

## Original Research Articles

# Path Analysis and Grey Correlation Analysis of Morphometric Traits to Body Weight of *Exopalaemon modestus*

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The Siberian prawn *Exopalaemon modestus* is a commercial freshwater shrimp with high nutritional value in China. To analyze the influence of morphometric traits on the body weight of *E. modestus* and identify effective indicators for breeding programs of *E. modestus*, we analyzed the correlations between 12 morphometric traits and body weight using correlation analysis, path analysis, multiple regression analysis, and grey relational analysis. Path analysis showed that body length (BL), carapace height (CH), carapace width (CW), and abdominal width (AW) significantly affect body weight (BW) directly ( $P < 0.05$ ). BL had the most significant direct effect (0.721) on body weight. The total determination coefficients of four significant morphometric traits on body weight were 0.906 ( $>0.85$ ), indicating that substantial morphometric traits explained 90.6% of BW variation. The regression equation was constructed as  $BW = -1.666 + 0.049BL + 0.045CH + 0.035CW + 0.021AW$  ( $P < 0.01$ ,  $R^2 = 0.906$ ). Grey relational analysis identified that the trait most correlated with BW was BL (relational grade = 0.952), followed by carapace length (CL), abdominal length (AL), and CH. Combined with path analysis and grey relational analysis, BL and CH were the key morphometric traits influencing the body weight of *E. modestus*. These results provided references for candidate morphometric traits for *E. modestus* selective breeding programs.

## INTRODUCTION

The Siberian prawn *Exopalaemon modestus* (Heller, 1862, Crustacea: Decapoda: Palaemonidae) is native to freshwater areas of eastern Asia, from the Amur and Ussuri basins in Siberia, through Korea, China, and Taiwan.<sup>1,2</sup> *E. modestus* is a commercial freshwater shrimp with high nutritional value.<sup>3</sup> In recent years, however, intensified water pollution and consumption demand-driven fishing pressure have degraded *E. modestus* germplasm resources, resulting in marked miniaturization of wild individuals and persistent population declines. According to statistics from the Food and Agriculture Organization of the United Nations (FAO), the capture production of *E. modestus* dropped from 98 thousand tonnes in 2019 to 48 thousand tonnes in 2022, which has declined by about half.<sup>4</sup> To support the growing demand and population conservation of *E. modestus*, it is essential to select and cultivate individuals with desirable traits through artificial breeding. Defining clear breeding objectives serves as the foundation of this selective breeding process.

To date, selection breeding programs in aquatic animals have frequently prioritized growth traits, particularly body

weight, due to its strong correlation with economic returns.<sup>5</sup> Previous researches have found average selection responses for body weight after one generation of selection to be 10.7% in *Marsupenaeus japonicus*,<sup>6</sup> and 21% in *Litopenaeus vannamei*.<sup>7</sup> But short-term environmental changes, such as variations in food supply, can cause rapid fluctuations in body weight,<sup>8</sup> affecting the accuracy of selective breeding. Moreover, the direct measurement of body weight results in animal sacrifice and hence in the loss of a large number of breeding candidates within the group. In contrast, morphometric indicators are relatively easy to measure accurately and more stable, especially with the development of computer vision technology and deep learning algorithms.<sup>9,10</sup> Numerous studies have reported that the morphometric traits of shrimp were closely correlated with body weight.<sup>11,12</sup> Therefore, understanding the correlations among morphological traits, and identifying morphometric traits closely related to body weight is of great significance for the indirect selection process.

Studies on the relationships between morphometric traits and body weight by correlation analysis, path analysis, and multiple regression analysis are now widely performed in aquatic animals. For example, head length and

body height were important for determining the body weight of rounded fish (species and hybrids of the *Colosoma* and *Piaractus* genera).<sup>13</sup> In contrast, body width was identified as a key factor affecting the *Lateolabrax maculatus* body weight.<sup>14</sup> Grey relation analysis, developed by Deng based on grey system theory, addresses the complex interrelationships among multiple factors.<sup>15</sup> This method has been widely applied in crop breeding<sup>16</sup> and has recently been used in aquatic animals such as *Procambarus clarkii*,<sup>11</sup> *Pinctada fucata*,<sup>17</sup> and *Siganus guttatus*.<sup>18</sup> The relationships of morphometric traits and body weight have yet to be reported in *E. modestus* with grey relation analysis.

