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Research Article

SEX IDENTIFICATION IN PEARLSPOT MADE SIMPLE: A STEP TOWARDS SUSTAINABLE BREEDING

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ABSTRACT

Pearlspot (*Etroplus suratensis*), the state fish of Kerala, is a promising candidate species for commercial aquaculture. However, sex identification poses a major challenge in the culture system, as sexual dimorphism is prominent only during the breeding season, complicating broodstock management. Traditional methods rely on the dissection of the species for sex confirmation, which is impractical for farmers. The present study introduces a visual morphology-based technique for accurate sex determination of this candidate species. By enabling precise identification of males and females, it empowers aquaculturists to optimise stocking ratios in breeding programs, thereby increasing yield, boosting production efficiency, enhancing conservation, and supporting sustainable pearlspot farming.

Keywords: *Etroplus suratensis*, State fish, Sex determination, Aquaculture, Fisheries management.

INTRODUCTION

Pearlspot (*Etroplus suratensis*), an Asian cichlid, is widely regarded as a major candidate species for aquaculture in various parts of India, particularly in Kerala. However, large-scale seed production of this food fish is constrained by the absence of reliable induced breeding techniques (Christo, M., & Divya, P. R., 2025). Although hatchery breeding and seed production techniques for *E. suratensis* have been successfully standardised, large-scale commercial seed production remains limited, and aquaculture operations continue to depend substantially on backyard hatcheries and wild-collected seed (Sukumaran *et al*., 2025; Hussain *et al*., 2022). Despite several nationwide attempts to commercialise pearlspot seed production, success has been limited (Vikas, P. A., & Subramannian, S., 2023). Major constraints include low fecundity, strong parental care behaviour, and difficulty in sex identification (Christo, M., & Divya, P. R., 2025). A key bottleneck restricting the expansion of pearlspot farming is the inadequate supply of quality seed for stocking in different grow-out systems, which is largely due to the lack of captive reproduction in hatcheries and farms (Vikas, P. A., & Subramannian, S., 2020; Smina *et al*., 2025).

Sex determination is a major constraint in some aquaculture species (Zhang, S. Y., & Zhang, L. B., 2020; Li, X. Y *et al*., 2022). Moreover, identifying the sex of individual animals is essential for understanding their behaviour and ecology (Li, X. Y *et al*., 2022) and has important implications for management and conservation (Horne *et al*., 2020). In aquaculture species, sex is a highly valuable trait, as pronounced sexual dimorphism is commonly observed in growth, size, morphology, colouration, and behaviour, making these organisms important models for studying sex determination mechanisms (Li, X. Y *et al*., 2022; Horne *et al*., 2020). However, reliable external sexing methods have not been established for many economically important finfish. Sex determination does not apply to sexually immature adults or sexually monomorphic species. Molecular sexing has become increasingly popular, yet it requires specialised skills, expensive laboratory equipment, and time-consuming sample processing. In contrast, morphometric analysis of sexual dimorphism offers a low-cost, rapid, and field-applicable alternative for sex determination (Wang *et al*., 2025). Given the central role of pearlspot in Kerala's aquaculture, accurate sex identification is critical for successful breeding programmes.

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Accurate sex determination is a fundamental prerequisite for artificial breeding and large-scale fry production in fish, which directly influences the success of the production cycle for each species. For pearlspot, the state fish of Kerala, distinguishing males from females remains a practical challenge for aquaculture practitioners. Pearlspot is a gonochoristic species with little external sexual dimorphism, making visual sexing difficult during the juvenile stage. In breeding programmes, the target sex ratio for stocked brooders is usually 1:1, but reliable sex identification is generally only possible in mature adults, especially during the breeding season (Vikas, P. A., & Subramannian, S., 2020). Pearlspot naturally forms pairs during the breeding season when they reach sexual maturity. In light of the above, the present study aims to determine the sex of pearlspot by carefully examining the external morphology of the genital papilla, even before sexual maturity is reached.

