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Probiotic-enriched black soldier fly larvae meal diets impact the relationship between morphometric traits and the growth of Redbreasted tilapia (Coptodon rendalli)

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Abstract

Fish morphometry (body shape) is a key variable that determines a fish's ability to feed, swim, reproduce, and perform other biological processes. Morphometric traits indicate fish health and overall well-being, yet their use in determining aquaculture production when probiotics are used in insect diets is not well understood. The relationships of fish morphometric traits with growth parameters have also not been well investigated. The current study describes the performance and relationship of body morphological variations and specific growth trajectories of Redbreasted tilapia fingerlings fed commercial probiotic-inoculated black soldier fly meal diets. The fish were reared for 72 days and fed various probiotic-inoculated diets described as P0 [0 CFU/kg] as a control, P1 [4×10^{10} CFU/kg] as treatment 1, P2 [8×10^{10} CFU/kg] as treatment 2, and P3 [12×10^{10} CFU/kg] as treatment 3. Significantly greater means for the morphometric traits of total length, eye diameter, distance between eyes, operculum length, and pectoral fin length were observed at T2 ($p < 0.05$). Similarly, the specific growth rate and weight gain were greater in T2, with T0 indicating the highest apparent feed conversion ratio ($p < 0.05$). Overall, the T2 inoculation level presented the greatest variability, with enhanced morphology-related growth in the present study. The study indicated that probiotic inoculation in BSFL diets enhances morphological variability and fish growth.

Introduction

Morphometric analysis, which is the quantitative analysis of body shape, length, and weight, forms the core of aquaculture and fisheries science, as it reveals the growth status, health, and ecological adaptation of fish populations (Kenneth et al. 2024). This involves the use of ecological informatics by providing precise tools for shape analysis, especially in fish (Talijan 2026). Fish deformities and body shape are important qualities for fish selection in aquaculture production; however, they are usually secondary to other growth parameters (Sutthakiet et al. 2020). Moreover, morphometric traits offer evidence of how well habitats support the functions of aquatic organisms (Hussein et al. 2025). In aquaculture fish selection programs, a trend has evolved whereby these traits are incorporated to predict fish performance. Morphometric traits significantly affect the preferences of consumers and their willingness to pay for fish (Mehar 2019). The most commonly used morphometrics include the length–weight relationship and condition indices to provide inferences on body condition, energy stores, physiological fitness, and quality of growth of cultured and wild fish stocks (Brosset et al. 2023). Morphometric measures are important in that they combine variables of size, shape, and energy reserves, thereby providing estimations of nutritional status, health, and biomass productivity that are often more sensitive than simple measures of body weight alone (Traverso et al. 2024). Additionally, morphometric traits are typically pseudo representations of fish condition, health, and biomass productivity, and they yield more information than just length or weight. They have positive impacts on growth performance, feed utilization, antioxidant status, immune functionality, and the gut microbiota in a number of cultured fish species, such as tilapia, seabass, and catfish (Wang et al. 2025). The use of geometric morphometric methods enables solid visualization and measurement of morphological

changes by separating the shape and non-shape parts and the ability to produce biologically significant morphological changes (Coure et al. 2025). In some cases, fish morphology has been used to determine fish escape from culture systems such as cages (Terje et al. 2026). Nonetheless, morphometric responses, including shifts in shape or condition indices compared with growth performance, are still poorly investigated. For example, while recent work on Atlantic salmon feeding trials explored shape variation under divergent protein sources, the short time frames, limited sample sizes, and limited definitive conclusions about diet-induced morphometric responses (Chollet-Villalpando et al. 2025).

Although fish growth parameters such as the apparent feed conversion ratio (AFCR), protein efficiency ratio (PER), specific growth rate (SGR), weight gain, and relative condition factor (*Kn*) have been extensively used over time, a significant gap in the research concerning their combination with deep morphometric responses when probiotics and insect meals are used in the world of aquaculture nutrition. These measures quantify gross changes in mass and feed efficiency but do not measure indirect morphological changes in body shape or structure, which can help to identify other effects of dietary treatments on fish physiology (Pamphile et al. 2024). This is further overstated in research that involves the use of insect meals to replace fish meal as a substitute ingredient and in probiotic-inoculated diets (Torres-Maravilla et al. 2024). Additionally, the effects of insect-based diets on growth performance and health parameters in different fish species have been demonstrated. However, limited emphasis has been given to morphological changes in fish. Insects such as black soldier fly (BSF) larvae, when used in aquaculture, improve their histology, reduce enterocyte steatosis in the pyloric caeca, and reduce IgM levels in the distal intestine of Atlantic salmon (*Salmo salar*) (Weththasinghe et al. 2021). Similarly, BSFL diets improve lipid deposition and fish welfare in gilt heads (Randazzo et al. 2021) and rainbow trout (*Oncorhynchus mykiss*) (Melenchón et al. 2022). Probiotics contribute to good fish performance, but this has been limited to putative (naturally isolated) probiotics. Fish rely on the microbiota to digest non-starch polysaccharides such as chitin (Egerton et al. 2018), and their digestion is greatly enhanced when probiotics are utilized (Hasan et al. 2023). Our present study examined the effects of exogenous probiotic inoculation of black soldier fly diets on morphometric responses, specific growth parameters, and their relationships with Redbreasted tilapia reared in concrete fish tanks. The findings will generate valuable scientific evidence, which will guide the imminent rearing of Redbreasted tilapia (*Coptodon rendalli*) and other cichlid species.

Methods

Study area, fish husbandry, and sampling

The study was carried out at the Domasi Aquaculture Centre in Zomba District, southern Malawi, which is located at coordinates of 11° 46' 703” S, 34° 13' 082” E.

Fish husbandry

In total, 480 Redbreasted tilapia fingerlings weighing 4.1 ± 0.10 grams were sourced from the Domasi Aquaculture Centre hatchery, Zomba district, Southern Malawi, and and used in the experiment. The fish were harvested and conditioned for 1 week in 2 m³ concrete fish tanks and fed a 40% crude protein exogenous diet.

Probiotic feed preparation

Exogenous Sanolife® PRO-2 probiotic mixtures (*Bacillus subtilis, Bacillus licheniformis, and Bacillus pumilis* obtained from INVE Aquaculture, Belgium) were used at T0 [0 CFU/kg] as a control, T1 [4×10^{10} CFU/kg] as treatment 1, T2 [8×10^{10} CFU/kg] as treatment 2, and T3 [12×10^{10} CFU/kg] as treatment 3. The probiotic concentrations were homogenized in 2 litres of fish oil. The formulated feed pellets were top-dressed evenly with the prepared mixture. The pellets were left to absorb the probiotic mixture for a period of 30 minutes to 2 hours at room temperature (25°C). Top dressing was applied at intervals of 3 days for the entire culture period, as this was the ideal maximum time at which the probiotics would remain attached to the pellets.

