**Effects of shell morphological traits on the weight trait of the orange strain of the
Manila clam**

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Abstract A new strain of Manila clam with orange shell color was produced after selection within a full-sib family for two generations. In the present study, the shell length, height, and width, and the live body weight of the orange strain were measured, and their correlation coefficients were calculated. The shell morphological traits were used as independent variables, and the live body weight was used as the dependent variable for calculating the path coefficients, correlation index, and determination coefficients. The results showed that the correlation coefficients between each shell morphological trait and the live body weight were all highly significant ($P < 0.01$). The correlation indices (R^2) of morphological traits against the live body weight of clams were larger than 0.85, indicating that the morphology traits were the main factors affecting the body weight. Multiple regression equations were obtained to estimate shell length X_1 (cm), shell height X_2 (cm), and shell width X_3 (cm) against live body weight Y (g): $Y = -2.62 + 0.34 X_1 + 0.145 X_2$, ($X_1 < 0.05$, $X_2 < 0.05$). The results suggest that the shell length could be used as the main trait for selective breeding and could indirectly make a large improvement in the weight trait.

Keywords Morphology - Weight - Orange strain

Introduction

In quantitative genetic studies of the selective breeding of marine bivalves, analysis of the effects of morphological traits on weight traits using path analysis and multiple regression is basic. The results are used to optimize the breeding strategy, which can effectively improve the economic value of shellfish^[1, 2]. The path analysis proposed by Wright (1921)^[3] has

become a powerful tool for analyzing the creature traits. This approach has three merits: firstly, there is variable standardization, which allows for comparison between independent variables and the dependent variables; secondly, the correlation coefficients among the different traits can be decomposed into direct and indirect effects; and lastly, the path diagram can be implemented, so that the relationships among the traits can be directly understood

The Manila clam, *Ruditapes philippinarum*, which is naturally distributed along the coast of China, is the most popular bivalve cultured in China because of its high nutritional value, delicate flavor and rapid growth. China is the most important Manila-clam-producing country in the world. In 2011, the annual production of Manila clams in China had increased to over 3,200,000 tons, accounting for an estimated 90% of the world production ^[4, 5]. For these reasons, studies on the breeding technology, healthy culture, and selective breeding, in terms of shell color and shell shape, of the Manila clam have been reported in recent years ^[6].

The Manila clam presents a large polymorphism for shell colors and patterns. The attractive and exclusive shell color of this species can give consumers an aesthetic appreciation that leads to higher prices in the international market. We have found that the orange shell color of the Manila clam occurs in only 0.4% of wild populations ^[7]. This makes it ideal for mass commercial production. As a result, the full-sib family with orange shell color has been produced since 2008, and 100,000 individuals of the orange strain have been cultured after a two-generation oriented selection from this full-sib family.

The objective of the present study was to evaluate the effects of morphological traits on the weight trait of the orange strain of Manila clam. The data obtained can provide valuable information for determining the main morphological traits affecting the weight trait and thereby help in the design of a reasonable breeding strategy for predicting the candidate traits. The results of this study provide a method to predict the live body weight of the Manila clam through measuring its shell morphological traits, which is laborsaving and cost effective.

Materials and Methods

Experimental Material

The clams with an orange shell color were sampled from an orange strain of Manila clam that had been selected for two generations (Fig.1).

Data and Analysis

The shell length (SL), shell height (SH), and shell width (SW) of 100 randomly sampled adult individuals (1 year of age) were individually measured using a vernier caliper (0.02 mm). Live body weight (LW) was measured individually using an electronic balance (accuracy, 0.0001 g).

