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Original Article

Influence of dietary *Schizochytrium limacinum* oil on growth performance, reproductive development, and testicular histo-architecture of genetically selected growth trait (SGT) *Clarias magur* male

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Abstract
Sustainable seed production of genetically selected growth-trait *Clarias magur* is a promising candidate for aquaculture diversification due to its superior growth rate and adaptability. However, it is a significant challenge to raise the quality of male brooders in captivity for commercial-scale seed production. The present study aimed to evaluate the effects of marine algal oil, *Schizochytrium limacinum* (SMAO), on growth performance, reproductive development, and testicular histoarchitecture of genetically selected growth trait (SGT) *Clarias magur* males through supplementation in broodstock diets at four different levels (SMAO 2%, SMAO 3%, SMAO 4%, and SMAO 5%), with fish oil (FO 3%) serving as the control, in a completely randomised design using 15 circular FRP tanks of 1000 L capacity, each holding 15 fish (ten males and five females) with a mean standard length of 24.3±0.86 cm and a mean weight of 96.04±4.14 g fish fed with 5% of body weight for 90 days. The SMAO 4% diet resulted in the highest weight gain (16.50±2.1 g) with the lowest feed conversion ratio (2.23±0.65). The highest gonad weight (0.55±0.12 g), gonadosomatic index (0.52), and abundant spermatozoa in the testis histology were observed in the SMAO 5% diet. The sperm density varied across SMAO treatments and the FO group, and higher spermatocrit value (94.39%) and sperm density (14.40×10^{9} cells mL^{-1}). In conclusion, SMAO supplementation at 4% enhances growth and captive maturation in male SGT magur, while 5% SMAO promotes higher sperm cell abundance in testes, supporting its use in sustainable broodstock development.

Keywords: captive maturation; histology; *Schizochytrium limacinum* oil; SGT *Clarias magur;* sperm quality

1 | INTRODUCTION

Captive broodstock development is crucial for the successful breeding and commercial production of large quantities of seeds of any fish species (Dubey *et al*. 2024; Khan *et al*. 2024). The diet fed to the broodstock during the entire process of captive maturation has a significant effect on fish reproduction, fecundity, gametogenesis, gamete quality and survival of larvae (Engdaw and Geremew 2024). Of these nutrients, lipids like highly unsaturated fatty acids (HUFAs) and polyunsaturated fatty acids (PUFAs) are crucial for reproductive success and larval development (Rumki *et al*. 2024). The dietary fatty acids were reported to affect the sperm morphology and sperm motility in common barbel, *Barbus barbus* (Alavi *et al*. 2009) and *Perca fluviatilis* (Henrotte *et al*. 2009). Among long-chain PUFAs (LC - PUFAs), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were reported to be present in male gametes in fish (Yıldız *et al*. 2021; Carneiro-Leite *et al*. 2023), and arachidonic acid (ARA) is also involved in steroidogenesis in reproduction in fish (Maranesi *et al*. 2018).

Fish oil is widely used as a lipid source in the formulation of aquatic feed (El-Sayed *et al*. 2022). However, due to the high price and limited availability of fish oil, and the growing attention to sustainable alternatives, vegetable oils (VOs) derived from plants have been introduced into feed formulations, although they are not rich in essential fatty acids (Milián-Sorribes *et al*. 2024). This shortcoming has spurred research into microalgae and single-cell organisms (SCOs) as potential sources of long-chain polyunsaturated fatty acids (LC-PUFAs) (Mazzocchi *et al*. 2021). Heterotrophic microalgae such as *Schizochytrium limacinum* can be used to replace part or all of the fish oil in broodstock diets, improving reproductive performance and sustainability (Rameez *et al*. 2020). Moreover, *Schizochytrium* marine algal oil (SMAO) is certified as GRAS (Generally Recognized as Safe) and is suitable for use in aquafeed formulations (Shah *et al*. 2018), as a lipid source to replace fish oil in aquafeeds. SMAO is reported to be rich in LC-PUFAs, including DHA, EPA and ARA, and can be used to complement aquafeed formulation to meet dietary requirements (Mazzocchi *et al*. 2021). The substitution of fish oil with SMAO can also reduce the number of organic pollutants that can be present in fish oil extracted from wild fish (Venegas-Calerón and Napier 2023).

Clarias magur (Hamilton, 1822), commonly known as magur, is an Endangered native air-breathing catfish, which has been taxonomically reclassified as a neotype of *Clarias batrachus* by Ng and Kottelat (2008). Genetically selected growth- trait *C. magur* (Rameez *et al*. 2020) developed by ICAR-Central Institute of Fisheries Education, Mumbai, India and exhibits significant potential for enhancing species diversification in Indian aquaculture (Jousy *et al.* 2018). Further, the large-scale production of magur seeds is hindered by the lack of good-quality milters for breeding and poor response to induced breeding by males (Sahoo *et al*. 2020; Priyadarshi *et al*. 2021). Chandan *et al.* (2024) reported the higher requirement of protein and lipid levels, especially LC-PUFAs, in their diet during the broodstock development of magur in captive conditions. Currently, there is no specific maturation feed formulated for magur catfish. The farmers mainly use commercial feeds that are available in the area and have a crude lipid content of approximately 4%, which is not sufficient for the nutritional needs of the species. Therefore, the present study aims to assess the effect of SMAO supplementation in the maturation diet of male *C. magur*.

2 | METHODOLOGY

2.1 Experimental animal

The selected growth-trait magur brooders of both sexes (225 nos) with an average body weight of 96.04±4.14 g and a total length of 23.9±0.38 cm, males and females were segregated, equally distributed and acclimatised in the FRP tank (1000 L) at ICAR-Central Institute of Fisheries Education (CIFE) wet laboratory to maintain optimal conditions. Before the experiment began, the fish were disinfected with potassium permanganate ($KMnO_4$) at a concentration of 5 ppm and salt at 3%. During the acclimatisation, the fish were fed with a commercial extruded floating feed. The feeding regime was divided into two portions: 30% of feed at 10:00 hrs and 70% at 22:00 hrs. Water exchange of 30% was conducted every three days, ensuring that various water quality parameters were kept at optimal levels.