Diet formulation

A 42% crude protein isonitrogenous and isocaloric BSFL-based probiotic-inoculated fish diet was formulated using locally available ingredients sourced from Mzuzu market, Mzuzu City, northern Malawi. BSFL was sourced from the BSF culture unit at the Department of Fisheries and Aquatic Sciences at Mzuzu University, Malawi. Prior to feed formulation, the BSFL and sourced ingredients were subjected to proximate analysis for crude protein, crude lipid, crude fibre, nitrogen-free extract, and ash contents while adopting the standard methods of AOAC (2000). The crude protein content was determined via the Kjeldahl system (Kjeltec™ 8400-Autoanalyzer, FOSS, Denmark), the crude lipid content was determined by the Soxhlet method, and the crude ash content was determined through combustion in a muffle oven, which involves oven incineration at 550°C for 5 hours. The moisture content was also determined through oven drying at 105°C. The pulverized ingredients were homogenized using a clean spade and extruded at approximately 90–120°C using an electric-powered pelletizer (Model: Y2-160 M-4, Dongxiang Hongxiang, China) to form 1 mm feed pellets. The formulated pellets were spread on clean synthetic tarpaulin and dried under shade for four days to reduce moisture. The inclusion levels of the formulated diet are indicated in Table 1. The formulated BSFL diet was subjected to proximate analysis before its application in the experiment. This was done to control for any quality checks in the feed, according to the dietary nutrient requirements of the fish. The feed was processed and stored in a well-aerated room at 25°C.

Table 1
Ingredient inclusion levels for the 42% CP probiotic inoculated BSFL fish feed.

Ingredient	**Inclusion level (%)**
Defatted black soldier fly larval meal	44
Deoiled soybean meal	20
Maize	5.8
Wheat flour	15
Wheat bran	12
Vitamin premix	1
Monocalcium phosphate	1
Stabilized Vitamin C	0.1
Salt	1
Chitinase enzyme	0.1
Total	**100**
Proximate composition (%)	
Crude protein	42.44
Crude lipid	8.24
Crude fibre	2.94
Digestible Energy (Kcal/kg)	4082.94

Premix analysis per 2000 g; vitamin An 11,500 IU, vitamin D $_3$ 3,000 IU, vitamin E 10,000 IU, vitamin K 2,000 mg, vitamin B_1 1,000 mg, vitamin B_2 4,000 mg, vitamin B6 500 mg, vitamin B12 6000 mcg, choline 150000 mg, iron 30000 mg, manganese 80000 mg, copper 5000 mg, zinc 50000 mg, cobalt 225 mg, iodine 1000 mg, selenium 150 mg, lysine 20000 mg, methionine 25000 mg, phosphorus 5250 mg and calcium 750 mg, calcium: 2.057%, phosphorus: 1.08%.

Experimental setup

Each concrete tank measuring 2×1×1 m was stocked with 40 fingerlings during the onset of the experiment. Probiotic inoculation levels were determined in triplicate (*n = 3*). The fish were fed *ad libitum* throughout the experiment. When feed utilization was not efficient, adjustments were made in relation to the proportionate average body weights. The experiment was carried out in a completely randomized design for 72 days. To maintain a clean environment, the fish tanks were routinely cleaned biweekly to eliminate any fecal matter and uneaten feed.

Fish sample collection

Body condition indices

The relative condition factor (*Kn*) was analysed using Le Cren's (1951) formula:

$Kn = W / aL^b$

The specific growth rate (% /day) was calculated via the following formula:

SGR (% /day) = Ln final weight – Ln initial weight /time (days) × 100

Weight gain was analysed via the formula described by Silva & Davy (1992).

Weight gain (g) = $Wt_2 - Wt_1$

Image acquisition and shape analysis

At the end of the experiment, a total of 30 fish fingerlings per inoculation level were harvested from the fish tanks and placed in 10-liter water-filled clean buckets. To minimize fish stress, the fish were immobilized in 40 mg/L dissolved clove solution following the protocols used by Anderson & McKinley (1997). This was conducted until stage 4 of anaesthesia was achieved (rare opercular movements, relaxed muscle tone, and no reaction to stimuli).

The Truss Network system standard protocol was used to analyse body morphometrics (Strauss & Bookstein 1982). The method is non-destructive and well refined for any morphological variation across fish of various subpopulations (Ines et al., 2024). It further helps in the estimation of, and compensation for, measurement error. The homologous landmarks are defined along the contours of the fish body while the distances between them are quantified. Each fish was laid on its left side against a white background, the fins were stretched out, and the fish were photographed from a fixed position of 40 mm at an elevated angle of 90°. The images were captured using a Canon DS126761 high-resolution digital camera (Canon Zoom Lens EF-S 18–55 mm, φ58 mm; Canon Inc. 3–30–2, Shinomaruko, Ohta-ku, Tokyo, Japan). In total, 20 anatomical landmarks were identified, which defined 20 distances as reflected in Fig. 1. The captured images per inoculation level were imported into TpsUtil software (v1.78) to generate an image file. The generated image files were imported into TpsDig software (v2.31) for landmarking (digitization). These software programs help in the actual placement and recording of X and Y landmarks, thus enabling the construction of a truss network.

The morphometric traits, landmarks, their numbers, and locations are described in Table 2. The upper part of the table describes the landmark, the morphometric trait, and its abbreviation, whereas the lower part describes the number and location.

Table 2
Morphometric distances and landmarks described along the body contour of Redbreasted tilapia used for body shape analysis. Please refer to Fig. 1 as well.

Body landmarks		
Landmark	**Morphometric trait**	**Abbreviation**
1 to 2	Head Length (cm)	HL
1 to 6	Total Length (cm)	TL
2 to 3	Dorsal Fin Length (cm)	DSFL
5 to 6	Caudal Fin Length (cm)	CFL
8 to 9	Anal Fin Length (cm)	AFL
10 to 11	Pelvic Fin Length (cm)	PVFL
12 to 13	Operculum Length (cm)	OL
14 to 15	Distance Between Eyes (cm)	DBE
16 to 17	Eye Diameter (cm)	ED
18 to 19	Pectoral Fin Length (cm)	PCFL
2 to 20	Body Depth (cm)	BD
Number	**Location**	
1	Anterior tip of the fish snout	
2	Anterior point of the dorsal fin	
3	Posterior tip of the dorsal fin	
4	Terminal part of the dorsal fin	
5	Anterior part of the caudal fin	
6	Posterior tip of the caudal fin	
7	Posterior end of anal fin	
8	Posterior tip of the anal fin	
9	Anterior part of the anal fin	
10	Posterior tip of the pelvic fin	
11	Anterior part of the pelvic fin	
12	Lower part of the operculum	
13	Upper part of the operculum	

Body landmarks		
Landmark	**Morphometric trait**	**Abbreviation**
14	Anterior part of the distance between eyes	
15	Posterior part of the distance between eyes	
16	Anterior point of eye diameter	
17	Posterior point of eye diameter	
18	Anterior part of the pectoral fin	
19	Posterior part of the pectoral fin	
20	Point of body depth measurement	

The body shape variation across PC1 covariance matrix scores (Fig. 2) was used to generate a wireframe (shape variation) diagram (Fig. 3) with demarcated distances. This was carried out using the generalized Procrustes analysis (GPA) for all landmarks under each section, which were pooled together via MorphoJ v1.70a software. To minimize the binary cross-entropy between the predicted heatmap h^k and the ground truth heatmap $\widehat{h}^k$ for each landmark *k*, the loss function was formalized via the following equation:

$$L = \frac{1}{KHW} \sum_{k} \sum_{xy} - h_{xy}^{x} . log \widehat{h}_{xy}^{k} - \left(1 - h_{xy}^{k}\right) . log \left(1 - \widehat{h}_{xy}^{k}\right)$$

where *k* denotes one of the K landmarks and where *x* and y represent the height (H) × width (W) of the sized image.