The mean values of the morphological and weight traits, the standard deviation, and the coefficient of variation (CV) were calculated using Excel software. The correlation coefficient and multiple regression analysis between the traits were evaluated using the SPSS13.0 software, and the multiple regression equations were established. The path coefficients,

correlation index, single-trait determination coefficients, and common-trait determination coefficients were derived using path analysis (Wright 1921). The formulas are as follows:

If the correlation variables y , x_1 , x_2 , x_3 exist in a linear equation, the regression equation is:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + e \dots \dots \dots (1)$$

where y is a dependent variable; x_1 , x_2 , and x_3 are independent variables; b_0 is the absolute term; b_1 , b_2 , and b_3 are the bias regression coefficients of y on x_1 , x_2 , and x_3 ; and e is the remaining item. The matrix notation of the k independent variable of the path analysis was obtained using a least-squares method and an identically equal distortion:

$$\begin{pmatrix} 1 & r_{12} & r_{13} & \dots & r_{1k} \\ r_{21} & 1 & r_{23} & \dots & r_{2k} \\ r_{31} & r_{32} & 1 & \dots & r_{3k} \\ \vdots & \vdots & \vdots & \dots & \vdots \\ r_{k1} & r_{k2} & r_{k3} & \dots & 1 \end{pmatrix} \begin{pmatrix} P_{01} \\ P_{02} \\ P_{03} \\ \vdots \\ P_{04} \end{pmatrix} = \begin{pmatrix} r_{01} \\ r_{02} \\ r_{03} \\ \vdots \\ r_{04} \end{pmatrix}$$

$$R^2 = \sum r_{x_i,y} \times P_i \quad (2)$$

$$d_i = P_i^2 \quad (3)$$

$$d_{ij} = 2r_{ij} \times P_i \times P_j \quad (4)$$

Results

Statistical Phenotypic Traits

The statistical phenotypic traits of shell length (SL), shell height (SH), shell width (SW), live body weight (LW), and the coefficient of variation are shown in Table 1. The results show that

the coefficient of variation of the weight trait was greater than those of the morphological traits.

Phenotypic Correlation Coefficients among the Traits

The phenotypic correlation coefficients of the traits are listed in Table 2. The correlation coefficients of the various traits showed significant differences ($P < 0.01$).

Path Coefficients and Correlation Indices of the Morphological Traits against the Weight Trait

The path coefficients and correlation indices are shown in Table 3. Path analysis was conducted by using the shell length, shell height, and shell width as independent variables and the live body weight as the dependent variable. The results show that the direct effect of the shell length on the live body weight was larger than the direct effects of the shell width and shell height.

The correlation indices of the relationships between the morphological traits and live body weight were decomposed to the direct effects through each trait and the indirect effects through other traits (Table 4). The main effect of the morphological traits on the live body weight was detected by comparing the correlation indices (r_{ij}), direct effect (P_i), and indirect effect ($r_{ij}P_j$). The results show that the shell length was highly correlated with the live body weight, as indicated by their having the highest values of r_{ij} and P_i . The indirect effect of shell height and shell width through the shell length was the second factor correlated with the live

body weight, as indicated by the larger $r_{ij}P_j$ of the shell length and the shell height.

The determination coefficients of the morphological traits relative to the weight trait are listed in Tables 5. The determination coefficients of individual morphological traits relative to the weight trait are on the diagonal. The results showed that the determination coefficient (43.7%) of the shell length relative to the live weight was the largest. The co-determination coefficients of the morphological traits against the weight trait are above the diagonal. The results showed that co-determination coefficient (21.2%) of the shell length and the shell height relative to the live weight was the largest. The correlation indices (R^2) of the morphological traits on the live body weight of the clams were larger than 0.85.

Establishment of Multiple Regression Equations

Multiple regression equations were obtained by testing the partial regression coefficient significance and gradually removing non-significant morphological traits to estimate the shell length X_1 (cm), shell height X_2 (cm), and shell width X_3 (cm) relative to the live body weight Y (g). $Y = -2.62 + 0.34 X_1 + 0.145 X_2$, ($X_1 < 0.05$, $X_2 < 0.05$). The partial regression coefficients through the test of significance showed that the selected morphological traits, except for shell width, had significant correlations with the live body weight at $P < 0.05$.

