

## Article

# Morphometric, Nutritional, and Blood Analyses in Hybrid Striped Bass (*Morone chrysops* x *Morone saxatilis*, Walbaum 1972) Reared in a Recirculating Aquaculture System (RAS) Implant in Sicily, Italy

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**Abstract:** Hybrid striped bass (HSB), a cross between white bass (*Morone chrysops*) and striped bass (*Morone saxatilis*), has garnered attention in aquaculture due to its adaptability, rapid growth, and high market value. This study investigates the morphometric, nutritional, and blood characteristics of HSB reared in a recirculating aquaculture system (RAS) in Sicily, Italy, over a 22-month grow-out period. The fish were managed under standardized feeding and water quality protocols, with weekly monitoring of the physicochemical parameters. A total of 21 clinically healthy fish, averaging  $571.33 \pm 129.32$  in body weight, were randomly sampled in the spring season from a commercial RAS facility equipped with biological filtration, UV sterilization, and seasonally regulated water parameters. The results revealed strong positive correlations between the morphometric parameters and blood indices, such as red blood cell (RBC) count, hemoglobin (Hb) levels, and hematocrit (Hct), highlighting their importance as health indicators. The proximate composition revealed an average moisture content of  $75.55 \pm 1.49$ , crude protein at  $20.29 \pm 0.26$ , total lipid at  $4.25 \pm 0.97$ , and ash content at  $1.69 \pm 0.17$ . Additionally, statistical analyses, including a principal component analysis (PCA), identified relationships between body size, nutritional content, and blood parameters, emphasizing the role of body size in influencing nutritional and health outcomes. The findings of this study are crucial for optimizing farming protocols and improving the health and productivity of HSB in RAS under Mediterranean conditions.

**Keywords:** nutrition; hematology; hybrid striped bass; recirculating aquaculture systems; morphometry

**Key Contribution:** This study presents a comprehensive assessment of hybrid striped bass reared in a Mediterranean recirculating aquaculture system, highlighting their physiological and nutritional profiles. The strong interlink between morphometrics, blood health markers, and tissue composition offers a novel approach to monitoring and enhancing fish performance in intensive aquaculture.



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## 1. Introduction

Hybrid striped bass (HSB), a crossbreed between white bass (*Morone chrysops*) and striped bass (*Morone saxatilis*), has gained significant interest in aquaculture due to its rapid growth, adaptability to intensive culture systems, and high market value [1]. The species has been successfully farmed in various aquaculture setups, including recirculating aquaculture systems (RASs), which offer a sustainable approach by optimizing water use, maintaining controlled environmental conditions, and reducing the risks associated with open-water farming [2,3]. RAS technology has been widely implemented in commercial aquaculture in the Mediterranean region, particularly in Italy, to enhance the sustainable production of fish species such as European seabass, gilthead seabream, and, recently, hybrid striped bass [4,5]. However, limited research has been conducted on the morphometric, nutritional, and blood profile variations of HSB cultured in RAS, particularly under Mediterranean climatic conditions. Evaluating these parameters is essential for optimizing growth performance, assessing health status, and ensuring high-quality fish production.

Morphometric analyses serve as a fundamental tool for assessing growth patterns, body condition, and overall fish health in aquaculture systems [6]. Morphometric measurements provide insights into fish growth efficiency and can serve as a reliable indicator of environmental or nutritional influences on fish physiology [7]. Similarly, a nutritional composition analysis, including proximate composition (protein, lipid, moisture, and ash content), is crucial for determining the dietary adequacy and flesh quality of cultured fish and other animals [8,9]. The biochemical composition of fish fillets influences consumer acceptance and market value, making it a vital aspect of aquaculture research [10]. Furthermore, a blood analysis is a widely used diagnostic tool for evaluating animal health status and physiological responses to farming conditions [11,12]. Hematological and biochemical parameters reflect the metabolic activity, stress levels, and immune competence of fish, which are critical factors in intensive aquaculture [13].

Despite the increasing adoption of RAS for fish farming, research gaps remain in understanding how this system influences the physiological and nutritional characteristics of HSB. Most existing studies on HSB have focused on open-water or pond-based aquaculture systems, leaving a significant knowledge gap regarding its performance in RAS. Additionally, while morphometric and hematological assessments have been extensively conducted for other aquaculture species, there is limited comprehensive data on these parameters for HSB grown in controlled environments like RAS, particularly in the Mediterranean region. Addressing these gaps is crucial for improving farming protocols, ensuring optimal fish health, and maximizing production efficiency. The primary objective of this study was to evaluate the morphometric characteristics, nutritional composition, and blood parameters of hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) reared in a recirculating aquaculture system in Sicily, Italy.