Huaihe River is one of the seven largest rivers in China and is rich in fishery resources; however, there is little information on the population of *E. modestus*. In the present study, we used correlation analysis, pathway analysis, regression analysis, and grey relation analysis to estimate the effects of morphometric traits on body weight and identify effective indicators that could be applied in selective breeding programs of *E. modestus* in the upper reaches of the Huaihe River. This study will provide a scientific basis for the selective breeding of *E. modestus*.

## MATERIALS AND METHODS

### EXPERIMENTAL MATERIALS

The Suyahu Reservoir (113°19'~114°19' E, 32°34'~33°11'N) is the largest plain artificial lake in Asia, located in the upper reaches of the Huaihe River, and regulates a drainage area of 4,498 km<sup>2</sup>.<sup>19</sup> A total of 199 specimens of *E. modestus* were collected from Suyahu Reservoir in January 2025. The body integrity of all samples was investigated to ensure the measurement accuracy of all morphometric traits and body weight.

### MORPHOLOGICAL TRAITS MEASUREMENT

Thirteen morphological traits for *E. modestus* were measured for further data analysis. These morphological traits were as follow: body length (BL), rostrum length (RL), carapace length (CL), carapace width (CW), carapace height (CH), abdominal length (AL), abdominal width (AW), abdominal height (AH), telson length (TeL), telson width (TeW), caudal fan length (FL), caudal fan width (FW) and body weight (BW). The former 12 indicators of morphometric traits were measured using IP54 digital display vernier calipers (Syntek: Deqing Shengtaixin Electronic Technology Co., LTD, China), accurate to 0.01 mm. Shrimp body surface moisture was cleaned with a dry towel and then weighed with an electronic balance, accurate to 0.01 g.

### PATH ANALYSIS

All traits' mean value and standard deviation (SD) were calculated using SPSS 20.0. The coefficient of variation (CV) for each of the 12 recorded morphometric traits and body weight was estimated as follows: CV = (SD/ mean) × 100%. The correlation analysis and path analysis were also performed using SPSS 20.0.

The direct path coefficients (path coefficient,  $P$ ) can be obtained directly, as described by Du and Chen (2010).<sup>20</sup> The determination coefficient was calculated using the formulas:

$$d_i = P_i^2$$

$$d_{ij} = 2r_{ij}P_iP_j$$

where  $d_i$  is the direct determination of  $i$ th trait on the body weight and  $d_{ij}$  is the correlated determination of  $i$ th trait on the body weight through  $j$ th trait ( $i \neq j$ );  $r_{ij}$  is the correlation coefficient between  $i$ th and  $j$ th trait, and  $P_i$  and  $P_j$  are the path coefficients of  $i$ th and  $j$ th trait on the body weight, respectively.

Stepwise multiple regression analysis was used to eliminate nonsignificant morphometric traits, and Student's  $t$ -test ( $\alpha=0.05$ ) was applied to evaluate significance. The multiple regression equation for body weight ( $Y$ ) was calculated as follows:

$$Y = a + b_1X_1 + b_2X_2 + b_3X_3 + \dots + b_iX_i$$

where  $Y$  is the dependent variable,  $a$  is the intercept,  $X_i$  are the independent variables, and  $b_i$  are the partial regression coefficients for  $X_i$  on  $Y$ .

### GREY RELATIONAL ANALYSIS

According to grey system theory,<sup>21</sup> body weight and 12 morphometric traits were selected to be grey systems. The body weight was a reference sequence ( $X_0$ ), while the 12 morphometric traits were comparison sequences ( $X_i$ ,  $i=1$  to 12).

