



Spot Polymorphism and Size do not indicate Sex Identity: Implications for the Random Selection Method for Natural Spawning of Spotted Barb (*Puntius binotatus*) in Pond

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Abstract

The relationship between the numbers of spot, sex and size of the spotted barb, *Puntius binotatus* was investigated in order to develop a phenotypic sex identification method for the broodstock management of this species. A total of 77 fish specimens with total length (TL) ranged from 4.0 to 9.6 cm were examined for spot polymorphism (3 spots and 4 spots) and sex. Chi's square test analysis showed spot polymorphism did not correlate with sex identity. The spot polymorphism appeared in both male and female fish at various sizes. These results suggest that such polymorphism is not a result of the morphological changes during the fish growth. The sex ratio in *P. binotatus* was found to be 1: 1.2 (male: female). Male reproductive organ (testis) was found fully developed first at TL 4.0 cm while ovary was observed in larger fish (TL 4.95 cm). The present study concluded that the spot polymorphism is not a reliable phenotypic sex identification method. Nevertheless, random selection of at least 77 fish with minimum size 4.0 cm as broodstocks would be sufficient for the natural spawning of *P. binotatus* in pond.

Keywords: Spot polymorphism, sex identification, spotted barb, *Puntius binotatus*.

Introduction

The spotted barb *Puntius binotatus* from Family Cyprinidae is an indigenous fish species in Asia¹. In some scientific literatures, this fish is synonymous with *Systemus binotatus*, *Capoeta binotata*, and *Barbodes binotatus*²⁻³. The *P. binotatus* is distributed in Brunei Darussalam, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Thailand and Vietnam, but introduced to Singapore and Palau⁴. It can be easily found in the mountain streams, rivers and lakes, and is omnivorous, feeding mainly on zooplanktons, insect larvae and some vascular plants⁵. Due to its common availability in these freshwater resources, the *P. binotatus* also plays an important role as a bio-indicator to habitat degradation or health status of environments⁶⁻⁹.

P. binotatus is a common ornamental fish species. However in Sabah, it is locally known as "Turongou" and has been a delicacy for the indigenous people, with additional preference to the egg-bearers. In order to promote this delicacy at commercial level, the first hatchery and pond culture for this fish has been setup¹⁰. However, its biological information which is critically important for hatchery production is limited. Although Rahmawati¹¹ reported the reproductive biology and characteristics of this fish in the wild of western Jawa (Indonesia), the sex identification for this fish remains difficult. The fish matures at small sizes hence its genital organs morphology is difficult to be examined by naked eyes. In recent, Dorado *et al.*¹² demonstrated the sexual dimorphism in body

shapes of the *P. binotatus* at Lake Buluan in Mindanao, Philippines. Based on the analysis using landmark-based geometric morphometric, the males were reported to possess significantly slender body shape and wider anal fin bases, while the female fish had bigger head region, deeper body depth and shorter tail region than the males. However, these differences were also difficult to be noticed by naked eyes as this study was actually conducted using digitization software to analyze the fish morphology. This information can be a good reference but it may not be friendly for the farmers to practice in separating both sexes of fish. Therefore, an easier method to determine sex should be found and developed for the broodstock management of this fish in farm.

There is an indigenous knowledge in Sabah believes that the spots on the trunk of this fish can be used to determine its sex. It was believed that the fish with four spots are females while those with three spots are males. However, there was no scientific evidence to support these statements. On the other hand, fish growth may influence the occurrence of the spot polymorphism. In fact, it was reported that the juveniles and sometimes adult possess 2 to 4 spots on its trunks¹³ and these spots may be disappeared in the larger specimens, except the one on the caudal peduncle^{11, 14}. Nevertheless, the relationship between the spot numbers with the fish sizes has never been elucidated. Therefore, the present study intends to clarify the relationship between the spot numbers, sex and size of the fish. Such information can be valuable for the brood stock management of captive breeding of *P. binotatus*.

