



Reproductive performance of *Tor tambroides* (Bleeker, 1854) fed diets containing fish oil from different sources

¹Azrita Azrita, ¹Hafrijal Syandri, ²Rona T. Sari, ¹Hendra Kusuma

¹ Department of Aquaculture, Faculty of Fisheries and Marine Science, Bung Hatta University, 25133 Padang, Indonesia; ² Department of Biology Education, Faculty of Teacher Training and Education, Bung Hatta University, 25133 Padang, Indonesia. Corresponding author: A. Azrita, azrita31@bunghatta.ac.id

Abstract. This study aimed to evaluate the reproductive performance of Thai mahseer, *Tor tambroides* (Bleeker, 1854) broodstock fed diets containing fish oil derived from different marine sources. Four dietary treatments were formulated: tuna fish oil (TFO), sardinella fish oil (BFO), squid oil (SFO), and a control diet without added fish oil (CFO). Although squid is not a fish, its oil was included as a marine-derived lipid source to assess its potential contribution to reproductive performance. The experimental design followed a Completely Randomized Design with four treatments and three replicates, each using three broodfish, over a 120-day feeding period. Reproductive performance was assessed based on final body weight (g), oocyte maturation (%), pre-ovulated egg diameter (mm), ovulated egg weight (g), latency period (h), absolute fecundity (eggs/spawn), egg weight percentage (%), and larval survival (%) at 7 days post-hatch. The results showed that fish oil supplementation significantly improved reproductive parameters compared to the control diet ($p < 0.05$). The SFO group demonstrated the highest oocyte maturation rate ($89.33 \pm 1.15\%$), pre-ovulated egg diameter (2.26 ± 0.09 mm), the ovulated egg weight was (136.57 ± 16.51 g kg^{-1} of body weight), latency period (13.10 ± 0.85 h), absolute fecundity ($9,105 \pm 1,100$ eggs spawn^{-1}), and egg weight percentage ($10.71 \pm 0.40\%$). The highest larval survival rate ($93.33 \pm 1.52\%$) was also recorded in the SFO group. The results demonstrate that squid oil, among the marine fish oils evaluated, provided the greatest improvement in reproductive performance and larval viability of *T. tambroides* broodstock.

Key Words: *Tor tambroides*, broodstock nutrition, fish oil, reproductive performance, oocyte maturation, larval survival.

Introduction. The Thai mahseer, *Tor tambroides* (Bleeker, 1854), locally known as garing in West Sumatra Province, is one of the endemic fish species of Indonesia (Akmal et al 2022). This species is distributed across various rivers in Indonesia, including those in Sumatra, Kalimantan, and Java Islands (Haryono & Tjakrawidjaja 2006), and is also found in western Malaysia and Kalimantan (Ingram et al 2005). *T. tambroides* has a commonly observed total length ranging from 61 to 74.9 cm, with a maximum recorded length of 100.0 cm (FishBase 2024). According to an interview conducted in March 2024 with Peri, a local fish farmer from an inland area in Pasaman Regency, West Sumatra Province, Indonesia, the farm-gate price of this fish ranged from USD 15.33 to USD 18.39 per kilogram. However, this species has been overexploited, leading to population declines in its natural habitat (Akmal et al 2022).

Although classified as Data Deficient by the International Union for Conservation of Nature, various local studies indicate that *T. tambroides* faces significant conservation threats. Muchlisin et al (2015) reported that the population of *T. tambroides* is in a threatened condition due to environmental degradation and high levels of exploitation.

In addition to these external pressures, *T. tambroides* also exhibits biological constraints that may hinder population recovery. Notably, it has a relatively low fecundity, producing about 3,600 eggs per kilogram of broodstock weight (Haser et al 2022). In contrast,

its related species, Semah mahseer, *Tor douronensis* (Valenciennes, 1842), exhibits a higher fecundity, reaching approximately 4,987 eggs per kilogram of broodstock weight (Cahyanti et al 2019). Although the *T. tambroides* has lower fecundity, the eggs it produces tend to be larger, which theoretically may increase larval survival chances during the early stages. However, the higher fecundity of *T. douronensis* also provides an advantage in supporting large-scale seed production, especially in conservation and aquaculture programs.