2.2 Experimental design and feeding trial

The experiment included four dietary lipid levels and one control in triplicate, by incorporating different inclusion levels of algal oil (SMAO 2%, SMAO 3%, SMAO 4%, and SMAO 5%) and a control with fish oil (FO 3%) in commercial feed containing 4% lipid. The fish oil (FO 3%) incorporated diet was used as the control, as most of the commercial farmers used 3% fish oil (FO) coated feed during broodstock husbandry for three months (March-May) before the spawning season during broodstock feed management. The commercial extruded floating feed of 0.8mm with 40% crude protein and 4% lipid was used as the base experimental diet. Extruded feed has a higher capacity to absorb oil during the coating process, compared to pelleted feed. The experimental feed of each treatment was coated with respective doses of SMAO oil and control with FO, followed by coating with binder gel (Blue gold gel – Blue weight Biosciences Pvt Ltd) at the rate of 5 mL kg^{-1} feed. Binder gel was used to protect the lipid from leaching out and also to reduce oxidation. Prepared experimental feed was allowed to dry in a dryer at

40°C for 3 hrs. Dried feed was stored in an airtight container and kept in 4°C at refrigerator for further use. The coating of feed with SMAO and FO was carried out based on the weekly requirement. The experimental design followed was completely randomised. A total of 15 circular fibre-reinforced plastic (FRP) tanks with 1000 L capacity were used for this experiment, and each FRP tank was stocked with 10 males and 5 females in a ratio of (2M:1F) fish with an average weight of 99.8±8.0 g per individual. The stocked fish were fed with 3% of their body weight twice a day, at 30% of feed at 10:00 hrs and 70% of feed at 22:00 hrs. During the experiment, partial water exchange was carried out every three-day interval. The water quality parameters, such as water pH, temperature, dissolved oxygen, total alkalinity, total hardness, ammonia, nitrite and nitrate, were measured and recorded fortnightly during the entire experimental period (APHA 2017).

2.3 Proximate composition of experimental diets and SGT magur carcass

Initially, the proximate composition of the experimental diets in different lipid levels was determined by using standard methods (AOAC 2000). Moisture content of the samples (50 g) was measured by drying the samples in a hot air oven at 100±2°C for 6 hrs (Hexatec, Model no. HIPL-023A). Crude protein (calculated as TN% × 6.25) was determined by using the micro-Kjeldahl method (Kelplus; PELICAN). The ether extract was measured through the Soxhlet extraction method (SOCS plus SAS-AS 08, PELICAN). Ash content was determined by incinerating the samples in a muffle furnace at 550°C for 6 hrs, and fibre content was measured by using the fibre tech apparatus (Tulin equipment, Model FRB 8, India).

2.4 Assessment of nutrient utilization efficiency

At the end of the experiment, fish were collected, and the following growth parameters were measured:

Weight gain = Final weight (g) – Initial weight (g)

Food conversion ratio (FCR) = Feed given (g) / Weight gain (g)

Survival rate (%) = (Total number of fish survived / Total number of fish stocked) × 100

2.5 Assessment of maturity performance

At the end of the 90-day feeding trial, SGT magur were starved for 24 hrs before sampling. Three fish from each tank were randomly selected and treated with clove oil (50 µL L^{-1}), and the selected fish were analysed to assess body weight. Subsequently, the weights of the gonads, liver, and viscera were measured to analyse the gonadosomatic index (GSI), hepato-somatic index (HSI), and gastro-somatic index (GaSI).

The final GSI was estimated by following the formula of Gabr *et al*. (1998): GSI (%) = Weight of the testis (g) / Total weight of the fish (g)) × 100

The HIS and GaSI were calculated as follows (Rajaguru 1992):

HSI (%) = (Weight of the liver (g) / Weight of the fish (g)) × 100

GaSI (%) = (Weight of gut (g) / Weight of fish (g)) × 100

2.6 Histological architecture of the liver and testis

The fish were anesthetised with clove oil (50 µL L^{-1}). The fish were dissected, and liver and testis were collected. The collected tissues per treatment were fixed in 10% NBF. Further histology slides were prepared as per Luna (1968), and slides were examined under an Olympus inverted microscope (FSX100) at 40X magnification to capture morphological changes using a bright-field inverted microscope (Optscopes/XD30A series, Germany).

2.7 Assessment of sperm quality

At the end of the experiment, the nine male brooders from each treatment group were dissected, and their testes were extracted. Before dissection, the genital area was gently dried with sterile tissue paper to avoid contamination with water, urine, blood, mucus, or faecal material, followed by rinsing of dissected testes with sterile saline to prevent cross-contamination. Small incisions were made in the lobes of the testes, allowing the milt to be expelled into a Petri dish (Adewumi *et al*. 2005). The milt was then collected using a 1 mL micropipette and promptly transferred to a 2 mL Eppendorf tube, where it was stored at 4°C for subsequent analysis of sperm quality. The assessment of sperm quality was slightly modified from the method described by Enzeline *et al*. (2022). The spermatocrit level was calculated by filling a micro haematocrit tube with milt until it reached four-fifths of the tube's capacity. The end of the tube was sealed, and the haematocrit tube was centrifuged for 5 minutes at 8000 rpm. The density of spermatozoa was measured using a haemocytometer. Initially, the milt was diluted 1000× (10 µL of milt in 990 µL of NaCl) with a non-activating solution (0.9% v/v NaCl) and examined in five observational fields under a microscope for each replication. The sperm motility duration was assessed under a microscope at a magnification of 200x. Two microliters of milt were placed on a glass slide, and activation was achieved by adding 5 µL of water to the milt. The evaluation of sperm motility was conducted by measuring the time from activation until the sperm became non-motile and expressed in minutes. This evaluation of sperm motility was performed at room temperature (25–26°C) immediately following the collection of milt.

2.8 Data analysis

The experimental dataset was analysed by using SPSS

(version 25.0) for the windows operating system. Data were tested for normality using the Shapiro–Wilk test ($p > 0.05$) and homogeneity of variance using Levene's test ($p > 0.05$) before one-way ANOVA. Data were subjected to one-way ANOVA, followed by post-hoc analysis using Tukey's multiple range test (TMRT) with a significance level of $p < 0.05$. Results are presented as the mean ± standard error.

3 | RESULTS

3.1 Water quality parameters

Water quality parameters of all the experimental tanks were observed to be within the optimal range conducive to the cultivation of *C. magur* with a water temperature of 27–29°C (28.36±1.37°C), pH between 7.2 and 7.5 (7.37±0.05), dissolved oxygen ranged from 5.4 to 5.9 mg L^{-1} (5.74±0.06 mg L^{-1}), total hardness (as $CaCO_3$) between 142 and 176 mg L^{-1} (165±2.86 mg L^{-1}), total alkalinity (as $CaCO_3$) between 170 and 199 mg L^{-1} (187 ± 8.22 mg L^{-1}), ammonia-N concentrations from 0.11–0.17 mg L^{-1} (0.14±0.03 mg L^{-1}), nitrite-N concentrations ranged from 0.04–0.09 mg L^{-1} (0.061±0.03 mg L^{-1}), and nitrate-N concentrations varied from 0.23–0.34 mg L^{-1} (0.28±0.02 mg L^{-1}).

