JOURNAL OF MARINE SCIENCES & ENVIRONMENTAL TECHNOLOGIES
Vol. 10, Issue No. 2 (December-2025)
https://doi.org/10.59743/jmset.v10i2.191

ISSN (Print): 2413-5267
ISSN (Online): 2706-9966

First Successful Hormonal Induced Spawning of European Seabass (*Dicentrarchus labrax*) in the Private Aquaculture Sector in Libya

Abdalla M. Elmgawshi[1]*, Sara A. A Almabruk[2], Mohamed F. Saadallah[1], Mohamed A. Araibi[3]

[1]Marine Biology Research Center, Tajoura, Libya.
[2]Higher Institution of Science & Technology-Cyrene, Libya.
[3]Saif Al-Bahr Aquaculture Company, Zuwara, Libya

***E-mail:** aelmgawshi@yahoo.com

نجاح أول تفريخ هرموني مستحث لأسماك القاروص الأوروبي (*Dicentrarchus labrax*) في القطاع الخاص للاستزراع المائي بليبيا

عبدالله المقاوشي[1]*، سارة المبروك[2]، مُحَمَّد سعدالله[1]، مُحَمَّد عريبي[3]

[1]مركز بحوث الأحياء البحرية، تاجوراء، ليبيا
[2]المعهد العالي للعلوم والتقنية – شحات، ليبيا
[3]شركة سيف البحر للزراعة المائية، زوارة، ليبيا

Received: 20 August 2025; Revised: 27 November 2025; Accepted: 10 December 2025

Abstract

This study documents the first successful hormonal-induced spawning of European seabass (*Dicentrarchus labrax*) in a private commercial hatchery in Libya. Wild broodstock (12 females, 9 males) collected from Farwa Lagoon were conditioned for three months under controlled photoperiod (12 L:12 D → 9 L:15 D) and stepped-down temperature (21 → 14 °C, ≈ 1638 DD). A single intramuscular injection of Lh-RHa (10 µg kg^{-1}) induced ovulation in all females within 72 h. Egg production averaged 16.3% of female body weight (4000 g total). Morphological indices (mean diameter 1000 µm) met established quality thresholds. Despite a female-biased sex ratio (1.3:1), hatching rate reached 50%, already surpassing the 30% typically recorded for imported eggs after transport. Economic analysis indicates a 61% reduction in seed cost (€0.10 vs €0.26 per viable fry). Results demonstrate that locally adapted protocols using wild broodstock can reliably supply high-quality seabass seed, offering a replicable model in Libyan hatcheries and reducing dependence on imported fry.

Keywords: *Artificial spawning, Dicentrarchus labrax, European seabass, hormonal induction, Lh-RHa.*

الملخص

تتناول هذه الدراسة توثيق أول تجربة ناجحة للتفريخ الهرموني المستحث لأسماك القاروص الأوروبي (*Dicentrarchus labrax*) في مفرخ تجاري خاص بليبيا. جُمعت الأمهات والفحول (12 أنثى و9 ذكور) من البيئة الطبيعية ببحيرة فروة، وتم إخضاعها لبرنامج تكييف استمر ثلاثة أشهر تحت ظروف ضوئية متدرجة (12 ساعة إضاءة: 12 ساعة ظلام، ثم 9 ساعات إضاءة: 15 ساعة ظلام)، مع خفض تدريجي لدرجة الحرارة من 21°م إلى 14°م، بما يعادل نحو 1638 وحدة حرارية يومية. أُعطيت الإناث حقنة عضلية واحدة من نظير الهرمون المطلق لمواجهة الغدد التناسلية Lh-RHa بجرعة (10 ميكروغرام/ كجم$^{-1}$)، ما أدى إلى حدوث الإباضة في جميع الإناث خلال 72 ساعة. بلغ متوسط إنتاج البيض 16.3% من وزن الأنثى، بكمية إجمالية بلغت 4000 جرام، فيما تراوح متوسط القطر 1000 ميكرومتر، مطابقًا لمعايير الجودة المعتمدة. ورغم انحياز نسبة الجنس لصالح الأناث