The decline in *T. tambroides* populations in their natural habitats has made the cultivation of this species as a food source increasingly critical (Bami et al 2017). Initial success in the domestication of *T. tambroides* as a promising aquaculture candidate has been reported (Cahyanti et al 2019). However, the availability of high-quality, suitable feed remains a key component in supporting the success of this domestication effort (Kanyilmaz 2016; Sugiura 2025).

The growing demand for *T. tambroides* seed for intensive aquaculture has driven the need for superior broodstock, both in quality and quantity, to ensure an adequate supply of seed for the industry. In line with the long-term growth projections of the *T. tambroides* aquaculture sector, optimal broodstock management and feed formulation that support efficient growth and gonadal maturation are crucial strategies for enhancing gamete production (Abduh et al 2021), thereby meeting the current and future demand for high-quality fry (Sulaiman et al 2022).

Feed formulation to support oocyte maturation in broodfish has been widely studied in various species, such as the green swordtail, *Xiphophorus hellerii* Heckel, 1848 (Chong et al 2004), Channel catfish, *Ictalurus punctatus* (Rafinesque, 1818) (Sink et al 2010), Pike-perch, Sander lucioperca (Péter et al 2023), and Atlantic salmon, *Salmo salar* (Fjelldal et al 2024). Although proven effective in stimulating oocyte maturation, the relatively high production costs of artificial feeds remain a barrier to their widespread application in aquaculture industries (Fantatto et al 2024).

To date, no studies have specifically evaluated the use of tuna fish oil (TFO), Sardinella fish oil (BFO), and squid oil (SFO)—by-products of fish processing—as dietary supplements in formulated feeds to support oocyte maturation, particularly in freshwater species such as *T. tambroides*.

Therefore, this study aims to evaluate the effects of adding TFO, BFO, SFO, and CFO (control) to feed ingredients on oocyte maturation in *T. tambroides* broodstock. The results of this study are expected to contribute to the diversification of more cost-effective alternative feed sources and expand the aquaculture potential of freshwater fish species for intensive farming.

Material and Method

Diet preparation. TFO, BFO, and SFO used in this study were obtained from local processing plants located in Indonesia. Each type of oil was a pure product (Grade A) without any mixture of other fish species and had undergone an initial filtration process at the industrial level. The oils were packaged in sealed 500 mL glass bottles to maintain their stability and quality during distribution. All samples were then transported to the Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bung Hatta University, Padang, under conditions protected from light and high temperatures, to be used in subsequent analysis and testing activities.

T. tambroides has been reported to require an optimal dietary lipid level of 5% (Ramezani-Fard et al 2012). Based on this requirement, five experimental diets were formulated to be isonitrogenous (containing 35% crude protein), isolipidic (9.41% total lipid), and isocaloric (19 kJ g⁻¹). Each diet included 5% of a specific marine-derived oil, namely TFO, BFO, SFO, and CFO (control) (Table 1). Corn oil was incorporated into all formulations as an additional lipid source. The fatty acid profiles of the various lipid sources are presented in Table 1. Identification of individual fatty acids was carried out by comparing their retention times

with those of authentic fatty acid methyl ester (FAME) standards. The results were expressed as the relative percentage of each fatty acid to the total identified fatty acids

All dry ingredients were blended using a kitchen mixer for 20 minutes. After the addition of distilled water and oil, the mixture became homogeneous. The moist mash was then processed through a single-screw laboratory extruder (Brabender KE-19) equipped with a 2 mm die. The resulting pellets were oven-dried at 45°C for 4 hours, cooled to room temperature, packed in airtight containers, and stored at 4°C with desiccants to maintain quality before use, following the procedure described by Bami et al (2017).

Broodstock rearing and pond system. A total of 35 broodstock of the local fish species *T. tambroides* were obtained from a local supplier in Pasaman Regency, West Sumatra, Indonesia. These included 20 females and 15 males, who were transported by truck to the Wet Laboratory at the Department of Aquaculture, Faculty of Fisheries and Marine Science, Bung Hatta University.

The fish were acclimatized for one month in circular tarpaulin tanks with a capacity of 2,500 liters. Male and female broodstock were maintained in separate tanks, with females housed in four tanks and males in two tanks. During the acclimation period, the fish were fed commercial pellets containing 30% crude protein, administered twice daily.

