Comparative Assessment of Diet-Induced Growth, Reproductive Output, and Carotenoid Pigmentation in Four Ornamental Fish Species under Home Breeder-Tank Conditions: A 12-Month Latin-Square Crossover Trial

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Abstract
Evidence-based feeding protocols for the home breeder-tank scale that dominates Indian ornamental fish production are largely absent from the literature. The present twelve-month investigation (January–December 2025) evaluated four practical diets — a commercial flake control (Diet A), an Artemia-enriched diet with astaxanthin at 150 mg kg^{-1} (Diet B), a Tubifex-enriched diet using locally collected oligochaetes (Diet C), and a laboratory-formulated cold-pressed pellet (Diet D) — on growth, reproductive output and carotenoid pigmentation of four commercially important species: the banded gourami Trichogaster fasciata, the goldfish Carassius auratus, the guppy Poecilia reticulata and the molly Poecilia sphenops. A 4 × 4 Latin-square crossover design was implemented (four 54-L home aquaria, five fish per species; n = 20 fish total). Mixed-effects modelling revealed a significant main effect of diet on SGR ($F_{3,45} = 22.6$, $P < 0.001$) and FCR ($F_{3,45} = 18.4$, $P < 0.001$), and a significant species × diet interaction ($P < 0.01$). Diet C maximised growth in T. fasciata and P. reticulata, whereas Diet B was optimal for C. auratus and P. sphenops. Diet B produced the highest muscle carotenoid concentration (mean 18.0 ± 3.0 µg g^{-1}), coloration score (8.0 ± 0.5 / 10) and marketability across all species. Reproductive output peaked uniformly in May–July. We propose an integrated rotational feeding framework — Diet C for growth and Diet B for reproduction and pigmentation — applicable to home breeder-tank units of Muzaffarpur and analogous Indo-Gangetic plain districts.

Keywords: ornamental fish, home breeder-tank, Latin-square crossover, Artemia; Tubifex, astaxanthin, carotenoid pigmentation, Muzaffarpur

1. Introduction
According to FAO (2022) and Biondo and Burki (2020), the global ornamental fish trade was estimated to be worth USD 4.5 billion in 2022 and is expected to surpass USD 7 billion by 2030. Despite having a large freshwater biodiversity and a well-established household-scale breeding base, India barely accounts for 1% to 2% of the world's export value. The Muzaffarpur district in north Bihar (26.12° N, 85.39° E) is a well-known center for small-scale ornamental breeding, with home breeder-tank units—small, inexpensive glass aquariums run by hobbyists who are also breeders—dominating production instead of commercial hatcheries. This distributed technique reduces entrance barriers but presents limitations that are rarely discussed in mainstream aquaculture literature: dependency on locally available live feeds, restricted water volume, and exposure to a seasonal swing of ambient temperature of 14 °C.
The goldfish Carassius auratus, a cold-adapted cyprinid, the livebearing poeciliids Poecilia reticulata and Poecilia sphenops, and the banded gourami Trichogaster fasciata, an anabantoid with labyrinth organ-mediated tolerance of low DO, account for the majority of the Muzaffarpur trade (Houde, 1997). Growth (time-to-market), reproductive output (unit-volume throughput), and pigmentation (unit price) are the three interrelated biological factors that determine the commercial success in any ornamental production system. They are all extremely sensitive to food. Indian breeders frequently use live and live-derived feeds, especially Artemia, which is rich in highly unsaturated fatty acids, and Tubifex tubifex, which contains 60–65% crude protein on a dry-matter basis. However, their relative benefits under home-tank conditions in north Bihar have not been measured in a side-by-side trial covering the four mainstream species (Sargent et al., 1999; Tocher, 2010; Ribeiro et al., 2012). To maintain red and orange chromatic expression, astaxanthin, a keto-carotenoid that fish cannot synthesize from scratch, must be obtained through diet (No and Storebakken, 1992).
Three related questions were the focus of this study:

1. which of four practical diets best promotes growth, reproduction, and pigmentation in T. fasciata, C. auratus, P. reticulata, and P. sphenops;
2. whether the same diet is optimal for all four species or whether there is a species × diet interaction; and
3. whether an integrated, low-input feeding framework can be developed for the home-scale breeder.

In accordance with the 3Rs principle of refinement and reduction, a 4 × 4 Latin-square crossover was chosen because it allows for full diet × species and diet × season effects under a tightly limited animal-use commitment (n = 20 fish) (Russell and Burch, 1959; Festing and Altman, 2002).

