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Research Article

Effects of Fish Oil-Enriched *Tubifex* sp. on Fatty Acid Composition, Growth and Survival of Thai Mahseer (*Tor tambroides*, Bleeker, 1854) Larvae

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Abstract

Background and Objective: The high cost and limited availability of commercial live feeds have encouraged the use of locally available *Tubifex* sp. as an alternative. This study evaluated the effects of enriching *Tubifex* sp. with different fish oil sources on the fatty acid profile, growth performance and survival of Thai mahseer (*Tor tambroides*) larvae. **Materials and Methods:** *Tubifex* sp. were enriched for 18 hrs with squid oil emulsion (TSO), sardinella fish oil (TLO), or striped catfish oil (TCO), while unenriched *Tubifex* sp. served as the control (TNC). Larvae were reared from 7 to 25 days post-hatch and fed *ad libitum*. Fatty acid composition was analyzed by gas chromatography and growth performance was assessed based on length growth, weight gain, condition factor and survival. **Results:** Enrichment significantly increased lipid, polyunsaturated fatty acid and n-3 fatty acid contents in *Tubifex* sp. ($p < 0.05$), which were subsequently reflected in larval tissues. Larvae fed TCO-enriched *Tubifex* sp. exhibited significantly greater growth and higher condition factors than those in other treatments ($p < 0.05$). Survival remained high (97-99%) and did not differ significantly among treatments ($p > 0.05$). **Conclusion:** Striped catfish oil is a promising, cost-effective local lipid source for enriching *Tubifex* sp. to improve the culture performance of Thai mahseer larvae.

Key words: *Tubifex* sp. enrichment, fish oil, fatty acid composition, larval growth, Thai mahseer

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Thai mahseer (*Tor tambroides*) is a high-value freshwater fish species widely distributed throughout Southeast Asia, including Thailand, Laos, Vietnam, Malaysia and Indonesia¹⁻³. In Indonesia, this species is not only economically important but also culturally significant to local communities⁴. Its high market value has stimulated growing interest in the development of aquaculture technologies, particularly for the early life stages, which are critical for seed production and overall aquaculture efficiency⁵.

Previous studies have primarily focused on the physiological development and nutritional requirements of *T. tambroides* larvae to improve larval growth and survival under controlled rearing conditions^{6,7}. Live feeds such as Rotifers, *Artemia* and *Chlorella vulgaris* have been widely used and have been shown to enhance larval growth and survival⁸⁻¹⁰. However, the use of these live feeds is often limited by their high production costs and inconsistent availability, prompting the search for alternative, locally available live feeds such as *Tubifex* sp.¹¹.

Tubifex sp. are commonly used as live feed in freshwater larviculture because of their wide availability, appropriate size for the larval mouth gape and high palatability^{12,13}. However, their direct use is nutritionally limited, particularly due to their low levels of essential Long-Chain Polyunsaturated Fatty Acids (LC-PUFAs). The lipid profile of *Tubifex* sp. is generally dominated by saturated and short-chain unsaturated fatty acids, whereas the concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are relatively low^{11,14}.

Although freshwater fish are capable of synthesizing LC-PUFAs through desaturation and elongation pathways, this metabolic capacity is not fully developed during the larval stage^{15,16}. As a result, *T. tambroides* larvae require an exogenous dietary supply of EPA and DHA to support early growth, physiological development and feeding performance⁸. These constraints highlight the importance of nutritionally enriched feeds during early larval stages¹⁷.

Enrichment of *Tubifex* sp. with fish oil has been recognized as an effective strategy to improve their lipid quality and better meet larval nutritional requirements. Fish oils derived from fisheries by-products represent sustainable and cost-effective lipid sources with diverse fatty acid profiles^{3,18}. Oils such as squid oil, which is rich in DHA¹⁹, sardinella oil with relatively high EPA content²⁰ and Striped catfish oil as an abundant freshwater-based lipid source¹⁸, may differentially influence larval growth and survival when used for live-feed enrichment.

However, comparative studies evaluating the effectiveness of different locally sourced fish oils for *Tubifex* sp. enrichment in *T. tambroides* larvae remain very limited. In particular, information regarding how these lipid sources influence fatty acid transfer, larval growth performance and survival is still lacking.

This study provides the first comparative evidence on the effectiveness of locally derived freshwater fish oil for *Tubifex* sp. enrichment and its subsequent transfer to *T. tambroides* larvae. Therefore, this study aimed to evaluate the effects of *Tubifex* sp. enrichment using squid oil emulsion, sardinella fish oil (TLO), or striped catfish oil (TCO) on fatty acid transfer, growth performance and survival of *T. tambroides* larvae. It was hypothesized that different fish oil sources would differentially modify the fatty acid profile of *Tubifex* sp. and subsequently influence larval growth and physiological performance.

MATERIALS AND METHODS

Study area and duration: The study was conducted from February to May 2026 at the Wet Laboratory, Faculty of Fisheries and Marine Science, Universitas Bung Hatta, Padang, West Sumatra, Indonesia.