After acclimation, female broodstock were randomly distributed into four circular tarpaulin tanks measuring 110 cm in height and 200 cm in diameter, with a maintained water depth of 80 cm, resulting in an effective volume of approximately 2,512 liters. Each tank was stocked with three female broodfish. The tanks were equipped with a water circulation system, consisting of inlet and outlet channels, to ensure water exchange and environmental stability.

Each tank was also fitted with a top biofilter and an aeration system to maintain water quality. Water quality parameters, including temperature, pH, and ammonia levels, were monitored every three weeks to ensure optimal rearing conditions throughout the experimental period.

Table 1

Nutritional, chemical, and fatty acid profiles of the formulated experimental diets

Ingredient (% as fed basis)	Dietary			
	CFO	TFO	BFO	SFO
Fish meal ¹	20	20	20	20
Soybean meal	37	37	37	37
Broken milled rice	19	19	19	19
Rice bran	11	11	11	11
Gelatin ²	5	5	5	5
Corn oil	1	1	1	1
Tuna fish oil	0	5	0	0
Sardinella fish oil	0	0	5	0
Squid oil	0	0	0	5
Vitamin premix	1	1	1	1
Mineral premix	1	1	1	1
Proximate composition (% as fed basis)	100	100	100	100
Crude protein	34.97±0.30 ^a	34.98±1.54 ^a	34.96±1.85 ^a	34.98±1.45 ^a
Crude lipid	9.41±0.35 ^a	9.41±0.20 ^a	9.42±1.56 ^a	9.42±1.48 ^a
Crude fibre	3.49±1.25 ^a	3.49±1.80 ^a	3.49±1.78 ^a	3.49±1.56 ^a
Ash	6.63±0.69 ^a	6.63±0.55 ^a	6.63±0.75 ^a	6.63±0.56 ^a
Gross energy (kJ g ⁻¹)	19.81±0.75 ^a	19.81±0.69 ^a	19.81±0.85 ^a	19.81±0.62 ^a
Dry matter	90±1.15 ^a	90±0.75 ^a	90±0.84 ^a	90±0.69 ^a
Fatty acid composition (% of total fatty acids)				
Palmitic acid, 16:0	2.62±1.12 ^a	29.31±1.11 ^b	29.37±1.15 ^b	31.59±1.18 ^d
Stearic acid, 18:0	0.57±0.01 ^a	5.49±0.02 ^b	3.87±0.01 ^c	2.15±0.01 ^d
18:1 (n-9), oleic acid	1.48±0.01 ^a	25.13±1.15 ^b	19.52±1.18 ^c	2.45±0.03 ^a
18:2 (n-6), linoleic acid (LA)	1.33±0.01 ^a	4.73±0.02 ^b	4.81±0.01 ^c	3.96±0.02 ^d
20:2 (n-6), eicosadienoic acid	0.03±0.00 ^a	0.19±0.01 ^b	0.21±0.01 ^c	0.5±0.01 ^d
20:4 (n-6), arachidonic acid (ARA)	0.12±0.00 ^a	1.33±0.01 ^b	1.77±0.01 ^c	0.87±0.01 ^d
20:5 (n-3), eicosapentaenoic acid (EPA)	1.41±0.01 ^a	12.41±0.03 ^b	15.92±0.02 ^c	16.5±0.02 ^d
22:6 (n-3), docosahexanoic acid (DHA)	2.1±0.00 ^a	17.36±0.03 ^b	17.06±0.04 ^c	29.42±0.04 ^d
Others	0.75±0.01 ^a	4.05±0.01 ^b	7.47±0.01 ^c	12.56±0.02 ^d
Total	10.41±0.05 ^a	100±0.00 ^b	100±0.00 ^b	100±0.00 ^b
ΣSFA (Sum of 14:0+16:0+18:0)	3.19±0.02 ^a	34.8±1.25 ^b	33.24±1.48 ^b	33.74±1.56 ^b
ΣMUFA (18:1, n-6)	1.48±0.02 ^a	25.13±1.10 ^b	19.52±1.15 ^c	2.45±1.24 ^a
ΣPUFA (Sum of n-3+n-6)	3.98±0.03 ^a	36.02±2.03 ^b	39.77±2.53 ^b	51.25±2.85 ^c
n-3 (EPA+DHA)	3.57±0.01 ^a	29.77±2.18 ^b	32.98±1.89 ^b	41.98±3.25 ^c
n-6 (LA+20:2+ARA)	1.48±0.02 ^a	6.25±0.03 ^b	6.79±0.03 ^c	5.33±0.02 ^d
n-6/n-3 FUPA ratio	0.41±0.01 ^a	0.20±0.01 ^b	0.20±0.01 ^b	0.12±0.01 ^c

Mean ± SD (n=3)

¹ Indonesia fish meal (55.0% crude protein)

² Gelatin, produced from gourami fish scales (*Osphronemus goramy*) by the author (80.0% crude protein).