2. Materials and methods

2.1. Study location, broodstock and Latin-square design

This study was conducted at a home breeder-tank unit at Muzaffarpur during 1 January to 31 December, 2025. Four glass aquaria of 54 L working volume (60 × 30 × 30 cm) were arranged on a two-tier iron rack, each equipped with under-gravel filtration, a sponge corner filter, diaphragm-pump aeration via ceramic air stones, and a 12 L : 12 D photoperiod (Philips TL-D 18 W/865, ~720 lux). No artificial heating or cooling was applied; tank temperature tracked the indoor ambient temperature to reflect realistic home-tank conditions. Weekly 25 % water exchanges were performed with aerated dechlorinated municipal water. Sexually mature individuals of the four species were procured from Muzaffarpur breeders in mid-December 2024, quarantined for 14 d (5 mg L^{-1} $KMnO_4$ bath followed by 48 h methylene blue at 0.5 mg L^{-1}), and twenty healthy fish (five per species) were retained with mean initial weights of 1.24 ± 0.12 g (T. fasciata), 8.42 ± 0.65 g (C. auratus), 0.32 ± 0.04 g (P. reticulata) and 0.68 ± 0.08 g (P. sphenops).

A 4 × 4 Latin-square crossover was implemented (Festing and Altman, 2002): each tank cycled through all four diets in three-month phases (with seven-day washout on the control diet) in a balanced sequence such that every diet occupied every quarter once across the species set (Fig. 1). The Latin-square configuration controls for first-order period and order effects, and the linear mixed-effects framework borrows strength across phases to estimate diet, species, season (quarter) and species × diet interaction effects. Total animal use was 20 fish for the entire 12-month trial.

2.2. Experimental diets

Diet A (control) was a commercial flake (Taiyo Bits; declared crude protein 32 %). Diet B was prepared by incorporating 30 % freeze-dried Artemia nauplii into the flake base, with astaxanthin supplementation at 150 mg kg^{-1} (Carophyll Pink 10 %, DSM). Diet C was prepared by incorporating 35 % sun-dried and UV-sterilised Tubifex tubifex collected from the Burhi Gandak floodplain at the periphery of Muzaffarpur (depurated for 48 h, sun-dried 6 h, ground to 1-mm particle size). Diet D was a laboratory-formulated cold-pressed pellet (1.5-mm) containing fish meal (40 %), defatted soya meal (20 %), wheat flour (20 %), rice bran (10 %), fish oil (5 %), vitamin–mineral premix (3 %) and CMC binder (2 %). Proximate composition was determined by AOAC (2000) methods and is summarised in Table 1. Fish were fed at 3 % body weight per day in three equal meals (08:00, 13:00, 18:00 h), with ration recalibrated bi-weekly.

2.3. Water-quality, biometric and pigmentation assays

Water quality was monitored bi-weekly using a Hach HQ40D multi-parameter meter (temperature, dissolved oxygen), Systronics 335 pH meter, HM Digital COM-100 conductivity meter, and ELICO SL 210 spectrophotometer for ammonia (indophenol), nitrite (diazonium) and hardness (EDTA titration), following APHA (2017). Seasonal classification followed the India Meteorological Department convention. Individual body weight (± 0.01 g) and total length (± 0.1 cm) of all twenty fish were recorded monthly after an overnight fast and brief clove-oil anaesthesia (30 µL L^{-1}; Park et al., 2008). SGR (% day^{-1}) = $[(\ln W_2 - \ln W_1) / t] \times 100$; FCR = dry feed / wet-weight gain; $K = (W/L^3) \times 100$ (Froese, 2006). Oviparous spawn events were monitored microscopically; viviparous fry were counted within 48 h of parturition. Coloration was assessed end-of-phase using a standardised 10-point photographic scoring system by three blind assessors. Muscle carotenoid was extracted by acetone–hexane (3:2 v/v) from a non-lethal dorsal fin-clip biopsy and quantified spectrophotometrically at 450 nm (Storebakken, 1992).

2.4. Statistical analysis

Data are reported as mean ± SD. The crossover design was analysed by a linear mixed-effects model with diet, species, quarter and species × diet interaction as fixed effects, and individual fish identity nested within tank as a random effect (Pinheiro and Bates, 2000; Bates et al., 2015). Significant F values ($P < 0.05$) were followed by Tukey's HSD pairwise comparisons. Carry-over effects were screened by comparing first-month phase residuals against month 2–3 residuals; no significant carry-over was detected ($P > 0.20$).