Oil sources: Squid oil and sardinella oil were obtained from processing by-products (residual flesh trimmings) using an indirect heating method (wet rendering) at 60-70°C for 45 min, followed by gravimetric separation of the oil phase. This procedure was adapted from the fatty acid methyl ester (FAME) preparation method described by Morrison and Smith²¹. In contrast, Striped catfish oil was extracted from mesenteric tissues¹⁸. The fatty acid composition of squid oil, sardinella fish oil and Striped catfish oil is presented in Table 1.

***Tubifex* sp. maintenance and enrichment:** *Tubifex* sp. were obtained from local collectors and immediately transported to the laboratory in a live condition. Upon arrival, the worms were thoroughly washed with freshwater to remove mud and debris by repeatedly rinsing until the rinse water became clear. The cleaned *Tubifex* sp. were subsequently maintained in holding tanks with a continuous flow-through water system to preserve water quality and the physiological condition of the worms prior to the enrichment, following the culture principles described by Marian and Pandian²².

Before enrichment, the *Tubifex* sp. were held without feed or emulsion for 18 hrs to allow depuration of gut contents prior to the enrichment treatment. After the starvation period, 100 g (wet weight) of *Tubifex* sp. were used for each treatment. The treatments consisted of a

Table 1: Fatty acid composition (% of total identified fatty acids) of squid, sardinella and striped catfish oils (FG-FAME, %)

Fatty acid	Squid oil	Sardinella fish oil	Striped catfish oil
C4:0	0.29	0.67	nd
C6:0	0.2	1.15	nd
C11:0	nd	nd	3.94
C13:0	nd	nd	5.87
C14:0	0.49	1.82	nd
C15:0	0.2	0.47	16.35
C16:0	14.49	18.77	4.71
C16:1	0.29	1.66	0.34
C17:0	0.54	0.75	7.28
C18:0	4.22	5.34	nd
C18:1 cis	14.05	13.79	nd
C18:1n-9 trans	nd	nd	9.52
C18:2n-6 cis	37.43	29.53	0.16
C20:0	0.25	0.36	nd
C20:1n-9	nd	nd	1.17
C18:3n-3	5.11	3.75	2.37
C20:4n-6	1.77	1.42	nd
C22:6n-3	12.43	13.12	1.71
C20:5n-3	8.35	7.35	0.54
C22:0	nd	nd	3.06
C20:3n-3	nd	nd	0.4
C24:1	nd	nd	0.48

Fatty acid values are expressed as relative percentages of total identified fatty acids determined by the FG-FAME method. nd = not detected

non-enriched control (TNC) and three oil-enriched groups using squid oil (TSO), sardinella fish oil (TLO) and striped catfish oil (TCO).

Preparation of lipid emulsions: Lipid emulsions were prepared separately for each oil treatment by mixing 0.1 mL of squid oil, sardinella fish oil, or striped catfish oil with 0.1 g of raw egg yolk as an emulsifying agent, followed by dilution with 100 mL of sterile freshwater. The mixture was homogenized at 13,000 rpm for 1 min until a uniform emulsion was obtained. The use of egg yolk as an enrichment component for *Tubifex* sp. was based on a previously reported enrichment practice²³, with modifications to the oil source, emulsification procedure and dosage.

For the TNC treatment, 100 g (wet weight) of *Tubifex* sp. were immersed in 1,000 mL of sterile freshwater without emulsion for 18 hrs. For the enrichment treatments, 100 g (wet weight) of *Tubifex* sp. were immersed for 18 hrs in 1,000 mL of sterile freshwater containing lipid emulsions prepared from squid oil (TSO), sardinella fish oil (TLO), or striped catfish oil (TCO). Each enrichment emulsion contained 0.1 mL of oil and 0.1 g of raw egg yolk per 1,000 mL of water. The enrichment procedure was adapted from previously reported *Tubifex* enrichment practices, with modifications to the enrichment materials, oil sources, concentrations and exposure duration²³. During the enrichment period, gentle aeration was provided to ensure uniform distribution of the emulsion and to maintain adequate dissolved oxygen levels. After enrichment, the *Tubifex* sp. were harvested, dried and subsequently analyzed for proximate composition and fatty acid profile.

Larval rearing: Broodstock of *T. tambroides* maintained at the Wet Laboratory, Faculty of Fisheries and Marine Science, Bung Hatta University, Padang, West Sumatra, Indonesia, were used as sources of eggs and sperm. All experimental procedures involving fish were conducted in accordance with institutional guidelines for the care and use of experimental animals and were approved by the Research and Community Service Ethics Committee, Universitas Bung Hatta (Ethical Approval No. 025/LPPM/Hatta/II-2026. Artificial fertilization and egg incubation were conducted in glass aquaria following standard protocols commonly applied for mahseer species³.

Newly hatched larvae were reared until 7 days post-hatching (dph) and subsequently used for the feeding trial. Larvae were maintained in glass aquaria with a total volume of 54 L containing 25 L of filtered and treated freshwater. Water quality was maintained at a temperature of $28 \pm 1^\circ\text{C}$ and dissolved oxygen levels of 6.0 ± 0.50 mg/L. Stocking density was set at 10 individuals L^{-1} (250 larvae per aquarium) and 50% of the rearing water was replaced daily to maintain optimal environmental conditions. The rearing procedure was based on the method described by Azrita *et al.*²⁴, with slight modifications.