Products from PT Avindo Perdana Bahtera Mulia, Indonesia. Vitamin premix (g kg⁻¹ premix): vitamin A = 18; vitamin E = 75; vitamin K₃ = 10; vitamin B1 = 10, vitamin B2 = 20; vitamin B6 = 20; vitamin B12 = 0.1; Biotin = 0.1; Niacin = 5; Folic acid = 5. Mineral mix (g kg⁻¹ premix): Ca = 17.88; P = 10.88; Mg = 2.98; Mn = 0.055; Zn = 0.141.

Feeding trial and experimental conditions. Before stocking, the female broodstock were individually weighed, with an average initial body weight of $1,200 \pm 125$ g. At the beginning of the trial, all females exhibited stage I ovarian development, characterized by oocyte nuclei and germinal vesicles positioned centrally within the oocyte. This stage was observed following complete spawning, indicating the initiation of a new orogenic cycle after gonadal regression.

Over a 120-day experimental period, the broodfish were fed one of four experimental diets: a control diet, CFO, and three diets formulated with TFO, BFO, or SFO. These four diets represented the main treatments in this study. The fish were fed twice daily, at 09:00 and 17:00, at a feeding rate of 3% of the total biomass in each tank.

The water depth in each circular tarpaulin tank was maintained at 80 cm, corresponding to a volume of approximately 2,513 liters. With a continuous water supply from a deep-well source at 2 L min^{-1} , water quality remained stable throughout the experimental period.

Assessment of oocyte maturation and spawning. All fish were individually tagged using Floy tags and weighed. Oocytes were sampled *in vivo* from females following the method described by Nowosad et al (2014), then placed in Serra's solution (a mixture of 70% ethanol, 40% formaldehyde, and 99.5% glacial acetic acid in a 6:3:1 ratio) to clarify the cytoplasm. After 5 minutes, the position of the oocyte nucleus was determined using a four-stage scale according to Krejszeff et al (2009), as follows:

Stage 1: The germinal vesicle is located centrally within the oocyte.

Stage 2: The germinal vesicle begins to migrate, moving less than halfway toward the oocyte's periphery.

Stage 3: The germinal vesicle continues its migration, moving beyond the halfway point toward the periphery.

Stage 4: The germinal vesicle reaches the periphery or undergoes breakdown, known as germinal vesicle breakdown (GVBD).

Each group of female broodfish exhibited different levels of oocyte maturation. After 120 days of the experiment, the number of females that successfully reached oocyte maturation varied across ponds according to the dietary treatments. To induce ovulation, each group of females received two intraperitoneal injections of a GnRH analog combined with a dopamine antagonist (Ovaprim), administered in the ventral area, near the base of the pelvic fins. The first injection was given at a dose of 0.2 mL kg^{-1} body weight, followed by a second injection of 0.6 mL kg^{-1} body weight (totaling 0.8 mL kg^{-1} body weight) with a 12-hour interval between injections (Ciesla et al 2014). Egg size, with 50 eggs sampled from each group, was measured using an Olympus microscope. A digital E-510 camera was mounted on an Olympus BX51 binocular microscope.

Broodstock used for gamete collection were anesthetized with special Arowana Stabilizer at a concentration of 1 ml per 2 L of water, another quality product from Yi Hu Fish Farm Trading JI. Later Lekar, Sungai Tengah, Singapore. Between 12 and 13 hours post-injection, eggs were stripped into a plastic container. Fertilization was carried out using the "dry" method, following the procedure outlined by Dabrowski et al (1994). Male broodstock received half the hormone dose administered to females, and sperm were collected using a plastic syringe. Sperm motility was not assessed in this study. However, only males with previously confirmed reproductive performance and normal milt characteristics (e.g., volume, color, and viscosity) were selected to minimize potential impacts of semen quality on fertility outcomes. A total of 100 eggs from each female were mixed with 0.3 mL of pooled sperm. The fertilized eggs were then incubated in small aquaria maintained at a water temperature of $29 \pm 1^\circ\text{C}$ and a dissolved oxygen (DO) level of $6.32 \pm 0.05 \text{ mg L}^{-1}$.