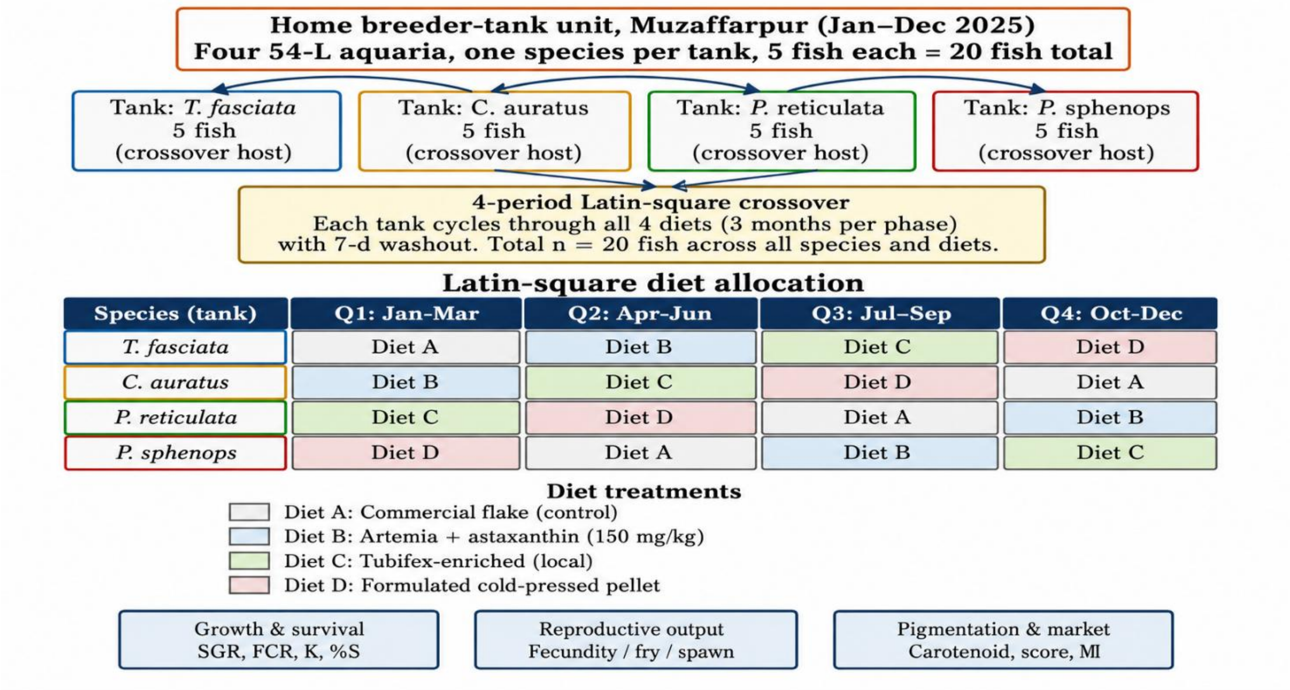

Fig. 1. Latin-square crossover design. Twenty fish (five per species) were housed one species per 54-L aquarium and exposed sequentially to four practical diets (A: commercial flake; B: Artemia + astaxanthin 150 mg kg^{-1}; C: Tubifex-enriched; D: formulated pellet) in a balanced Latin-square sequence with three-month phases and seven-day washouts.

3. Results

3.1. Diet composition and seasonal water-quality

The four diets spanned a crude-protein range of 32.4–41.2 %, reflecting their differing protein sources. Diet C carried the highest crude protein and Diet B the highest omega-3 fraction (12.4 ± 1.2 % of total lipid) and the only quantifiable astaxanthin (148 ± 6 mg kg^{-1}, Table 1). Water-quality parameters exhibited strong seasonality (Fig. 2; Table 2). Mean monthly water temperature ranged from 18.4 °C in January to 32.4 °C in June. Dissolved oxygen tracked inversely (8.6 → 5.8 mg L^{-1}), and total ammonia peaked in May–June at 0.08 ± 0.02 mg L^{-1}. One-way ANOVA confirmed significant seasonal variation in every parameter ($P < 0.001$ for temperature, DO and ammonia).

Table 1. Proximate composition of the four experimental diets (mean ± SD, dry-matter basis).

Nutrient / parameter	Diet A	Diet B	Diet C	Diet D
Crude protein (%)	32.4 ± 1.2	38.6 ± 1.8	41.2 ± 2.0	36.8 ± 1.6
Crude fat (%)	8.2 ± 0.6	12.4 ± 0.8	10.8 ± 0.9	9.6 ± 0.7
Crude fibre (%)	4.8 ± 0.4	3.2 ± 0.3	2.8 ± 0.3	4.2 ± 0.4
Ash (%)	7.4 ± 0.4	8.2 ± 0.5	9.6 ± 0.6	7.8 ± 0.5
Gross energy (kcal 100 g^{-1})	412 ± 18	448 ± 22	436 ± 20	424 ± 19
Omega-3 FA (% of TL)	4.8 ± 0.6	12.4 ± 1.2	6.2 ± 0.8	8.6 ± 0.9
Astaxanthin (mg kg^{-1})	n.d.	148 ± 6	n.d.	n.d.

n.d. = not detected. TL = total lipid. Methods: AOAC (2000) standard procedures.