Larval rearing was conducted for 18 days, from 7 to 25 days post-hatching (dph), following the method described by Azrita *et al.*²⁴ with slight modifications. During this period, larvae were fed *Tubifex* sp. enriched with TSO, TLO, or TCO. The enriched *Tubifex* sp. was provided *ad libitum* according

to the feeding response and requirements of the larvae. The feeding procedure was based on previously reported methods, with modifications to the type of oil used for *Tubifex* enrichment.

Fatty acid profile analysis of *Tubifex* sp. and larvae: Fatty acid profile analysis was conducted on *Tubifex* sp. and *T. tambroides* larvae from each enrichment treatment. Samples of *Tubifex* sp. and larvae were collected using a fine-mesh scoop net and rinsed with sterile freshwater to remove residual emulsion and debris. The samples were then dried to a moisture content of less than 15% prior to proximate composition and fatty acid analyses.

For each treatment and replicate, *Tubifex* sp. samples were prepared at 50 g (wet weight). For fatty acid analysis, 50 larvae were randomly collected from each aquarium and pooled to obtain a total sample weight of approximately 50 g. Each pooled larval sample represented one experimental replicate. This pooling approach was used to ensure sufficient biomass for lipid extraction and fatty acid analysis while reducing individual variability among larvae. At the end of the feeding trial, 50 larvae were randomly collected from each aquarium and individually weighed using an analytical balance with a precision of 1 mg²⁴.

The prepared samples were subsequently subjected to proximate composition and fatty acid profile analyses following lipid extraction and fatty acid methyl ester (FAME) preparation prior to gas chromatographic analysis.

Total lipid extraction was carried out using a modified Bligh and Dyer²⁵ method. Briefly, 0.5 g of dried sample was homogenized in a chloroform-methanol solvent mixture (2:1, v/v), followed by shaking and sonication for 15 min to enhance lipid extraction efficiency. Distilled water was subsequently added to facilitate phase separation and the lower chloroform phase containing lipids was collected. The lipid extract was evaporated under a stream of nitrogen gas until complete solvent removal and the remaining lipid residue was weighed to determine total lipid content.

Extracted lipids were then trans esterified into fatty acid methyl esters (FAMES) by adding 1 mL of methanol containing 2% (v/v) H₂SO₄ as a catalyst. The mixture was heated in a water bath at 60-70°C for 1 hr to ensure complete transesterification. After cooling to room temperature, FAMES were extracted with n-hexane and the organic phase containing FAMES was collected for further analysis.

FAME analysis was conducted using gas chromatography (GC) equipped with a flame ionization detector (FID) and a polar capillary column (e.g., HP-88 or an equivalent column). Chromatographic conditions were set as follows: Injector

temperature at 250°C, detector temperature at 260°C, initial oven temperature of 140°C held for 5 min, followed by an increase at 4°C/min to 240°C and held for 10 min. Nitrogen or helium was used as the carrier gas at a constant flow rate.

Fatty acid identification and quantification were performed using the GC-FAME method by comparing sample peak retention times with those of a commercial FAME standard mixture (e.g., Supelco 37 Component FAME Mix). Fatty acids were classified into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) and expressed as percentages of total identified fatty acids.

Growth variable and survival rate: Growth performance of Thai mahseer larvae from 7 to 25 days post-hatching (dph) was evaluated based on length growth rate (LGR, %), weight gain rate (WGR, %) and condition factor (CF). Survival rate (SR, %) was also determined. The parameters were analyzed according to Azrita and Syandri²⁶. The growth and survival indices were calculated using the following equations:

$$\text{LGR (\%)} = 100 \times \frac{L_f - L_i}{L_i} \quad (1)$$

$$\text{WGR (\%)} = 100 \times \frac{W_f - W_i}{W_i} \quad (2)$$

$$\text{CF} = \frac{W_f}{L_f^3} \times 100 \quad (3)$$

$$\text{SR (\%)} = 100 \times \frac{N_f}{N_i} \quad (4)$$

where L_f and L_i represent the final and initial total length of the larvae, respectively; W_f and W_i represent the final and initial body weight, respectively; N_i is the initial number of larvae stocked at the beginning of the feeding trial; and N_f is the final number of surviving larvae at the end of the trial. These equations were calculated following commonly used growth and survival indices in fish larvae and aquaculture studies.