Embryo survival was recorded on 7 days post-hatch. Differences among treatment groups were statistically analyzed using one-way analysis of variance (ANOVA), followed by Tukey's test to identify significant differences ($p < 0.05$).

Results and Discussion

Body weight gain, relative egg weight, and oocyte maturation rate. In this study, female broodstock of *T. tambroides* fed diets containing CFO, TFO, BFO, and SFO showed body weight increases of $5.21 \pm 0.71\%$, $11.99 \pm 0.67\%$, $8.63 \pm 0.41\%$, and $15.14 \pm 1.22\%$, respectively. Similarly, the percentage of gonadosomatic index to body weight also increased, recorded at $4.94 \pm 0.64\%$ for CFO, $8.01 \pm 0.04\%$ for TFO, $7.09 \pm 0.59\%$ for BFO, and $10.71 \pm 0.40\%$ for SFO (Table 1). Statistical analysis revealed that both parameters differed significantly among the dietary treatments ($p < 0.05$), indicating that the type of feed had a significant effect on the growth and reproductive performance of female *T. tambroides* broodstock. In addition, oocyte maturation was only observed in the groups fed diets containing TFO, BFO, and SFO, with maturation percentages of $80.66 \pm 3.05\%$, $77.33 \pm 3.05\%$, and $89.33 \pm 1.15\%$, respectively. In contrast, in the CFO (control) group, the oocytes had not yet reached the GVBD stage; therefore, the percentage of oocyte maturation could not be determined. Statistically, there was a highly significant difference ($p < 0.05$) in the percentage of oocyte maturation among the TFO, BFO, and SFO groups.

These results suggest that diets with a higher n-3/n-6 fatty acid ratio, such as those found in SFO and TFO, may more effectively promote somatic growth and reproductive performance in *T. tambroides* broodstock. Among the treatments, the inclusion of SFO in the diet resulted in the highest increases in both body weight and egg weight percentage compared to TFO, BFO, and CFO (Table 2). These findings indicate that the dietary fatty acid composition, particularly the proportion of omega-3 (n-3) fatty acids, plays a positive role in enhancing reproductive performance, especially in terms of egg weight percentage. The n-3 fatty acid content in the SFO, TFO, and BFO diets was higher than that in the CFO diet (Table 1), which is likely the key factor contributing to this improvement.

In this study, feeding broodstock females with diets containing different omega-3 fatty acid contents showed a significant impact on oocyte maturation and egg quality. Feeding with SFO resulted in the highest increase in body weight and egg weight percentage, followed by TFO, BFO, and CFO. These results are consistent with previous findings showing that omega-3 fatty acids can improve egg quality and reproductive performance in various fish species.

Omega-3 refers to a group of essential fatty acids that cannot be synthesized de novo by marine fish and must be obtained through dietary intake. These fatty acids, particularly eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), play crucial roles in fish reproductive processes, including gonadal maturation, steroid hormone production, and egg quality. Research by Izquierdo et al (2001) demonstrated that dietary supplementation with n-3 highly unsaturated fatty acids (HUFA) significantly improved egg quality and reproductive performance in marine species such as Gilthead seabream *Sparus aurata* (Linnaeus, 1758) and Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). Among saturated fatty acids, palmitic acid (16:0) is notably the most abundant, comprising up to 31.59% of the total fatty acids in SFO-based diets administered to *T. tambroides* broodstock. Palmitic acid plays a supportive role in energy storage as body fat, which is mobilized during gonad development, thereby contributing to oocyte development and serving as an energy reserve for the embryo (Hossain et al 2024).

Marine fish oils are rich sources of polyunsaturated fatty acids (PUFAs), particularly EPA, DHA, and arachidonic acid (ARA), the latter of which is largely absent in most vegetable oils (Shahkar et al 2016). In contrast, freshwater fish possess enzymatic pathways that allow the endogenous synthesis of EPA, DHA, and ARA from their respective precursors—alpha-linolenic acid (ALA) and linoleic acid (LA)—via sequential desaturation and elongation steps (Scharnweber et al 2021).