3.2 Proximate composition of experimental diets and SGT magur male carcass

The proximate composition of the experimental diets (isonitrogenous) coated with varying levels of SAMO oil was analysed, and the results are presented in Table 1. The findings indicate that no significant differences were observed in moisture, crude protein, total ash, crude fibre, and NFE across the treatments, except in ether extract, where ether extract percentage increased as the level of SAMO oil increased. Similarly, the proximate compositions of the SGT magur carcass were measured following the 90 days of feeding trials; the treatment group exhibited significant variation in moisture, protein and lipid levels. However, no significant differences were observed in total ash and total fibre content among the fish from different treatment groups (Table 2).

TABLE 1 Proximate composition of experimental diet containing graded levels of marine algal oil derived from *Schizochytrium limacinum* and fish oil.

Treatments	Amount (%)					
	Moisture	Crude protein	Ether extract[1]	Ash	NFE	Fibre
SMAO 2%	6.53±0.04	40.74±0.51	5.92±0.07^{d}	5.39±0.09	36.19±0.63	3.33±0.07
SMAO 3%	6.17±0.09	41.66±0.35	7.13±0.03^{c}	5.74±0.03	37.66±0.24	3.77±0.12
SMAO 4%	6.47±0.03	40.50±0.16	8.29±0.02^{b}	5.51±0.04	35.97±0.31	3.73±0.12
SMAO 5%	6.03±0.04	41.48±0.30	9.34±0.06^{a}	5.66±0.04	36.76±0.43	3.57±0.03
FO 3%	6.15±0.03	40.38±0.50	7.08±0.04^{c}	5.57±0.04	37.50±0.55	3.63±0.07

NFE = Nitrogen Free Extract; values are expressed as mean ± SE ($n = 3$).
Values with different superscripts indicate a significant difference ($p < 0.05$).
Lipid % in commercial extruded feed (CEF) = 4%.
[1]Analysed value.

TABLE 2 Proximate composition of fish carcass fed with experimental diet containing graded levels of marine algal oil, *Schizochytrium limacinum* (SMAO) and fish oil (FO) after a 90-day feeding trial.

Treatments	Amount (%)				
	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Fibre (%)
SMAO 2%	72.01±0.41^{d}	18.40±0.30^{b}	5.36±0.03^{b}	3.34±0.03	4.50±0.06
SMAO 3%	73.26±0.21^{c}	18.67±0.07^{b}	5.54±0.05^{b}	3.62±0.06	4.47±0.09
SMAO 4%	74.91±0.20^{b}	19.12±0.78^{b}	6.70±0.04^{a}	3.49±0.01	4.57±0.09
SMAO 5%	75.88±0.18^{a}	19.82±0.14^{a}	7.24±0.29^{a}	3.47±0.03	4.57±0.12
FO 3%	71.82±0.04^{d}	18.58±0.53^{b}	5.60±0.06^{b}	3.39±0.01	4.50±0.12

Values are expressed as mean ± SE; values in the same column with different superscripts are significantly different ($p < 0.05$).

3.3 Growth traits and reproductive indices

SMAO inclusion significantly influenced the growth and feed efficiency of SGT magur (Table 3). The weight gain was recorded significantly higher in the 4% SMAO group compared to other treatment groups, whereas no significant difference was exhibited between the 4%, 2% and

3% SMAO-fed groups. Similarly, the feed conversion ratio (FCR) was significantly lower in the 4% SMAO-fed group compared to other groups. Further, no significant difference was observed between the 2%, 3%, and 3% FO fed groups. The gonadosomatic index (GSI) was significantly highest in the 4% SMAO-included diet. Likewise, the hepatosomatic index (HSI) exhibited a significantly higher value in the 4% SMAO group and significance with the 3% FO-included diet. No significant differences were observed in the gastro-somatic index, GaSI, across the groups.

3.4 Sperm quality

The sperm quality parameters of SGT magur males fed graded levels of marine algal oil derived from *S. limacinum* (SMAO) and fish oil (FO) after 90 days of feeding trials are presented in Table 4. Sperm pH showed no significant difference across all treatments, indicating that dietary lipid source and inclusion level did not influence the acid-base balance of seminal plasma. The osmolality value varied significantly among treatments. Meanwhile, SMAO 2% and FO 3% had no significant differences and were observed within SMAO groups. Spermatocrit value differed significantly across treatments, following a clear dose-dependent pattern with increasing SMAO inclusion, but SMAO 3% and SMAO 2% had significant differences. Sperm density exhibited a significant difference between all the treatments of SMAO groups and the FO group. Sperm motility duration showed no significant variation among treatments, with values ranging from 1.29±0.31 min (SMAO 2%) to 1.38±0.54 min (SMAO 3%).

TABLE 3 Bio-growth and gonadal maturation indices of SGT magur male brooders reared in different experimental groups for a period of 90 days.

Treatments	Initial weight (g)	Final weight (g)	Weight gain (g)	FCR	GSI	HSI	GaSI
SMAO 2%	99.0±9.2	113.13±5.87^{a}	13.63±0.47^{b}	2.66±2.9^{a}	0.30±0.04^{c}	0.89±0.10^{c}	0.56±0.1
SMAO 3%	100.4±3.8	114.40± 5.36^{a}	14.20±0.80^{b}	2.48±2.9^{a}	0.40±0.04^{b}	1.03±0.11^{b}	0.58±0.09
SMAO 4%	99.8±8.0	115.4±6.49^{ab}	16.50±2.10^{a}	2.23±0.65^{b}	0.56±0.03^{a}	1.54±0.18^{a}	0.65±0.08
SMAO 5%	98.2±4.5	113.6±1.15^{bc}	15.33±0.88^{ab}	2.31±1.9^{a}	0.52±0.08^{ab}	1.24±0.20^{a}	0.46±0.04
FO 3%	102.6±4.5	117.8±2.89^{ab}	15.67±2.3^{ab}	2.35±0.3^{ab}	0.46±0.09^{b}	1.11±0.13^{b}	0.49±0.24

FCR = Feed conversion ratio; GSI = Gonado Somatic Index; HSI = Hepato Somatic Index; GaSI = Gastro-Somatic Index
Values are expressed as mean ± SE (n = 3)
Values with different superscripts in each column indicate significant differences at $p < 0.05$.

TABLE 4 Sperm Quality of SGT magur male fed with graded levels of marine algal oil derived from *Schizochytrium limacinum* and Fish oil after 90 days of feeding trials.