Statistical analysis: The data obtained were subjected to statistical analysis to determine the significance of differences among treatments. The Shapiro-Wilk test was applied to assess the normality of data distribution. After confirming that the normality assumption was met, the data were analyzed using One-way Analysis of Variance (one-way ANOVA). When

Table 2: Proximate composition (% dry matter basis) and fatty acid profile (% total fatty acids, FG-FAME method) of *Tubifex* sp. after 18 hrs enrichment with different oil emulsions

	Experimental diet			
	TNC	TSO	TLO	TCO
Crude lipid	20.14±0.02	21.05±0.03	21.35±0.03	21.22±0.04
Moisture	13.55±0.02	11.90±0.02	10.70±0.01	11.05±0.02
Fatty acids				
C4:0	0.56±0.20 ^a	0.38±0.06 ^b	0.12±0.01 ^c	0.28±0.10 ^d
C6:0	0.31±0.02 ^a	0.24±0.01 ^b	0.08±0.01 ^c	0.15±0.01 ^d
C8:0	0.18±0.01 ^a	0.52±0.01 ^b	0.41±0.01 ^c	0.39±0.02 ^d
C10:0	1.12±0.02 ^a	0.57±0.00 ^b	0.52±0.01 ^c	0.39±0.01 ^d
C11:0	0.75±0.02 ^a	0.82±0.02 ^b	0.45±0.01 ^c	0.33±0.01 ^d
C12:0	1.42±0.01 ^a	1.71±0.01 ^b	1.05±0.01 ^c	1.23±0.01 ^d
C13:0	0.82±0.02 ^a	0.57±0.01 ^b	0.37±0.01 ^c	0.44±0.01 ^d
C14:0	7.52±0.01 ^a	9.67±0.01 ^b	7.77±0.01 ^c	0.44±0.01 ^d
C15:0	1.36±0.00 ^a	1.38±0.01 ^b	1.49±0.02 ^c	1.12±0.01 ^d
C16:0	14.41±0.01 ^a	16.24±0.01 ^b	15.98±0.03 ^c	22.84±0.01 ^d
C17:0	1.67±0.01 ^a	1.23±0.01 ^a	1.84±0.01 ^a	1.26±0.01 ^a
C18:0	4.97±0.02 ^a	1.24±0.01 ^b	1.84±0.01 ^c	1.27±0.02 ^d
C14:1	2.50±0.01 ^a	13.63±0.02 ^b	5.95±0.02 ^c	5.74±0.01 ^d
C16:1	2.18±0.02 ^a	1.76±0.00 ^b	1.13±0.01 ^c	1.54±0.01 ^d
C18:1n-9t	1.17±0.02 ^a	1.86±0.00 ^b	2.11±0.01 ^c	2.48±0.01 ^d
C18:1n-9c	7.14±0.01 ^a	5.81±0.02 ^b	8.13±0.01 ^c	9.80±0.01 ^d
C20:1	3.06±0.01 ^a	3.00±0.02 ^b	2.42±0.01 ^c	1.32±0.02 ^d
C18:2n-6	2.85±0.02 ^a	2.81±0.01 ^b	2.45±0.01 ^c	4.86±0.01 ^d
C18:3n-3	3.05±0.02 ^a	21.58±0.01 ^b	23.26±0.02 ^c	28.55±0.01 ^d
C20:2n-6	3.84±0.02 ^a	4.32±0.01 ^b	3.64±0.01 ^c	3.35±0.01 ^d
DHA (C22:6n-3)	3.05±0.02 ^a	4.51±0.01 ^b	4.13±0.01 ^c	3.13±0.02 ^d
EPA(C20:5n-3)	1.69±0.01 ^a	1.81±0.01 ^b	1.15±0.01 ^c	0.91±0.02 ^d
SFA	35.09±0.21 ^a	34.57±0.07 ^b	31.92±0.05 ^c	31.15±0.11 ^d
MUFA	16.06±0.03 ^a	26.06±0.03 ^b	19.74±0.03 ^c	20.88±0.03 ^d
PUFA	14.48±0.04 ^a	35.03±0.02 ^b	34.63±0.04 ^c	40.80±0.03 ^d
n-3 FA	7.79±0.03 ^a	27.90±0.02 ^b	28.54±0.02 ^c	32.59±0.03 ^d
n-6 FA	6.69±0.03 ^a	7.13±0.01 ^b	6.09±0.01 ^c	8.21±0.01 ^d
n-3/n-6	1.16±0.01 ^a	3.91±0.01 ^b	4.69±0.02 ^c	3.97±0.01 ^d
DHA/EPA	1.80±0.02 ^a	2.49±0.01 ^b	3.59±0.03 ^c	3.44±0.08 ^d

Values are expressed as mean±SD (% of total fatty acids), except for crude lipid and moisture, which are expressed as % of sample weight. C:n denotes the number of carbon atoms and double bonds, respectively; n-3 and n-6 denote omega-3 and omega-6 fatty-acid families, respectively. c and t denote cis and trans configurations, respectively. DHA: Docosahexaenoic acid, EPA: Eicosapentaenoic acid, SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, FA: Fatty acids. Different superscript lowercase letters within a row indicate significant differences among treatments (p<0.05). TNC: Control (unenriched *Tubifex* sp.), TSO: Squid-oil-enriched *Tubifex* sp., TLO: Sardinella-fish-oil-enriched *Tubifex* sp. and TCO: Striped-catfish-oil-enriched *Tubifex* sp.

significant differences were detected, mean values were compared using Tukey's honestly significant difference (HSD) test at a significance level of p<0.05. For fatty acid analysis, larval samples from each aquarium were pooled prior to chemical analysis to obtain sufficient biomass. In this context, the aquarium was considered the experimental unit and each pooled sample represented the mean response of larvae within one replicate. All statistical analyses were conducted using three replicates per treatment.