Although sexual dimorphism based on genital papilla morphology has previously been described in pearlspot, the reliability of this method has not been validated under captive breeding conditions. In the present study, morphology-based sex assignments were confirmed through individual tagging and subsequent reproductive behaviour, providing validation of the method's accuracy. The study, therefore, aimed to develop and validate a practical, non-invasive protocol for sex identification using external morphological traits, with applications in broodstock management, conservation programmes, and sustainable aquaculture.

MATERIALS AND METHODS

The study was conducted at the Government Fish Farm, ADAK Ayiramthengu, Kollam, Kerala, India (9°07′26″N, 76°28′51″E). The experimental fish were obtained from cage culture systems. A total of 50 pearlspot brooders were collected and maintained in a 1-ton FRP tank. Five male-female pairs were selected based on external examination of the genital papilla. Mature males were identified by the presence of a narrow, pointed genital papilla, whereas females possessed a broader and blunt ovipositor. Only sexually mature individuals exhibiting distinct genital papilla morphology were chosen as broodstock for breeding.

Selected individuals were tagged intraperitoneally using Biomark HPR Lite 9 mm passive integrated transponder (PIT) tags. For each tagged fish, tag ID, total length (cm), body weight (g), and sex were recorded. Detailed information on tagging, morphometrics, and sex is presented in Table 1. Each selected pair was stocked separately in individual RCC tanks (4 m × 2 m × 1.5 m), provided with suitable substratum for egg attachment and a sand layer for pit formation. The sand substrate was demarcated and stabilised using bricks to facilitate natural spawning behaviour. Breeding trials were conducted using five independent male-female pairs, each representing a replicate. Consistency was assessed based on the occurrence of characteristic reproductive behaviours, including pair formation, spawning, and parental care. Comparable behavioural patterns were observed across all five replicates, providing consistent confirmation of the morphology-based sex experiment. During the breeding trials, water quality parameters were maintained at salinity around 15 ppt, dissolved oxygen above 3.5 mg L^{-1}, pH 7.0-8.0, and temperature 24-32 °C. Broodstock were fed twice daily with a formulated pellet diet containing 32% crude protein and 5% lipid. These conditions were maintained throughout the study to support normal reproductive behaviour and successful spawning. To further validate sex determination, ten additional pairs were sacrificed, and gonadal examination was performed through dissection. The observed anatomical sex was cross-verified with prior morphological assessments. This combined approach of morphological selection, anatomical validation, replication of breeding trials, and individual tagging and segregation ensured minimised experimental error and enhanced the reliability of the results.

Table 1. Tagging details, length, weight, and sex of the pearlspot used in the study.

Sl. No.	Tag ID	Length (cm)	Weight (gm)	Sex
1	TAG 982.126057501736	15.9	92	Female
2	TAG 982.126057501757	15.5	93	Male
3	TAG 982.126057501741	15.3	81	Female
4	TAG 982.126057501761	14.2	72	Male
5	TAG 982.126057501769	14.3	65	Female
6	TAG 982.126057501778	16.5	114	Male
7	TAG 982.126057501749	15.7	106	Female
8	TAG 982.126057501737	14.8	65	Male
9	TAG 982.126057501800	17	110	Female
10	TAG 982.126057501792	16.2	108	Male

RESULT AND DISCUSSION

In males, a distinct notch was clearly visible externally through morphological examination, appearing as two separate parts along the genital papilla. In females, this notch was either absent or markedly less pronounced, with the genital region maintaining a more uniform, rounder shape (Figure 1). Upon dissection, individuals exhibiting the prominent notch were confirmed as males. Those lacking a distinct notch were identified as females, thereby validating the external morphological assessment (Figure 2). In the RCC tanks, the tagged pairs showed clear pairing behaviour, further supporting the accuracy and reliability of the present methodology for sex identification in pearl spot (Figure 3).