Statistical analysis

Multivariate morphometric analysis and growth parameters

A multivariate statistical approach of principal component analysis (PCA) was used to determine the physiological state of Redbreasted tilapia across inoculation levels and estimate the extensive relationships between all the morphometric traits and growth parameters. To maximize variance in loadings, orthogonal rotation was performed via varimax rotation (Cozzolino et al. 2019). Multiple variables were summarized using the shared correlation structure to derive principal components. Each variable was scaled and centered. To study the statistical effect of individual explanatory variables on the morphometric body condition index, initial tests for multicollinearity between morphometric traits relying on the variance inflation factor (VIF) with values < 5 were carried out (Zuur et al. 2007). A generalized linear modelling (GLM) approach with a Gaussian distribution and a log link function (Xiao et

al., 2018) was used to estimate the distributions of growth parameters and morphometric traits across inoculation levels. The extracted growth parameters and morphometric traits were subjected to normality and homogeneity of variance checks using Shapiro–Wilk's test and Levene's test, respectively. One-way analysis of variance (ANOVA) was conducted to test for statistically significant differences between inoculation levels for morphometric traits and selected growth variables. Tukey's *honestly significant difference (HSD) post hoc test* was used to separate statistically significant differences across probiotic inoculation levels. Single-variable linear regression was conducted to determine the functional relationships between the extracted morphometric traits and growth parameters. Statistical significance was considered at $\alpha < 0.05$. Statistical analyses were conducted in Python programming v3.12.2 (Rossum 2025) via the SciPy (Virtanen et al. 2020), Matplotlib (Hunter 2007), Scikit-learn (Pedregosa et al. 2011), and PtitPrince libraries.

The linear regression and principal component analysis equation models are described in equations **i** and **ii**, respectively.

$$\widehat{\gamma} = \beta_0 + \beta_i X$$

(i)

where $\widehat{\gamma}$ represents the observed values of the growth parameter, β_0 represents the intercept, β_i represents the coefficient, and *X* represents all the observed values of the morphometric traits.

$$PC_k = a_{k1}X_1 + a_{k2}X_2 + a_{k3}X_3 + \cdots\ \cdots\ \cdots\ + a_{kn}X_n$$

(ii)

where PC_k represents the k^{th} principal component, $X_1,\ X_2,\ X_3 \ldots\ \ldots\ \ldots\ .X_n$ represents the morphometric traits or growth variables, $a_{k1},\ a_{k2},\ a_{k3}$..... a_{kn} represents the coefficients or loadings of morphometric traits and growth variables, and *n* represents the number of morphometric traits or growth variables.

Results

Growth parameters

The PCA biplot scores revealed that morphometric traits and growth-related parameters were interstructured, with PC1 as the major factor, with an explanation of 51.54% (Fig. 4). This finding indicated that total growth performance was the most prevalent influence on phenotypic differentiation across treatments. PC2, which was associated with the AFCR and SGR, accounted for 23.90% of the total variance. The observed morphological divergence indicated an inclination toward greater inoculation, which was correlated with increased growth, indicating treatment-dependent morphological divergence. Weight gain, SGR, and the AFCR were retained after rotation.

The ANOVA results for the retained growth parameters are shown in Fig. 5. The T2 inoculation level had the highest mean values for SGR (3.10 ± 0.07) and weight gain (9.12 ± 0.21), with T0 having the lowest mean values of 1.92 ± 0.06 and 4.40 ± 0.14, respectively (B & C). This was indicative of increased fish growth when T2-inoculated feed was used to feed the fish. In contrast, T2 had the lowest mean AFCR (1.56 ± 0.03), with T0 having the highest AFCR (2.26 ± 0.05) (A). The lowest AFCR was associated with better feed utilization by the fish. Statistically significant differences across inoculation levels were shown for SGR ($p < 0.001$), weight gain ($p < 0.001$) and AFCR ($p < 0.001$).

Morphometric traits

The correlogram heatmap indicates that all morphometric variables were highly correlated, with the lowest correlation reflected between the operculum and distance between eyes, with a Pearson's correlation coefficient (R) of 0.80 (Fig. 6). The majority of morphometric traits were strongly correlated with each other, indicating covariance with size in general. Intercorrelations between total length, operculum length, pelvic fin length, pectoral fin length, and head length presented the highest Pearson's correlation coefficient, $R = 0.99$. The results from PCA indicated that the data were suitable for PCA reduction, with a Kaiser–Meyer–Olkin test value of 0.87 and a Bartlett's test of sphericity, $p < 0.001$, indicating that the variables were related.

The PCA biplot revealed a distinct separation of the effects of inoculation on morphometric traits (Fig. 7). PC1 and PC2 accounted for 93.87% and 3.47% of the total variation, respectively. PC1 was highly related to total length, weight gain, body depth, and fin dimensions.

These findings indicated that overall growth-related morphometric traits were affected mainly by the inoculation of probiotics. PC2 represents the secondary shape variation. The variables that were extracted after PCA orthogonal rotation included operculum length, eye diameter, pectoral length, total length, and distance between eyes.

The results for the extracted traits are shown in Table 3. The highest mean values for TL, ED, DBE, OL, and PFL were recorded at the T2 inoculation level. Statistically significant differences in morphometric traits were observed across all inoculation levels ($p < 0.001$). Additionally, statistically significant polynomial contrasts were indicated across inoculation levels for all morphometric traits ($p < 0.001$).