Treatment	pH	Osmolality (mOsm/kg)	Spermatocrit value (%)	Sperm density (X 10^{9} Cells/mL)	Sperm Motility duration (mins)
SMAO 2%	7.33±0.06	286.67±7.27^{c}	89.94±0.31^{bc}	8.47±0.23^{c}	1.29±0.31
SMAO 3%	7.40±0.08	315.00±6.43^{b}	89.69±0.54^{bc}	9.47±0.15^{bc}	1.38±0.54
SMAO 4%	7.33±0.05	321.67±3.84^{b}	91.98±0.29^{ab}	10.77±0.55^{b}	1.35±0.29
SMAO 5%	7.38±0.06	332.00±3.61^{a}	94.39±0.37^{a}	14.40±0.60^{a}	1.36±0.37
FO 3%	7.42±0.08	289.00±3.22^{c}	89.12±0.86^{c}	7.66±0.23^{c}	1.32±0.86

Values are expressed as mean ± SE (n = 3).
Values with different superscripts in each column indicate significant differences at $p < 0.05$.

3.5 Histology micrograph of liver and testis

After a 90-day feeding period, the liver histology of SGT magur that were given diets supplemented with SMAO at different levels and the control group revealed no signs of pathological damage or lipidosis in hepatocytes (Figure 1A–1E). In addition, all the treatment groups (SMAO and FO) showed moderate vacuolation of hepatocytes, characterized by an eccentric round nucleus. Furthermore, instances of enlarged hepatocytes, anucleated hepatocytes without significant lipid accumulation in their cytoplasm, and some hepatocytes in a degenerative stage were noted across all treatment groups.

After 90 days of feeding, liver histology of fish fed with SMAO-supplemented diets showed no evidence of pathological damage or lipidosis in hepatocytes (Figure 1A–1E). The groups supplemented with SMAO and FO both exhibited moderate hepatocellular vacuolation alongside an eccentric round nucleus in hepatocytes. In addition, the groups supplemented with SMAO and FO both displayed Instances of enlarged hepatocytes, anucleated hepatocytes showing no significant lipid accumulation in their cytoplasm, and some hepatocytes went

degenerative stage were observed in all the treatments. Testicular histology revealed that the SMAO 5% treatment had a greater quantity of spermatozoa cells, accompanied by tubular lumen breakdown, and the absence of spermatogonia cells in the testes when compared to other treatments (Figure 2A–2E). Conversely, the FO 3%, SMAO 2%, and SMAO 3% treatments exhibited a lower number of spermatozoa but a higher presence of spermatids and spermatogonia, while the SMAO 4% treatment exhibited a satisfactory number of spermatozoa and spermatids, and a limited number of spermatogonia.

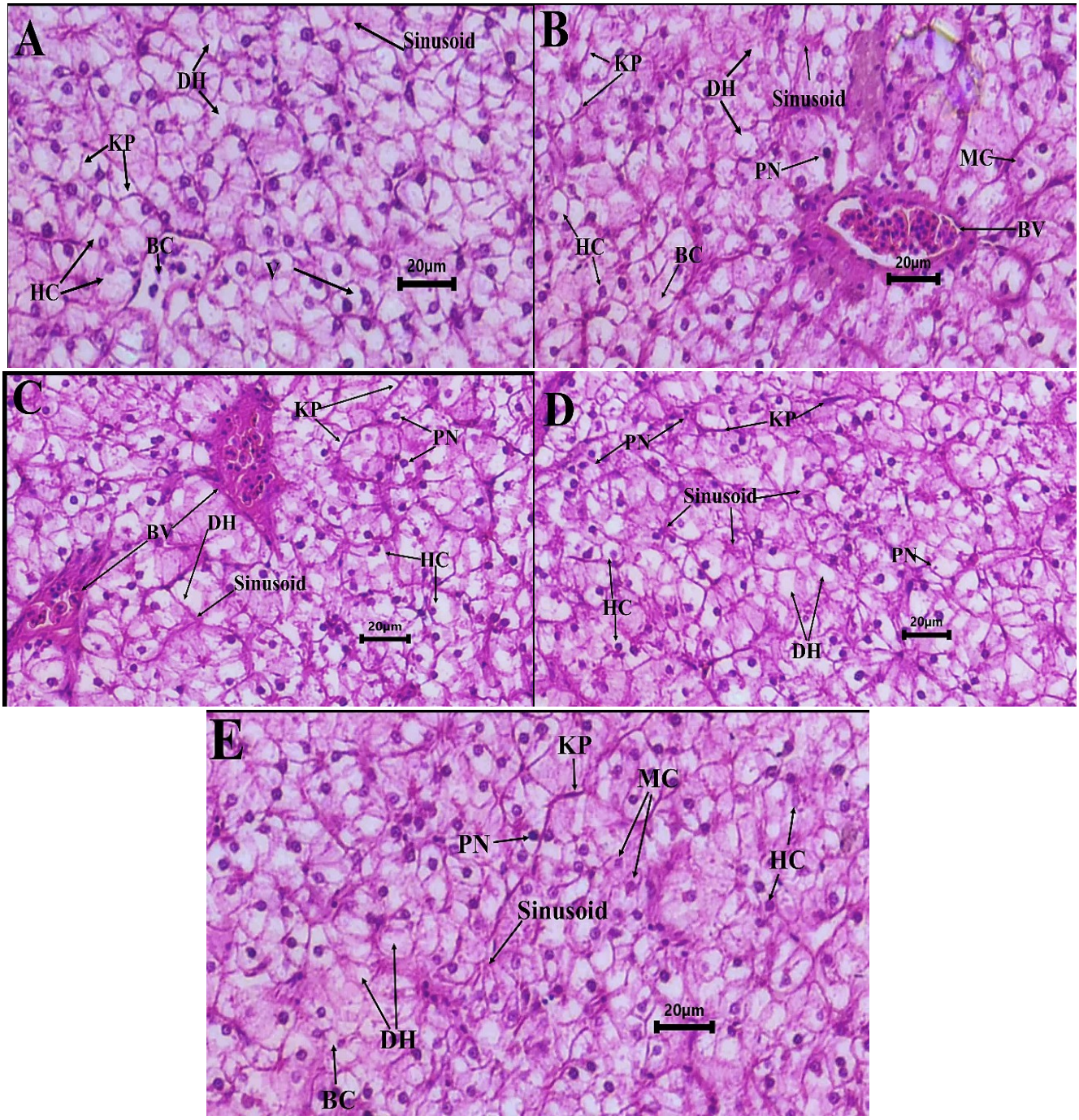

FIGURE 1 (A–E): Liver histology (H&E, 40×). A = 2%, B = 3%, C = 4%, D = 5% SMAO, and E = 3% FO group displayed mild hepatocellular degeneration, which was marked by cytoplasmic degeneration and changes in cellular structure. Instances of enlarged hepatocytes, anucleated hepatocytes, hepatocellular vacuolation, and congested blood vessels (as seen in B) were noted. HC-Hepatocytes, BC-Basophilic Cytoplasm, BV-Blood vessels or congested blood vessel, MC-Macrophage, KP-Kupffer cell, DH – Degenerated Hepatocytes (scale 20 um).