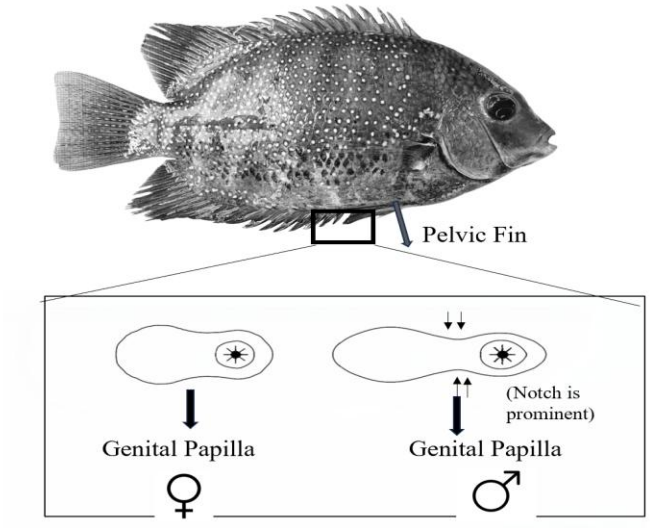

Figure 1. Image showing sex differentiation in female and male pearlspot.

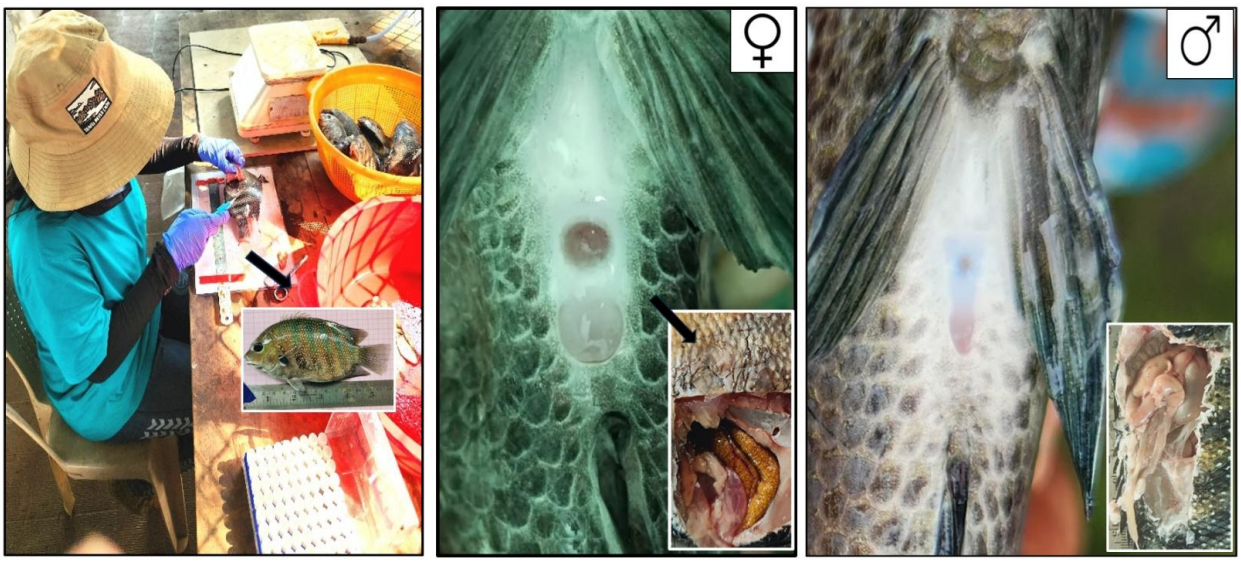

Figure 2. Visual sex determination in pearlspot broodstock through gonad dissection.