Table 3
Mean (± SE) values of morphometric traits across inoculation levels

Inoculation level (cfu/kg)					**Polynomial contrasts**		
Morphometric trait	T0	T1	T2	T3	Linear	Quadratic	Cubic
TL (mm)	61 ± 0.49[a]	71 ± 0.00[b]	92.30 ± 0.80[d]	74.6 ± 0.16[c]	< 0.001	< 0.001	< 0.001
ED (mm)	4.21 ± 0.04[a]	4.48 ± 0.02[b]	6.20 ± 0.00[d]	5.86 ± 0.02[c]	< 0.001	< 0.001	< 0.001
DBE (mm)	6.51 ± 0.16[a]	8.93 ± 0.02[b]	12.57 ± 0.11[d]	9.2 ± 0.00[c]	< 0.001	< 0.001	< 0.001
OL (mm)	7.77 ± 0.12[a]	8.59 ± 0.03[b]	12.84 ± 0.02[d]	12.53 ± 0.02[c]	< 0.001	< 0.001	< 0.001
PFL (mm)	6.67 ± 0.52[a]	10.24 ± 0.06[b]	21.97 ± 0.21[d]	19.93 ± 0.03[c]	< 0.001	< 0.001	< 0.001

*Values in rows with different superscripts are statistically significant at $p < 0.05$ according to Tukey's HSD *post hoc test*. *NOTE: TL – Total Length, ED – Eye Diameter, DBE – Distance between Eyes, OL – Operculum Length, and PFL – Pectoral Fin Length*

Hierarchical clustering of the inoculation levels (T0, T1, T2, and T3) on the basis of the mean morphometric parameters (Euclidean distance and Ward linkage method) is depicted by the dendrogram in Fig. 8. Two major clusters are reflected: Cluster 1 (T0 and T1) and Cluster 2 (T2 and T3). The clusters converge at a far greater Euclidean distance (~ 45), suggesting great overall morphometric dissimilarity between the low inoculation levels (T0 and T1) and the high inoculation levels (T2 and T3).

T0 and T1 are relatively small distances from each other (~ 14), implying that the fish in T0 and T1 have morphometric profiles that can be considered relatively similar. T2 and T3 are concentrated at the intermediate level (~ 22), hence being similar to more pronounced morphometric reactions. The results indicate that the morphological response to inoculation with T2 was dose dependent, and the greatest increase in morphometric parameters was found for all the parameters. The dendrogram supports multivariate separation as well as robust inoculation-induced structural partitioning of morphometric traits at the level of inoculation.

The regression results between the PCA-extracted growth parameters and morphometric traits are shown in Table 4. Positive Pearson's correlation (R) values were observed between growth parameters and morphometric traits. Similarly, positive coefficients (*b*) were observed between the growth parameters of weight gain and SGR across all the morphometric traits. Morphometric traits positively influenced weight gain and SGR. However, the AFCR negatively influenced morphometric traits, as the results indicated negative coefficients (*b*). Overall, the regression model was statistically significant ($p < 0.05$).

Table 4
Linear regression of extracted growth parameters and extracted morphometric traits

Morphometric trait vs Growth parameter	R	R^2	$R^2_{Adjusted}$	a	b	p value
TL (cm) vs WG (g)	0.38	0.14	0.12	-1.01	0.06	0.017
ED (cm) vs WG(g)	0.34	0.11	0.09	-0.22	0.71	0.034
DBE (cm) vs WG(g)	0.37	0.14	0.11	0.58	0.31	0.018
OL (cm) vs WG (g)	0.34	0.11	0.09	0.67	0.27	0.033
PFL (cm) vs WG (g)	0.34	0.12	0.09	2.07	0.09	0.032
TL (cm) vs SGR (%)	0.39	0.14	0.13	0.01	0.05	0.014
ED (cm) vs SGR (%)	0.36	0.13	0.12	0.60	0.68	0.023
DBE (cm) vs SGR (%)	0.38	0.14	0.12	1.50	0.28	0.016
OL (cm) vs SGR (%)	0.36	0.13	0.11	1.45	0.26	0.022
PFL (cm) vs SGR (%)	0.36	0.13	0.11	2.79	0.09	0.021
AFCR vs TL (cm)	0.34	0.11	0.09	86.04	-7.79	0.033
AFCR vs ED (cm)	0.38	0.14	0.12	6.14	-0.66	0.016
AFCR vs DBE (cm)	0.31	0.09	0.07	11.30	-1.38	0.050
AFCR vs OL (cm)	0.38	0.14	0.12	12.99	-1.74	0.017
AFCR vs PFL (cm)	0.37	0.14	0.11	21.70	-4.82	0.020

Discussion

This study focused on testing the hypothesis that morphometric traits and growth parameters such as the SGR, AFCR, *Kn*, and weight gain of Redbreasted tilapia are improved when the fish are fed probiotic-inoculated black soldier fly meal diets. Most studies have focused on the utilization of energy-dense variables such as fatty acids, proteins, and hormones to determine morphometric trait performance in fish. Morphometric traits act as representations of the energy-dense performance of a fish. The results from our study revealed that the fish growth parameters were closely linked to the morphometric traits, although the AFCR negatively contributed to the performance of the morphometric traits. The high SGR, weight gain, and AFCR could be linked to the enhanced digestive efficiency as a result of probiotics producing extracellular enzymes such as proteases, cellulases, amylases, and lipases. The enzymes efficiently breakdown proteins such as chitin, carbohydrates, and lipids, leading to increased nutrient absorption and hence facilitating faster growth (Hasan et al. 2021). Similarly, during the stunting periods of Milkfish for compensatory cell growth, the high SGR and morphometric growth were similar to those reported in the present study (Lingam et al. 2019). This finding supports the assumption that nutrient

deprivation in fish affects their morphological traits (Pavlov 2015). Moreover, the morphological traits and growth parameters of SGR and weight gain decreased with limited probiotic inoculation. Similarly, the use of probiotics in insect aquafeeds increased shrimp fish weight and survival, as reflected in this study (> 95%) (Toledo et al. 2019). Probiotics improve fish weight gain and feed utilization indices in addition to intestinal morphological modifications in fish, especially Nile tilapia (Tabassum et al. 2021), increasing protein intake and hence increasing SGR and weight gain, corroborating the findings of this study (Du et al. 2021). Similar to this work, the application of *Tinebrio molitor* in Nile tilapia fish diets, including *Saccharomyces cerevisiae*, did not significantly alter the SGR (Anany et al. 2025). The high SGR and overall weight and performance values may be related to the synthesis of growth-promoting substances such as bacteriocins and other vitamins, particularly vitamin B complex, which improve the fish gut microbiota and function as natural growth promoters. The use of bacteriocins (Pereira et al. 2022) and multivitamins (Asadollahi et al. 2025) as alternatives to antibiotics in aquaculture increases fish growth and overall fish morphometric traits, as seen in our study. In homogenous fish rearing environments, morphological variation decreases with somatic growth (Lorena et al. 2023). In contrast, our study revealed increased variability in the morphometry of fish with somatic growth. This could be attributed to the variation in probiotic inoculation levels, which improved nutrient availability, leading to increased growth of various morphometric traits. The addition of the appropriate amount of probiotics should improve fish growth and morphometric trait performance. The addition of undesirable probiotic inoculation levels in basal diets for fish growth can lead to low growth rates and yields coupled with low survival rates, as reflected in the rearing of *Clarias gariepinus* (Hadijah et al. 2024). In our study, although T3 had a greater quantity than did T2, it was outperformed by the inoculation level T2. These findings suggest that the dosage for effective performance in Redbreasted tilapia in BSFL fish diets is ideal at the T2 level. The higher growth rate and morphometric traits in the probiotic-fertigated diets could be linked to the high digestive enzyme activities resulting from secretion by the diverse microbiota in the fish gut. The source of a fish can affect overall morphological trait performance. Fish that are usually sourced from the wild for culture do not show variability in morphometric growth (Delfina 2017). The fish utilized in this study were on-farm hatchery-raised fish with indications of morphological trait changes. However, morphometric trait analysis integrates external and internal phenotypic signals that increase the reliability of the findings on fish performance (Kulzer et al. 2026), auditory mechanisms (Robins et al. 2025) and shell morphology in freshwater mussels (Fassatoui et al. 2024). Moreover, the use of various morphometric traits effectively predicts variables such as total length, as used in our study, and regression models increase the number of morphometric traits that predict fish total length (Mathias et al. 2026) and weight (Saleh et al. 2024; Wasso et al. 2025). Several authors acknowledge that morphometric traits such as body depth and eye size, as reflected in our study, indicate significant changes in fish morphological changes and can also predict fish growth parameters such as weight gain (Third & Parsons 2024). Although geometric morphology can help in assessing homogeneity in morphological features (Binashikhbubkr et al. 2024), heterogeneity in fish morphometric traits across probiotic inoculation levels is notable. While our study revealed significant changes in morphometric traits and growth, some studies have shown contrasting results (no significant differences) in morphometric trait changes and fish growth, as indicated by Long (2026). These differences could be