(1.3 أنثى: 1 ذكر)، وصلت نسبة الفقس إلى 50%، متجاوزة النسب المعتادة للبيض المستورد بعد النقل (≈ 30%). وأظهر التحليل الاقتصادي أن التكلفة انخفضت بنسبة 61%، إذ بلغت 0.10 يورو لكل يرقة يتم تفريخها محلياً مقارنة بـ 0.26 يورو للزريعة للمستوردة. تشير النتائج إلى أن تطبيق بروتوكولات محلية باستخدام أمهات جمعت من البحر، يمكن أن يوفر زريعة قاروص عالية الجودة بشكل مستدام، ويُعتبر نموذجًا عمليًا قابلاً للتكرار في مفرخات الأسماك بليبيا، مما يساهم في تقليل الاعتماد على الزريعة المستوردة.

الكلمات الدالة: Dicentrarchus labrax، التحفيز الهرموني، التفريخ الصناعي، سمكة القاروص، نظير الهرمون المطلق لمواجهة الغدد التناسلية.

1. Introduction

Marine aquaculture development in North Africa faces a fundamental constraint: the absence of reliable local sources for marine fish fry (El-Sayed, 2020). This dependency on imported seed stock creates significant economic burdens, supply chain vulnerabilities, and barriers to industry expansion (Ben-Attia *et al*., 2021). European seabass (*Dicentrarchus labrax*) exemplifies this challenge, representing a high-value species with substantial market demand across the Mediterranean region, yet local hatchery production remains virtually non-existent in most North African countries (FAO, 2023).

European seabass production in the Mediterranean exceeded 180,000 tonnes annually by 2022, generating market revenues of approximately €1.2 billion (FAO, 2023; Pavlidis *et al*., 2021). However, North African countries contribute less than 2% of regional production despite possessing over 4,000 km of suitable coastline and growing domestic markets (El-Sayed, 2020). Libya, with its extensive 1,900 km Mediterranean coast, operates only two marine fish farms, highlighting a dramatic development gap compared to established producers like Greece (>300 operations) and Turkey (>250 facilities) (Pavlidis *et al*., 2021; Mylonas & Zohar, 2021).

The economic impact of fry importation is substantial and multifaceted. Marine seabass fry typically cost €0.15-0.25 per unit, representing 15-20% of total production costs in grow-out operations (Costa-Pierce, 2022). Beyond direct costs, importation creates logistical challenges including transportation mortality, biosecurity risks, and supply disruptions during seasonal peaks or regional conflicts (Brigolin *et al*., 2021). Local fry production could potentially reduce seed stock costs by 40-60% while improving supply reliability and reducing disease introduction risks (El-Sayed, 2020; Ben-Attia *et al*., 2021).

The primary technical obstacle to establishing marine hatcheries lies in achieving reliable artificial spawning. European seabass, like most marine finfish, rarely reproduce naturally in captivity due to environmental and physiological disruptions of normal reproductive cycles (Carrillo *et al*., 2020; Duncan *et al*., 2023). Confinement stress, altered photoperiod and temperature regimes, and social dynamics typically prevent complete gonadal maturation and synchronized spawning behavior (Carrillo *et al*., 2020; Mylonas & Zohar, 2021). Consequently, commercial hatcheries worldwide rely on hormonal induction techniques to

stimulate final oocyte maturation, ovulation, and spermiation (Zohar & Mylonas, 2001; Mylonas *et al*., 2010).

Luteinizing hormone-releasing hormone analogues (Lh-RHa) have emerged as the preferred method for spawning induction in European seabass, demonstrating superior efficacy compared to traditional gonadotropins like human chorionic gonadotropin (HCG) (Mylonas *et al*., 2010; Falahatkar *et al*., 2020). Lh-RHa activates the hypothalamic-pituitary-gonadal axis more precisely, inducing endogenous luteinizing hormone release while minimizing adverse physiological effects (Mylonas *et al*., 2010; Zohar & Mylonas, 2001). Successful protocols typically achieve egg production rates of 10-20% of female body weight and hatching rates of 70-90% under optimal conditions (Moretti *et al.*, 1999; Asturiano *et al*., 2023; Pereira *et al*., 2018).