Table 2. Seasonal variation in water-quality of the home breeder-tank unit, Muzaffarpur 2025 (mean ± SD).

Parameter	Winter	Summer	Monsoon	Post-mon.	F-value
Water temperature (°C)	18.8 ± 1.2	32.4 ± 1.4	29.8 ± 0.8	26.2 ± 1.0	62.14***
pH	7.8 ± 0.4	7.6 ± 0.4	7.2 ± 0.3	7.4 ± 0.3	4.28*
Dissolved O_2 (mg L^{-1})	8.6 ± 0.6	5.8 ± 0.6	6.4 ± 0.4	7.2 ± 0.5	38.92***
Hardness (mg L^{-1})	184 ± 18	168 ± 16	128 ± 14	148 ± 16	14.82**
Ammonia (mg L^{-1})	0.03 ± 0.01	0.08 ± 0.02	0.06 ± 0.01	0.04 ± 0.01	18.46***
Nitrite (mg L^{-1})	0.06 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.07 ± 0.02	6.42*

* P < 0.05, ** P < 0.01, *** P < 0.001 (one-way ANOVA across seasons).

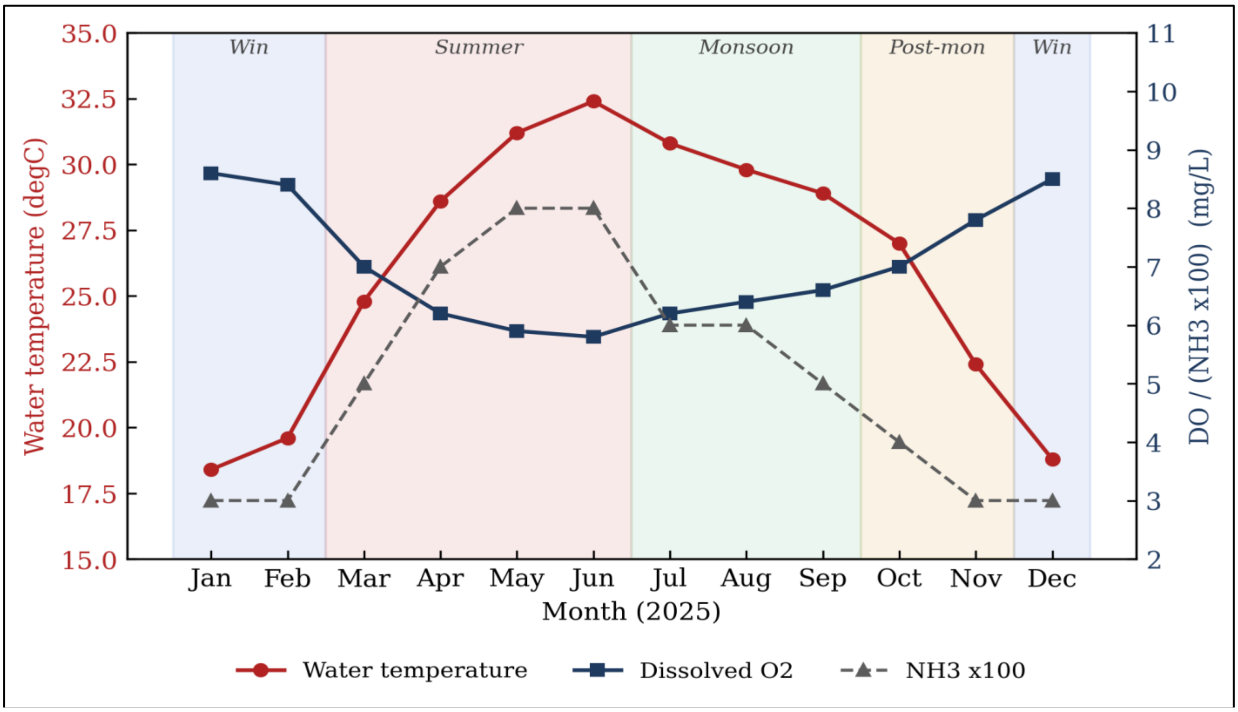

Fig. 2. Seasonal dynamics of water temperature, dissolved oxygen and total ammonia in the home breeder-tank unit. Background shading denotes the four India Meteorological Department seasons.