Material and Methods

A total of 77 fish specimens with wide range of sizes were collected from the ponds managed by Innovasi Sedia Private Ltd. Details of the pond culture conditions were described in Lim *et al.*¹⁰. In brief, the ponds water was sourced from mountain streams by gravity, and the temperature ranged 27 – 29 °C and pH 7 – 9 from morning to evening. Approximately 200 wild *P. binotatus* were caught from different locations and cultured in these ponds as the founder fish. These fish were fed twice daily until satiation with commercial starter pellets for tilapia (Dindings Ltd., Malaysia). Such extensive culture has been continued for 2 years long, and natural spawning occurred in the ponds. The young fish were cultured together with the founders in the same ponds. The collected specimens for the present study were recognized as the offspring of the founder fish based on their smaller body sizes.

The specimens were transferred to the wet laboratory of the Borneo Marine Research Institute (BMRI) and maintained in several aquaria with aeration. All specimens were dissected to examine their gonad for sex determination. Before the dissection, the fish were anesthetized with 200 ppm MS222. The numbers of spot on both sides of fish trunk lateral was counted from the near-to-gill area to the peduncle, and the total length (TL) was measured. The data were then analyzed by using Chi's square (χ^2) test in the SPSS v.19 software.

Results and Discussion

The TL of all fish specimens was ranged from 4.0 to 9.6 cm. Two major types of spot polymorphism were recorded: Type 1 (both side of body possessed 3 spots) and Type 2 (both sides of body possessed 4 spots). The examples of three- and four-spots fish are shown in figure 1a and 1b, respectively. Based on the gonad examination, the female fish was identified by the egg-carrying ovary (figure 1c); the male fish was determined by the appearance of the whitish testis (figure 1d) while those that possessed neither ovary nor testis were categorized as the *sexunknown*. The smallest size of the male and female fish was at TL 4.0 and 4.95 cm, respectively. These sizes are smaller than those observed by Rahmawati¹¹ in the wild *P. binotatus* of western Jawa (female TL 5.6 cm, male TL 5.0 cm). The onset of maturation in fish is not genetically determined but a function of growth rate and size¹⁵. Therefore, the *P. binotatus* should be first matured when they achieved at least TL 4.0 cm. Early first maturation of the fish in hatchery as opposed to the wild ones could be mainly due to the fish feeding and nutritional conditions. In the culture pond, the fish were fed twice daily until satiation. In addition, they could feed on the natural food that available in pond, including planktons and mosquito larvae. Compared to those in nature that relies solely on natural food and are exposed to predators, the cultured fish should be able to utilize more nutrients and energy for spawning hence the maturation take place once they achieved the right size. Since the *P. binotatus* matured at minimum TL 4.0 cm, the *sex*

unknown fish recorded in this study were those of in spent condition but not immature.

The number of fish based on sex and the spot numbers on their body is shown in table 1. Of the total 77 fish specimens, 25 females, 29 males and 23 *sexunknowns* were identified and showed no significant difference among each other ($\chi^2=1.273$, $df=2$, $P=0.529$) as $P>0.05$. The ratio of female to male fish was 1: 1.16. On the other hand, the number of fish with 3 and 4 spots was 28 and 49 respectively, and the number of 4 spots fish was significantly higher than the 3 spots fish ($\chi^2=5.727$, $df=1$, $P=0.017$) as $P<0.05$. For the correlation test between the variables of spot numbers and sex, the values of $\chi^2=1.417$, $df=2$ and $P=0.492$ ($P>0.05$). This result indicates that the numbers of spot does not determine sex identity of the fish. Nevertheless, the possibility remained that the spot polymorphism could be a visual cue to the fish for mate selection, such as the 4-spots female may prefer the male with same spot numbers than the one with 3-spots. In fact, it is well known that guppies *Poecilia reticulata* select mates depending on the color pattern polymorphism¹⁶. Further examination is necessary to elucidate this hypothesis.