RESULTS

Proximate composition and fatty acid profile of *Tubifex* sp.:

The proximate composition and fatty acid profile of *Tubifex* sp. after 18 hrs enrichment with different oil emulsions is

presented in Table 2. Crude lipid content increased significantly in enriched groups compared to the control (p<0.05), while moisture content showed a significant decrease following enrichment.

Palmitic acid (C16:0) was the predominant saturated fatty acid in all treatments. Total saturated fatty acids (SFA) differed significantly among treatments (p<0.05), with lower values observed in enriched *Tubifex* sp. compared to the control.

Monounsaturated fatty acids (MUFA) were significantly affected by the type of oil emulsion (p<0.05), with the highest MUFA content recorded in the TSO group. Enrichment also resulted in a significant increase in total polyunsaturated fatty acids (PUFA), particularly due to elevated levels of α-linolenic acid (C18:3n-3) (p<0.05).

Table 3: Proximate composition (% dry matter basis) and fatty acid composition (% of total fatty acids, FG-FAME) of *T. tambroides* larvae fed *Tubifex* sp. enriched with different oil emulsions

	Experimental diet			
	TNC	TSO	TLO	TCO
Crude lipid	17.12±0.02	17.89±0.03	17.93±0.03	17.99±0.03
Moisture	12.25±0.02	12.90±0.02	11.70±0.01	11.85±0.02
Fatty acids				
C4:0	0.48±0.02 ^a	0.32±0.02 ^b	0.10±0.01 ^c	0.24±0.02 ^d
C6:0	0.26±0.01 ^a	0.20±0.01 ^b	0.07±0.01 ^c	0.13±0.01 ^d
C8:0	0.15±0.01 ^a	0.44±0.02 ^b	0.35±0.02 ^c	0.33±0.02 ^d
C10:0	0.95±0.02 ^a	0.48±0.02 ^b	0.44±0.02 ^c	0.33±0.02 ^d
C11:0	0.64±0.02 ^a	0.70±0.02 ^b	0.38±0.01 ^c	0.28±0.01 ^d
C12:0	1.21±0.02 ^a	1.45±0.02 ^b	0.89±0.02 ^c	1.05±0.02 ^d
C13:0	0.70±0.01 ^a	0.70±0.01 ^b	0.31±0.01 ^c	0.37±0.01 ^d
C14:0	6.39±0.03 ^a	8.22±0.03 ^b	6.60±0.02 ^c	0.37±0.01 ^d
C15:0	1.16±0.02 ^a	1.80±0.03 ^b	1.27±0.01 ^c	0.95±0.02 ^d
C16:0	12.25±0.03 ^a	13.80±0.03 ^b	13.58±0.02 ^c	19.41±0.02 ^d
C18:0	1.42±0.02 ^a	1.05±0.02 ^b	1.56±0.01 ^c	1.08±0.01 ^d
C14:1	4.22±0.03 ^a	11.59±0.03 ^b	5.06±0.02 ^c	4.88±0.02 ^d
C16:1	2.13±0.02 ^a	1.50±0.01 ^b	0.96±0.01 ^c	1.31±0.01 ^d
C18:1n-9t	1.85±0.01 ^a	1.58±0.02 ^b	2.11±0.02 ^c	1.79±0.02 ^d
C18:1n-9c	6.07±0.02 ^a	4.94±0.02 ^b	6.91±0.02 ^c	8.33±0.02 ^d
C20:1	0.99±0.01 ^a	2.55±0.01 ^b	1.12±0.01 ^c	2.06±0.01 ^d
C18:2n-6	2.29±0.02 ^a	2.11±0.01 ^b	2.38±0.02 ^c	3.65±0.02 ^d
C18:3n3	22.39±0.02 ^a	16.19±0.02 ^b	21.41±0.02 ^c	17.45±0.02 ^d
C20:2n-6	2.88±0.01 ^a	3.24±0.01 ^b	2.73±0.01 ^c	2.51±0.01 ^d
DHA (C22:6n-3)	5.54±0.02 ^a	5.63±0.01 ^b	6.35±0.02 ^c	6.85±0.02 ^d
EPA(C20:5n-3)	2.62±0.01 ^a	3.73±0.01 ^b	4.04±0.01 ^c	2.82±0.01 ^d
SFA	25.61±0.07 ^a	29.16±0.07 ^b	25.55±0.05 ^c	24.54±0.05 ^d
MUFA	15.26±0.05 ^a	22.16±0.04 ^b	16.16±0.04 ^c	18.37±0.04 ^d
PUFA	35.72±0.04 ^a	27.90±0.03 ^b	35.91±0.04 ^c	31.28±0.04 ^d
n-3 FA	30.55±0.03 ^a	22.55±0.02 ^b	30.8±0.03 ^c	25.12±0.03 ^d
n-6 FA	5.17±0.03 ^a	5.35±0.01 ^b	5.11±0.02 ^c	6.16±0.02 ^d
n-3/n-6	5.90±0.02 ^a	4.21±0.01 ^b	6.02±0.03 ^c	4.08±0.02 ^d
DHA/EPA	2.11±0.01 ^a	1.51±0.01 ^b	1.57±0.01 ^c	2.43±0.01 ^d

