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Effects of a luteinizing hormone-releasing hormone analogue (LHRHa) on the reproductive of Asian seabass (*Lates calcarifer*)

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Abstract

Asian seabass (*Lates calcarifer*), locally found in the southern waters of Iran and commercially known as barramundi, is an economically valuable species due to its rapid growth, tolerance to high stocking density, resistance to adverse environmental conditions, high nutritional value, and palatability. Despite these advantages, its commercial aquaculture in Iran has not yet been sufficiently developed. Expanding the aquaculture industry of this valuable species requires accurate knowledge of its biology and biotechnical aspects of spawning and seed production. In the present study, different doses of luteinizing hormone-releasing hormone (LHRH) analogue were tested to optimize the spawning response in male and female broodstock. The results indicated that in females with oocytes of 400 µm in diameter, injection of 50 µg/kg body weight successfully induced ovulation, while in males; a dose of 25 µg/kg body weight was suitable for spermation.

Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), fish and fishery products account for approximately 20% of animal protein consumed worldwide, with their intake growing faster than other protein sources (Rosati *et al.,* 2025). Over the past few decades, there has been a marked increase in global demand for high-value aquaculture species, especially those exhibiting fast growth, resilience to variable environmental conditions, and desirable flesh characteristics (Valenza-Troubat *et al.,* 2022). Among such species, Asian seabass (*Lates calcarifer*), locally found in southern Iran and internationally known as barramundi, has attracted considerable attention due to its biological and economic characteristics (Pattarapanyawong *et al.,* 2021; Rosati *et al.,* 2025). As a euryhaline species, it demonstrates the ability to thrive across a wide salinity range, encompassing both freshwater and marine habitats, while maintaining high growth performance, tolerance to intensive rearing conditions, and production of flesh with superior organoleptic and nutritional properties (Khemis *et al.,* 2015).

However, intensive aquaculture operations frequently introduce disinfectants, veterinary pharmaceuticals, and residual feed into marine ecosystems, leading to nutrient pollution, oxygen depletion, and harmful algal blooms (Rosati *et al.,* 2025). In their natural habitats, the spawning of Asian seabass (*Lates calcarifer*) in the wild is synchronized with seasonal changes in salinity, temperature, and photoperiod (Emran *et al.,* 2024; Budd *et al.,* 2025). In captivity, the absence of natural environmental cues often results in delayed or incomplete gonadal development, impaired ovulation, reduced spawning success, and lower larval output (Al-Emran *et al.,* 2024; Budd *et al.,* 2025). Therefore, implementing effective strategies for artificial reproduction is essential to ensure sustainable aquaculture production.

Hormonal induction has become a widely employed strategy to overcome reproductive constraints in marine fish. Among the most frequently used agents are luteinizing hormone-releasing hormone analogues (LHRHa), which effectively stimulate final oocyte maturation, ovulation, and spermiation. LHRHa exerts its effect by activating the hypothalamic–pituitary–gonadal (HPG) axis, thereby promoting gonadotropin release and subsequent gamete maturation (Mohammadzadeh *et al.,* 2021; Yeganeh *et al.,* 2022). In some cases, dopamine antagonists are used in combination with LHRHa to suppress inhibitory effects and enhance reproductive responses. Several studies have demonstrated the effectiveness of LHRHa in Asian seabass and other marine species (Heidari *et al.,* 2025). However, responses vary depending on factors, such as oocyte diameter, broodstock size, hormonal dose, and environmental conditions (Alcántar-Vázquez *et al.,* 2016). Optimizing these factors is crucial for maximizing spawning success, fertilization rate, and larval quality, while minimizing stress and cost (Alcántar-Vázquez *et al.,* 2016).

This study aims to evaluate the effectiveness of LHRHa injections in inducing final maturation and spawning in Asian seabass broodstock in southern Iran. Specifically, the study investigates: (i) the optimal hormonal dose for males and females, (ii) the relationship between oocyte diameter and responsiveness to hormone, (iii) the spawning performance, fertilization rate, and hatching success, and (iv) practical implications for hatchery operations and sustainable aquaculture. The findings provide critical insights for developing efficient, cost-effective, and reproducible broodstock management protocols for Asian seabass.

Materials and Methods

Broodstock collection and maintenance

To provide broodstock for the pilot phase of this study, 30 fish (20 males and 10 females) were purchased from Nixa Development Company. This ratio was chosen due to the protandrous nature of the species, where males may later change sex to females after one spawning season. Additionally, in seabass hatcheries, the sex ratio is commonly maintained at 2:1 (male: female) to overcome the limited sperm availability in marine fish. For transportation, broodstock were placed in 500 litre sealed polyethylene tanks with aeration cylinders, ensuring 100% survival after a 12-hour transfer to the National Shrimp Research Institute in Bushehr. Broodstock were held in either fiberglass tanks or concrete ponds depending on the season. Three greenhouse ponds (10 × 15 m, 2 m depth) were constructed for overwintering. Water quality parameters were controlled year-

round, with temperature maintained at 30–32°C and salinity at 30 ppt during the spawning season. From December 6 to February 5, water was maintained at 22–24°C and salinity at 22–23 ppt to support gonadal development. Broodstock were fed 3% body weight/day during warm months and 1% during the cold season with formulated broodstock pellets (Beyza 21 Co., Fars province). Squid was introduced as the primary diet two months before spawning (mid-March).