## 2. Materials and Methods

### 2.1. Study Design

The guidelines suggested by the Guide for the Care and Use of Laboratory Animals and Directive 2010/63/EU for animal research were followed in the review and approval of animal husbandry and experimentation protocols. The HSB used in this study were reared in an RAS under controlled environmental conditions. Fish were randomly selected for sampling. A total of 21 HSB specimens, with an average body weight of 570 g, were analyzed. All fish were deemed healthy after their general condition and health state were assessed by an exterior examination to find any anomalies or infestations. An RAS with 22 tanks (8 m in diameter and 60 m<sup>3</sup> in capacity) was used to breed fish from a Sicilian farm in Acate (Ragusa, Sicily, Italy). An antibacterial UV system (200–280 nm) and biological and

drum filters were used to filter the water. The fish were reared in an RAS for 22 months before blood sampling. Blood samples were collected during the spring season. The water quality parameters were monitored regularly throughout the experimental period to ensure optimal rearing conditions. Specifically, key parameters such as temperature, dissolved oxygen, pH, ammonia, nitrite, and nitrate were measured on a weekly basis using standard procedures. Any deviations from the acceptable ranges were promptly corrected. The average values of water quality parameters are presented in Table 1. The fish were fed VERONESI BASIC 6G, a commercial feed that contains 42% protein, 18% fat, 3.2% fiber, 9% ash, 1.4% phosphorus, 150 mg/kg of vitamin C, and 150 mg/kg of vitamin E twice a day (morning and evening). First, 0.6 mL L<sup>-1</sup> of phenoxyethanol (2-PE) was used to anesthetize the fish [14]. A balance (Kern 440–49 N, Balingen, Germany) was used to weigh each fish separately as soon as they were anesthetized, and an ictiometer (Scubla SNC, 600 mm, Remanzacco, Italy) was used to measure their overall length. Each fish had its caudal vein punctured using a sterile 2.5 mL plastic syringe fitted with a 22-gauge needle to collect blood samples between 08:00 and 09:00 in the morning, following a fasting period (24 h). For the evaluation of the hematological analysis, each blood sample was placed in triplicate into microtubes containing ethylenediamine tetraacetic acid (EDTA) (ratio of 1.26 mg/06 mL) (Miniplast 0.6 mL; LP Italiana Spa, Milano, Italy) as an anticoagulant agent. Each analysis was completed within five hours of the blood being drawn. The guidelines suggested by the Guide for the Care and Use of Laboratory Animals and Directive 2010/63/EU for animal research were followed in the review and approval of animal husbandry and experimentation protocols.

**Table 1.** Water quality parameters for striped bass (*M. saxatilis*) in a recirculating aquaculture system (RAS).

| Parameters             | Mean ± SD   | Min–Max   |
|------------------------|-------------|-----------|
| T (°C)                 | 23.5 ± 1.2  | 21.0–25.0 |
| DO (mg/L)              | 6.2 ± 0.4   | 5.5–7.0   |
| pH                     | 7.1 ± 0.2   | 6.8–7.4   |
| NH <sub>3</sub> (mg/L) | 0.07 ± 0.02 | 0.03–0.10 |
| NO <sub>2</sub> (mg/L) | 0.18 ± 0.04 | 0.10–0.22 |
| NO <sub>3</sub> (mg/L) | 190 ± 20    | 160–210   |
| CTD (µS/cm)            | 1450 ± 300  | 400–2000  |

Note: T (temperature); DO (dissolved oxygen); pH; NH<sub>3</sub> (ammonia); NO<sub>2</sub> (nitrite); NO<sub>3</sub> (nitrate); CTD (conductivity).

## 2.2. Morphometric and Biometric Measurements

The morphometric indices, including total length, were measured from the tip of the snout to the longest caudal fin ray using a measuring tape. The standard length was determined as the distance from the tip of the snout to the posterior margin of the hypural plate, excluding the caudal fin. Head length was measured from the tip of the snout to the posterior edge of the operculum. This measurement was taken using a digital caliper. The maximum height of the fish was measured as the greatest vertical distance from the dorsal to the ventral side of the body, typically at the dorsal fin base. A digital caliper was used for precise measurements. The measurements were recorded in centimeters (cm). The biometric measurements, such as the total weight of each fish, were recorded to the nearest gram (g) using a digital weighing balance. The eviscerated weight was determined after removing all internal organs, including the gastrointestinal tract, liver, and gonads. The weight was measured using a digital weighing balance. The liver was carefully excised and weighed separately using a digital weighing balance.

The condition factor (*K*) was calculated to assess the overall health and well-being of the fish using the following formula:

$$K = \left( TW / SL^3 \right) \times 100$$

where  $TW$  is the total weight (g), and  $SL$  is the standard length (cm).

The cranial index ( $CI$ ) was determined to describe the proportional size of the head relative to the standard length using the following formula:

$$CI = (HL/SL) \times 100$$

where  $HL$  is the head length, and  $SL$  is the standard length.

The relative profile ( $RP$ ) was calculated to determine the body shape index using the following formula:

$$RP = (MH/SL) \times 100$$

where  $MH$  is the maximum height, and  $SL$  is the standard length.

The hepatosomatic index ( $HSI$ ) was calculated to evaluate the liver size relative to the total body weight using the following formula:

$$HSI = (LW/TW) \times 100$$

where  $LW$  is the liver weight, and  $TW$  is the total weight.

The carcass yield ( $CY$ ) was calculated as the percentage of the eviscerated weight relative to the total weight using the following equation:

$$CY = (EW/TW) \times 100$$

where  $EW$  is the eviscerated weight, and  $TW$  is the total weight.