Table-1
The number of specimens observed based on the spot numbers and sex

Spot number	Sex			Total
	Female	Male	Unknown	
3 spots	11	11	6	28
4 spots	14	19	16	49
Total	25	30	22	77

Figure 2 shows the correlation between the variables of spot numbers and fish TL. It was found that the distribution of the plots was overlapping and did not show distinctive clustering across any sizes. Both types of spot polymorphism can be found in all sizes of fish hence the occurrence of spot polymorphism is not due to the fish growth. On the other hand, it could be due to the genetic variation among different populations of fish. In fact, Orton *et al.*¹⁷ demonstrated that the dorsal-lateral spot numbers in a marine kyphosid fish *Girella nigricans* in California is significantly difference between populations from proximal island and mainland, and also between the geographic extremes in the sampled range. Therefore, the co-existence of *P. binotatus* with different types of spot polymorphism in this study could be the result of the origin of broodstocks, which were collected from different places in Sabah with apparent geographical differences. Nevertheless, the size range of fish examined in the present study was narrow (4.0 – 9.6 cm), compared to the largest size of fish from wild (20 cm) which was ever recorded¹⁸. It is possible that the number of spot may reduce when the fish has grown into larger size than the sizes examined in the present study, as suggested by Rahmawati¹¹ and Herre¹⁴. Further observation is needed to clarify this hypothesis.

Figure 3 demonstrates the number of female and male fish in each size class. Based on the χ^2 -test analysis, the number of

female and male fish in each size class showed no significant difference ($df=1$, $P>0.05$). This indicates that the *P. binotatus* does not undergo sex reversal within the observed size classes.

Indeed, there has been no record on sex changing in cyprinids with growth.