Due to different dimensions among different influence factors, the first step was linear normalization of raw data. The following equation performed the data preprocessing:

$$X'_i = \frac{X_i - \bar{X}_i}{\sigma}$$

where  $X'_i$  is the value after standardization;  $X_i$  is the value of each morphometric traits;  $\bar{X}_i$  is the average value of  $X_i$ ;  $\sigma$  is the standard deviation of  $X_i$ ;  $i$  is the morphometric traits number ( $i = 1$  to 12).

Then, the grey relational coefficient was calculated as:

$$\xi_i = \frac{\min\Delta_i + \rho \max\Delta_i}{\Delta_i + \rho \max\Delta_i}$$

where  $\xi_i$  is the grey relational coefficient, which is the relationship between the best and the actual normalized data;  $\Delta_i$  is the absolute value between the reference sequence and comparison sequences,  $\Delta_i = |X_0 - X_i|$ ;  $\min\Delta_i$  and  $\max\Delta_i$  are the minimum and the maximum value of the second level, respectively;  $\rho$  is the distinguishing coefficient ( $\rho = 0.5$ ).

Finally, the grey relational grade was calculated as follows:

$$r_i = \frac{1}{n} \sum \xi_i$$

where  $r_i$  is the grey relational grade, and  $n$  is the number of performance characteristics ( $n=199$ ).

**Table 1. Descriptive statistics of morphometric traits and body weight in *E. modestus* (N = 199).**

Traits	Min	Max	Mean	SD	CV
BW/g	0.45	1.71	0.87	0.24	27.60%
BL/mm	27.42	50.56	40.15	3.53	8.79%
RL/mm	6.05	18.88	11.98	2.29	19.10%
CL/mm	1.76	12.97	9.89	1.18	11.89%
CW/mm	3.71	7.38	5.38	0.67	12.43%
CH/mm	3.55	7.88	5.93	0.71	11.98%
AL/mm	23.25	41.32	29.95	3.09	10.32%
AW/mm	3.64	10.96	5.27	0.69	13.18%
AH/mm	4.59	13.84	6.08	0.87	14.26%
FL/mm	5.89	12.96	9.50	1.23	13.00%
FW/mm	8.84	17.64	12.57	1.69	13.45%
TeL/mm	5.01	9.45	7.19	0.81	11.26%
TeW/mm	1.06	2.39	1.63	0.25	15.52%

## RESULTS

### Statistical analysis and correlation coefficients of morphological traits in *E. modestus*

The mean, SD, and CV for the 12 morphometric traits and body weight of wild *E. modestus* are presented in [Table 1](#). The average body weight of *E. modestus* was  $0.87 \pm 0.24$  g. Among the morphometric traits, RL exhibited the highest CV at 19.10%, while BL had the lowest CV at 8.79%. By contrast, the CV of body weight was much higher than any of the morphometric traits, which was 27.60%.

### CORRELATION COEFFICIENTS AMONG THE MORPHOLOGICAL TRAITS IN *E. MODESTUS*

The correlation coefficients among the 13 morphological traits (including the 12 morphometric traits and body weight) of *E. modestus* are shown in [Figure 1](#). Significant correlations were detected in all comparisons among all morphometric traits ( $P < 0.01$ ), except between TeW and FW, which had no significant correlation ( $P > 0.05$ ). All 12 morphometric traits were remarkably correlated with body weight ( $P < 0.01$ ), with the correlation coefficient ranging from 0.44 (between BW and FW) to 0.94 (between BW and BL).