However, a critical knowledge gap exists regarding the application of established spawning protocols under North African conditions. Mediterranean hatcheries primarily utilize domesticated broodstock maintained under controlled conditions year-round, while developing aquaculture regions often rely on wild-caught fish with different genetic backgrounds, seasonal conditioning, and stress responses (Zupa *et al*., 2020; Carnevali *et al*., 2022). Environmental factors such as regional temperature patterns, water chemistry, and seasonal photoperiod cycles may require protocol modifications for optimal results (Felip *et al*., 2009; Mylonas *et al*., 2023). Additionally, infrastructure limitations and economic constraints in developing regions necessitate adaptations to standard hatchery practices (El-Sayed, 2020).

Libya's aquaculture landscape presents both opportunities and constraints for marine hatchery development. The country's National Aquaculture Development Strategy (2020-2030) identifies marine fry production as a strategic priority for reducing import dependence and supporting coastal economic development. Currently, the Saif Al-Bahr facility in Abu Kamash represents the only operational marine hatchery attempting artificial spawning protocols, making its success critical for demonstrating commercial viability and attracting additional investment in the sector.

The significance of establishing proven spawning protocols extends beyond immediate commercial applications. Successful documentation of locally-adapted techniques would provide essential technical references for government policy development, private sector investment decisions, and regional research collaboration (Mañanós *et al*., 2020). Furthermore, Libya's experience could serve as a model for similar developing aquaculture regions throughout North Africa and the Eastern Mediterranean, where comparable environmental conditions and economic constraints exist (El-Sayed, 2020; Ben-Attia *et al*., 2021).

2. Research Objectives

This study was designed to evaluate the effectiveness of hormonal spawning induction in European seabass under realistic commercial hatchery conditions in Libya. The specific objectives were to:

- Assess the efficacy of Lh-RHa treatment (10 μg/kg body weight) for inducing spawning in wild-caught European seabass broodstock collected from Libyan coastal waters
- Quantify reproductive performance including egg production rates, morphological quality parameters, and hatching success compared to established Mediterranean benchmarks
- Document operational protocols and infrastructure requirements for commercial-scale implementation under regional conditions
- Identify critical success factors and limitations to guide future protocol optimization and facility development
- Success criteria were established as: egg production ≥10% of female body weight, hatching rates ≥40%, and documentation of reproducible protocols suitable for commercial application. This research represents the first peer-reviewed documentation of successful artificial spawning in Libya's private aquaculture sector and provides foundational data for developing standardized protocols adapted to North African conditions.

3. Materials and Methods

3.1. Broodstock Collection and Transportation

European seabass (*Dicentrarchus labrax*) broodstock were collected from Farwa Lagoon (33°05'N, 11°45'E), located on the western coast of Libya, during December 2020. Collection was conducted using monofilament gillnets (mesh size: 40-60 mm, depth: 2-8 m) deployed inside the lagoon in shallow areas (water depth: 1–6 m) known for seasonal seabass aggregations. A total of 21 adult fish were selected based on visual assessment of sexual maturity indicators: body length >35 cm for females and >30 cm for males, corresponding to estimated ages of 3-5 years for females and 2-4 years for males (Pavlidis *et al*., 2021).

Fish were transported to the Saif Al-Bahr commercial hatchery facility in Abu Kamash (33°01'N, 11°56'E), approximately 14 km west of Zuwara city, using aerated transport tanks (500 L capacity) with oxygen supplementation maintained at >6 mg/L. Transportation time was minimized to <3 hours to reduce handling stress. Water temperature during transport was

maintained at ambient seawater temperature (19-21°C) with gradual adjustment to facility conditions upon arrival.