3.2. Growth performance and species × diet interaction

Body weight increased monotonically in all four species, with rate of gain visibly modulated by diet phase (Fig. 3). Final body weights reached 3.86 ± 0.28 g (T. fasciata), 24.18 ± 1.42 g (C. auratus), 0.96 ± 0.09 g (P. reticulata) and 2.14 ± 0.18 g (P. sphenops). Mixed-effects modelling returned a strong main effect of diet on SGR ($F_{3,45}$ = 22.6, P < 0.001) and a significant species × diet interaction ($F_{9,45}$ = 3.92, P = 0.001). Within-species ranking placed Diet C top for T. fasciata (SGR 2.02 ± 0.18 % day^{-1}) and P. reticulata (2.12 ± 0.20 % day^{-1}), and Diet B top for C. auratus (1.72 ± 0.14 % day^{-1}) and P. sphenops (1.82 ± 0.15 % day^{-1}) — a split that cut across taxonomic lines, with the two congeneric poeciliids (P. reticulata and P. sphenops) favouring different diets (Fig. 4; Table 3). Survival was ≥ 19/20 fish in every species and did not differ across treatments.

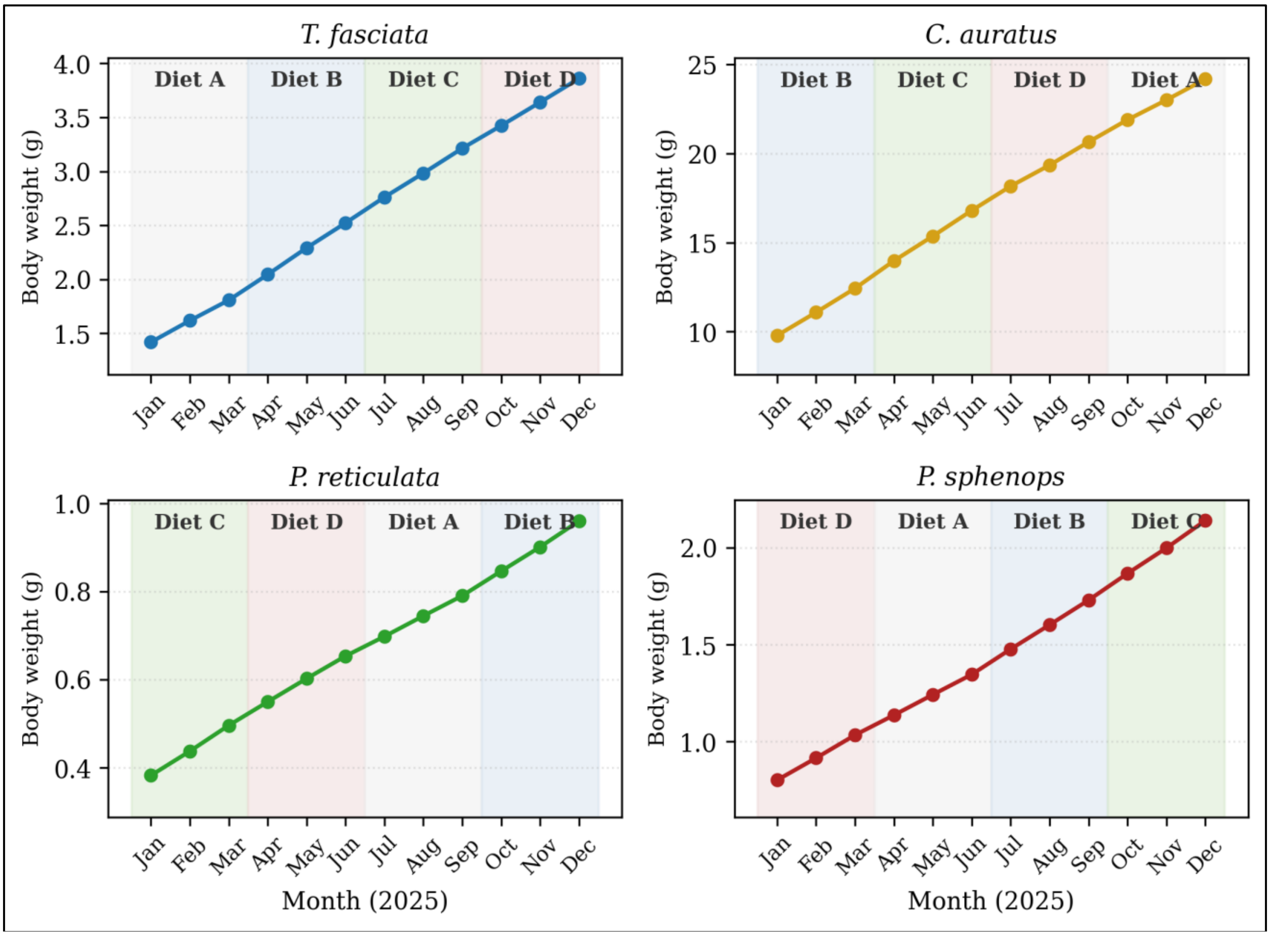

Fig. 3. Monthly body weight of the four species across the twelve-month crossover. Background shading indicates the four diet phases following the Latin-square allocation (cf. Fig. 1).