### PATH AND DETERMINATION COEFFICIENTS OF MORPHOMETRIC TRAITS ON BODY WEIGHT IN *E. MODESTUS*

The direct and indirect effects of the different morphometric traits on body weight in *E. modestus* were evaluated through path analysis, as summarized in [Table 2](#). Of the 12 morphometric traits, four traits (BL, CH, CW, and AW) showed significant direct effects on body weight ( $P < 0.05$ ). The direct impact of these traits on body weight ranged from 0.060 (AW) to 0.721 (BL), and the indirect effects ranged from 0.221 (BL) to 0.714 (CH). Only the direct impact of BL was greater than the indirect effects on body

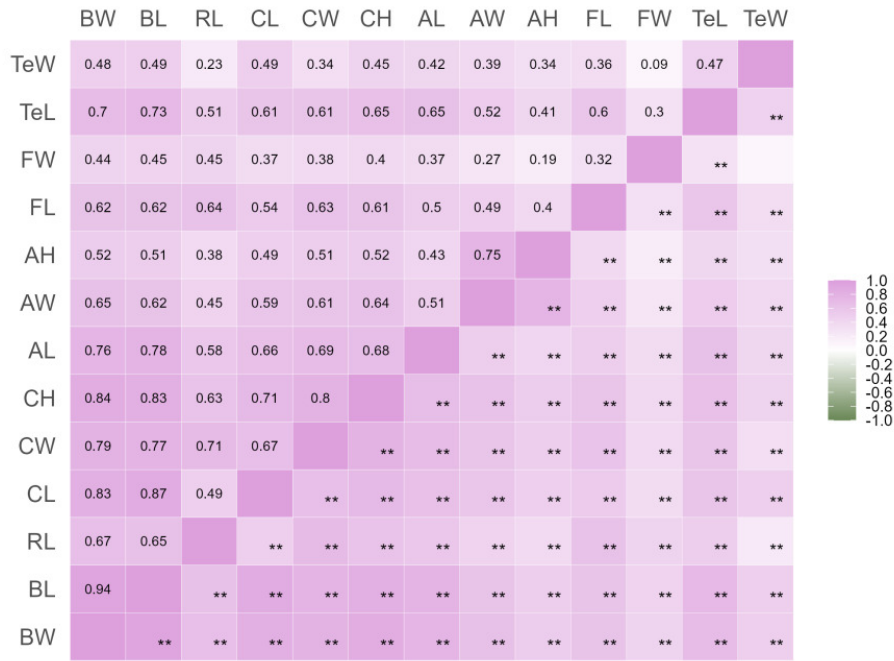
weight. The determination coefficients of the morphometric traits on body weight of wild *E. modestus* are presented in [Figure 2](#). The sum of the determination coefficients of morphometric traits on body weight was 0.906. Of these, the determination coefficient of BL was the largest (0.520), whereas that of AW was the lowest (0.004). The co-determinant coefficient of BL and CH on body weight was the highest (0.157), while that of CW and AW on body weight was the smallest (0.007).

### MULTIPLE REGRESSION EQUATIONS FOR MORPHOMETRIC TRAITS AND BODY WEIGHT IN *E. MODESTUS*

Multiple regression analysis was performed to investigate the influence of morphometric characteristics on body weight in *E. modestus*. The partial regression coefficients of four morphometric traits (BL, CH, CW, and AW) showed a significant regression relationship to body weight ( $P < 0.05$ ; [Table 3](#)). The best-fit multiple regression equation of *morphometric traits* and body weight was constructed as follows:  $BW = -1.666 + 0.049BL + 0.045CH + 0.035CW + 0.021AW$  ( $R^2 = 0.906$ ). The coefficient of the regression equation for body weight was significant ( $P < 0.01$ ; [Table 4](#)).

### GREY RELATIONAL ANALYSIS OF MORPHOMETRIC TRAITS ON BODY WEIGHT

The raincloud plot showed the grey relational grades between 12 morphometric traits and body weight of *E. modestus* ([Figure 3](#)). The bar graph showed the grey relational analysis of 12 morphometric traits on the body weight of *E. modestus*. The relational grade of the factors on body weight ranged between 0.850 and 0.952. The highest grey relational grade was found in BL, with the order of association with body weight being  $CL > AL > CH > CW > AW > TeL > AH > RL > FL > FW > TeW$ .



