered formalin for 24 h, dehydrated in a graded ethanol series and later embedded in paraffin. The tissues were then sectioned at 4–5 µm using a microtome

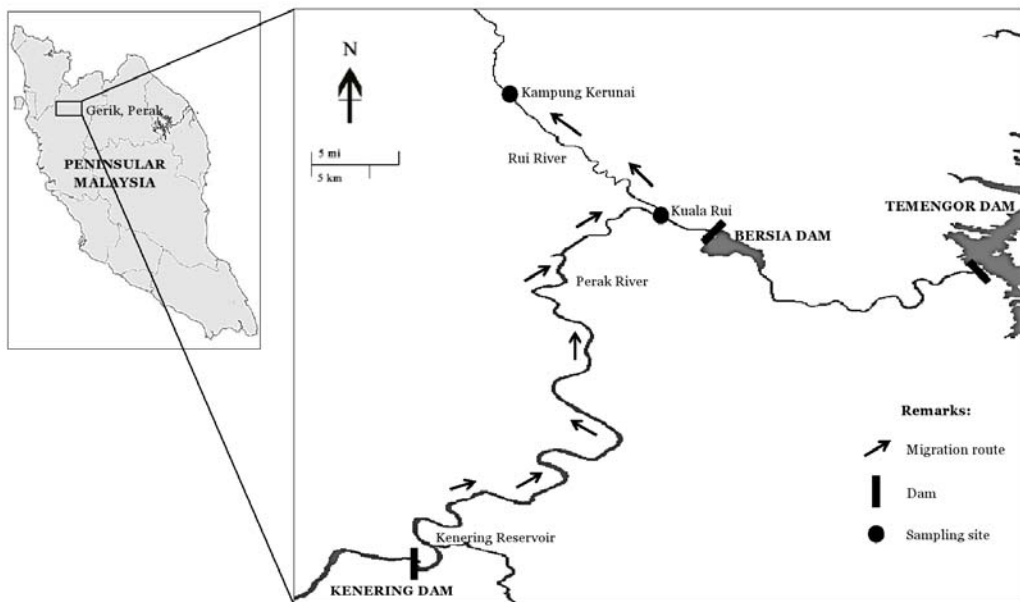


FIGURE 1. Map of the study area, the Kuala Rui and Kampung Kerunai sites along the Rui River

and stained with haematoxylin and eosin (H&E) before being examined under a light microscope to determine their developmental stages.

The different maturation stages (stages 2–5) of the female gonad that were of interest in this study are defined as follows<sup>7,8</sup>. Stage 2 (develop stage) oocytes demonstrated small, spherical to oval shape, rounded and large nucleus with dense basophilic ooplasm (perivitellogenic oocytes). Stage 3 (early maturation stage) gonads are small, spherical to oval shaped, have a rounded and large nucleus with a dense basophilic ooplasm and the appearance of yolk vesicle formation at the periphery of the oocytes. Stage 4 (fully-matured) gonads are characterized by the abundance of vitellogenic oocytes with numerous yolk and fat granules in the ooplasm with presence/absence of germinal centre at the oocyte centre. Stage 5 (spent) gonads have oocytes with a shrunken appearance, less

yolk granules in the ooplasm or the presence of atretic oocytes (hydrated oocytes).

As for the male gonads, a mature gonad (characteristic of the ripe and spawning class or class 4) has compacted seminiferous tubules with spermatids and spermatozooids. These tubules usually appear engorged and huge. In contrast, a spent gonad (post-spawning or class 5) is flaccid with semi-empty seminiferous tubules with noticeable spermatogenesis cells.

It is also important to note that the tiny scale barb exhibited isochronal or total spawning characteristics in which only a fraction of the eggs were released during repeated spawning acts<sup>9</sup>, and so several stages were expected in the male and female gonads obtained from the study area.