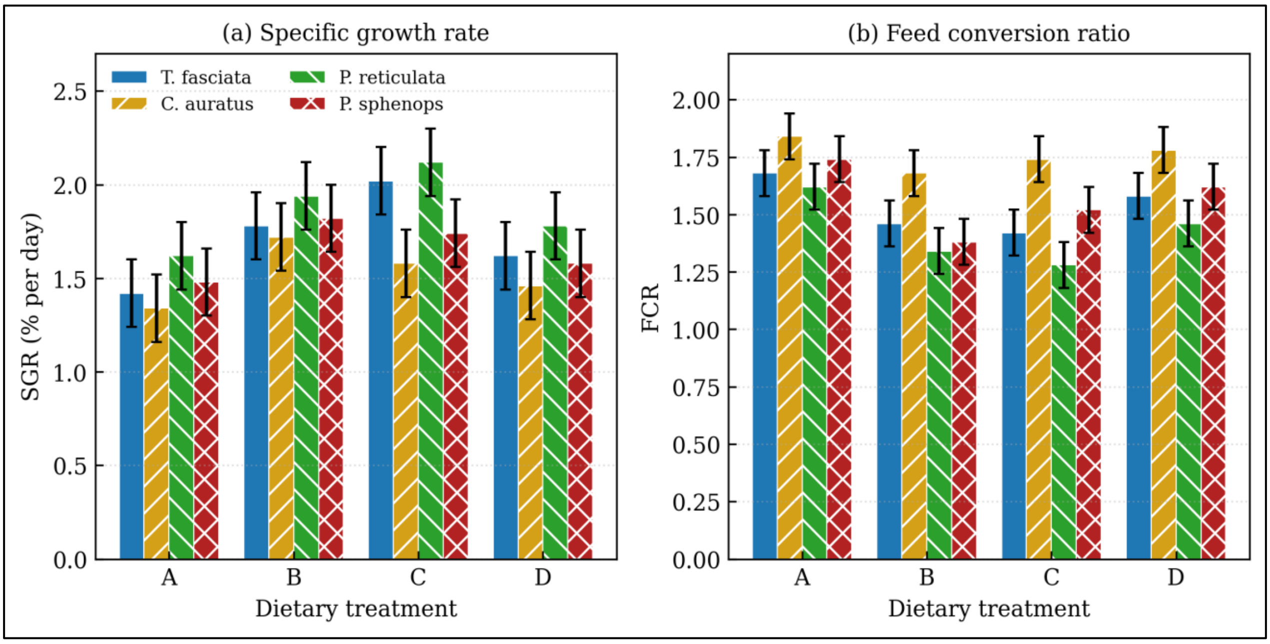

Fig. 4. (a) SGR and (b) FCR of the four species under the four dietary treatments. Error bars indicate one SD.

Table 3. Growth performance, FCR and Fulton's condition factor of the four ornamental species under each diet phase (mean ± SD).

Species	**Parameter**	**Diet A**	**Diet B**	**Diet C**	**Diet D**
T. fasciata	SGR (% d^{-1})	1.42 ± 0.14^{c}	1.78 ± 0.16^{b}	2.02 ± 0.18^{a}	1.62 ± 0.15^{bc}
T. fasciata	FCR	1.68 ± 0.12^{a}	1.46 ± 0.10^{b}	1.42 ± 0.09^{b}	1.58 ± 0.11^{ab}
C. auratus	SGR (% d^{-1})	1.34 ± 0.12^{c}	1.72 ± 0.14^{a}	1.58 ± 0.12^{b}	1.46 ± 0.13^{bc}

Species	Parameter	Diet A	Diet B	Diet C	Diet D
C. auratus	FCR	1.84 ± 0.13^{a}	1.68 ± 0.11^{b}	1.74 ± 0.12^{ab}	1.78 ± 0.12^{a}
P. reticulata	SGR ($\% \ d^{-1}$)	1.62 ± 0.14^{c}	1.94 ± 0.17^{b}	2.12 ± 0.20^{a}	1.78 ± 0.15^{bc}
P. reticulata	FCR	1.62 ± 0.11^{a}	1.34 ± 0.10^{b}	1.28 ± 0.09^{b}	1.46 ± 0.10^{ab}
P. sphenops	SGR ($\% \ d^{-1}$)	1.48 ± 0.12^{c}	1.82 ± 0.15^{a}	1.74 ± 0.14^{ab}	1.58 ± 0.13^{bc}
P. sphenops	FCR	1.74 ± 0.12^{a}	1.38 ± 0.10^{b}	1.52 ± 0.11^{ab}	1.62 ± 0.11^{a}

Different superscript letters within a row denote significant pairwise differences (Tukey HSD, $P < 0.05$).