### 2.3. Automatic Hematological Analysis

Immediately following blood collection, the hemogram was evaluated automatically using the blood cell counter HeCo Vet C (SEAC, Florence, Italy). This device makes use of a unique fish lysing reagent (SEAC, Code 71010460) that has been used in the past to examine the hematological profile of this species [15], as well as other fish species [16–19]. The same operator performed triple analyses on each blood sample. All samples underwent a manual hematological examination to confirm the accuracy of the automated approach.

### 2.4. Proximate Body Composition

The proximate composition of fish, including moisture content, protein concentration, lipid content, and ash content, was analyzed following the standard protocols outlined by the Association of Official Analytical Chemists [20]. All measurements were conducted in triplicate to assess the nutritional variations across different seasons. For fish of larger size (~570 g/fish), the samples were handled carefully to maintain consistency and representativeness. Each fish was thoroughly rinsed with distilled water and euthanized humanely. The scales and internal organs were carefully removed. To obtain fillet samples, the fish were placed on a clean cutting board, and fillets were sliced lengthwise on both sides of the backbone using a sharp knife. Special attention was paid to extracting the maximum amount of muscle tissue while minimizing contamination with bone, skin, or other tissues. The fillets were then homogenized to ensure uniformity before a proximate analysis.

### 2.5. Statistical Analysis

All data, including the morphometric, biometric, hematological, and proximate composition parameters, were analyzed by calculating the mean and standard deviation (SD) using Microsoft Excel 2016. A correlation analysis between the parameters was performed using GraphPad Prism (version 7.0). Additionally, a principal component analysis (PCA)

was conducted in GraphPad Prism to identify patterns and relationships among the studied parameters. Statistical significance was considered at  $p < 0.05$ .

### 3. Results

#### 3.1. Morphometric and Body Indices

Table 2 presents various morphometric and body parameters of HSB reared in RAS. The morphometric measurements indicate an average total length of 37.50 cm and a standard length of 28.33 cm, with a head length of 9.86 cm and a maximum body height of 8.62 cm. The fish have an average total weight of 571.33 g, while the eviscerated weight averages 495.05 g, and the liver weight varies between 6 g and 18 g, averaging 10.76 g. The condition factor was recorded as 0.149, while the cranial index and relative profile were 0.261 and 0.228, respectively. The hepatosomatic index was measured at 1.845, and the carcass yield was 86.68%.

**Table 2.** Morphometric and body condition indices of hybrid striped bass (HSB) reared in a recirculating aquaculture system (RAS).

| Parameters                | Mean $\pm$ SD       | Min    | Max    |
|---------------------------|---------------------|--------|--------|
| Total length (cm)         | 37.50 $\pm$ 2.57    | 33     | 42.5   |
| Standard length (cm)      | 28.33 $\pm$ 1.04    | 27.5   | 36.5   |
| Head length (cm)          | 9.86 $\pm$ 0.87     | 8      | 11.5   |
| Max. height (cm)          | 8.62 $\pm$ 0.82     | 7      | 10     |
| Total weight (g)          | 571.33 $\pm$ 129.32 | 300    | 802    |
| Eviscerated weight (g)    | 495.05 $\pm$ 109.94 | 268    | 692    |
| Liver weight (g)          | 10.76 $\pm$ 3.87    | 6      | 18     |
| Condition factor (K)      | 0.149 $\pm$ 0.027   | 0.090  | 0.190  |
| Cranial index (CI)        | 0.261 $\pm$ 0.011   | 0.240  | 0.280  |
| Relative profile (RP)     | 0.228 $\pm$ 0.012   | 0.210  | 0.260  |
| Hepatosomatic index (HSI) | 1.845 $\pm$ 0.433   | 1.100  | 2.900  |
| Carcass yield (CY)        | 86.680 $\pm$ 1.461  | 83.600 | 89.300 |

#### 3.2. Hematological and Proximate Body Composition

The hematological parameters (Table 3), including the white blood cell (WBC) count, was 28.82, while the red blood cell (RBC) count was 3.16. Hemoglobin (Hb) levels averaged 7.54 g/dL, and the hematocrit (Hct) percentage was 45.40%, reflecting the oxygen-carrying capacity of the blood. The values for other blood indices were as follows: mean corpuscular volume (MCV) was 145.38 fL, mean corpuscular hemoglobin (MCH) was 24.03 Pg, and mean corpuscular hemoglobin concentration (MCHC) was 17.22%. The proximate body composition of HSB provides essential insights into their nutritional status (Table 4). The moisture content averaged 75.55%. The crude protein was 20.29%. The total lipid content was 4.25%, and, lastly, the ash content was 1.69%.