A total of 122 fish (46 and 16 of males and females, respectively, from the Kuala Rui site and 44 and 16 of males and females, respectively, from the Kampung

**TABLE 1.** Biological information of the tiny scale barb during their mass migration in Kuala Rui and Kampung Kerunai from October to December 2013

Parameters	Sex	Kuala Rui		Kampung Kerunai	
No. of examined fish	Male	46		44	
	Female	16		16	
		Mean±SD	range	Mean±SD	range
Body weight (g)	Male	51.65 ± 1.94 <sup>a</sup>	36.83 – 67.11	51.93 ± 1.39 <sup>a</sup>	39.15 – 66.42
	Female	56.02 ± 2.21 <sup>a</sup>	48.35 – 63.39	56.42 ± 13.11 <sup>a</sup>	43.31 – 69.53
Total length (cm)	Male	18.12 ± 0.21 <sup>a</sup>	15.90 – 19.40	18.41 ± 0.12 <sup>a</sup>	16.90 – 19.50
	Female	17.91 ±0.32 <sup>a</sup>	16.60 – 19.30	18.3 ± 1.4 <sup>a</sup>	16.90 – 19.70
*The stages occurred in female gonads					
Stage 2		100 (135.6±38.8) <sup>a</sup>		88.9 (146.2±39.3) <sup>a</sup>	
Stage 3		33.3 (186.8±63.4) <sup>a</sup>		44.4 (229.9±60.2) <sup>a</sup>	
Stage 4		100 (633.5±124.4) <sup>a</sup>		100 (721.5±119.2) <sup>b</sup>	
Stage 5		0 (0.0±0.0) <sup>a</sup>		100 (411.0±139.9) <sup>b</sup>	
*The stages occurred in male gonads					
Stage 4		100 (NA)		60 (NA)	
Stage 5		0 (NA)		40 (NA)	

\*Reading in “percentage of occurrence (diameter of oocytes for female and spermatocytes in male in mean±SD  $\mu\text{m}$ )”

<sup>a,b</sup> Different letters represent a significant differences ( $p < 0.05$ ) between measurement in Kuala Rui and Kampung Kerunai of the same row. However, for the stages occurred in female gonads, the different letters only represent for the diameter of oocytes, but not for percentage of occurrence.