3.2. Quarantine and Health Management

Upon arrival, all broodstock underwent a standardized quarantine protocol designed to eliminate ectoparasites and treat physical injuries sustained during capture and handling. Fish were subjected to a formalin bath treatment (200 ppm formaldehyde solution) for 30 minutes in aerated quarantine tanks while maintaining dissolved oxygen saturation at 100% using supplemental oxygen injection (Dhert *et al*., 1992). During treatment, fish behavior and respiratory distress indicators were continuously monitored.

Following formalin treatment, fish were examined for external injuries, scale loss, and parasitic infections. Fish showing signs of severe stress, major injuries, or abnormal behavior were excluded from the breeding program. The quarantine period lasted 14 days with daily health monitoring and feeding assessment.

3.3 Housing and Culture System

After quarantine, the broodstock group (n=21) was housed in two circular fiberglass tanks, each with a working volume of 7000 L (diameter: 3 m, depth: 1 m). Initially, the system operated as an open-flow design with continuous seawater supply from underground wells. Raw seawater was pumped to overhead storage tanks (28,000 L capacity) and distributed to broodstock tanks via gravity flow at a rate of 1-2 tank volumes per hour.

In September 2021, the open system was converted to a recirculating aquaculture system (RAS) to improve water quality control and enable precise environmental manipulation. The RAS components included:

- Mechanical filtration: Sand filter (50 μm retention) and drum filter (25 μm retention) for suspended solids removal
- Biological filtration: Moving bed biofilm reactor (MBBR) with plastic carriers (500 m^2/m^3 surface area) for nitrification
- UV sterilization: 120W UV lamp (254 nm wavelength) for pathogen control
- Temperature control: 5 kW chiller unit with digital temperature controller (±0.5°C accuracy)
- Water circulation: Centrifugal pumps (2000 L/h capacity) with variable flow control
- Backup systems: Emergency generator and backup water reservoir (5000 L)

Each tank was equipped with continuous aeration (30 L/min air flow rate) provided by regenerative blowers and ceramic diffusers positioned to create gentle water circulation without excessive turbulence.

3.4 Water Quality Management

Water quality parameters were monitored and controlled according to optimal ranges established for European seabass broodstock conditioning (Mylonas *et al*., 2010):

- Temperature: Maintained at 21-23°C initially, gradually reduced to 14°C during pre-spawning conditioning
- Salinity: 37±1 ppt, measured daily using optical refractometer
- Dissolved oxygen: >6 mg/L, monitored using YSI dissolved oxygen probe
- pH: 7.8-8.2, measured daily using digital pH meter
- Ammonia (NH_3-N): <1.0 mg/L, tested every 48 hours using spectrophotometric method
- Nitrite (NO_2-N): <0.25 mg/L, tested weekly using standard colorimetric analysis
- Nitrate (NO_3-N): <50 mg/L, monitored weekly

Water exchange rates were maintained at 10-15% daily volume replacement to ensure optimal water quality stability while conserving heated/chilled water during temperature conditioning phases.

3.5 Pre-Spawning Conditioning Protocol

3.5.1. Nutritional Conditioning

Three months prior to hormonal induction (September-December 2021), broodstock were subjected to an intensive nutritional conditioning regime designed to optimize gonadal development and egg quality. The feeding schedule followed a 7-day rotation cycle:

- Day 1: Commercial marine broodstock diet (45% protein, 15% lipid) enriched with vitamin E (200 mg/kg) and vitamin C (500 mg/kg)
- Day 2: Fresh whole sardines (*Sardina pilchardus*), 3-4% body weight
- Day 3: Chopped cuttlefish (*Sepia officinalis*), 2-3% body weight
- Day 4: Vitamin-enriched commercial diet (as Day 1)
- Day 5: Cooked shrimp (*Penaeus* spp.), 2% body weight
- Day 6: Fresh squid (*Loligo vulgaris*), 2-3% body weight
- Day **7**: Fasting (no feeding)

All natural feeds were obtained from local fishing vessels within 24 hours of capture and stored at -20°C until use. Feeds were thawed in seawater immediately before feeding and inspected for quality. Feeding was conducted twice daily (08:00 and 16:00) with feed quantities adjusted based on water temperature and observed feeding response. Feeding continued until apparent satiation, indicated by cessation of active feeding behavior after 10-15 minutes.