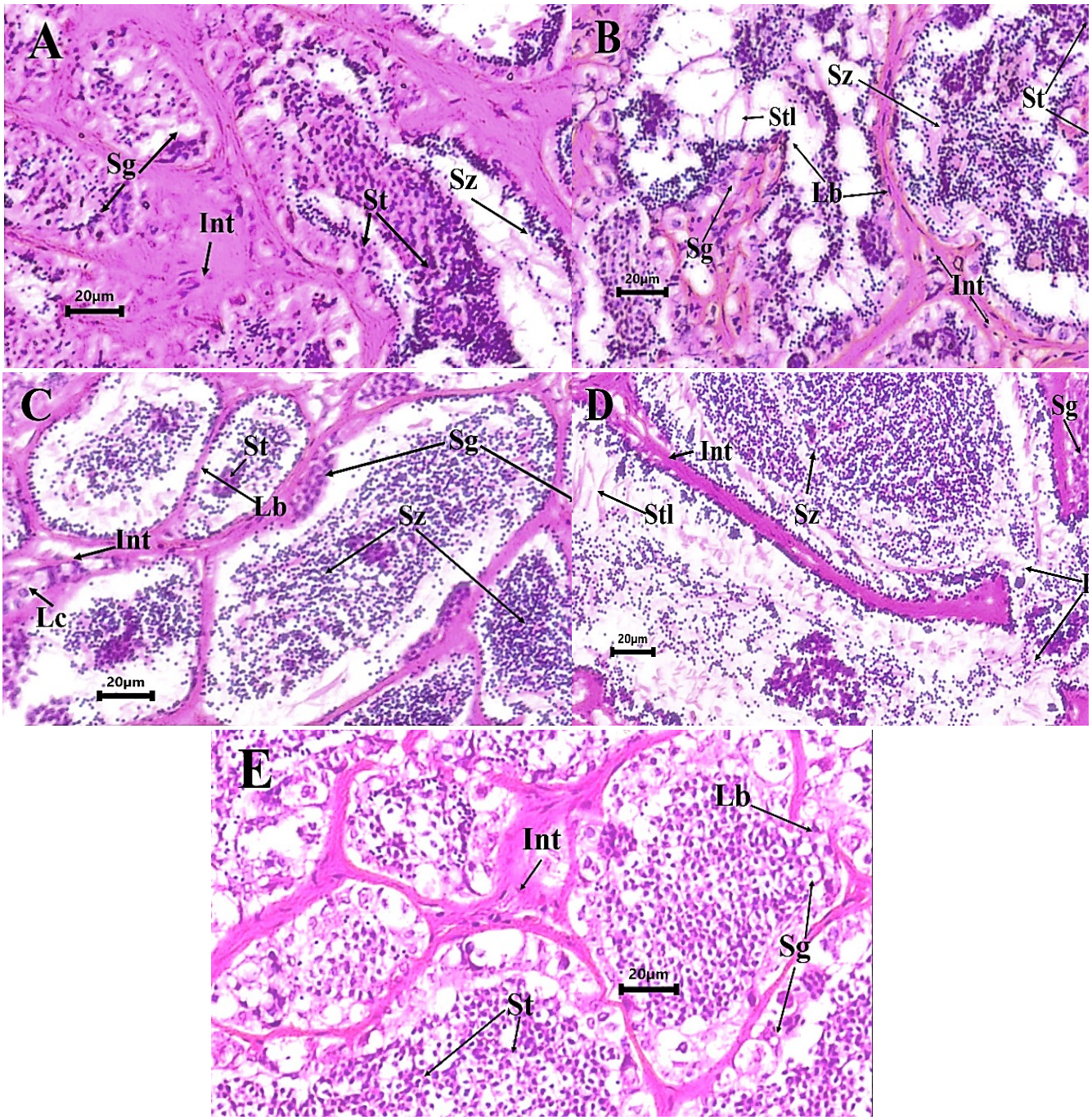

FIGURE 2 (A–E) Histological examination of testes (H&E, 40×) revealed that testes from fish receiving 2% and 3% SMAO (A, B) contained sperm cysts at various developmental stages. In contrast, testes from fish that were fed 4% and 5% SMAO (C, D) demonstrated advanced spermatogenesis, with spermatozoa present in each cyst, accompanied by lumen breakdown. Additionally, testes from fish that consumed 3% fish oil (FO) (E) displayed earlier germ-cell stages, predominantly spermatogonia and spermatids, suggesting a delay in spermiation compared to those with higher SMAO levels. Int - Interstitial tissue; Sg - Spermatogonia; St - Spermatids; Sz - Spermatozoa; Lb - Lumen breakdown; Stl- sperm tail, Lc- Leydig cell, B - Blood cell. (scale 20 um).

4 | DISCUSSION

4.1 Proximate composition of SGT magur male carcass

The effects of dietary *S. limacinum* marine algal oil (SMAO) on nutrient utilisation, and captive maturation of male magur brooders were evaluated. The HPG axis regulates reproduction (oogenesis, spermatogenesis, spawn-

ing) and adequate energy is required during gametogenesis and maturation (Xue *et al*. 2020). Lipids are a major energy source for milt quality and captive maturation; broodstock dietary lipids and fatty acid composition critically influence male reproductive maturation (Butts *et al*. 2015; Billard and Cosson 2020; Martins *et al*. 2020; Santos *et al*. 2021; Figueroa Villalobos *et al*. 2025). The present study observed an increase in dietary lipid levels as SMAO inclusion percentage increased in feed. However, carcass lipid levels remained unchanged among all the treatments, possibly due to lipid deposition in the gonad rather than the muscle (Perez *et al*. 2022).

4.2 Growth traits and reproductive indices

FCR is the key indicator of feed utilization in relation to the growth of animals (Haque *et al*. 2021; Paul *et al*. 2022). In this study, higher FCR were noted in all the treatments, indicating utilisation of feed for gonadal development rather than somatic growth, which directly influences the FCR. A previous study also reported that *C. magur* shows higher FCR during the maturation period compared to the growing stage, suggesting greater energy utilisation for gonadal growth, gamete maturation, and physiological homeostasis (Gupta *et al*. 2021). The highest weight gains were observed in 4% SMAO compared to other inclusion levels. The increased DHA and EPA content in SMAO is likely to facilitate enhanced lipid metabolism and energy utilisation during gonadal development (Jovanovic Gasovic 2023). The diets supplemented with higher n-3 LC-PUFA at a proper ratio could promote gonadal growth and increase their GSI in freshwater fish (Asghar *et al*. 2024). Similarly, higher gonad weight and GSI value have been recorded in SAMO 5% compared to other treatments. A lower GSI was observed in SMAO 2%, suggesting a low level of inclusion of SMAO. Notably, phospholipids or polyunsaturated fatty acids (PUFAs) enriched in DHA and EPA diets have been shown to significantly enhance gonadal development, GSI and overall reproductive output in species like *Nandus nandus* (Reza *et al*. 2013); *Colisa fasciatus* (Hossen *et al*. 2014) and *Labeo rohita* (Rumki *et al*. 2024).