attributed to the addition of probiotic inoculation to the feed used. Various morphometric traits are affected differently when fish are subjected to different rearing conditions and diets. For example, in our study, the extracted traits of operculum length, distance between eyes, eye diameter, and total length contrasted with body depth and snout length in a study conducted by Majeed et al. (2026).

Conclusion

The results of the present study provide evidence that the morphometric traits and selected growth parameters of Redbreatsed tilapia are influenced by the addition of probiotics to black soldier fly meal diets. This study highlights the novelty of the use of selected probiotics and black soldier flies and how they improve fish morphometric traits, growth, and their relationships. The study further revealed that morphometric and selected growth parameters improved with increased probiotic addition. This effect was most pronounced in the T2 probiotic inoculation treatment. This study revealed that morphometric traits and selected growth parameters were integrative and influenced when probiotics were added to black soldier fly diets. The use of morphometric variation to determine fish fitness and performance is recommended when commercial probiotics at the right inoculation levels are used.

Declarations

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Competing Interests

The authors have non-financial interests to disclose.

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Author Contribution

T.Z. Conceptualized and conducted the research, performed data analysis and visualization, provided the software, and wrote the main manuscript text. E.C., K.M. and J.K. supervised, wrote and reviewed the

manuscript. G.L.A., P.A., I.B., P.S.K and N.N. wrote the manuscript. All authors reviewed the manuscript.

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Data Availability

The data are available upon request.

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Figures

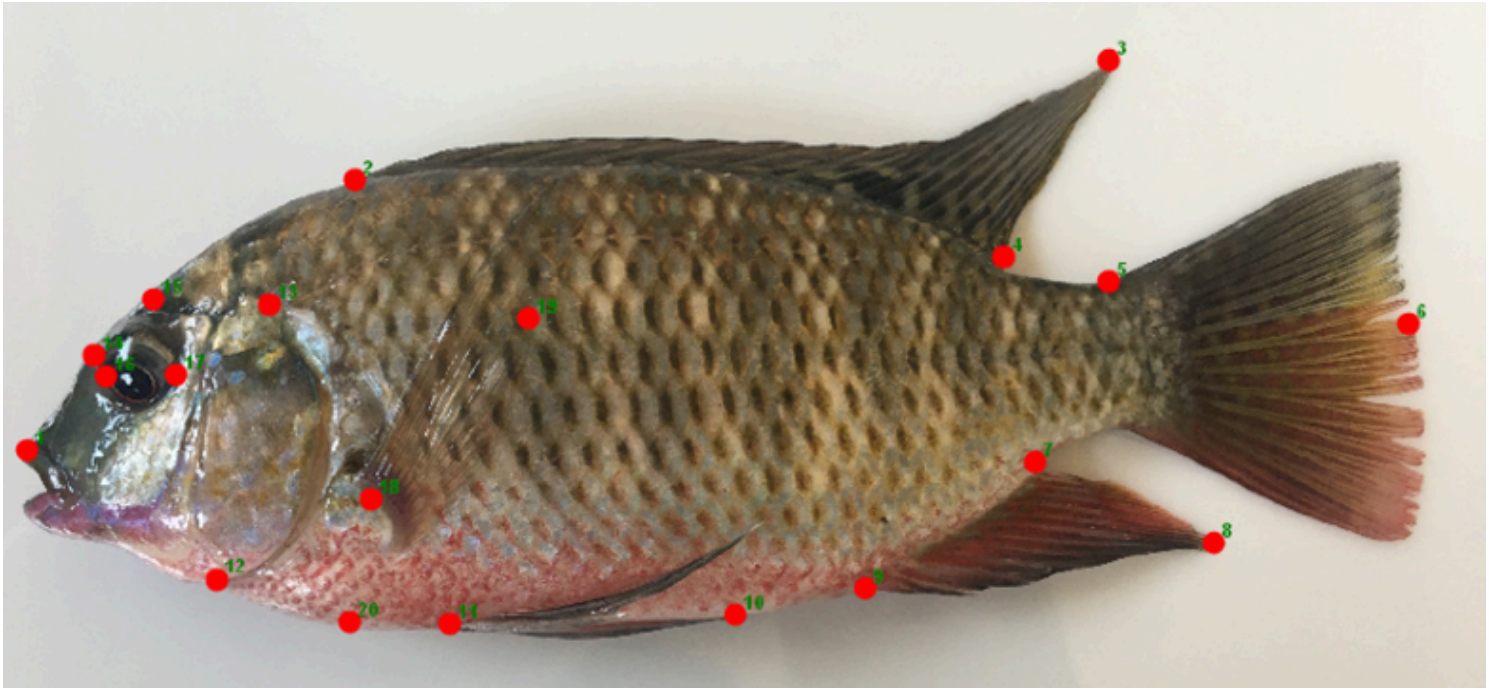


Figure 1

Positions of the biological landmarks (red dots) of Redbreastedtilapia fingerlings taken from inoculation level T3. The landmarks were generated from TpsDig2 v2.31 software.