**Figure 1. Phenotype correlation coefficients among the 12 morphometric traits and body weight in *E. modestus* (N = 199). \*\* represents a significant difference (P < 0.01), blank represents no significant difference (P > 0.05).**

**Table 2. Path analysis of morphometric traits on body weight in *E. modestus* (N = 199).**

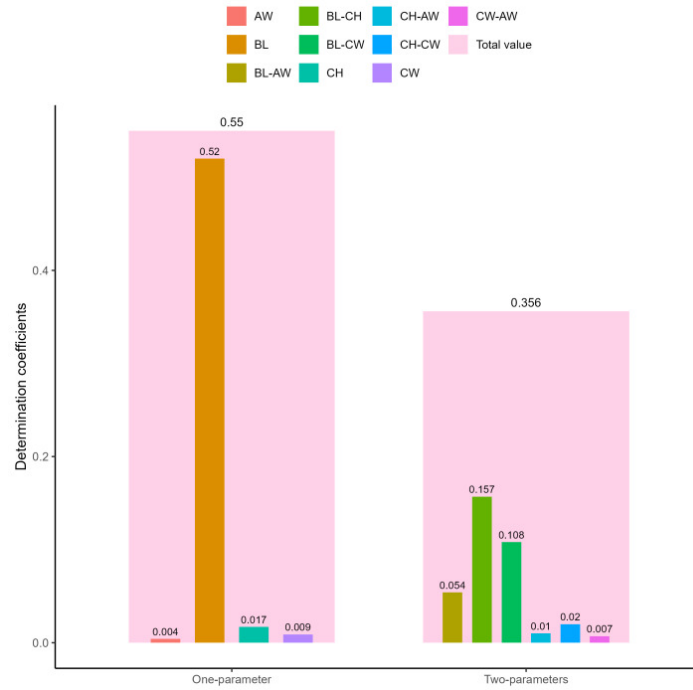
Traits	Correlation coefficient	Direct effect	Indirect effect				Σ
			BL	CH	CW	AW	
BL	0.942	0.721		0.109	0.075	0.037	0.221
CH	0.845	0.131	0.598		0.078	0.038	0.714
CW	0.796	0.097	0.557	0.105		0.036	0.699
AW	0.649	0.060	0.447	0.084	0.059		0.589

**DISCUSSION**

Body weight is widely used as a direct indicator for selection and is closely correlated with morphometric traits in many aquatic animals. In the current study, we analyzed the relationship between 12 morphometric traits and body weight of *E. modestus* in Suyahu reservoir from the upper reaches of the Huaihe River. The CV for these traits varied from 8.79% to 27.60%; the abundant variation was consistent with the previous findings in the Dianshan Lake population (9.05%-28.30%),<sup>22</sup> and the Taihu Lake population (14.565%-35.095%),<sup>23</sup> both located in the lower reaches of the Yangtze River. Furthermore, the CV of the Suyahu population was lower than that of corresponding traits in both the Dianshan Lake and Taihu Lake populations.<sup>22,23</sup> These differences may be explained by variations in population growth stages during sampling months, potential influences from fishing gear selectivity, and differential nutritional conditions across the respective aquatic environments. Among the 13 morphological traits, body weight had the largest CV (27.60%). This result aligns with previous findings in *E. modestus*<sup>22,23</sup> and in other species.<sup>11-14</sup>

Traits with greater CV often exhibit higher levels of variability and respond better to selective breeding; hence, body weight is used as a target breeding trait. In *Pampus argenteus*, selective breeding significantly boosted body weight by nearly 30% over three generations, far surpassing improvements in body length (8.9%) and fork length (8.1%).<sup>24</sup>