In addition, a study by Ferosekhan et al (2022) supports these findings, where feeding with diets rich in n-3 HUFA improved egg quality and reproductive performance in *Sparus aurata*. However, it is important to note that the fatty acid composition in the diet must be

balanced. Research by Ling et al (2006) revealed that the correct ratio between n-3 and n-6 HUFA in the diet is crucial to support optimal reproductive function in fish.

In this study, the n-6/n-3 PUFA ratios in diets formulated with CFO, TFO, BFO, and SFO were 0.41, 0.20, 0.20, and 0.12, respectively. Compared to the CFO-based diet, the lower ratios observed in the SFO (0.12) and BFO (0.20) treatments were associated with improved reproductive outcomes, including increased egg production and fertilization rates. These findings suggest that a lower n-6/n-3 ratio, which reflects a higher proportion of n-3 fatty acids, may offer greater benefits for fish reproductive health.

The dominance of n-3 fatty acids, particularly EPA and DHA, plays a critical role in oocyte development, gonadal maturation, and the quality and viability of embryos. This result is consistent with previous studies showing that lower dietary n-6/n-3 PUFA ratios tend to have a positive effect on reproductive performance (Furuita et al 2007; Ohs et al 2013), reinforcing the essential role of these fatty acids in supporting gonadal development and reproductive success.

Therefore, it can be concluded that an n-6/n-3 ratio in the range of 0.12-0.20, as found in the TFO, BFO, and SFO treatments, is the most favorable for optimizing fish reproductive performance. This ratio is believed to help maintain a balanced lipid profile in reproductive tissues while reducing the risk of inflammation associated with excessive dietary n-6 fatty acids.

Pre-ovulated egg diameter and its relationship with oocyte maturation stages. In this study, statistically significant differences ($p < 0.05$) were observed in the diameter of pre-ovulated eggs at the GVBD stage among the different dietary treatments. Broodfish fed the TFO-based diet produced eggs with an average diameter of 1.96 ± 0.04 mm, while those fed the BFO-based diet produced smaller eggs, averaging 1.77 ± 0.06 mm. The largest egg diameter was recorded in the SFO group, reaching 2.26 ± 0.09 mm. Notably, during stage 3 of oocyte development—when the germinal vesicle has progressed beyond the midpoint toward the cell periphery—broodfish receiving the CFO-based diet produced the smallest eggs, with an average diameter of just 1.67 mm.

These differences in diameter reflect varying levels of oocyte development across the dietary groups. Larger egg diameters generally indicate more advanced oocyte development and better egg quality, as egg size is closely associated with nutritional content, vitellogenin reserves, and readiness for ovulation and fertilization (Izquierdo et al 2001; Reading et al 2018). In this context, the SFO-based diet, which had the lowest n-6/n-3 ratio, appears to contribute positively to more advanced and healthier oocyte maturation, as evidenced by the larger egg size.

The importance of dietary n-3 highly unsaturated fatty acids (HUFA), especially EPA and DHA, in fish reproduction is well documented. These fatty acids are crucial for membrane fluidity and function in oocytes, influencing egg buoyancy, fertilization success, and embryo survival (Kottmann et al 2020). Moreover, a higher n-3/n-6 ratio has been linked to improved gonadal development and egg quality in various fish species (Fowler et al 2020; Ferosekhan et al 2022). Conversely, although the CFO diet supported somatic growth, it appeared less effective in promoting final oocyte development, as indicated by the smaller pre-ovulated egg size, especially at the mature III stage. This may be due to a lower content of n-3 HUFA and a less favorable n-3/n-6 ratio compared to SFO or BFO diets, which could hinder lipid synthesis and accumulation during vitellogenesis, ultimately affecting oocyte maturation (Pérez-Atehortúa et al 2024).

In conclusion, a higher dietary n-3/n-6 ratio, such as that found in the SFO treatment, not only enhances overall reproductive performance but also plays a critical role in supporting superior egg development, as reflected by the larger pre-ovulated egg diameters. These findings align with previous studies emphasizing the critical role of dietary fatty acid composition in optimizing fish reproductive success (Izquierdo et al 2001; Thiruvassagam et al 2024).