4.3 Sperm quality

The SMAO-fed group of SGT magur males confirms that a minimum dietary supplement of DHA and EPA is required to sustain steroidogenesis, gonadal development, and sperm quality in SGT magur male broodstock, consistent with findings in other freshwater teleosts (Izquierdo *et al*. 2001; Singh *et al*. 2021). The dose-dependent enhancements in sperm density and spermatocrit value with increasing SMAO levels reflect the regulatory role of n-3 LC-PUFAs on androgenic hormone biosynthesis and sperm cell production efficiency per unit seminal fluid volume (Lahnsteiner *et al*. 1998; Carneiro-Leite *et al*. 2023). The higher sperm concentration in SMAO 5% over all other treatments indicates that the DHA source manages the spermatogenic output, as DHA is preferentially incorporated into sperm membranes and is essential for flagella function and spermatogenic cell proliferation (Bera *et al*. 2025; Figueroa *et al*. 2025), which is further supported by Cardona *et al*. (2022), who demonstrated comparable reproductive performance between *Schizochytrium* sp. derived DHA and fish oil diets in rainbow trout. The sperm motility period suggests that sperm motility kinetics are less responsive to dietary lipid manipulation than structural elements, as motility is primarily governed by calcium signalling, membrane potential, and seminal plasma biochemistry (Alavi *et al*. 2015; Gallego and Asturiano 2019). Overall, the reliable dominance of SMAO over FO 3% across the reproductive parameters, together with the well-documented sustainability and bioavailability of microalgal DHA across commercially important aquaculture species (Li *et al*.2009; Sprague *et al*. 2017), strongly supports the substitution of fish oil with *S. limacinum*-derived algal oil in SGT magur broodstock diets to optimize male reproductive performance.

4.4 Histology micrograph of liver and testis

Histological analyses of liver tissues in the present study showed no adverse effects across all treatments, with hepatocytes maintaining their normal morphology. Even at higher SMAO concentrations, the absence of lipid accumulation or structural damage to hepatocytes indicates the safety of SMAO as a dietary lipid source. In *Sperata aurata*, liver safety was also observed when fish oil was substituted with a blend of *S. limacinum* (Karapanagiotidis *et al*. 2022). Dietary n-3 fatty acids (notably DHA) improve the n 3: n 6 ratio, enhance liver morphology and metabolic function, and stimulate lipid metabolism and lipoprotein lipase synthesis (Yao *et al*. 2022; Thiruvasagam *et al*. 2024). The histological observations of the testis of *C. magur* under 40X magnification revealed distinct differences among the treatments. SMAO 2% shows the early stage with abundant spermatogonia and fewer spermatozoa. SMAO 4% and 5% group shows advanced maturation stage with dense spermatozoa and extensive lumen breakdown, which aligns with the histological section of a fully matured magur male observed by (Majhi *et al*. 2020). The results are consistent with the evidence that the function and motility of sperm are dependent on the fatty acid composition and mitochondrial fatty acid oxidation for energy (Bell *et al*. 1996; Lahnsteiner 2010; Dziewulska and Domagała 2013; Köprücü *et al*. 2015).

5 | CONCLUSIONS

This study suggests that SMAO is an effective and sustainable alternative to fish oil for broodstock development and aquaculture of genetically selected growth trait *C. magur*. SMAO supplementation, particularly at 4%,

enhances growth and maturation performance and SMAO at 5% gives a higher sperm cell number per lumen. These benefits address critical challenges in magur farming, such as poor reproductive efficiency and the scarcity of male broodstock. Future research should explore the long-term effects of SMAO supplementation, female reproductive assessment, and economic analyses and cost effective are the challenges and are required before commercial recommendations.

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ETHICAL APPROVAL
The experimental protocol received approval (Approval No. 524/GO/ReRcBi Bt/S/02/CCSEA/IAEC/CIFE-04-2025) from the Institute Animal Ethics Committee, ICAR - CIFE, Mumbai. The standard framework laid by the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests (Animal Welfare Division) of the Government of India, was followed to ensure ethical treatment of the experimental animal.

CONFLICT OF INTEREST
The author declares no conflict of interest.

AUTHORS' CONTRIBUTION
Gokul S - conducted this experiment, methodology, investigation, Data curation, Formal analysis, writing original draft; Dr Thongam Ibemcha Chanu: Conceptualisation, methodology, Supervision, Data curation, writing original draft; Dr. Babitha Rani A. M: Data curation, writing review and editing; Dr Prem Kumar: Writing review and editing; Dr. Debajit Sarma: Methodology, Resource. Rohit Kumar: Validation, Writing review and editing; Harshavarthini M: Validation, Visualization; Velmurugan A: Writing review and editing; Harini G: Analysis and Data curation; Kamil Akamad: Writing review and editing.

AI USE DECLARATION
No AI was used to generate, analyze, or interpret data, or to determine the study outcomes.

DATA AVAILABILITY STATEMENT
Data will be available on reasonable request.

REFERENCES
Adewumi AA, Olaleye VF, Adesulu EA (2005) Egg and sperm quality of the African catfish, *Clarias gariepinus* (Burchell) broodstock fed differently heated soybean-based diets. Research Journal of Agricultural and Biological Sciences 1(1): 17–22.

Alavi SMH, Ciereszko A, Hatef A, Křišťan J, Dzyuba B, Boryshpolets S, Linhart O (2015) Sperm morphology, physiology, motility, and cryopreservation in Percidae (pp. 163–191). In: Biology and culture of percid fishes: principles and practices. Springer Netherlands, Dordrecht.

Alavi SMH, Pšenička M, Policar T, Rodina M, Hamáčková J, … Linhart O (2009) Sperm quality in male *Barbus barbus* L. fed different diets during the spawning season. Fish Physiology and Biochemistry 35(4): 683–693.

AOAC (2000) Official Methods of Analysis, 17th edition. Association of Official Analytical Chemists, Arlington, VA, USA.

Asghar M, Khan N, Fatima M, Arslan M, Davies SJ, ul Haque N (2024) Growth, body composition, lipid profile and peroxidation, physiological stress, and blood metabolic indicators in *Labeo rohita* fed a fish oil replacement with blended linseed and sunflower oils. Aquaculture International 32(5): 6947–6966.