**Table 3.** Hematological parameters of hybrid striped bass (HSB) reared in a recirculating aquaculture system (RAS).

| Parameters                          | Mean $\pm$ SD      | Min  | Max  |
|-------------------------------------|--------------------|------|------|
| WBC ( $\times 10^3 / \mu\text{L}$ ) | 28.82 $\pm$ 4.93   | 20.5 | 36.4 |
| RBC ( $\times 10^6 / \mu\text{L}$ ) | 3.16 $\pm$ 0.28    | 2.46 | 3.8  |
| Hb (g/dL)                           | 7.54 $\pm$ 0.85    | 5.7  | 9    |
| Hct (%)                             | 45.40 $\pm$ 4.81   | 34.1 | 52.3 |
| MCV (fL)                            | 145.38 $\pm$ 12.44 | 109  | 160  |
| MCH (Pg)                            | 24.03 $\pm$ 2.25   | 20.6 | 28   |
| MCHC (%)                            | 17.22 $\pm$ 3.22   | 13.9 | 28   |

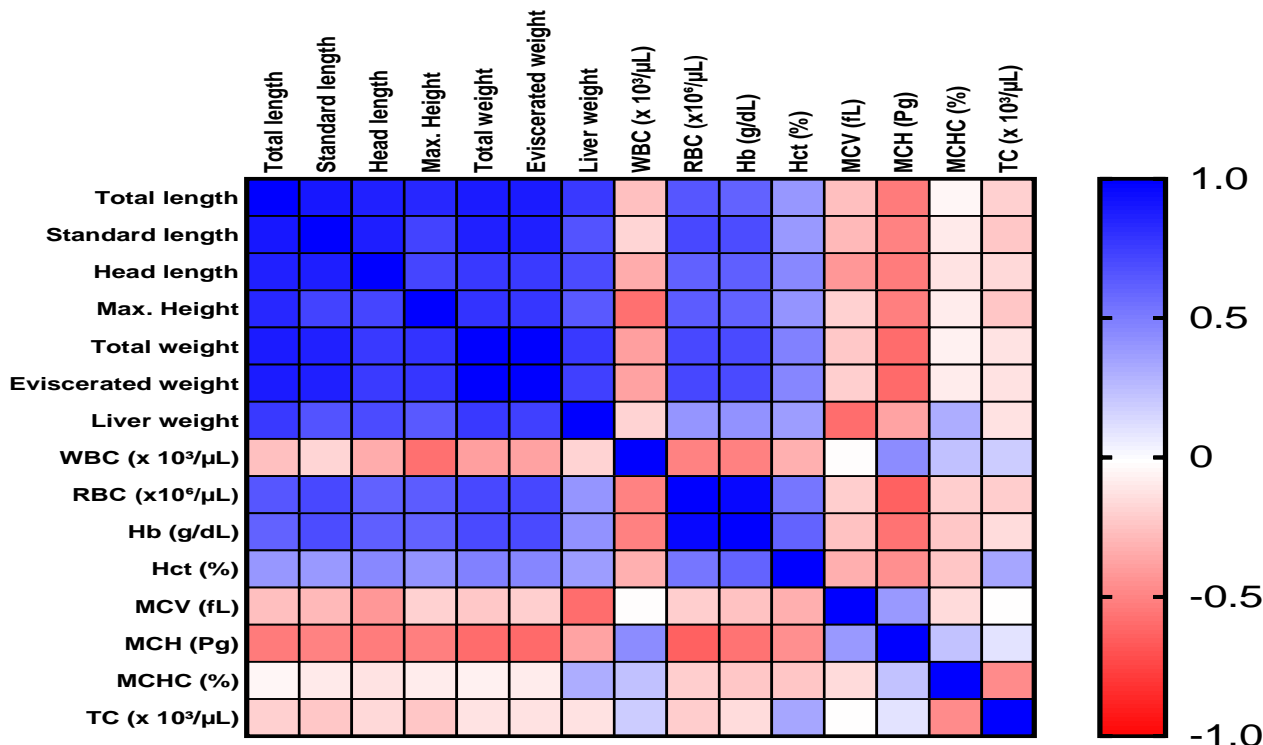
Note: white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

**Table 4.** Proximate body composition of hybrid striped bass (HSB) reared in a recirculating aquaculture system (RAS).

| Parameters           | Mean ± SD    | Min   | Max   |
|----------------------|--------------|-------|-------|
| Moisture content (%) | 75.55 ± 1.49 | 73.31 | 77.44 |
| Crude protein (%)    | 20.29 ± 0.26 | 19.75 | 20.78 |
| Total lipid (%)      | 4.24 ± 0.97  | 2.73  | 5.66  |
| Ash content (%)      | 1.69 ± 0.17  | 1.33  | 1.97  |

3.3. Correlation Between Morphometric Measurements and Blood Parameters in HSB

The correlation heatmap (Figure 1) illustrates the relationships between the body morphometric measurements and the blood parameters in HSB. The morphometric parameters, such as total length, standard length, head length, maximum height, total weight, and eviscerated weight, exhibit strong positive correlations among themselves, as seen by the deep blue shading. Liver weight, although positively correlated with body size, shows a weaker association compared to other morphometric traits. Among the blood parameters, RBC, Hb, and Hct are positively correlated with each other, indicating that fish with higher RBC counts also tend to have higher Hb levels and Hct values. However, MCV, MCH, and MCHC show a negative correlation with RBC count, suggesting that fish with higher RBC counts have smaller and less hemoglobin-dense red blood cells. Interestingly, the WBC and TC show weak correlations with body morphometric traits but exhibit a slight negative correlation with RBC count, which may suggest an inverse relationship between immune response and red blood cell abundance in this species. Overall, the heatmap highlights strong correlations within the morphometric parameters and within the hematological parameters but limited interactions between these two groups, except for a few moderate correlations between liver weight and blood parameters.