Morphometric traits were significantly correlated with body weight. They could therefore serve as an indirect basis for selecting for body weight in selective breeding, improving the accuracy and reliability of breeding efforts.<sup>24</sup> Correlation analysis indicated that all 12 morphometric traits showed highly significant positive correlations with body weight (P < 0.01), but the correlation coefficients >0.80 were observed only for BL, CL, and CH. In the Dianshan Lake population,<sup>22</sup> the correlation coefficient BL, AL, and RL were higher than 0.80. In contrast, in the Taihu Lake population,<sup>23</sup> BL, AL, CL, AW, CW, FL and FW had correlation coefficient higher than 0.80. It was found that the correlation between same morphometric traits and body weight was dissimilar at diverse populations. Similar results have been found in Pacific abalone *Haliotis discus hannai*.<sup>25</sup>



**Figure 2. Determination coefficients of morphometric traits on body weight in *E. modestus* (N = 199). The values represent the determination coefficients.**

**Table 3. Regression coefficient test of morphometric traits to the body weight in *E. modestus* (N = 199).**

Model	Partial regression coefficient	Standard error	t value	P
Constant	-1.666	0.062	-26.763	0
BL	0.049	0.003	17.026	0
CH	0.045	0.015	2.896	0.004
CW	0.035	0.014	2.448	0.015
AW	0.021	0.01	2.011	0.046

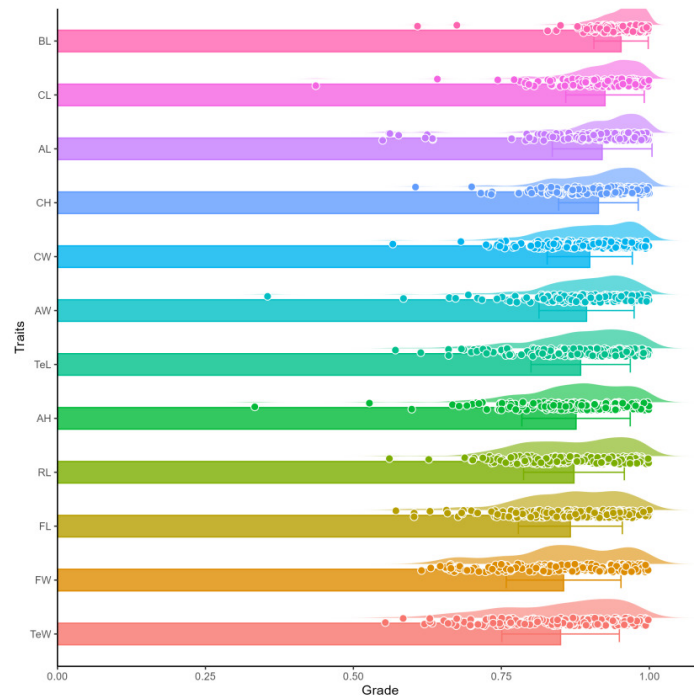
**Table 4. Analysis of variance of multiple regression equation of *E. modestus* (N = 199).**

Index	Sum of squares	df	Mean square	F	P
Regression analysis	10.448	4	2.612	462.827	0.000
Residual	1.095	194	0.006		
Total	11.543	198			

Correlation analysis can only obtain a simple association between morphometric traits and body weight. It cannot clarify the specific scale of their role or degree of influence on body weight. This study employed path analysis to identify effective indicators to address the limitation of correlation analysis in reliably elucidating relationships between variables. This method applies a stepwise multiple linear regression model to decompose correlation coefficients into: direct path coefficients (measuring the independent effect of a predictor on body weight), and indirect path coefficients (quantifying effects mediated through other variables), thereby enabling direct comparison of factor contributions.<sup>13</sup> In this study, BL, CH, CW, and AW

showed significant direct effects on body weight ( $P < 0.05$ ). Not all the morphometric traits significantly correlated with body weight were retained. Similar results to ours have been found in other species, such as *Macrobrachium nipponense*,<sup>12</sup> and *L. maculatus*.<sup>14</sup> These findings confirmed the necessity of path analysis for morphological traits assessment.