Values are expressed as mean±SD (% of total fatty acids), except for crude lipid and moisture, which are expressed as % of sample weight. C:n denotes the number of carbon atoms and double bonds, respectively; n-3 and n-6 denote omega-3 and omega-6 fatty-acid families, respectively. c and t denote cis and trans configurations, respectively. DHA: Docosahexaenoic acid, EPA: Eicosapentaenoic acid, SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, FA: Fatty acids. Different superscript lowercase letters within a row indicate significant differences among treatments (p<0.05). TNC: Control (unenriched *Tubifex* sp.), TSO: Squid-oil-enriched *Tubifex* sp., TLO: Sardinella-fish-oil-enriched *Tubifex* sp. and TCO: Striped-catfish-oil-enriched *Tubifex* sp.

Total n-3 fatty acids increased significantly in enriched treatments, leading to higher n-3/n-6 ratios compared to the control (p<0.05). The contents of DHA and EPA, as well as the DHA/EPA ratio, differed significantly among treatments (p<0.05), indicating that oil enrichment effectively modified the nutritional quality of *Tubifex* sp.

Proximate and fatty acid composition of larvae: The proximate composition and fatty acid profile of *T. tambroides* larvae fed *Tubifex* sp. enriched with different oil emulsions are presented in Table 3. The crude lipid content of the larvae increased significantly after feeding with enriched *Tubifex* sp. compared to the initial larvae (p<0.05), while moisture content showed only minor variations among treatments.

The fatty acid composition of the larvae was significantly affected by dietary treatments (p<0.05). Palmitic acid (C16:0) was the predominant saturated fatty acid in all groups, with the highest total saturated fatty acid (SFA) content observed in larvae from the TSO treatment. Monounsaturated fatty acids (MUFA) increased significantly in larvae fed enriched *Tubifex* sp., particularly in the TSO group, which showed higher proportions of myristoleic acid (C14:1) and oleic acid (C18:1n-9c).

Polyunsaturated fatty acids (PUFA) represented the major fatty acid fraction across all treatments. α-Linolenic acid (C18:3n-3) and docosahexaenoic acid (DHA) were the main contributors to total PUFA. Significant differences in total PUFA and total n-3 fatty acid contents were observed among treatments (p<0.05), with the highest values recorded in the

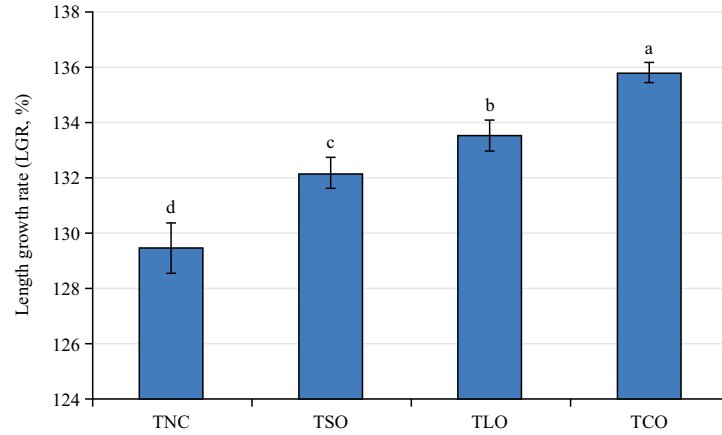


Fig. 1: LGR (%) of *T. tambroides* larvae after feeding on *Tubifex* sp. enriched with various diets at the conclusion of the trial

Data are expressed as Mean $\pm$ SD (n = 3). Bars sharing different letters indicate significant differences ($p < 0.05$), C: Control (unenriched *Tubifex* sp.), TSO: Squid-oil-enriched *Tubifex* sp., TLO: Sardinella-fish-oil-enriched *Tubifex* sp. and TCO: Striped-catfish-oil-enriched *Tubifex* sp.

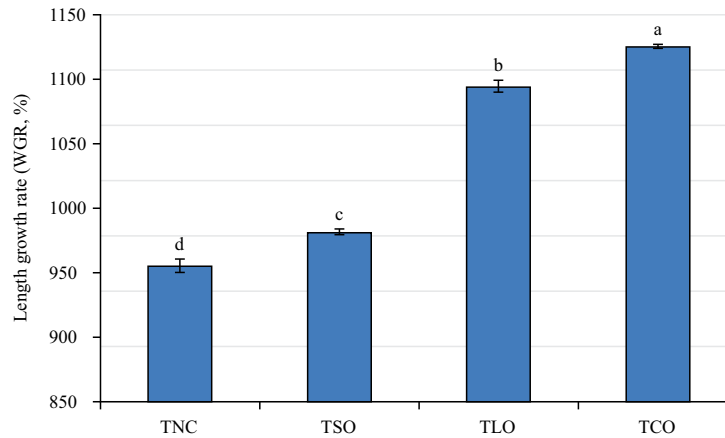


Fig. 2: WGR (%) of *T. tambroides* larvae after feeding on *Tubifex* sp. enriched with various diets at the conclusion of the trial

Data are expressed as Mean $\pm$ SD (n = 3). Bars sharing different letters indicate significant differences ($p < 0.05$), C: Control (unenriched *Tubifex* sp.), TSO: Squid-oil-enriched *Tubifex* sp., TLO: Sardinella-fish-oil-enriched *Tubifex* sp. and TCO: Striped-catfish-oil-enriched *Tubifex* sp.