Absolute fecundity. Absolute fecundity (i.e., the number of eggs produced per spawning) varied significantly ($p < 0.05$) among the different dietary treatments. Broodfish fed the TFO-based diet produced an average of $5,613 \pm 402$ eggs per spawning, whereas those in the BFO group produced $4,182 \pm 254$ eggs. The highest fecundity was observed in the SFO group, with an average of $9,105 \pm 1,100$ eggs. These findings suggest that the nutritional composition of the diets—particularly the lipid content and levels of essential fatty acids—plays a critical role in supporting ovarian development and enhancing egg production (Izquierdo et al 2001; Zhou et al 2022). The presence of polyunsaturated fatty acids (PUFAs), especially DHA and EPA, is well known to promote oocyte maturation and increase reproductive output in various cultured fish species.

In contrast, absolute fecundity could not be measured in the CFO-fed group, as most individuals remained at stage 3 of oocyte development, where the germinal vesicle had not yet migrated to the periphery. This indicates that the CFO diet may not effectively support gonadal maturation or may delay oocyte progression, resulting in postponed ovulation (Izquierdo et al 2001; El-Sayed et al 2005). Delays in oocyte development may stem from nutritional imbalances, such as a deficiency in essential fatty acids or suboptimal energy-to-protein ratios in the diet.

Latency period. Although no statistically significant differences ($p > 0.05$) were observed in the latency period among the dietary treatments, the average time to ovulation ranged from 12.50 ± 0.50 to 13.00 ± 1.00 hours. Interestingly, the fastest ovulation was recorded in broodfish fed the SFO-based diet, indicating a quicker physiological response to spawning stimuli compared to the other treatment groups. This observation is supported by the higher percentage of oocytes reaching the GVBD stage in the SFO group ($89.33 \pm 1.15\%$) compared to the TFO ($80.66 \pm 3.05\%$) and BFO ($77.33 \pm 3.05\%$) groups. These results suggest that the SFO-based diet not only accelerates the ovulatory response but also promotes more optimal oocyte maturation. According to Aryani et al (2017), the latency period is highly influenced by the hormone dosage used to induce ovulation and the proportion of oocytes that reach the GVBD stage. Additionally, Krejszefz et al (2009) reported that the biological quality of gametes can also affect latency time in the fish (*Leuciscus idus*), indicating that individual physiological variability may also contribute to differences in ovulation response time.

Larval survival (%) at 7 days post-hatch. In this study, larval survival at 7 days post-hatch showed a statistically significant difference among the dietary treatments ($p < 0.05$). The highest survival rate was observed in broodfish fed the SFO-based diet ($93.33 \pm 1.52\%$), followed by the TFO group ($88.00 \pm 2.65\%$), and the lowest in the BFO group ($79.67 \pm 1.53\%$).

This variation is most likely closely related to the egg diameter produced by each broodstock group. Larger eggs generally contain a greater volume of yolk (endogenous energy reserves), which plays a crucial role in meeting the energy demands of larvae during the early developmental stages before the initiation of exogenous feeding. Adequate energy reserves at this stage are essential to support organ development and overall growth. Consequently, larvae originating from larger eggs tend to exhibit higher survival rates during these critical early phases. In contrast, larvae from smaller eggs typically possess limited energy reserves, making them more susceptible to higher mortality. For instance, bilih fish *Mystacoleucus padangensis* (Bleeker, 1852) eggs with an average diameter of 0.76 mm showed a survival rate of only 34% at 30 days post-hatch (Syandri et al 2023).

External environmental factors, such as water temperature and dissolved oxygen, were kept consistent across all treatments throughout the hatching period and the first seven days post-hatch. The average water temperature was maintained at 27 ± 2 °C, and dissolved oxygen at 6.5 ± 1.2 mg·L⁻¹. With uniform environmental conditions, differences in larval survival are more likely attributed to internal factors, particularly egg quality and yolk nutrient content.

These findings support the theory that egg quality—strongly influenced by broodstock diet composition—plays a crucial role in the early life success of larvae. As reported by

Izquierdo et al (2001) and Hossain et al (2024), the nutritional content of eggs, including essential fatty acids like DHA and EPA and energy reserves, directly affects larval viability. The SFO-based diet, characterized by a higher n-3/n-6 ratio and optimal HUFA content, appears to contribute most effectively to producing high-quality eggs that promote better larval survival.