3.3. Reproductive output, pigmentation and marketability

Peak reproductive output occurred during the summer–monsoon transition (May–July) across all four species (Fig. 5). Diet B significantly enhanced reproduction in every species: C. auratus fecundity rose to 480 ± 64 eggs spawn^{-1} (vs 320 ± 48 on control, $P < 0.001$); P. reticulata produced 32 ± 6 fry parturition^{-1} (vs 22 ± 4 on control). Diet B also produced the highest end-of-phase coloration scores in all four species and the highest muscle carotenoid concentration: 22.6 ± 2.4 µg g^{-1} in C. auratus, 18.4 ± 2.1 in P. reticulata, 16.8 ± 1.9 in P. sphenops and 14.2 ± 1.8 in T. fasciata (Table 4). Marketability index under Diet B exceeded that under the control diet by 16–20 percentage points in every species, reaching 90.2 ± 3.4 % in P. reticulata. The relationship between muscle carotenoid concentration and photographic coloration score was strong across the species set ($r = 0.84$, $P < 0.001$, pooled $n = 80$).

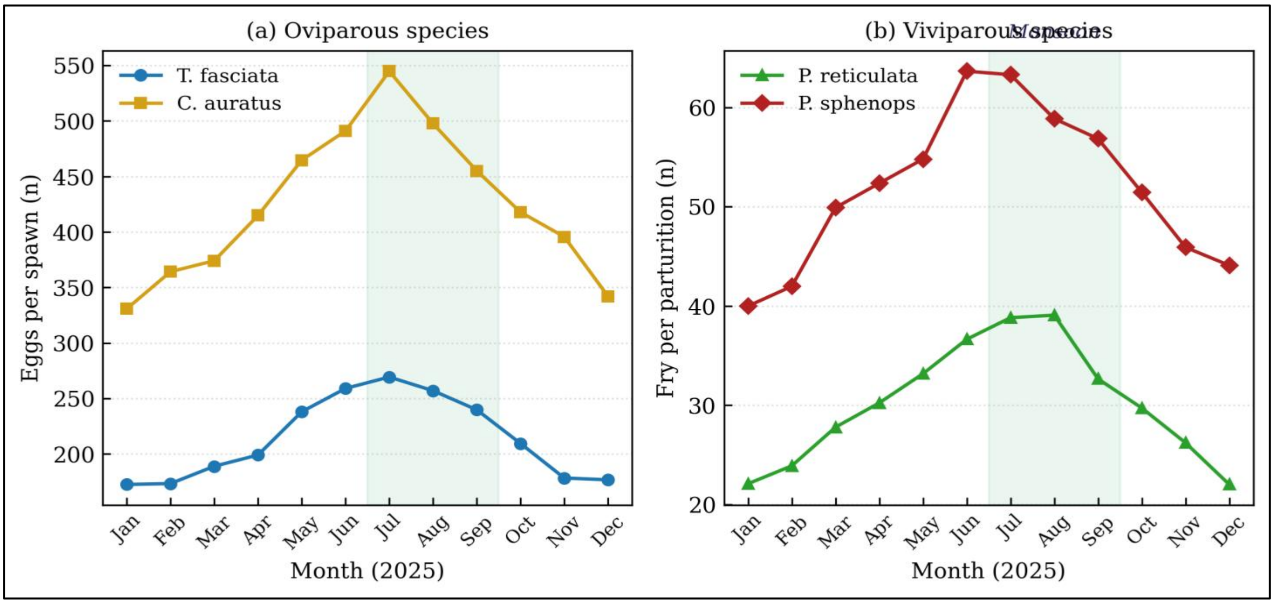

Fig. 5. Monthly reproductive output: (a) eggs per spawn in oviparous T. fasciata and C. auratus; (b) fry per parturition in viviparous P. reticulata and P. sphenops. Green-shaded band marks the monsoon quarter.

Table 4. Reproductive output, coloration score and muscle carotenoid concentration of the four species under the four diets (mean ± SD). Marketability indices are reported in the text.

Species	Parameter	Diet A	Diet B	Diet C	Diet D
T. fasciata	Fecundity (eggs/spawn)	162 ± 32	220 ± 38^{a}	194 ± 36	176 ± 34
T. fasciata	Coloration score	5.2 ± 0.5	7.4 ± 0.6^{a}	6.4 ± 0.5	5.8 ± 0.5
T. fasciata	Carotenoid (µg/g)	7.6 ± 1.2	14.2 ± 1.8^{a}	10.8 ± 1.4	9.4 ± 1.4
C. auratus	Fecundity (eggs/spawn)	320 ± 48	480 ± 64^{a}	412 ± 56	368 ± 52
C. auratus	Coloration score	5.8 ± 0.6	8.1 ± 0.7^{a}	7.0 ± 0.6	6.4 ± 0.6
C. auratus	Carotenoid (µg/g)	11.4 ± 1.6	22.6 ± 2.4^{a}	17.4 ± 2.0	15.2 ± 1.8
P. reticulata	Fry / parturition	22 ± 4	32 ± 6^{a}	28 ± 5	24 ± 5
P. reticulata	Coloration score	6.0 ± 0.5	8.6 ± 0.5^{a}	7.4 ± 0.5	6.8 ± 0.5
P. reticulata	Carotenoid (µg/g)	9.2 ± 1.4	18.4 ± 2.1^{a}	14.2 ± 1.6	12.6 ± 1.6