**Figure 1.** Correlation heatmap of body morphometric and blood parameters in hybrid striped bass (HSB). Blue indicates positive correlations, while red represents negative correlations, with darker colors signifying stronger relationships. Abbreviations: White Blood Cells (WBC); Red Blood Cells (RBC); Hemoglobin (Hb); Hematocrit (Hct); Mean Corpuscular Volume (MCV); Mean Corpuscular Hemoglobin (MCH); Mean Corpuscular Hemoglobin Concentration (MCHC); Total Count (TC).

### 3.4. Correlation Matrix of Morphometric Traits and Proximate Composition in HSB

Figure 2 displays the correlation matrix between the morphometric traits and the proximate composition parameters in HSB. As expected, body size measurements, such as total length, standard length, head length, maximum height, total weight, and eviscerated weight, are strongly and positively correlated with each other, as shown by the deep blue shades. Liver weight also follows this trend but exhibits a slightly weaker correlation with other morphometric traits. Among the proximate composition parameters, moisture content shows a moderate negative correlation with total lipid content, suggesting that fish with higher moisture levels tend to have lower fat reserves. Additionally, moisture content is weakly negatively correlated with total length, which could indicate a tendency for larger fish to have slightly reduced water content in their muscle tissue. Crude protein content shows no strong correlation with morphometric parameters, suggesting that protein levels remain relatively stable regardless of fish size. However, total lipid content is negatively correlated with body size measurements and strongly negatively correlated with ash content, meaning that fish with higher fat content tend to have lower mineral composition.

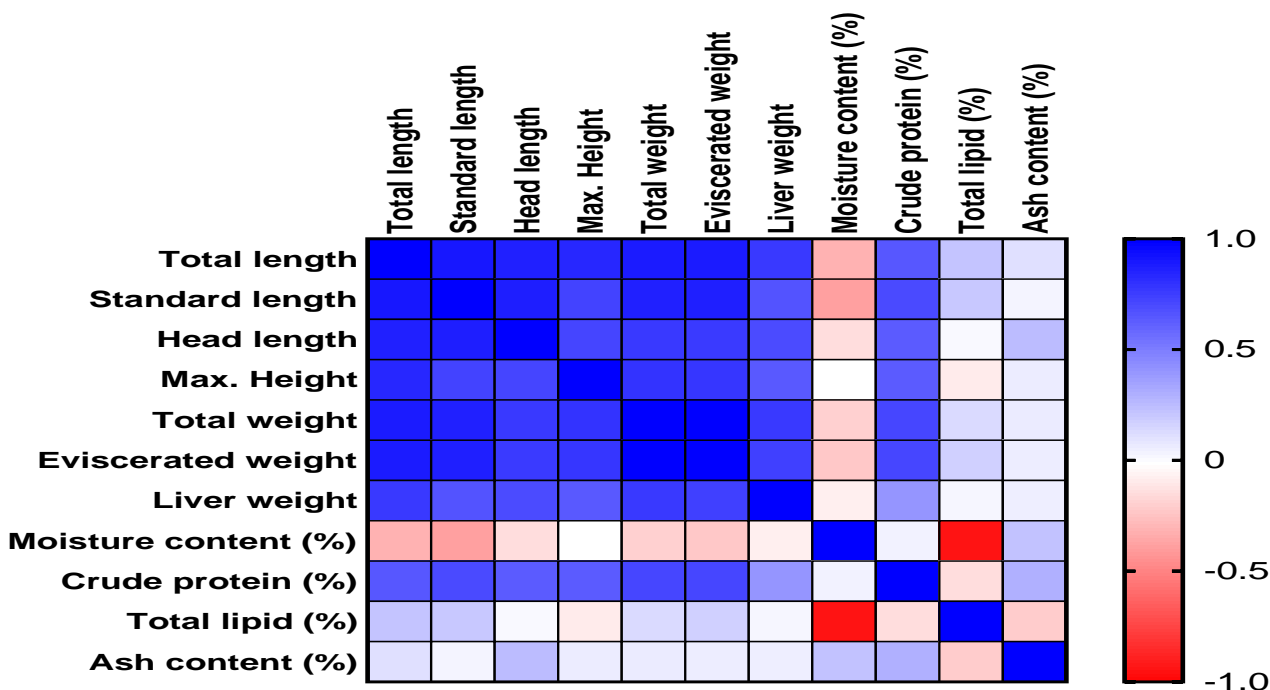


Figure 2. Correlation heatmap of body morphometric and proximate composition parameters in hybrid striped bass (HSB). Strong positive correlations (blue) are observed among morphometric traits, while notable negative associations (red) appear between moisture and lipid content as well as lipid and ash content.