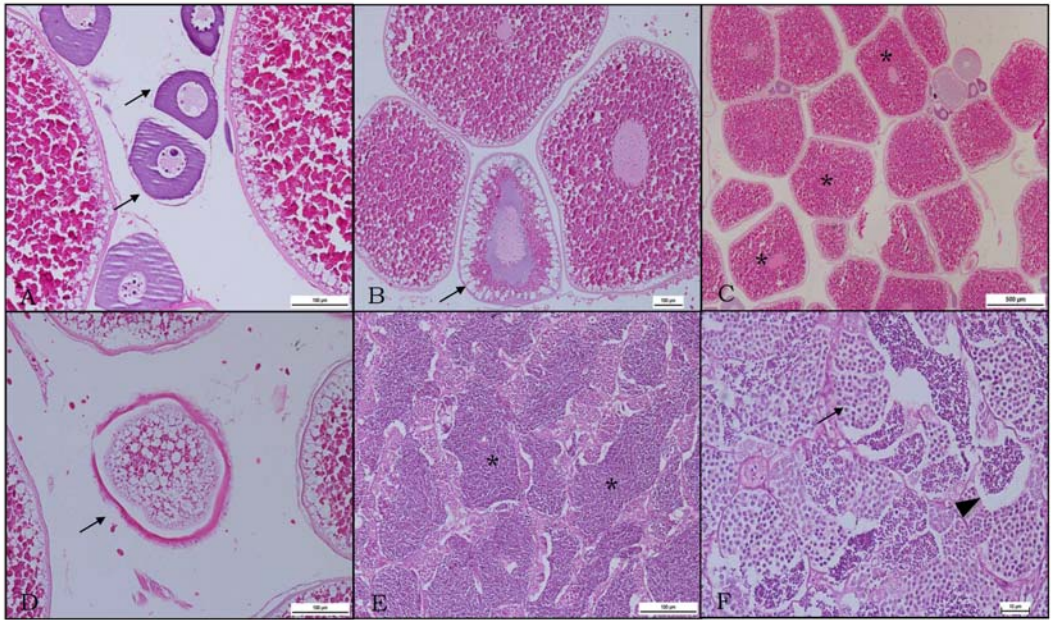
NA data not available

Kerunai site) were caught. The biological information of the tiny scale barb during their mass migration in both sampling sites from October to December 2013 is presented in Table 1.

All of the females (100%) sampled at the Kampung Kerunai and Kuala Rui sites showed the occurrence of stage 4 or fully matured oocytes in their gonads with smaller percentage of stage 3 (44.4% and 33.3% of oocytes respectively) and stage 2 (88.9% and 100% of oocytes respectively) gonads (Table 1). In addition, 100% of the female fish sampled at the Kampung

Kerunai showed the occurrence of stage 5 gonads with a shrunken appearance and the presence of atretic oocytes, compared to 0% occurrence in Kuala Rui. Moreover, the diameter of oocytes for stage 4 and 5 significantly bigger in Kampung Kerunai compared to Kuala Rui, but not for stage 2 and 3. All of these observations suggest that the spawning happened between these two sampling sites.

As for the males, 100% of the fish collected from the Kuala Rui showed the occurrence of gonads that were engorged or ripe and ready to spawn or stage 4.



**FIGURE 2.** (A) Dense homogenous basophilic ovioplasm (arrow) and nucleus at the centre of oocytes. Stage 2; perivitellogenic oocytes. Kuala Rui sample, HE, X200. (B) Early yolk vesicle formation at the periphery of oocyte (arrow). Stage 3; early mature oocytes. Kampung Kerunai sample, HE, X100. (C) Dense yolk vacuoles and fat granules accumulated in the ovioplasm (star) with/no presence of germinal centre. Stage 4; vitellogenic oocytes. Kuala Rui sample, HE, X40. (D) Thick muscle and shrinkage appearance (arrow) of oocytes. Stage 5; atretic oocytes. Kampung Kerunai sample, HE, X200. (E) Compacted spermatid in the seminiferous tubules (star). Class 4; ripe and spawning class. Kuala Rui sample, HE, X200. (F) Spermatogenesis cells (arrow) and semi empty seminiferous tubule (arrow head). Class 5; spent class. Kampung Kerunai sample, HE, X400.

Spermatocytogenesis cells were also observed at the peripheral area of the tubules and consisted of spermatogonia and primary spermatocytes. In contrast, 60% of the male fish sampled from the Kampung Kerunai had ripe gonads of the spawning class while the other 40% of the males had spent gonads or stage 5, indicating that a portion of the sperm had been released, and thus spawning had taken place in the lower part of the station. Representative examples of the different stages of female and male gonads observed during the study are shown in Fig. 2.

In general, this study confirmed that the Rui River, especially the area from Kuala

Rui to Kampung Kerunai plays an important role in the tiny scale barb population as it serves an area for their spawning activity, as previously suggested<sup>4</sup>. In addition, the fractional spawning behaviour exhibited by the species over a certain period of time is viewed as advantageous to help reduce intraspecific competition and allow optimal utilization of the breeding ground and physico-chemical quality of the water<sup>10, 11</sup>. Thus, by control the harvesting activities of this fish along the river during their migration season might help in maintaining their population for the future. Furthermore, conserving this area remains essential to

ensure the continuity of the species and fish supplies to the locals in the future.

Presently, the large scale mining activity in the upper stream of the river is of some concern as the water quality would deteriorate and eventually affect the quality of the fish in particular and the ecosystem in general. Future studies should also address the status of the pollution, such as trace heavy metals, and monitor the habitat quality of the study area.

### ACKNOWLEDGEMENTS

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