Discussion

The levels of phenotypic correlation among the traits of the orange strain were highly significant. Because of this, the correlation coefficients could be used as conditions for path

analysis. The results showed that the correlation indices for all the morphological traits relative to the live body weight trait were greater than 0.85 and approximately the same as the determination coefficients. A previous study showed that if the correlation indices among the traits are greater than 0.85, the main factors affecting the relative trait have been found ^[8]. As a result, it is concluded that the morphological traits (shell length, shell height, and shell width) were the main factors affecting the live body weight.

The use of the morphological traits for selective breeding can conveniently and quickly facilitate genetic improvement, thus speeding up the process of breeding. The study of the effects of the morphological traits on the weight trait of the orange strain of Manila clams, *Ruditapes philippinarum*, using path analysis can determine the main morphological traits affecting the weight trait. Analysis of the relationship between the morphological traits and the weight has been reported in other shellfish species, including great scallop, *Pecten maximus*, pearl oyster, *Pinctada martensi*, *Siliqua minima*, purplish Washington clam, *Saxidomus purpuratus* ^[9-11]. However, the previous results showed that the effects of the morphological traits on the weight trait were variable among the different shellfish species. For example, in Zhikong, scallop, *Chlamys farreri*, it is reported that the shell height is the main factor affecting the weight trait ^[12]. In purplish Washington clam, *Saxidomus purpuratus*, the shell width is the main factor affecting the weight trait ^[11]. In the present study, the direct effect of the shell length on the live body weight was greater than that of the shell width and shell height, indicating that the live body weight was most significantly affected by the shell length. If we could reasonably predict live body weight by measuring shell length, then we

could save on the enormous labor costs.

In this study, multiple regression equations were obtained to estimate shell length X_1 (cm), shell height X_2 (cm), and shell width X_3 (cm) relative to the live body weight Y (g). The regression forecast indicated that the estimated values and the actual observed values showed no significant differences. This suggests that the equations can be easily and reliably applied to the selective breeding of the orange strain of Manila clams.

In conclusion, the genetic correlations are significant among the growth traits including shell length, shell height, shell width, and live body weight. The shell length could be used as the main trait for selective breeding, which can indirectly make a large improvement in the weight trait.

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Fig. 1 *The orange strain of Manila clam*

246 Table 1. *Statistical analyses of various traits of the orange strain of Ruditapes philippinarum*

Traits	(SL) X ₁	(SH) X ₂	(SW) X ₃	(LW) Y(g)
	(emmm)	(emmm)	(emmm)	
Mean	9.624	7.092	3.804	0.158
SD	1.897	1.361	0.788	0.087
(CV%)	19.711	19.275	20.715	55.063

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249 Table 2. *Phenotype correlation coefficients between the traits of the orange strain of*

250 *Ruditapes philippinarum*

Traits	LW	SL	SH	SW
LW	1	0.957**	0.935**	0.932**
SL	-	1	0.958**	0.955**
SH	-	-	1	0.946**
SW	-	-	-	1

251 Note: **highly significant correlation ($P<0.01$)

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253 Table 3. *Path coefficients and correlation index of the morphological traits on the weight trait*

254 *of the orange strain of Ruditapes philippinarum*

Weight Traits	Path coefficients and correlation index			
	P_1	P_2	P_3	R^2
LW	0.661	0.167	0.142	0.920

Table 4. *Effects of Morphological Traits on the Live Weight of the Orange Strain of Ruditapes philippinarum*

Weight trait	Morphological traits	Correlation coefficient	Direct effect P_i	Indirect effect $r_{ij} P_j$			
				SL	SH	SW	Total Σ
r_{ij}							
Live body weight	SL	0.957**	0.661**		0.160	0.136	0.296
	SH	0.935**	0.167	0.633		0.134	0.767
	SW	0.932**	0.142	0.631	0.158		0.789

Note: ** highly significant correlation ($P < 0.01$) and shell length was closely linked to the weight trait

Table 5 *Determinant and co-determinant coefficients of the morphological traits on the weight trait of the orange strain of Ruditapes philippinarum*

Traits	LW		
	SLX_1	SHX_2	SWX_3
SL	0.437	0.212	0.178
SH		0.028	0.045
SW			0.020
Σd Total		0.920	
R^2 Correlation index		0.920	