3.5.2. Environmental Conditioning

Environmental parameters were gradually modified to simulate natural pre-spawning conditions experienced by wild European seabass in the Mediterranean during winter months (Felip *et al*., 2009):

3.5.2.1. Photoperiod Manipulation:

- Initial photoperiod: 12L:12D (September)
- Final photoperiod: 9L:15D (December)
- Light intensity: 200-300 lux at water surface using LED lighting
- Gradual reduction: 30 minutes per week over 12-week period

3.5.2.2. Temperature Conditioning:

- Initial temperature: 21°C (September)
- Final temperature: 14°C (December)
- Reduction rate: 0.5°C per week
- Temperature stability: ±0.5°C daily variation

This conditioning protocol was designed to synchronize gonadal maturation with natural spawning cues while maintaining fish health and minimizing stress responses.

3.6 Gonadal Maturity Assessment

Broodstock sexual maturity was evaluated on November 25, 2021, using standardized protocols for European seabass (Moretti *et al*., 1999). Fish were anesthetized using 2-phenoxyethanol (2-PE) at a concentration of 300 mg/L in aerated seawater. Anesthesia depth was monitored by loss of equilibrium and reduced gill ventilation rate, with total anesthesia time limited to 5 minutes per individual.

***3.6.1. Male Assessment*:** Males were identified by gentle abdominal pressure toward the genital pore, with mature individuals releasing creamy white milt. Milt quality was assessed visually for color (white/cream), consistency (thick/fluid), and volume.

***3.6.2. Female Assessment*:** Female maturity was determined by catheter sampling of oocytes using a flexible plastic catheter (2 mm diameter) inserted 2-3 cm into the genital opening. Oocyte samples were examined under a compound microscope (Olympus CX21) connected to a digital imaging system. Oocyte diameter was measured using calibrated ocular micrometer with measurements taken from 30 randomly selected oocytes per female. Females with mean oocyte diameter >650 μm were considered ready for hormonal induction (Zohar & Mylonas, 2001).

3.7. Hormonal Induction Protocol

3.7.1. Hormone Preparation

Luteinizing hormone-releasing hormone analogue (Lh-RHa, [Des-Gly^{10}, D-Ala^{6}]-LHRH ethylamide, Bachem AG, Switzerland, catalog #H-4070, purity >95%) was used for spawning induction. The hormone was stored at -20°C in original packaging and protected from light until use.

On December 19, 2021, hormone solution was prepared under sterile conditions by dissolving 5000 µg Lh-RHa in 500 mL sterile physiological saline (0.9% NaCl, pH 7.2-7.4) to achieve a final concentration of 10 µg/mL.

3.7.2. Injection Protocol

At 12:00 PM on December 19, 2021, mature females (n=12) identified during previous assessment were re-anesthetized using 2-PE (300 mg/L) and individually weighed using a digital scale (±1 g accuracy). Each female received a single intramuscular injection of Lh-RHa at a dosage of 10 µg/kg body weight (equivalent to 1 mL hormone solution per kg body weight).

Injections were administered using sterile disposable syringes (5 mL capacity) fitted with 25-gauge needles (25 mm length). The injection site was the dorsal musculature, posterior to the second dorsal fin and anterior to the caudal peduncle, at a depth of approximately 10-15 mm depending on fish size. The needles were changed between individuals to prevent cross-contamination.

Males (n=9) were not subjected to hormonal treatment due to their apparent natural readiness indicated by milt expression during handling. This decision was based on limited hormone availability and standard protocols suggesting male hormonal treatment is less critical when natural spermiation is evident (Mylonas & Zohar, 2007).

3.7.3. Post-Injection Management

Following hormone injection, all broodstock (12 females + 9 males) were transferred to a dedicated spawning tank (7000 L circular fiberglass tank) equipped with a floating egg collection system. The collection system consisted of a perforated PVC cylinder (diameter: 50 cm, height: 30 cm) covered with 500-µm nylon mesh and positioned to collect floating eggs via gentle surface water flow.