### 3.5. Correlation Matrix of Morphometric Parameters, Condition Indices, and Carcass Yield in HSB

Figure 3 presents the correlation matrix between morphometric parameters, condition indices, and carcass yield in HSB. As observed in previous figures, morphometric traits, including total length, standard length, head length, maximum height, total weight, and eviscerated weight, exhibit strong positive correlations among themselves, reflecting the proportional growth across different body dimensions. Liver weight also follows this trend but with slightly weaker correlations. Among the condition indices, the condition factor (K) shows weak-to-moderate negative correlations with body length and weight parameters, suggesting that larger fish may not necessarily have higher body condition values. The hepatosomatic index (HSI), which reflects liver size relative to total body weight, shows a

weak negative correlation with total length and weight, indicating that larger fish do not always have proportionally larger livers.

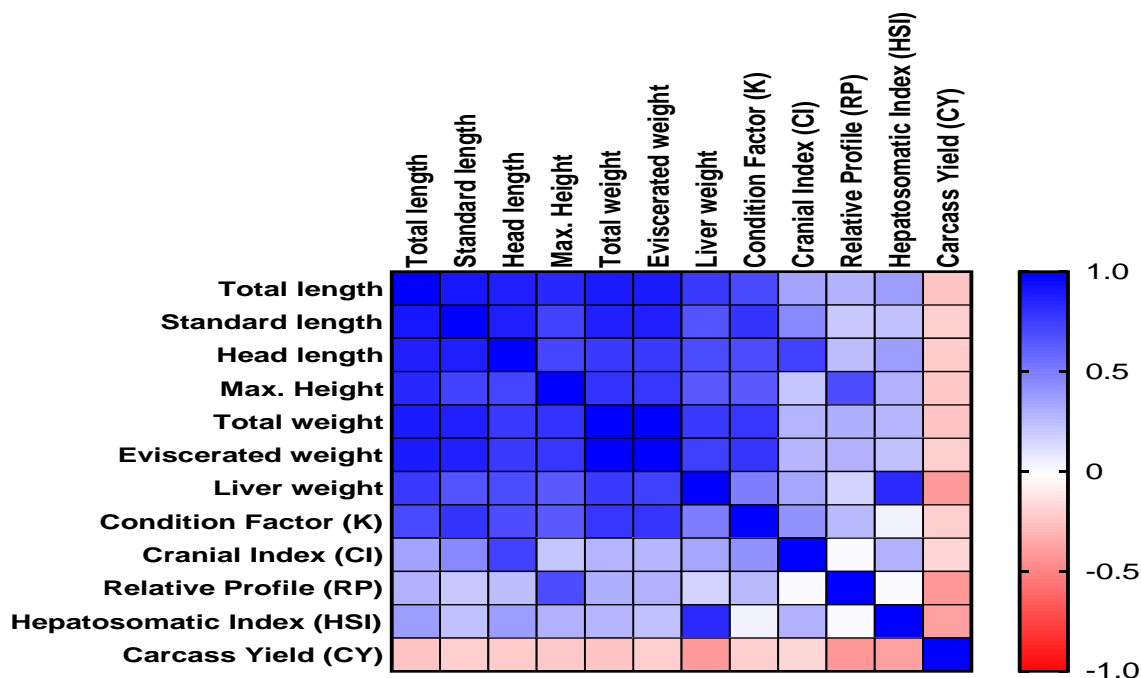


Figure 3. Correlation heatmap of body morphometric parameters, condition indices, and carcass yield in hybrid striped bass (HSB).

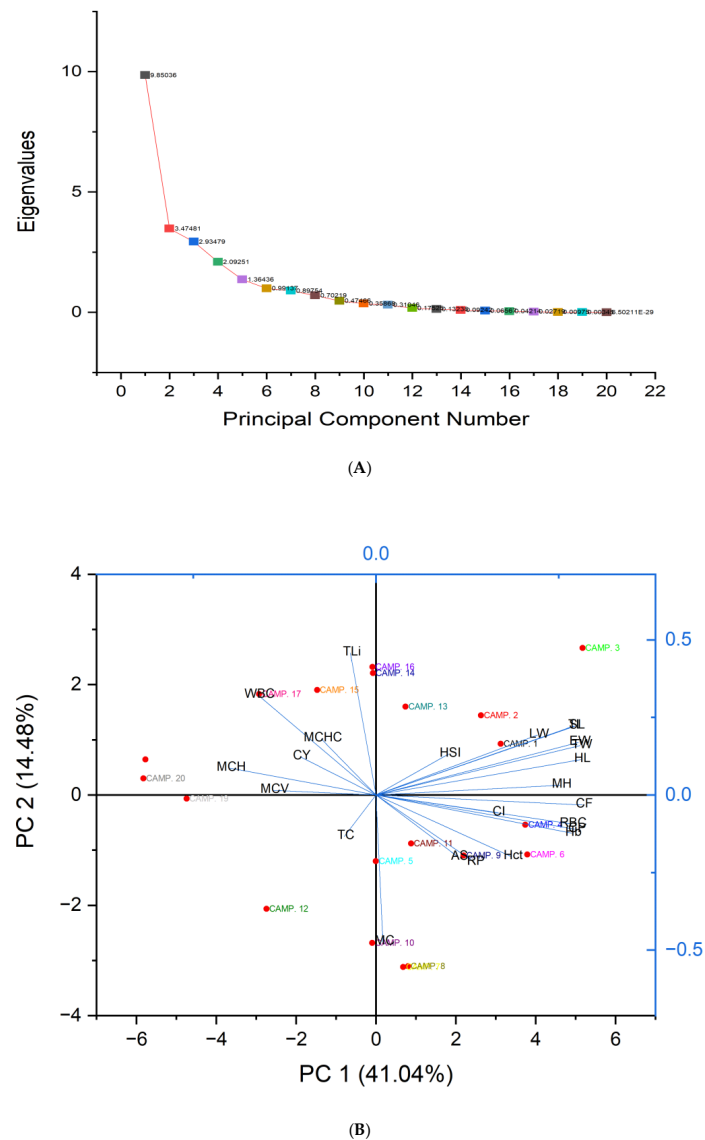
The carcass yield (CY) parameter, which represents the proportion of usable fish meat, exhibits moderate negative correlations with the hepatosomatic index (HSI) and condition factor (K), as well as with standard length and total weight. This suggests that fish with larger livers, higher condition scores, or larger body sizes may have slightly lower carcass yield percentages, potentially due to greater allocation to non-edible components.