Bell MV, Dick JR, Thrush M, Navarro JC (1996) Decreased 20:4n−6/20:5n−3 ratio in sperm from cultured sea bass, *Dicentrarchus labrax*, broodstock compared with wild fish. Aquaculture 144: 189–199.

Bera P, Dhara H, Mandal S, Sau SK, Das S (2025) Nutritional strategies for improving reproductive performance in fishes (pp. 409–430). In: Aquaculture: Enhancing Food Security and Nutrition. Springer Nature Switzerland, Cham.

Billard R, Cosson MP (2020) The energetics of fish sperm motility (pp. 153–173). In: Controls of sperm motility. CRC Press.

Butts IAE, Baeza R, Støttrup JG, Krüger-Johnsen M, Jacobsen C, … Tomkiewicz J (2015) Impact of dietary fatty acids on muscle composition, liver lipids, milt composition and sperm performance in European eel. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 183: 87–96.

Cardona E, Segret E, Cachelou Y, Vanderesse T, Larroquet L, … Skiba-Cassy S (2022) Effect of micro-algae *Schizochytrium* sp. supplementation in plant diet on reproduction of female rainbow trout (*Oncorhynchus mykiss*): maternal programming impact of progeny. Journal of Animal Science and Biotechnology 13(1): 33.

Carneiro-Leite L, Silva LPD, Pazzini HB, Lobato SIR, Borges LP, … Ninhaus-Silveira A (2023) Effect of dietary omega-3 polyunsaturated fatty acids supplementation of *Astyanax lacustris* males on semen quality. Neotropical Ichthyology 21: e230077.

Chandan GM, Reang D, Jahageerdar S, Sahu NP, Jayant M, … Shamna N (2024) Dietary protein requirement in genetically selected magur (*Clarias magur*) broodstock: expression of reproduction-related genes. Animal Feed Science and Technology 318: 116141.

Dubey SK, Rajts F, Gogoi K, Das RR, Padiyar A, Belton B, Bhadury P (2024) Mass scale seed production of indigenous small fish species: a promising solution to scale nutrition-sensitive aquaculture (pp. 109–134). In: Perspectives and applications of indigenous small fish in India. Springer Nature Singapore.

Dziewulska K, Domagała J (2013) Spermatozoa concentration influences cryopreservation success in sea trout (*Salmo trutta* m. *trutta* L.). Theriogenology 80(6): 659–664.

El-Sayed AFM, Izquierdo M (2022) The importance of vitamin E for farmed fish: a review. Reviews in Aquaculture 14(2): 688–703.

Engdaw F, Geremew A (2024) Broodstock nutrition in Nile tilapia and its implications on reproductive efficiency. Frontiers in Aquaculture 3: 1281640.

Enzeline V, Nasrullah H, Sudrajat AO, Zairin M Jr, Alimuddin A, Widanarni W (2022) Spermatogenesis and sperm quality of male African catfish fed with *Bacillus* sp. NP5 probiotic supplemented diet. Aquaculture, Aquarium, Conservation and Legislation 15(1): 339–349.

Figueroa Villalobos E, Pereira WA, Pérez-Atehortúa M, Sandoval-Vargas L, Romero J, … Villasante A (2025) Influence of dietary fatty acids on fish sperm tolerance to cryopreservation. Reviews in Aquaculture 17: e12968.

Gabr HR, Hanlon RT, Hanafy MH, El-Etreby SG (1998) Maturation, fecundity and seasonality of reproduction of two commercially valuable cuttlefish, *Sepia pharaonis* and *S. dollfusi*, in the Suez Canal. Fisheries Research 36: 99–115.

Gallego V, Asturiano JF (2019) Fish sperm motility assessment as a tool for aquaculture research: a historical approach. Reviews in Aquaculture 11(3): 697–724.

Gupta G, Srivastava PP, Kumar M, Varghese T, Chanu TI, … Jana P (2021) Modulation effects of dietary zinc on reproductive performance and gonadotropin (FSH and LH) expression in threatened Asian catfish *Clarias magur* (Hamilton, 1822) broodfish. Aquaculture Research 52: 2254–2265.

Haque R, Sawant PB, Sardar P, Xavier KAM, Varghese T, Naik VA (2021) Synergistic utilization of shrimp shell waste-derived natural astaxanthin with its commercial variant boosts physio-metabolic responses and enhances colouration in discus (*Symphysodon aequifasciatus*). Environmental Nanotechnology, Monitoring and Management 15: 100405.

Henrotte E, Kaspar V, Rodina M, Pšenička M, Linhart O, Kestemont P (2009) Dietary n-3/n-6 ratio affects the biochemical composition of Eurasian perch (*Perca fluviatilis*) semen but not indicators of sperm quality. Aquaculture Research 41(9): e31–e38.

Hossen MS, Reza AM, Rakhi SF, Takahashi K, Hossain Z (2014) Effects of polyunsaturated fatty acids (PUFAs) on gonadal maturation and spawning of striped gourami *Colisa fasciatus*. International Aquatic Research 6(2): 1–13.

Izquierdo MS, Fernandez-Palacios H, Tacon AGJ (2001) Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197(1–4): 25–42.

Jousy N, Jahageerdar S, Prasad JK, Babu PG, Krishna G (2018) Body weight at harvest and its heritability estimate in *Clarias magur* (Hamilton, 1822) reared under mono and polyculture systems. Indian Journal of Fisheries 65(2): 82–88.

Jovanovic Gasovic S (2023) Metabolic and regulatory network analysis of DHA-producing recombinant Yarrowia lipolytica. Doctoral thesis. Universität des Saarlandes, Germany.

Karapanagiotidis IT, Metsoviti MN, Gkalogianni EZ, Psofakis P, Asimaki A, … Zarkadas I (2022) Effects of replacing fishmeal by *Chlorella vulgaris* and fish oil by *Schizochytrium* sp. and *Microchloropsis gaditana* blend on growth performance, feed efficiency, muscle fatty acid composition and liver histology of gilthead seabream (*Sparus aurata*). Aquaculture 561: 738709.

Khan SA, Sherzada S, Ahmad QUA, Hussain A, Hussain N, Nowosad J (2024) Captive breeding and early developmental dynamics of *Cirrhinus mrigala*: implications for sustainable seed production. Animals 14(19): 2799.

Köprücü K, Yonar ME, Özcan S (2015) Effect of dietary n-3 polyunsaturated fatty acids on antioxidant defense and sperm quality in rainbow trout (*Oncorhynchus mykiss*) under regular stripping conditions. Animal Reproduction Science 163: 135–143.

Lahnsteiner F, Berger B, Weismann T, Patzner RA (1998) Determination of semen quality of the rainbow trout, *Oncorhynchus mykiss*, by sperm motility, seminal plasma parameters, and spermatozoal metabolism. Aquaculture 163(1–2): 163–181.