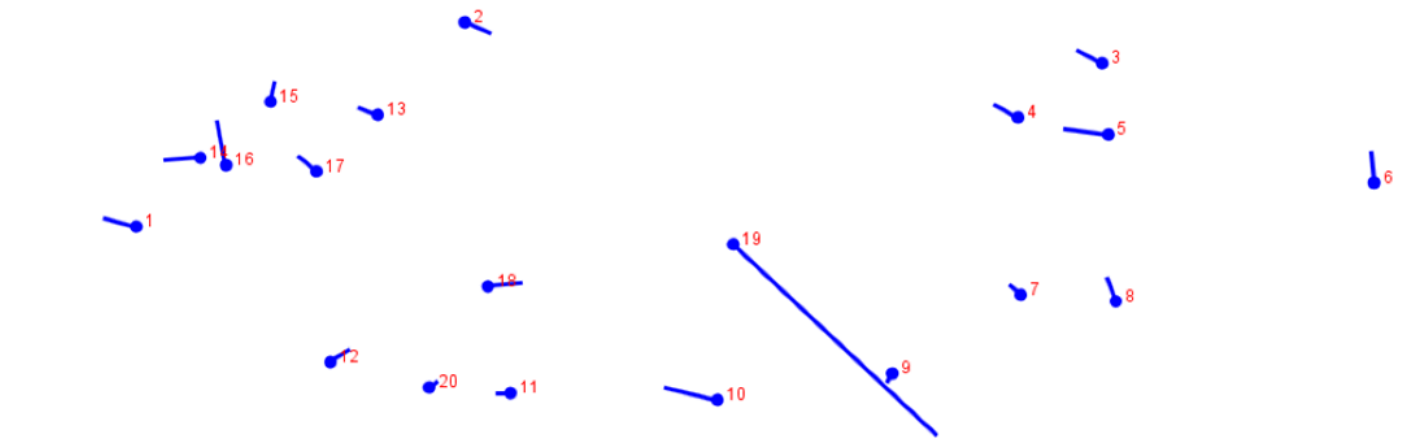


Figure 2

Body shape variation across PC 1 covariance matrix scores

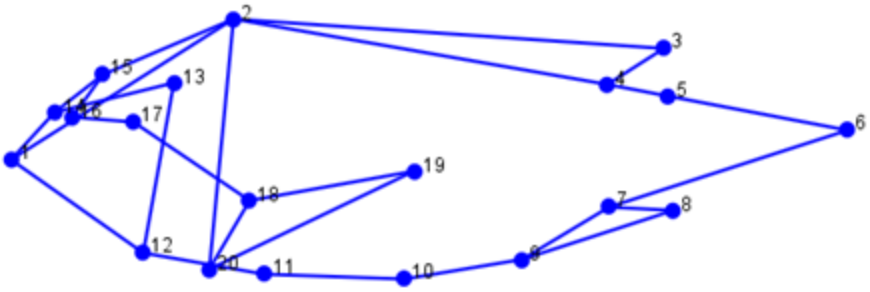


Figure 3

A truss network of interconnected morphometric trait distances of Redbreasted tilapia