Coefficients of determination for morphometric traits influencing body weight were calculated based on correlations and path coefficients. A cumulative determination coefficient  $\geq 0.85$ , comprising individual trait contributions and pairwise co-determinant terms, indicates that the primary independent variables affecting body weight have



**Figure 3. Grey relational grades of morphometric traits on body weight in *E. modestus* (N = 199).**

been identified.<sup>26</sup> In this study, the sum of the determination coefficients for BL, CH, CW, and AW was 0.906, demonstrating that selected morphometric traits were the principal traits affecting body weight for *E. modestus*. The sum determination of BL on body weight was 0.839, which made up 92.6% of the total determinations of four morphometric traits on body weight. This identifies BL as the key determinant of body weight in *E. modestus*. Consistent results were observed across both the Dianshan Lake<sup>22</sup> and Taihu Lake populations.<sup>23</sup> These results confirm that a larger geometric volume is conducive to nutrient storage in shrimp bodies.

Grey relational analysis is an analytical method to describe the correlation between various factors by assessing their degree of geometric similarity. Compared with path analysis, grey relational analysis is particularly suitable for small sample sizes while effectively assessing the influence between variables.<sup>21</sup> This analytical method was increasingly employed in morphological traits<sup>11,17,18</sup> and physiological traits<sup>27,28</sup> in aquatic animals. The current study conducted grey relational analysis on *E. modestus* to investigate the relationship between morphometric traits and body weight for the first time. The results suggest that BL was the morphometric trait with the greatest influence on body weight of *E. modestus*, followed by CL, AL, and CH. However, the main factors affecting body weight were BL, CH, CW, and AW by using path analysis. Although the order of morphometric traits affecting body weight differed slightly between the two methods, both identified two common traits: BL and CH. Notably, BL had the most significant impact on body weight in both methods.

Different analytical methods are grounded in distinct principles and have specific data requirements. Path analysis is suitable for large sample data, but grey relational

analysis is ideal for small sample data. Furthermore, path analysis can analyze the relationships among independent variables, whereas grey relational analysis evaluates independent variables individually, without regard to their significance or collinearity.<sup>29</sup> Estimating relationships among morphological traits is critical in quantitative genetics and selective breeding. Consequently, selecting appropriate statistical approaches or synthesizing multiple methods is essential to identify key factors influencing target traits.

In conclusion, the relationships between morphometric traits and body weight of *E. modestus* were further tested in this study. By comprehensively using path analysis and grey relational analysis, this study identified key traits, particularly BL and CH, that significantly influence the body weight of *E. modestus*. In the practical breeding program of *E. modestus*, BL should be used as the main selective trait, and CH should be used as an assisted selective trait to improve the body weight indirectly. This study advances the morphological understanding of *E. modestus* and contributes valuable insight toward improving aquaculture productivity and resource management.

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#### AUTHORS' CONTRIBUTION

Conceptualization: Jiahui Liu (Lead). Methodology: Jiahui Liu (Equal), Zhiguo Hu (Equal). Writing – original draft: Jiahui Liu (Lead). Writing – review & editing: Jiahui Liu (Equal), Rongjing Huang (Equal). Funding acquisition: Jiahui Liu (Equal), Rongjing Huang (Equal). Resources: Jiahui Liu (Equal), Rongjing Huang (Equal). Investigation: Zhiguo Hu (Equal), Chaoqun Su (Equal), Gaoyou Yao (Equal). Formal Analysis: Zhiguo Hu (Equal), Chaoqun Su (Equal). Data curation: Jia Li (Equal), Wenhui Shao (Equal), Linying Dong (Equal). Supervision: Rongjing Huang (Lead).

#### COMPETING OF INTEREST – COPE

No competing interests were disclosed.

#### ETHICAL CONDUCT APPROVAL – IACUC

The authors confirm that every effort has been made to alleviate the suffering of the test shrimp, including the following details: All test shrimp were anesthetized before being sampled. The authors comply with the Convention on Biological Diversity and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

#### INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

#### DATA AVAILABILITY STATEMENT

All are available upon reasonable request.

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