Lahnsteiner FA (2010) A comparative study on the composition and importance of free amino acids in semen of gilthead sea bream, *Sparus aurata*, and perch, *Perca fluviatilis*. Fish Physiology and Biochemistry 36(4): 1297–1305.

Li MH, Robinson EH, Tucker CS, Manning BB, Khoo L (2009) Effects of dried algae *Schizochytrium* sp., a rich source of docosahexaenoic acid, on growth, fatty acid composition, and sensory quality of channel catfish *Ictalurus punctatus*. Aquaculture 292(3–4): 232–236.

Luna LG (1968) Manual of histologic staining methods of the Armed Forces Institute of Pathology. Armed Forces Institute of Pathology, Washington, DC. 258 pp.

Majhi SK, Kumar S, Maurya PK, Mohindra V, Lal KK (2020)

Non-invasive method for collection of *Clarias magur* spermatozoa for breeding. MethodsX 7: 100929.

Maranesi M, Castellini C, Dall'Aglio C, Petrucci L, Mattioli S, … Zerani M (2018) Effects of polyunsaturated fatty acids on animal reproduction: male and female performances and endocrine mechanisms. Phytochemistry Reviews 17(4): 801–814.

Martins G, Diogo P, Santos T (2020) Microdiet formulation with phospholipid modulates zebrafish skeletal development and reproduction. Zebrafish 17: 27–37.

Mazzocchi A, De Cosmi V, Risé P, Milani GP, Turolo S, … Agostoni C (2021) Bioactive compounds in edible oils and their role in oxidative stress and inflammation. Frontiers in Physiology 12: 659551.

Milián-Sorribes MC, Martínez-Llorens S, Peñaranda DS, Jauralde I, Jover-Cerdá M, Tomás-Vidal A (2024) Growth, survival and intestinal health alterations in Mediterranean yellowtail (*Seriola dumerili*) due to alternatives to fishmeal and fish oil. Current Issues in Molecular Biology 46(1): 753–772.

Ng HH, Kottelat M (2008) The identity of *Clarias batrachus* (Linnaeus, 1758), with the designation of a neotype (Teleostei: Clariidae). Zoological Journal of the Linnean Society 153(4): 725–732.

Paul M, Sardar P, Sahu NP, Varghese T, Nazeemashahul S, … Krishna G (2022) Optimal dietary protein requirement of juvenile GIFT tilapia (*Oreochromis niloticus*) reared in inland ground saline water. Journal of Environmental Biology 43(2): 205–215.

Perez MJ, Rodriguez C, Cejas JR, Martin MV, Jerez S, Lorenzo A (2007) Lipid and fatty acid content in wild white seabream (*Diplodus sargus*) broodstock at different stages of the reproductive cycle. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 146(2): 187–196.

Priyadarshi H, Das R, Singh AA, Patel AB, Pandey PK (2021) Hormone manipulation to overcome a major barrier in male catfish spawning: the role of oxytocin augmentation in inducing voluntary captive spawning. Aquaculture Research 52: 51–64.

Rajaguru A (1992) Biology of two co-occurring tonguefishes, *Cynoglossus arel* and *C. lida* (Pleuronectiformes: Cynoglossidae), from Indian waters. Fishery Bulletin 90: 328–367.

Rameez R, Jahageerdar S, Chanu TI, Jayaraman J, Bangera R (2020) Genetic variation among full-sib families and the effect of non-genetic factors on growth traits at harvest in *Clarias magur* (Hamilton, 1822). Aquaculture Reports 18: 100411.

Reza AHMM, Rakhi SF, Hossen MS, Takahashi K, Hossain Z (2013) Enhancement of reproductive performances of Gangetic leaffish *Nandus nandus* through up-regulation of serum Ca^{2+} concentration and improved morphological alterations of liver and ovary with dietary polyunsaturated fatty acids. Fish Physiology and Biochemistry 39(4): 779–791.

Rumki SP, Moniruzzaman M, Hossain MS, Saha TK, Tabassum A, … Hossain Z (2024) Effects of dietary polyunsaturated fatty acid-enriched diets on breeding, embryonic development and organogenesis of rohu carp *Labeo rohita* (Hamilton 1822). Reproduction and Breeding 4(3): 155–163.

Sahoo L, Barat A, Sahoo SK, Sahoo B, Das G, … Swain SK (2020) Genetic diversity and population structure of endangered Indian catfish *Clarias magur* revealed by mtDNA D-loop marker. Turkish Journal of Fisheries and Aquatic Sciences 21: 9–18.

Santos D, Rocha E, Malhão F, Lopes C, Gonçalves JF, Madureira TV (2021) Multi-parametric portfolio to assess the fitness and gonadal maturation in four key reproductive phases of brown trout. Animals 11(5): 1290.

Shah MR, Lutzu GA, Alam A, Sarker P, Kabir Chowdhury MA, … Daroch M (2018) Microalgae in aquafeeds for a sustainable aquaculture industry. Journal of Applied Phycology 30(1): 197–213.

Singh SK, Baidya S, Das P, Biswas P (2021) Functional role of dietary supplements on reproductive physiology of fishes (pp. 243–258). In: Recent updates in molecular endocrinology and reproductive physiology of fish: an imperative step in aquaculture. Springer Singapore,

Sprague M, Betancor MB, Tocher DR (2017) Microbial and genetically engineered oils as replacements for fish oil in aquaculture feeds. Biotechnology Letters 39(11): 1599–1609.

Thiruvasagam T, Chidambaram P, Ranjan A, Komuhi NB (2024) Significance of fatty acids in fish broodstock nutrition. Animal Reproduction Science 268: 107573.

Venegas-Calerón M, Napier JA (2023) New alternative sources of omega-3 fish oil (pp. 343–398). In: Advances in food and nutrition research, volume 105. Academic Press.

Xue X, Hall JR, Caballero-Solares A, Eslamloo K, Taylor RG, … Rise ML (2020) Liver transcriptome profiling reveals that dietary DHA and EPA levels influence suites of genes involved in metabolism, redox homeostasis and immune function in Atlantic salmon (*Salmo salar*). Marine Biotechnology 22(2): 263–284.

Yao X, Lin Y, Shi M, Chen L, Qu K, … Xie S (2022) Effect of *Schizochytrium limacinum* supplementation to a low fish-meal diet on growth performance, lipid metabolism, apoptosis, autophagy and intestinal histology of *Litopenaeus vannamei*. Frontiers in Marine Science 9: 1090235.

Yıldız M, Ofori-Mensah S, Arslan M, Yamaner G, Ekici A, … Tacer-Tanas Ş (2021) Effects of different dietary lipid resources on sperm quality and reproductive success in rainbow trout (*Oncorhynchus mykiss*). Aquaculture Research 52(8): 3804–3814.

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