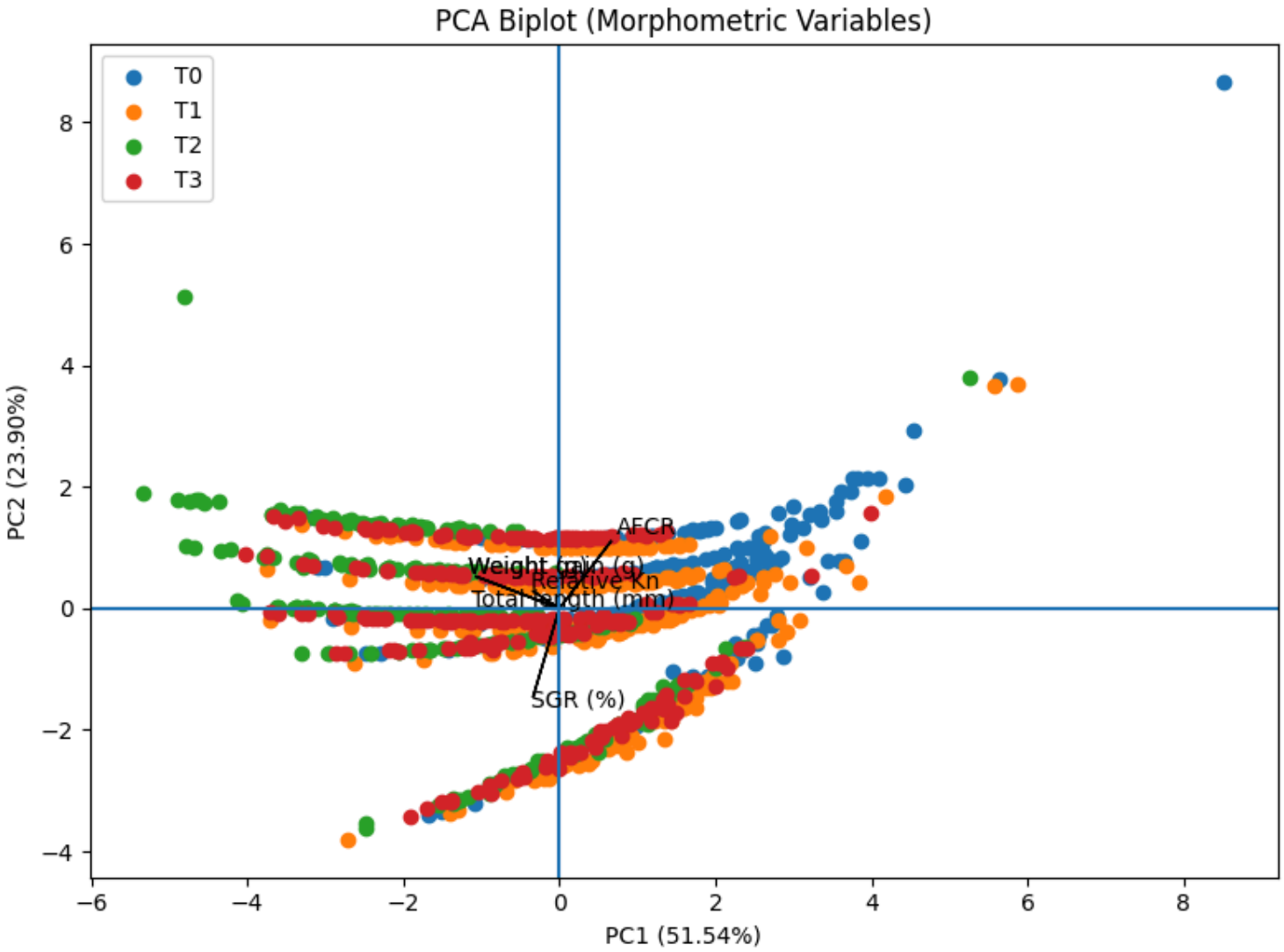


Figure 4

PCA biplot for growth parameter scores

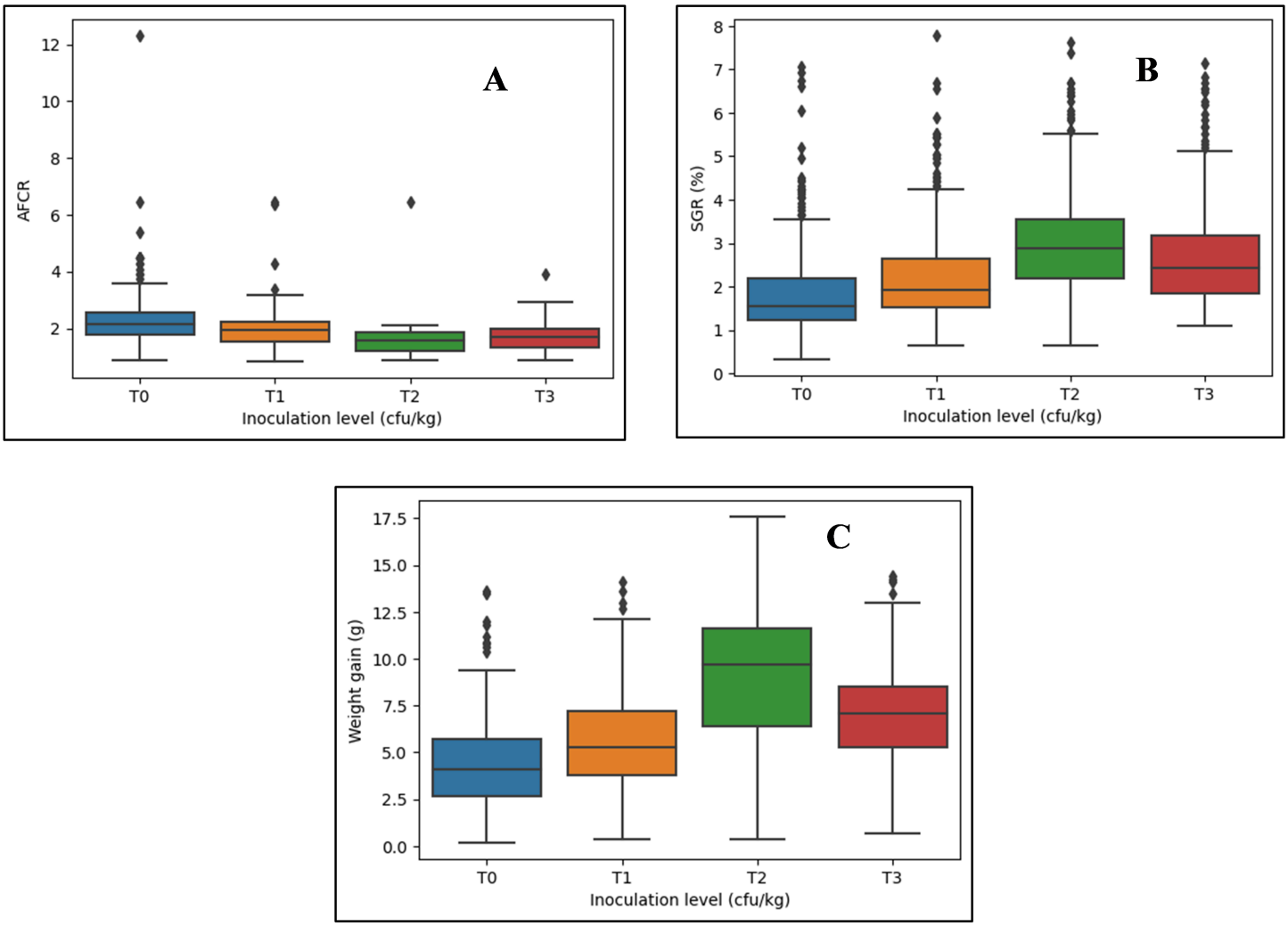


Figure 5

Boxplots for growth parameters (mean ± SE). **A** is the AFCR, **B** is the SGR, and **C** is weight gain.