Environmental conditions in the spawning tank were optimized for spawning behavior:

- Complete darkness maintained by covering tank and eliminating all light sources.
- Water temperature: 14±0.5°C.
- Gentle aeration (10 L/min) to maintain water circulation without excessive turbulence.
- No feeding provided for 72 hours post-injection.
- Minimal disturbance with observation limited to twice-daily egg collection checks.

3.8. Egg Incubation System

3.8.1. Incubation Tank Setup

Eight conical-bottom fiberglass tanks (total volume: 1800 L each, working volume: 1500 L) were prepared for egg incubation. Tank interiors were smooth-finished to minimize mechanical damage to developing embryos. Each tank was equipped with:

- Aeration system: Ceramic air stones positioned at tank bottom providing gentle upward water flow (5 L/min air flow rate per tank)
- Temperature control: Integrated with facility chiller system maintaining 14±0.2°C
- Water circulation: Continuous gentle flow (2 L/min) to maintain egg suspension without excessive turbulence
- Effluent screening: 500-μm nylon mesh screens at tank outlets to prevent egg/larval loss
- Light exclusion: Complete darkness maintained using opaque tank covers

3.8.2. Water Quality Management During Incubation

The egg incubation system operated as a closed recirculating system with the following specifications:

- Filtration: Sand filter → drum filter → biological filter → UV sterilizer sequence
- Protein skimming: Venturi-type protein skimmer for dissolved organic removal
- Water exchange: 5% daily volume replacement with temperature-matched water
- Monitoring frequency: Temperature (continuous), dissolved oxygen (twice daily), pH and salinity (daily)

3.8.3. Incubation Density and Loading

Fertilized eggs were distributed among incubation tanks at a density of 4000 eggs/L, equivalent to approximately 360 g total egg mass per 1500 L working volume. This density was selected based on established protocols for European seabass to ensure adequate water circulation, oxygenation, and space for embryonic development while maximizing production efficiency (Moretti *et al.*, 1999).

3.9. Data Collection and Analysis

3.9.1. Spawning Response Metrics

- Latency period: Time from hormone injection to first egg collection (hours)
- Spawning duration: Total time period of egg collection (hours)
- Individual female response: Number of females contributing to spawning (visual assessment of continued egg release)

3.9.2. Egg Production Assessment

- Total egg mass: Gravimetric measurement of collected eggs (±0.1 g accuracy)
- Egg production rate: Calculated as (total egg mass/total female body weight) × 100
- Individual variation: Estimated contribution per female based on relative body weights

3.9.3. Egg Quality Evaluation

Egg quality was assessed using established morphological criteria (Bromage & Roberts, 1995):

- Diameter measurement: Random sampling of 100 eggs, measured using calibrated microscope micrometer
- Shape assessment: Spherical vs. irregular morphology (% of sample)
- Transparency: Clear vs. opaque classification (% of sample)
- Oil droplet evaluation: Number and size distribution per egg
- Floatation test: Buoyancy assessment in 37 ppt seawater
- Contamination screening: Visual inspection for debris, bacteria, or parasites

3.9.4. Hatching Assessment

- Hatching initiation: Time from fertilization to first larval emergence (hours)
- Hatching duration: Period of active hatching (hours)
- Hatching rate: (Mass of hatched larvae/total fertilized egg mass) × 100
- Synchrony: Temporal distribution of hatching events

3.9.5. Statistical Analysis

Due to the single-trial nature of this study and infrastructure limitations preventing replication, descriptive statistics (mean ± standard deviation) were calculated for all measured parameters. Where applicable, results were compared to established literature benchmarks using single-sample comparisons. Individual variation among females was

estimated based on proportional body weight contributions, acknowledging limitations in directly tracking individual spawning contributions.

3.10. Ethical Considerations

This study was conducted under the operational permit of Saif Al-Bahr commercial hatchery facility. Wild broodstock collection was conducted with appropriate local fishing permits and followed sustainable collection practices. Fish handling and experimental procedures were designed to minimize stress and followed established aquaculture industry standards for broodstock management.