### 3.6. PCA Analysis

Figure 4 presents the scree plot and PCA biplot for the body morphometric, hematological, and biochemical parameters of HSB. The scree plot (top panel) illustrates the eigenvalues associated with each principal component (PC), helping to determine the number of significant PCs. The sharp decline in eigenvalues after the first two components (PC1 and PC2) suggests that these two components explain the majority of the variance in the dataset, justifying their selection for further interpretation. Specifically, PC1 accounts for 41.04% of the total variance, while PC2 explains 14.48%, together capturing 55.52% of the total variability in the dataset. The subsequent components contribute progressively less variance, indicating diminishing importance.

The PCA biplot (bottom panel) provides a visual representation of the relationships among the measured variables and their contributions to the principal components. The direction and length of the vectors indicate the strength and contribution of each variable to PC1 and PC2. The morphometric traits, including total length, standard length, maximum height, and total weight, are clustered together and strongly associated with PC1, suggesting that size-related parameters primarily drive the first principal component. Conversely, the hematological parameters, including WBC, RBC, Hb, Hct, and MCHC, exhibit a distinct orientation, indicating that they contribute more to the variance along PC2. Biochemical parameters such as total lipid, moisture content, and ash content also display distinct loadings on the PCA axes, indicating their independent contributions to the overall variability. Interestingly, the total lipid content is positioned opposite to the

moisture content, highlighting a potential inverse relationship between these two factors. Additionally, the carcass yield (CY) vector aligns with PC1 but opposes certain hematological parameters, suggesting a trade-off between body size and some blood characteristics. The condition factor (K) and hepatosomatic index (HSI) occupy intermediate positions, indicating a moderate influence on both PC1 and PC2.



**Figure 4.** Principal component analysis (PCA) of body morphometric, hematological, and biochemical parameters in hybrid striped bass (HSB). The scree plot (A) illustrates the decreasing eigenvalues of principal components, confirming that PC1 and PC2 explain the majority of the variance. The biplot (B) visualizes the relationships between variables, showing that body morphometric traits strongly contribute to PC1, while hematological and biochemical parameters display varied influences along PC2. Abbreviations: Total length (TL); Standard length (SL); Head length (HL); Max. Height (MH); Total weight (TW); Eviscerated weight (EW); Liver weight (LW); White Blood Cells (WBC); Red Blood Cells (RBC); Hemoglobin (Hb); Hematocrit (Hct); Mean Corpuscular Volume (MCV); Mean Corpuscular Hemoglobin (MCH); Mean Corpuscular Hemoglobin Concentration (MCHC); Total Count (TC); Moisture content (MC); Crude protein (CP); Total lipid (Tli); Ash content (AC); Condition Factor (CF); Cranial Index (CI); Relative Profile (RP); Hepatosomatic Index (HSI); Carcass Yield (CY).

#### 4. Discussion

RASs offer a sustainable and efficient method for fish farming by maintaining optimal water quality, reducing negative environmental impact, and enhancing fish health and growth