Table 1. Biometric characteristics of Asian seabass broodstock at purchase and after one year of rearing.

Fish gender	Weight (kg)		Length (cm)		Condition factor	
Time	June 2021	May 2022	June 2021	May 2022	June 2021	May 2022
Male	1.75±0.40	3.60±0.30	42±12	58±8	2.36	1.84
Female	3.90±0.65	7.85±0.70	56±14	79±10	2.22	1.60

Induced Breeding

At the onset of the spawning season (June), broodstock were transferred to the hatchery. Ovarian biopsies were performed under anesthesia (0.40 ml/l 2-phenoxyethanol) to evaluate oocyte maturity. Females with spherical oocytes >400 µm were selected. Males were screened based on sperm quality, assessed by motility in seawater. Selected broodstock were injected with LHRHa hormone: females received 50 µg/kg body weight, and males received 25 µg/kg. Fish were stocked in circular fiberglass tanks at a 2:1 sex ratio. Spawning occurred 36–40 hours post-injection. Eggs were collected at night due to high water temperature and rapid hatching.

Eggs were disinfected with 100 mg/l iodine for 5 minutes, then incubated at 28–33°C in 300 litre tanks with gentle aeration. Fertilization rate was determined by observing cleavage in 100 floating eggs. Hatching occurred within 12–18 hours depending on temperature. Hatched larvae were transferred to 5-ton larval rearing tanks.

Results

Hormonal induction successfully triggered spawning in all selected broodstock. Females with oocytes >400 µm and males with motile sperm responded positively to LHRHa injection. Spawning occurred 36 hours post-injection. Fertilization rate exceeded 90%, while hatching success reached up to 58%.

Discussion

The present study clearly demonstrates that LHRHa is highly effective in inducing final oocyte maturation, ovulation, and spermiation in Asian seabass under controlled aquaculture conditions. In captivity, natural environmental factors, such as seasonal salinity shifts, temperature fluctuations, and photoperiod changes, are often absent, leading to impaired gonadal maturation and reduced reproductive performance (Garcia *et al.,* 2020). Hormonal induction with LHRHa successfully activated the hypothalamic–pituitary–gonadal (HPG) axis, increasing plasma gonadotropin levels and triggering synchronized gamete release (Antonopoulou *et al.,* 2016; Mohammadzadeh *et al.,* 2022; Heidari *et al.,* 2025).

A key determinant of responsiveness to hormonal induction was oocyte diameter (White, 2024). Females with oocytes larger than 400 µm responded effectively to 50 µg/kg LHRHa, while smaller oocytes failed to mature or ovulate. This is consistent with earlier reports showing that the gonadal stage and oocyte size are critical predictors of spawning success in Asian seabass and other marine fish species (Schipp *et al.,* 2007; Alcántar-Vázquez *et al.,* 2016; White, 2024). In males, a dose of 25 µg/kg effectively induced spermiation, supporting findings that male broodstock generally require lower doses for gamete release than females. The precise timing of hormone administration resulted in spawning 36–40 hours later, in agreement with established protocols for semi-artificial spawning in seabass (Mylonas *et al.,* 2010; Hollander-Cohen *et al.,* 2024).

The study confirmed that environmental conditions critically affect reproductive success. Spawning was optimized by maintaining water temperatures of 30–32°C and salinity at 30 ppt, closely simulating the species' natural habitat. Overwintering at 22–24°C likely facilitated gonadal development, ensuring broodstock readiness for hormonal induction. These results emphasize the need to integrate environmental regulation with hormonal treatments to maximize spawning efficiency.

High fertilization rates (>90%) and hatching success (up to 58%) were achieved, demonstrating the practical applicability of

LHRHa in aquaculture settings. Compared to natural or purely environmental induction methods, hormone-assisted spawning significantly improves predictability, reduces handling stress, and ensures synchronization of gamete release (Soudagar *et al.,* 2016). This has direct implications for hatcheries, particularly for off-season breeding, large-scale fingerling production, and cage farming in southern Iran and other regions. Dietary management also contributed to reproductive performance. Feeding broodstock with high-protein formulated pellets during warm months and incorporating squid two months prior to spawning likely enhanced gonadal maturation and gamete quality. Nutrition is known to affect both fecundity and egg quality in marine fish (Khemis *et al.,* 2015), suggesting that optimized feeding regimes are an essential complement to hormonal induction.

Despite the successful outcomes, some limitations exist. The study was conducted on a relatively small number of broodstock and tested a single hormonal protocol. Further research should explore alternative dosing strategies, slow-release hormone formulations, combinations with dopamine antagonists, and the effects of repeated injections on egg quality and larval survival. Additionally, long-term monitoring of broodstock health, early sexual maturation, and sex reversal dynamics would provide valuable insights for sustainable production.

In conclusion, this study confirms that LHRHa is a reliable tool for inducing spawning in Asian seabass, particularly when applied to appropriately selected broodstock under controlled environmental conditions. The findings offer practical guidelines for optimizing broodstock management, improving hatchery efficiency, and supporting the expansion of commercial Asian seabass aquaculture. Moreover, the integration of hormonal induction, environmental manipulation, and nutrition management provides a comprehensive framework for sustainable broodstock reproduction, potentially applicable to other valuable marine species in the region.

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Conflict of Interest

There is no conflict of interest among the authors.

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