4. Results and Discussion

4.1. Sexual Maturity of Broodstock

The selected wild broodstock showed optimal sexual maturity based on weight and age (Table 1). The ideal body weight for male European sea bass collected from the wild is approximately 600 g, corresponding to ages between 2–4 years, while optimal female weights range from 1000 to 1500 g for ages 5–8 years (Buke, 2002). Sexual maturity in females typically begins after the second year of age, while in males it starts after the first year (Felip *et al*., 2009).

Table 1. Weights of females and males used in the trial

No.	Weight of Female (g)	Weight of Male (g)
1	1520	840
2	2290	1040
3	1930	1260
4	2530	1000
5	2250	1020
6	2000	1190
7	1890	1210
8	2360	1430
9	2120	1500
10	2000	-
11	1750	-
12	1900	-

Microscopic examination of oocytes from female ovaries revealed diameters ranging from 740 to 810 µm, indicating readiness for hormonal induction. According to literature, hormone effectiveness is optimal when oocyte diameter exceeds 650 µm (Moretti *et al*., 1999).

4.2. Egg Collection and Quality Evaluation

Eggs were collected on the morning of December 22, 2021, at 08:00. A total of 4000 g of eggs was obtained from 12 females with a combined weight of 24,540 g, yielding an egg production rate of 16.3% of female body weight, a satisfactory result for a first commercial trial.

Microscopic analysis showed excellent morphological characteristics: average egg diameter was 1000 µm, exceeding the 950 µm threshold for high viability (Vatsos *et al*., 2021). Eggs were spherical in shape with fully developed yolk, which are typical indicators of complete vitellogenesis and final oocyte maturation (Mañanós *et al*., 1997; Zohar & Mylonas, 2001; Fernandez-Palacios *et al*., 2020).

Eggs exhibited high transparency with no dark spots or deformations, suggesting intact chorion and healthy cell integrity (Bromage & Roberts, 1995; Navarro-Martín *et al*., 2019).

Between 1–6 oil droplets were observed per egg, typical for marine species, aiding buoyancy and embryonic energy supply (Carrillo *et al*., 2009; Lubzens *et al*., 2010; Vatsos *et al*., 2021). No visible signs of parasitic infestation or microbial contamination were observed on the surface of the eggs, which is likely attributed to the favorable environmental conditions during ovulation and the proper hygienic practices applied (Dhert *et al*., 1992; Mladineo & Poljak, 2022).

4.3 Fertilization and Hatching Rates

Hatching began at 17:06 on December 25, 2021, and completed by 07:00 on December 26. A total of 2000 g of unhatched eggs was recorded, resulting in a hatching rate of approximately 50%. This 50% hatch falls within the 40–60% range reported for first-time Lh-RHa trials with wild-caught Mediterranean seabass (Zanuy *et al*., 1995; Falahatkar *et al*., 2020) and is significantly above the 30% hatch typical of imported eggs shipped to North Africa (Brigolin et al., 2021), already representing a net gain of ~20% viable larvae. Logistic regression on these data predicts that increasing the ratio to 1.5 males per female would raise hatch to ~70%, providing a quantitative target for future trials.

The low hatching rate is likely due to the unbalanced sex ratio (9 males to 12 females). Literature recommends a 2:1 male-to-female ratio for effective fertilization (Zohar & Mylonas, 2001). Additionally, the absence of hormonal stimulation in males may have caused asynchrony between sperm release and ovulation (Mañanós *et al*., 1997).

4.4 Effects of Broodstock Nutrition and Environmental Stimuli

Nutrition is a critical factor in egg quality during induced spawning. In this trial, a varied diet including enriched commercial feed and natural sources (sardines, cuttlefish, squid, shrimp) was used. Proper nutrition supports vitellogenesis, chorion development, buoyancy, and embryogenesis (González-Rodríguez *et al*., 2020; Ghasemi *et al*., 2023; Zupa *et al*., 2021).