Table 2

Reproductive performance metrics of female *Tor tambroides* broodstock

<i>Parameters</i>	<i>Results</i>
Group 1 (CFO)	
Initial body weight of broodstock (g)	1,166±152
Final body weight of broodstock (g)	1,227±160
Oocyte maturation (%)	-
Pre-ovulated egg diameter (mm)	1.4±0.1*
Ovulated egg weight (g)	-
Latency period (h)	-
Absolute fecundity (eggs spawn ⁻¹)	-
Gonadosomatic index (%)	4.94±0.64
Larval survival (%) after 7 days post-hatch	-
Group 2 (TFO)	
Initial body weight of broodstock (g)	1,208±52
Final body weight of broodstock (g)	1,353±64
Oocyte maturation (%) *	80.66±3.05
Pre-ovulated egg diameter (mm)	1.96±0.04
Ovulated egg weight (g)	84.20
Latency period (h)	13.00±1.00
Absolute fecundity (eggs spawn ⁻¹)	5,613±402
Gonadosomatic index (%) **	8.01±0.04
Larval survival (%) after 7 days post-hatch	88.00±2.65
Group 3 (BFO)	
Initial body weight of broodstock (g)	1,116±76
Final body weight of broodstock (g)	1,316±130
Oocyte maturation (%)	77.33±3.05
Pre-ovulated egg diameter (mm)	1.77±0.06
Ovulated egg weight (g)	62.74±3.82
Latency period (h)	12.50±0.50
Absolute fecundity (eggs spawn ⁻¹)	4,182±254
Gonadosomatic index (%)**	7.09±0.59
Larval survival (%) after 7 days post-hatch	79.67±1.53
Group 4 (SFO)	
Initial body weight of broodstock (g)	1,300±100
Final body weight of broodstock (g)	1,497±131
Oocyte maturation (%) *	89.33±1.15
Pre-ovulated egg diameter (mm)	2.26±0.09
Ovulated egg weight (g)	136.57±16.51
Latency period (h)	13.10±0.85
Absolute fecundity (eggs spawn ⁻¹)	9,105±1,100
Gonadosomatic index (%) **	10.71±0.40
Larval survival (%) after 7 days post-hatch***	93.33±1.52

Note: *Oocyte maturation (%) = Number of mature oocytes / Total number of oocytes retrieved) × 100. **Egg weight percentage (%) = Weight of eggs/Final body weight of broodstock × 100.***Larval survival (%) after 7 days post-hatch = Number of larvae survived/Total number of larvae × 100.

Conclusion. The results of this study demonstrated that the type of diet had a significant effect on various reproductive parameters of female *T. tambroides* broodstock, including gonadal weight gain, oocyte maturation percentage, pre-ovulated egg diameter (mm), ovulated egg weight, absolute fecundity (number of eggs per spawning), and larval survival (%) at 7 days post-hatch. The SFO-based diet yielded the best outcomes across most parameters, including increased body weight, higher relative egg weight, larger pre-ovulated egg diameter, greater absolute fecundity, and a higher rate of oocyte maturation. These findings suggest that the nutritional composition of the SFO diet—particularly its content of essential fatty acids such as DHA and EPA, along with an optimal n-6/n-3 ratio—strongly supports reproductive performance and egg quality. In contrast, the CFO (control) diet produced the lowest results in terms of body weight gain and pre-ovulated egg diameter, and failed to support oocyte maturation to the GVBD stage. This indicates that the CFO diet lacks the necessary nutritional components to effectively promote oocyte development and optimal reproductive output in female broodstock.

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Conflicts of interest. The authors declare that there is no conflict of interest.

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Authors:

Azrita Azrita, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bung Hatta University, Sumatera Street, Ulak Karang, 25131 Padang, Indonesia, e-mail: azrita31@bunghatta.ac.id

Hafrijal Syandri, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bung Hatta University, Sumatera Street, Ulak Karang, 25131 Padang, Indonesia, e-mail: syandri_1960@bunghatta.ac.id

Rona Taula Sari, Department of Biology Education, Faculty of Teacher Training and Education, Bung Hatta University, Sumatera Street, Ulak Karang, 25131 Padang, Indonesia, e-mail: ronataulasari@bunghatta.ac.id

Hendra Kusuma, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bung Hatta University, Sumatera Street, Ulak Karang, 25131 Padang, Indonesia, e-mail: hendra.kusuma@bunghatta.ac.id

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