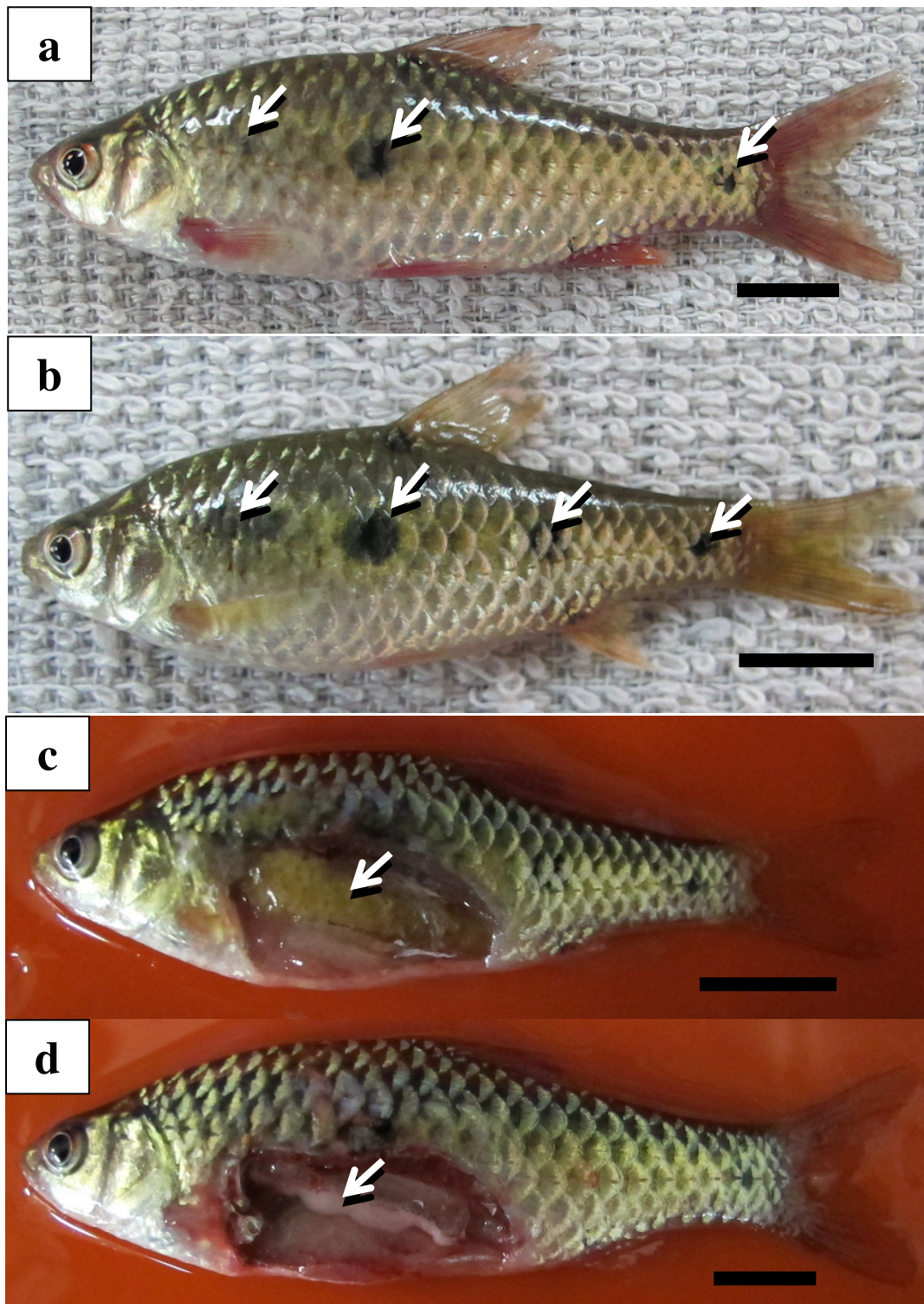


Figure-1

Spotted barb, *Puntius binotatus* (a) 3 spots; (b) 4 spots; (c) female; (d) male. The arrows in (a)-(b) show the spot on body surface, while those in (c)-(d) show the fish gonads. Scales: 1.0 cm

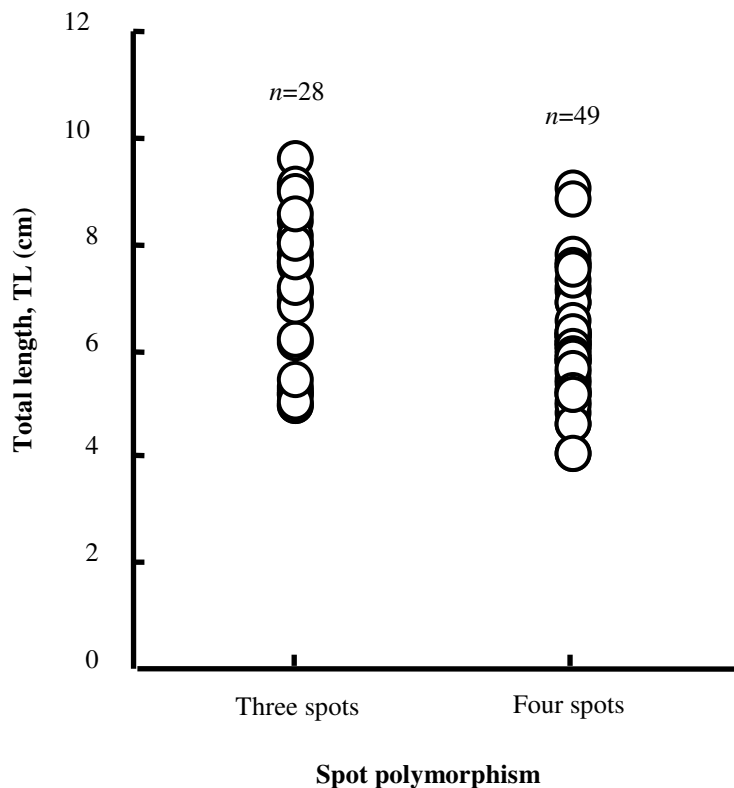


Figure-2
 Distribution pattern of the fish based on the types of spot polymorphism and total length