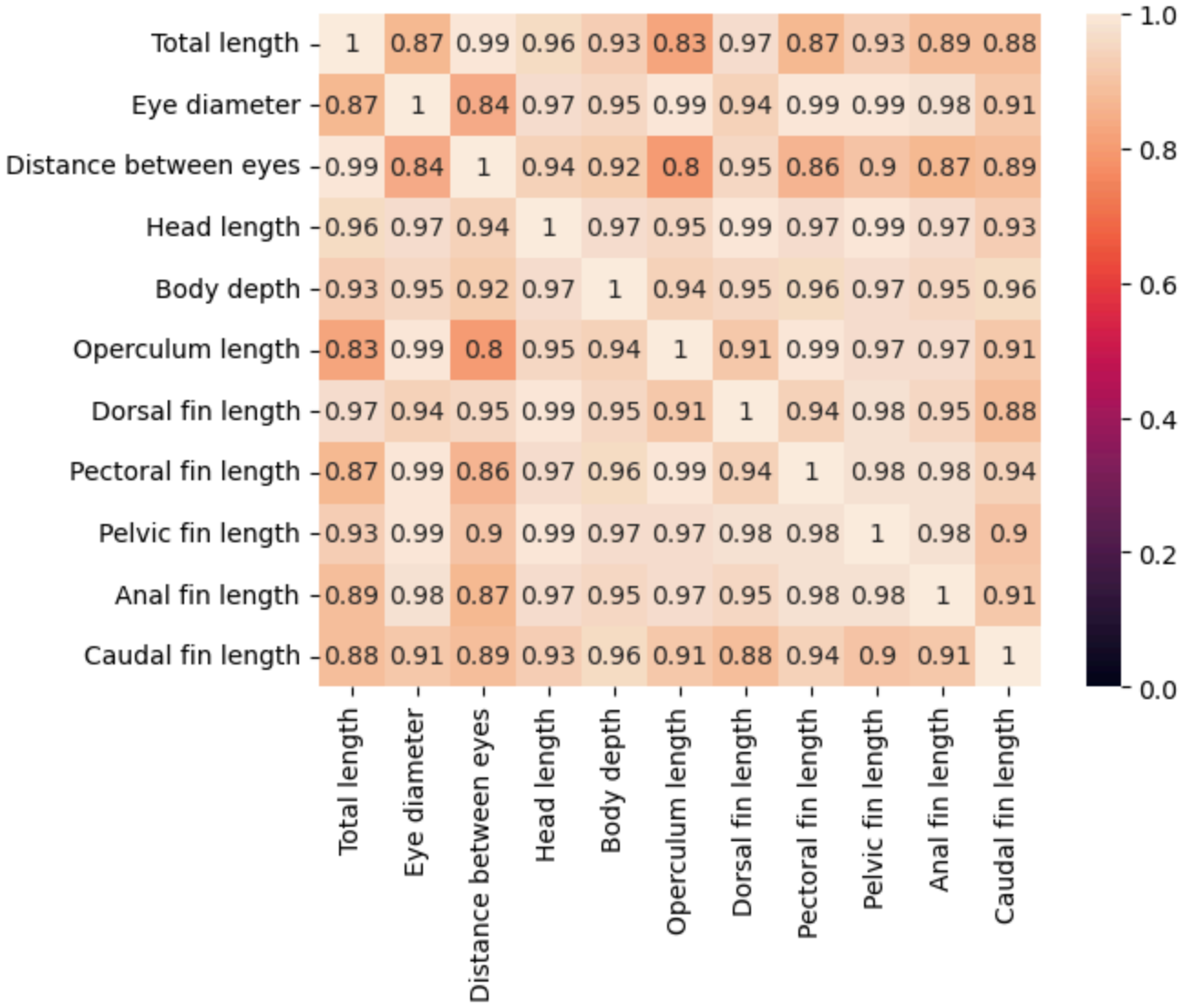


Figure 6

Correlogram heatmap for Coptodon rendalli morphometric traits

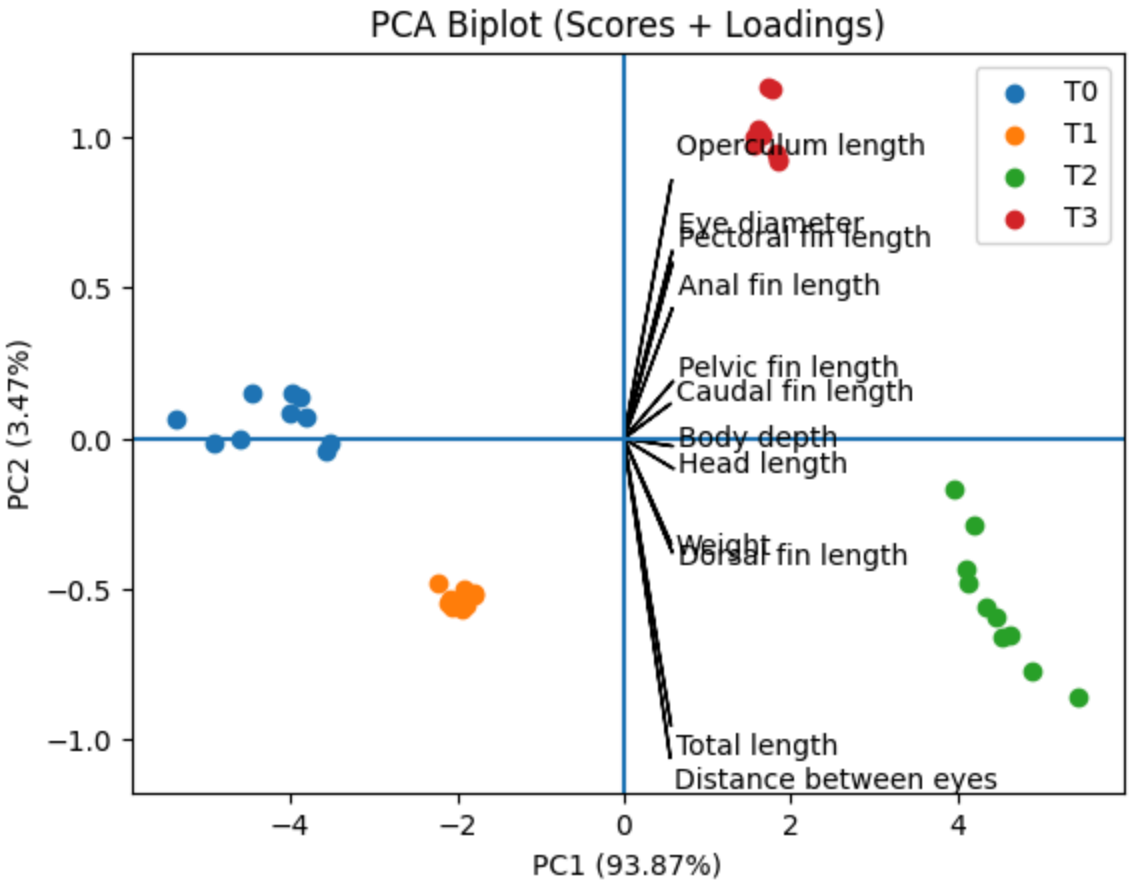


Figure 7

PCA biplot of scores and morphometric loadings

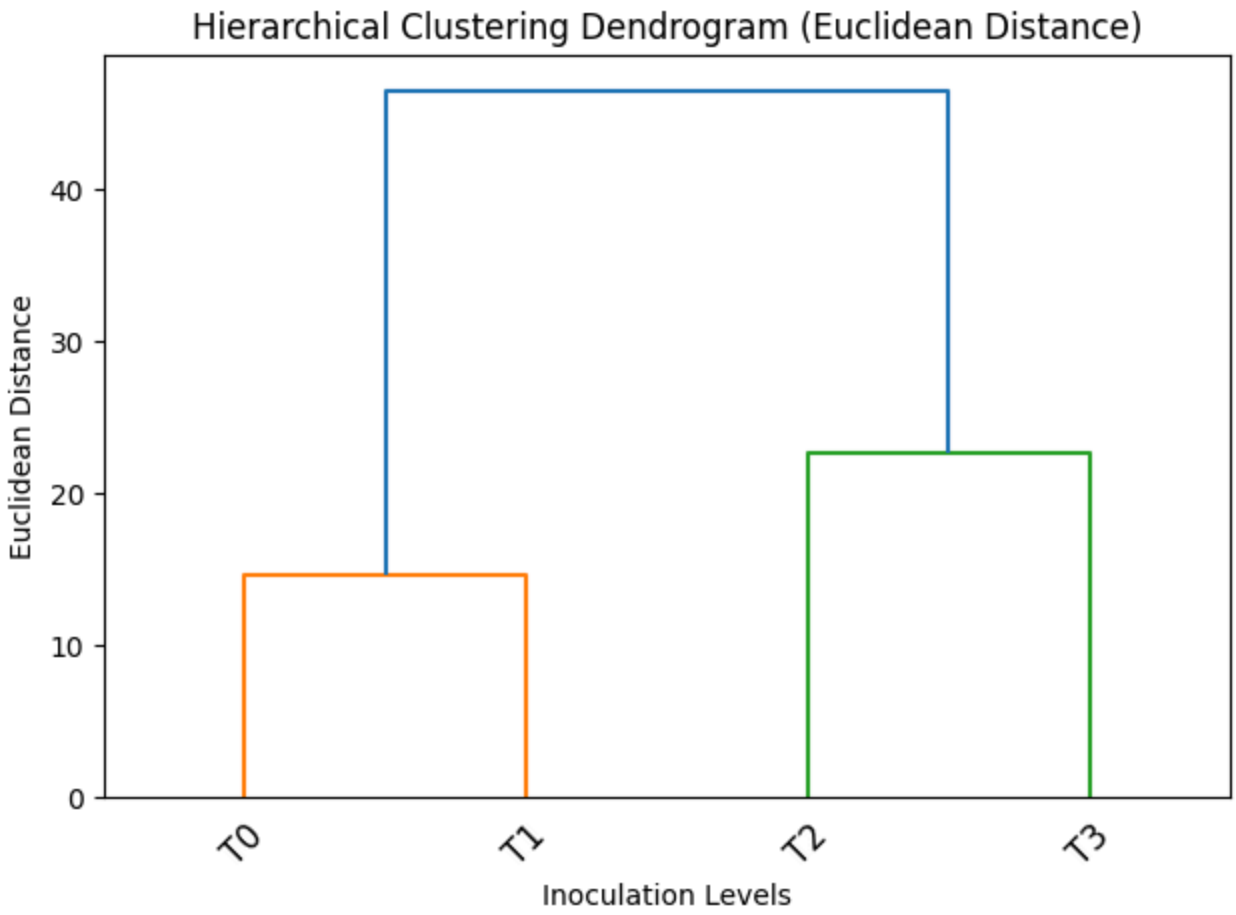


Figure 8

Hierarchical clustering dendrogram with Euclidean distances

Supplementary Files

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- image1.png