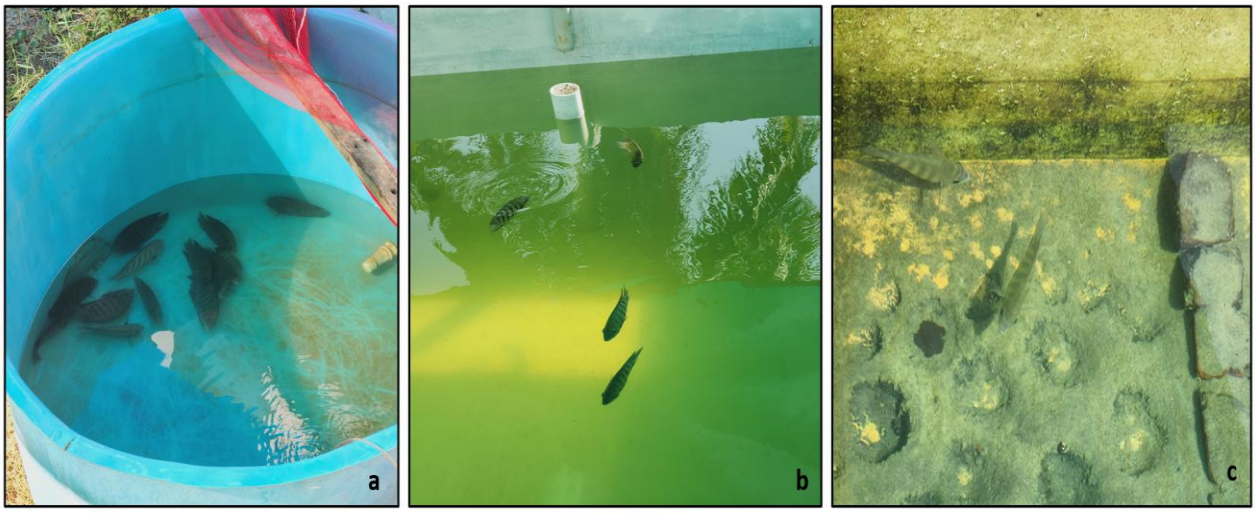

Figure 3 (a). Broodfish held in an FRP tank for sexual identification; (b) Stocking of brood fish in an RCC tank; (c) Male and female Brooders with egg.

Accurate sex identification is essential for successful artificial breeding and broodstock management in aquaculture, and phenotypic approaches are generally preferred for their simplicity, cost-effectiveness, and rapid applicability in the field (Wang *et al*., 2025). Sexual dimorphism has attracted attention in biological research for its ecological and practical significance (Horne *et al*., 2020). Although widespread across many species, the mechanisms governing sex determination are highly diverse. Fishes, in particular, exhibit remarkable variability in sex-determination systems, making them ideal models for investigating these processes (Lu, G., & Luo, M., 2020; Ma, Y. *et al*., 2022; Wu, Y. *et al*., 2022; Chen, J. *et al*., 2022)

Sexual dimorphism in fishes is expressed through a wide range of phenotypic traits, including differences in body size, shape, colouration, physiology, and behaviour (Gui J. F. *et al*., 2021). These variations arise from the combined influence of genetic and environmental factors during growth and development (Chelini, M. C. *et al*., 2019). In aquaculture, the extent of sexual dimorphism is particularly important because species exhibiting pronounced sex differences offer advantages for selective breeding and production efficiency. However, in many fish species, sex chromosomes are poorly differentiated, making chromosomal identification difficult. In addition, environmental factors such as temperature can influence sex differentiation, resulting in discrepancies between genotypic and phenotypic sex (Chen, J. *et al*., 2022). Conventional methods for sex identification, including molecular marker-based techniques, can accurately determine sex at the genetic level, but their application is often constrained by high costs, technical complexity, and the requirement for sophisticated laboratory infrastructure (Xiao, Y. *et al*., 2024). Similarly, anatomical and histological examination of gonadal tissue provides reliable results but involves invasive procedures such as dissection, making them unsuitable for live broodstock in breeding programmes (Li, X. Y. *et al*., 2022). These limitations, coupled with the need for skilled personnel and time-intensive procedures, restrict their use in routine aquaculture practices. Consequently, there is a growing demand for simple, rapid, and non-invasive methods for sex identification that can be applied effectively in both laboratory and field conditions. Efficient seed production and reproductive performance in pearlspot aquaculture depend on proper broodstock management (Kumar, P. *et al*., 2025; Khanna, A. R. *et al*., 2026; Malik, M. A. *et al*., 2026), where accurate sex identification becomes an important practical consideration.