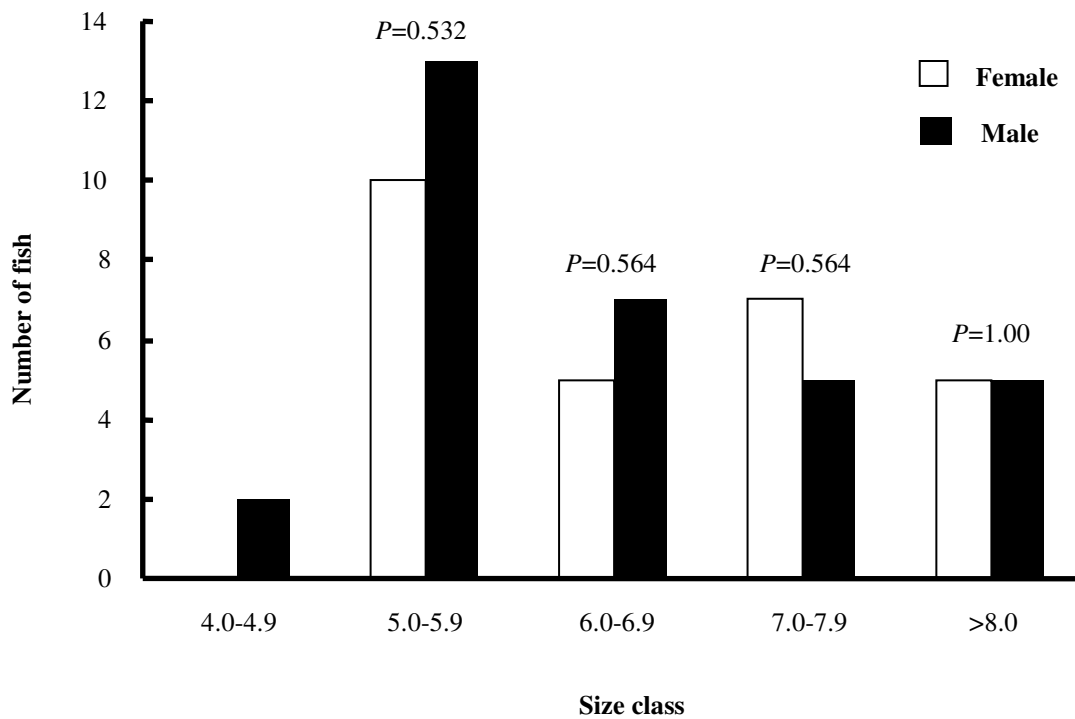


Figure-3
 Comparisons between the number of female and male fish in each size class. The P value of Chi's square test for comparing the number of female and male fish in each size class is shown above the bar graph

In short, the spot polymorphism does not indicate sex in the *P. binotatus* and such polymorphism could be due to the genetic variation for the fish from different populations. Therefore, the indigenous knowledge has no scientific basis and will not be beneficial for sex identification of this fish species. Nevertheless, this fish does not experience sex reversal at least up to TL 9.6 cm, and probably throughout its life history. In addition of that, it possess a nearly 1 to 1 sex ratio (in this present study, male: female = 1: 1.16; in Rahmawati, male: female = 1:1.15) with at least 77 or more fish are selected. These results suggest that random selection of at least 77 fish at minimum TL 4.0 cm as broods stocks would be sufficient for the natural spawning of *P. binotatus* in pond, instead of selecting the fish by sex.

Conclusion

The indigenous knowledge on the sex identification for the spotted barb *P. binotatus* based on the numbers of spot on its body is not practicable as the spot numbers, also the size in length, do not indicate the fish sex identity. Nonetheless, selection of the broodstock for natural spawning in pond can be done with randomly select minimum 77 fish with sizes of at least 4.0cm TL, according to its population nature with approximate 1: 1 of sex ratio.

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