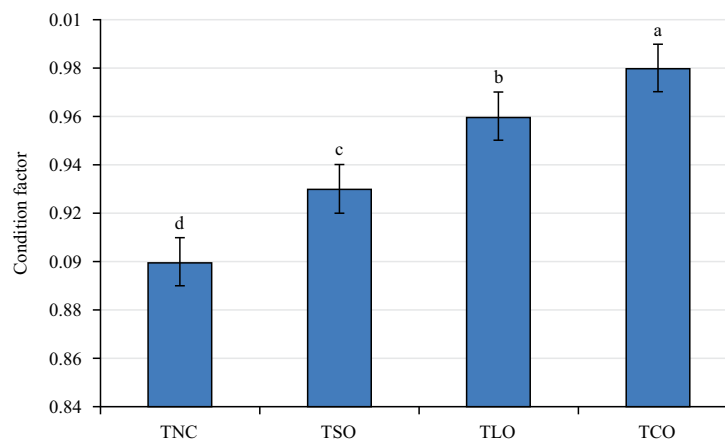


Fig. 3: Condition factor of *T. tambroides* larvae fed *Tubifex* sp. enriched with different diets at the end of the experimental period

Data are presented as Mean $\pm$ SD (n = 3). Bars sharing different letters indicate significant differences ($p < 0.05$), C: Control (unenriched *Tubifex* sp.), TSO: Squid-oil-enriched *Tubifex* sp., TLO: Sardinella-fish-oil-enriched *Tubifex* sp. and TCO: Striped-catfish-oil-enriched *Tubifex* sp.

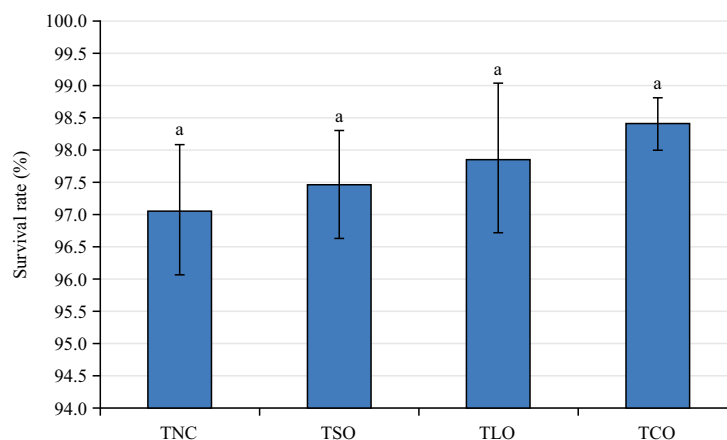


Fig.4: Survival rate (%) of *T. tambroides* larvae fed *Tubifex* sp. enriched with different diets at the end of the feeding trial

Values represent Means $\pm$ SD (n = 3). Bars sharing the same letter indicate no significant difference ($p > 0.05$), NC: Control (unenriched *Tubifex* sp.), TSO: Squid-oil-enriched *Tubifex* sp., TLO: Sardinella-fish-oil-enriched *Tubifex* sp. and TCO: Striped-catfish-oil-enriched *Tubifex* sp.

initial larvae and larvae from the TLO treatment. Correspondingly, the n-3/n-6 fatty acid ratio varied significantly among treatments, with higher ratios observed in the initial larvae and the TLO group.

The contents of DHA and EPA, as well as the DHA/EPA ratio, differed significantly among treatments ($p < 0.05$). The highest DHA/EPA ratio was observed in the initial larvae, while lower ratios were recorded in larvae fed enriched *Tubifex* sp.

Larva growth and survival: The growth performance of *T. tambroides* larvae from 7 to 25 days post-hatching (dph) was significantly affected by dietary treatments ($p < 0.05$). At 25 dph, larvae fed *Tubifex* sp. enriched with TCO attained the highest total length (25.00 ± 1.56 mm) and body weight (127.48 ± 1.69 mg), followed by those receiving TLO (24.27 ± 0.56 mm; 124.36 ± 15.01 mg) and TSO (24.13 ± 0.33 mm; 123.90 ± 15.67 mg), while the lowest values were observed in the TNC group (23.73 ± 1.16 mm; 121.20 ± 16.75 mg). Accordingly, length growth rate (LGR, %) and weight gain rate (WGR, %) differed significantly among treatments, with the highest values recorded in the TCO group and the lowest in the TNC group ($p < 0.05$) (Fig. 1 and 2).

A similar pattern was observed for final body weight. Larvae in the TCO group recorded the highest body weight and differed significantly from all other treatments ($p < 0.05$). The TLO group showed significantly higher body weight than the TSO and TNC groups, while the lowest value was observed in the TNC group. In addition, the condition factor ranged from 0.90 to 0.98 and differed significantly among treatments ($p < 0.05$) (Fig. 3).