Environmental factors like temperature and photoperiod significantly influence natural reproductive cycles. Decreased temperatures and shorter photoperiods during winter act as natural cues for spawning (Mylonas *et al*., 2023; Felip *et al*., 2009). Reproductive season typically begins at temperatures between 11–13°C and reduced daylight (Bromage & Roberts, 1995; Moretti *et al*., 1999).

In commercial hatcheries, environmental control systems simulate winter conditions to induce reproductive maturity. The optimal thermal range is 13–15°C, with a minimum of 9°C and a maximum of 18°C (Moretti *et al*., 1999; Mylonas & Zohar, 2007). Salinity also influences egg buoyancy and fertilization success, with 37‰ being the ideal level (Buchet *et al*., 2008; Vatsos *et al*., 2021).

4.5 **Effect of Darkness During Incubation**

Complete darkness was maintained throughout egg incubation, a common hatchery practice that benefits embryo development and hatching rates. Darkness reduces physiological stress and prevents phototoxicity in early embryonic stages (Villamizar *et al*., 2011). Light may induce premature activity, increase energy consumption, and delay cell division (Bolla &

Holmefjord, 1988). Recent studies confirm improved hatch synchrony and survival under dark conditions (Migaud *et al*., 2020).

4.6 Hormonal Induction Efficacy

The results of this study showed that applying a single injection protocol with a dose of 10 µg/kg of the luteinizing hormone-releasing hormone analogue Lh-RHa effectively stimulated ovulation in European seabass females. This was clearly reflected in the amount of eggs produced, which reached 16.3% of the total body weight. This level of egg production falls within the optimal range for successful artificial spawning programs, as several studies indicate that egg production in mature European seabass females typically ranges between 10% and 20% of body weight (Mylonas *et al*., 2010; Pereira *et al*., 2018; Zohar & Mylonas, 2001).

The eggs showed high quality: proper diameter, shape, transparency, and oil droplet count, indicating full oocyte maturation and hormonal responsiveness. Egg morphology is a primary indicator of spawning success (Fernández-Palacios *et al*., 2005; Valdebenito *et al*., 2013; Mylonas *et al*., 2023).

Physiologically, Lh-RHa activates the hypothalamic-pituitary-gonadal (HPG) axis, inducing endogenous LH release and triggering final oocyte maturation and ovulation. Lh-RHa provides more precise timing compared to traditional hormones like human chorionic gonadotropin HCG, which may cause overstimulation or asynchronous responses (Falahatkar *et al*., 2020; Krol *et al*., 2023).

Moreover, Lh-RHa is environmentally safe, leaving no active residues and posing minimal immunological risks. It is favored in both commercial and research hatchery protocols for its safety and sustainability (Mylonas & Zohar, 2007; Papadaki *et al*., 2022).

This study confirms the effectiveness of the hormonal protocol and supports existing literature advocating the targeted use of Lh-RHa to improve spawning success and egg quality under controlled hatchery conditions (Vatsos *et al*., 2021; Mylonas *et al*., 2023).

5. Conclusion

This study represents the first scientific documentation of successful induced spawning of European sea bass (*Dicentrarchus labrax*) using hormonal stimulation in a private commercial hatchery in Libya. The use of Lh-RHa effectively stimulated gonadal maturation and ovulation in wild-caught females, resulting in the production of high-quality eggs under real hatchery conditions.

An egg production rate of 16.3% of female body weight was achieved, with a hatching rate of 50%, despite the low number of males, indicating potential for improvement through better broodstock management. These findings demonstrate the viability of using wild broodstock for hatchery-based spawning and highlight the need for tailored local spawning protocols. Planned experiments will test: (H_1) increasing male ratio to 1.5:1 raises hatch to ≥70%; (H_2) a

split-dose Lh-RHa protocol (5 + 5 μg kg^{-1}, 12 h apart) reduces latency by 20% without affecting egg quality (Pereira *et al.,* 2018).

Acknowledgments

The team acknowledges the valuable support of the farm manager and all staff at Saif Al-Bahr Fish Farm (Abu-Kamash, Libya) during this study.

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