performance [21]. This study is crucial for advancing sustainable aquaculture by demonstrating the benefits of RASs in optimizing the health and nutritional value of hybrid striped bass. Moreover, the controlled environment of RASs facilitates the precise monitoring and management of key factors, such as water temperature, dissolved oxygen, and nitrogenous wastes, which are critical for minimizing stress and disease outbreaks. The morphometric measurements of HSB in this study demonstrate a strong correlation between environmental conditions and fish growth performance. The observed growth parameters align with previous studies indicating that RASs provide a stable and controlled environment that mitigates external stressors and promotes uniform growth [22,23]. Compared to conventional pond or cage systems, the controlled parameters in RASs, such as temperature, oxygen levels, and water quality, likely contributed to the observed consistency in morphometric traits [24]. Morphometric measurements play a vital role in aquaculture and resource management. Identifying stable morphometric characteristics is essential, as variations in feed and environmental conditions can influence these traits [25]. These measurements also serve as valuable indicators for selective breeding programs, facilitating the identification of desirable phenotypes that optimize growth and health in cultured populations [26–28]. Peter et al. [29] compared pike-perch juvenile production in an exclusive RAS culture versus a pond larviculture followed by RAS transfer. While the pond-nursed fry initially grew slower, they outperformed the RAS-nursed fry post-transfer, though RAS fish later regained superior growth with better feed conversion. Despite lower early survival, RASs demonstrated long-term growth advantages, supporting their viability for pike-perch aquaculture. Another study by Abanikanda et al. [30] investigated sex-based differences in the growth and morphometric traits of *Clarias gariepinus* over nine months, finding that males exhibited higher values for most traits, while females had superior heart girth, growth index, and condition factors. Fish morphometric traits are important biological indicators used to assess the effectiveness of different aquaculture systems [26]. These traits are mainly shaped by environmental conditions such as temperature (Salinas et al., 2019), water quality, and light exposure [31,32]. Additionally, aquaculture factors like diet composition, stocking density [33], and overall farm management, along with genetic influences, including certain genes, also play significant roles [34]. Fish hematology is a powerful diagnostic and monitoring tool that helps researchers, fish farmers, and environmental scientists ensure fish health and sustainability [17]. In our study, the hematological parameters fell within the optimal range for healthy HSB populations, suggesting that RAS conditions effectively reduce physiological stress. The consistency of hematological indices further underscores the potential of RASs to maintain fish welfare, which is a key factor in sustainable aquaculture production. Several studies have investigated fish hematology under different culture environments. For instance, Badran et al. [35] examined the hematological responses of flathead grey mullet in various culture systems, reporting that RASs led to higher Hb levels and WBC counts, indicating improved fish health. Similarly, Hisano et al. [36] compared the hematological profiles of Nile tilapia fingerlings reared in biofloc technology (BFT) and an RAS over 60 days, finding no significant differences ( $p > 0.05$ ) between the two systems. This suggests that both BFT and RAS provide comparable physiological conditions for fish [37]. Additionally, Tabarrok et al. [38] observed no significant variations in hematocrit, hemoglobin, albumin, total protein, and total cholesterol among common carp reared in different aquaculture systems. Studying the proximate composition of fish is essential for understanding its nutritional value, including moisture, protein, fat, and ash contents. This analysis helps evaluate fish quality, optimize feed formulations, and assess the impact of environmental and dietary factors on fish health and growth [8]. In the current study, HSB exhibited a moisture content of 75.55%, a crude protein of 20.29%, a total lipid content of 4.25%, and an ash content of 1.69%, reflecting its nutritional composition and quality. The

proximate composition of fish varies widely across species, shaped by a combination of internal factors and external environmental influences. Different studies were conducted on the proximate composition of fish reared in RASs. For example, Zafar et al. [39] found that higher stocking densities negatively affected the proximate composition of Nile tilapia, with significantly lower crude lipid and carbohydrate contents in the fish. This indicates that increased biomass can influence the nutritional quality of fish, highlighting the importance of optimal stocking density in RAS. Barbacariu et al. [40] compared European catfish reared in RASs and earthen ponds, finding no significant growth differences but higher fat content and distinct intestinal microbiota in RAS fish. This current study sample size (21 fish) limits the statistical power and wider applicability. It was conducted only in spring, so seasonal variations were not assessed. Additionally, fish sex was not distinguished, which may overlook sex-related differences. Future research should use larger, seasonally varied samples with sex differentiation to improve understanding and management.

## 5. Conclusions

This study provides valuable insights into the morphometric, nutritional, and hematological characteristics of HSB reared in an RAS in Sicily, Italy. The results highlight the strong correlations between body size and health indicators, such as red blood cell count, Hb levels, and Hct, suggesting that these parameters can serve as reliable indicators of fish health and growth performance in RASs. The proximate composition analysis reveals a favorable nutritional profile for HSB, with a high moisture content and reasonable protein and lipid levels, making it a viable species for aquaculture in controlled environments. The PCA analysis further reinforces the significant role of body size in influencing both nutritional and blood parameters, suggesting that larger fish tend to exhibit different hematological profiles. Overall, the findings underscore the potential of RAS technology for sustainable HSB farming in Mediterranean climates, with the identified correlations offering a foundation for optimizing farming practices, ensuring fish health, and maximizing production efficiency. Future studies should focus on long-term performance and the impact of different RAS management strategies on HSB's physiological and nutritional traits.

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**Institutional Review Board Statement:** The guidelines suggested by the Guide for the Care and Use of Laboratory Animals and Directive 2010/63/EU for animal research were followed in the review and approval of animal husbandry and experimentation protocols. Our study was concurrently performed with the competent authority and an authorized veterinarian during the official sanitary routine inspection of each farm. In Italy, the sanitary routine inspection of the farms does not require an authorization, an ID, or a protocol number.

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**Data Availability Statement:** For access to the data presented in this study, kindly contact the corresponding author.

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