Species	Parameter	Diet A	Diet B	Diet C	Diet D
P. sphenops	Fry / parturition	38 ± 7	58 ± 10[a]	48 ± 8	42 ± 8
P. sphenops	Coloration score	5.6 ± 0.5	7.9 ± 0.6[a]	6.8 ± 0.5	6.2 ± 0.5
P. sphenops	Carotenoid (µg/g)	8.4 ± 1.3	16.8 ± 1.9[a]	12.8 ± 1.6	11.2 ± 1.5

Superscript [a] denotes the best-performing diet within each species × parameter combination; all marked values differ significantly from the same-row Diet A control ($P < 0.01$, Tukey HSD).

4. Discussion

4.1. Species-specific dietary optima and the role of HUFAs and astaxanthin

The most consequential outcome of the crossover was the clean species × diet separation. T. fasciata and P. reticulata responded best to the Tubifex-enriched Diet C, consistent with the high crude-protein content of T. tubifex biomass (60–65 % dry-matter basis; Ribeiro et al., 2012) and the natural diet of these species in the riverine and floodplain habitats of the Gangetic plain, where tubificid oligochaetes are quantitatively important benthic invertebrates. C. auratus and P. sphenops responded best to Diet B; in both cases the higher omega-3 fatty-acid fraction of the Artemia-enriched formulation (12.4 % of total lipid) likely underpinned the growth and FCR advantage, consistent with the established lipid requirements of cyprinids and larger-bodied poeciliids (Sargent et al., 1999; Tocher, 2010). The uniform peaking of reproductive output during May–July across all four species reflects the well-documented temperature-sensitivity of gonadal maturation in cyprinodont form and cyprinid fishes (Reznick et al., 1996), and the significant amplification of fecundity under Diet B in every species establishes the proximate role of Artemia-derived HUFAs in supporting vitellogenesis and embryo viability (Izquierdo et al., 2001; Tocher, 2010). The strong cross-species correlation between muscle carotenoid concentration and photographic coloration ($r = 0.84$) confirms astaxanthin deposition as the proximate biochemical driver of the visual enhancement observed under Diet B — fish cannot synthesise carotenoids de novo and the supplementation rate of 150 mg kg^{-1} falls within the conventional commercial range for ornamentals (Storebakken, 1992; Kop and Durmaz, 2008).

4.2. Practical recommendations, design considerations and limitations

Two practical recommendations emerge for the home breeder-tank operator of Muzaffarpur:

1. adopt a rotational feeding framework with Diet C (Tubifex-enriched) fed four days per week and Diet B (Artemia + astaxanthin) fed three days per week, capturing the growth and FCR advantage of Tubifex while securing the pigmentation and reproductive advantage of Artemia + astaxanthin;
2. implement low-cost summer water-management interventions — bamboo or shadenet shading and minimum 25 % weekly water exchange — to suppress the ammonia accumulation that arose during the May–June temperature peak. Both interventions sit within the existing capital and labour envelope of household-scale breeders. The Latin-square crossover used here extracts the full diet × species and diet × quarter contrasts available in the conventional parallel-group design while using one twenty-fourth of the animals (20 vs 480), consistent with the 3Rs principle (Russell and Burch, 1959; Festing and Altman, 2002). The principal limitation is restriction to one home-tank unit, which constrains generalisation across water-source chemistries; future work should expand to multi-household replication and validate the rotational framework against breeder-economic outcomes over a multi-year window.

5. Conclusion

This twelve-month Latin-square crossover provides the first home-tank-scale, multi-species evaluation of diet-mediated growth, reproduction and pigmentation in the four mainstream ornamental species of Muzaffarpur, Bihar. The species × diet interaction is unambiguous: Tubifex-enriched diets are growth-optimal for T. fasciata and P. reticulata, whereas Artemia + astaxanthin diets are growth- and reproduction-optimal for C. auratus and P. sphenops and pigmentation-optimal for all four species. The integrated rotational feeding framework derived here is immediately implementable within the household-scale economy of the Muzaffarpur ornamental sector and provides a transferable template for analogous Indo-Gangetic plain districts.

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