In this context, external morphological traits have been explored as practical alternatives, although such criteria are limited to a relatively small number of fish species. In general, sexual dimorphism in fishes is manifested through differences in body size, with females often larger and heavier due to reproductive adaptations, such as increased gonadal development and fecundity, whereas in some species, males may attain larger size due to sexual selection and competition (Horne, C. R. *et al*., 2020; Sharma, L., & Singh, R., 2024). Additional differences include variations in body shape, fin morphology, and the presence of specialised structures such as gonopodia and nuptial

tubercles, which are typically more pronounced in males and play key roles in courtship and reproductive success (Sharma, L., & Singh, R., 2024). Colouration is another important dimorphic trait, with males often exhibiting brighter and more conspicuous colours during the breeding season to attract mates, whereas females tend to display more subdued colouration for camouflage and energy conservation. Subtle variations in body proportions, such as increased abdominal depth in females and more streamlined morphology in males, further contribute to sex differentiation (Wang, Q. *et al*., 2025; Sharma, L., & Singh, R., 2024). Despite these general patterns, many economically important species exhibit only temporary dimorphism, where distinguishing features become apparent primarily during the breeding season. This is particularly evident in pearlspot, which is largely monomorphic, with sexual maturity typically attained within the first year and clear dimorphic traits expressed mainly during the reproductive period (Christo, M., & Divya, P. R., 2025). Previous studies have highlighted the challenges associated with external sex identification in this species, which can lead to increased handling stress and reduced efficiency in broodstock management (Vikas, P. A., & Subramannian, S., 2023). Nevertheless, certain reliable morphological cues emerge during the breeding season. The genital papilla-like projection located anterior to the genital aperture shows distinct differences between sexes, becoming enlarged, swollen, and blunt in females, while remaining slender and pointed in males (Roshni, K. *et al*., 2021). In addition to genital morphology, differences in body colouration provide supplementary cues for sex identification in pearlspot. Males typically exhibit more intense and iridescent colouration, characterised by greenish-blue hues with pearly white spots and prominent dark bands, along with a slight reddish tint in the fin rays during the breeding period. In contrast, females are generally smaller and display comparatively dull, muddy yellowish colouration (Christo, M., & Divya, P. R., 2025). Sex assignments based on external morphology of genital papilla were subsequently verified through gonadal examination of ten male-female pairs. Morphological and anatomical sex identification showed complete agreement in all examined individuals, resulting in an accuracy of 100% under the conditions of the present study. However, reliance on a single trait may not always yield accurate results, particularly outside the breeding season.

Therefore, the integration of genital papilla morphology and body colouration offers a simple, reliable, and non-invasive approach for accurate sex identification in pearlspot, thereby enhancing broodstock selection and improving the efficiency of breeding programmes in aquaculture systems. Although the number of individuals examined was relatively limited, all morphology-based sex experiments were consistently confirmed through breeding observations and gonadal examination. Nevertheless, further studies involving larger sample sizes from different populations and size classes would be valuable for assessing the broader applicability and robustness of the proposed identification method.

CONCLUSION

Identifying male and female pearlspot remains challenging, particularly outside the breeding season, due to the absence of clear sexual dimorphism. The present study introduces a simple and novel non-invasive method for sex identification based on the external morphology of the urogenital papilla. A distinct notch in the genital aperture was consistently observed in males, whereas females exhibited a smooth, rounded aperture. Validation through dissection of ten pairs showed complete agreement with morphological identification, confirming the reliability of the method. Additionally, differences in body colouration during the spawning season provided supportive cues for preliminary differentiation. The novelty of this study lies in establishing a rapid, field-applicable, and non-destructive technique that eliminates the need for invasive procedures. This method can be directly adopted by farmers and hatchery managers for accurate broodstock selection and optimisation of breeding ratios. Future studies should focus on validating this approach across larger populations and across different developmental stages, particularly in juveniles, and on exploring its integration with image-based or automated identification systems. It is recommended that this method be incorporated into standard aquaculture practices to enhance breeding efficiency, improve yield, and support the sustainable conservation of pearlspot.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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