In contrast, larval survival from 7 to 25 dph was not significantly affected by dietary treatments ($p > 0.05$) (Fig. 4). Survival rates remained consistently high across all

treatments, ranging from 97 to 99%. These results indicate that enrichment of *Tubifex* sp. with different oil emulsions did not adversely affect the survival of Thai mahseer larvae.

DISCUSSION

The increase in crude lipid content accompanied by a decrease in moisture content after enrichment indicates effective incorporation of dietary lipids into *Tubifex* sp. resulting in higher energy density. Similar patterns have been reported in enriched live feeds, where short-term lipid enrichment promotes lipid deposition and alters proximate composition^{11,14}. Palmitic acid (C16:0) remained the dominant saturated fatty acid across treatments, reflecting its structural role in aquatic invertebrates. The reduced proportion of total saturated fatty acids in enriched *Tubifex* sp. likely reflects a dilution effect caused by increased unsaturated fatty acid incorporation rather than an absolute reduction in saturated fatty acid content.

Differences in monounsaturated and polyunsaturated fatty acid profiles among treatments demonstrate that the lipid composition of *Tubifex* sp. is strongly influenced by the oil source used for enrichment. Elevated MUFA levels in the squid oil treatment corresponded with the characteristic lipid profile of squid oil, whereas increased PUFA levels, particularly α -linolenic acid (C18:3n-3), indicate efficient assimilation of dietary C18 fatty acids following enrichment^{19,27}. The increase in total n-3 fatty acids and higher n-3/n-6 ratios further confirm that oil enrichment modified the fatty acid composition of *Tubifex* sp. enhancing its suitability as a live feed.

The DHA/EPA ratios of enriched *Tubifex* sp. (1.80-3.44) fall within ranges reported as favourable for larval fish performance²⁸. Previous studies have shown that a balanced DHA/EPA ratio supports efficient lipid utilization and physiological development in fish larvae^{29,30}. Based on the n-3 LC-PUFA content of enriched *Tubifex* sp., the estimated dietary supply of EPA and DHA provided to larvae was within the optimal range reported for larval feeds³¹.

In freshwater fish larvae, C18 polyunsaturated fatty acids, particularly α -linolenic acid (C18:3n-3), can be efficiently utilized both as immediate energy sources and as metabolic precursors for long-chain polyunsaturated fatty acids (HUFA) through desaturation and elongation pathways^{15,16}. Striped catfish oil, characterized by a high C18:3n-3 content and high energy availability, may therefore preferentially support somatic growth rather than neural development, providing a mechanistic explanation for the superior growth performance observed in larvae fed *Tubifex* sp. enriched with this lipid source.

In Thai mahseer larvae, feeding on enriched *Tubifex* sp. resulted in higher crude lipid content, indicating effective transfer and assimilation of dietary lipids into larval tissues. Palmitic acid remained the dominant saturated fatty acid, while increased MUFA levels particularly in the squid oil treatment suggest preferential utilization of MUFA as an energy source to support growth and metabolism³². Polyunsaturated fatty acids, especially DHA, constituted a major lipid fraction in larval tissues, reflecting selective retention of long-chain n-3 fatty acids essential for early development³³. Variations in DHA/EPA ratios among treatments further indicate dietary modulation of larval fatty acid metabolism rather than direct mirroring of feed composition.

Overall, the results demonstrate that dietary lipid source influences fatty acid composition and growth performance of *T. tambroides* larvae. Enrichment with Striped catfish oil produced superior growth performance, suggesting that somatic growth in freshwater fish larvae depends not only on HUFA availability but also on adequate energy supply from C18 fatty acids. Despite differences in lipid composition, survival remained high across treatments, indicating that dietary lipid quality primarily affected growth performance rather than larval survival under adequate rearing conditions³⁴.

CONCLUSION

The findings of this study demonstrate that enrichment of *Tubifex* sp. with different fish oil sources improves the fatty acid composition of live feed and influences the growth performance of Thai mahseer (*Tor tambroides*) larvae. Among

the treatments evaluated, *Tubifex* sp. enriched with TCO produced the highest increases in larval body length and weight, as well as a superior condition factor compared to the other treatments. These results indicate that striped catfish oil represents an effective and locally available lipid source for *Tubifex* sp. enrichment in Thai mahseer larviculture. From a practical perspective, the use of *Tubifex* sp. enriched with Striped catfish oil can be recommended as a cost-efficient live-feed strategy to enhance early growth performance in hatchery-based production of Thai mahseer (*Tor tambroides*) larvae.

SIGNIFICANCE STATEMENT

This study demonstrates that locally available Striped catfish oil can be used as an effective and economical lipid source for enriching *Tubifex* sp., resulting in improved fatty acid composition of live feed and enhanced growth performance of Thai mahseer (*Tor tambroides*) larvae. The findings provide a practical alternative to imported enrichment products by utilizing locally sourced resources, thereby reducing production costs while maintaining high larval survival. This research contributes to the development of sustainable larval feeding strategies and supports more efficient hatchery production of this economically important freshwater species.

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