Aquaculture 516 (2020) 734532

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Estimating heritability for meat composition traits in the golden shell strain of Pacific oyster (*Crassostrea gigas*)

Sai Wan[a], Qi Li[a,b,*], Hong Yu[a], Shikai Liu[a], Lingfeng Kong[a]

[a] Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, 5 Yushan Road, Qingdao, 266003, China
[b] Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Wenhai Road, Qingdao, 266237, China

ARTICLE INFO

Keywords:
Crassostrea gigas
Meat composition traits
Parentage assignment
Near-infrared reflectance spectroscopy
Microsatellite marker
Genetic parameters

ABSTRACT

The Pacific oyster (*Crassostrea gigas*) is a widely cultured shellfish species with great economic value; however, estimation of heritabilities for meat composition traits meat composition have not been studied with molecular pedigree. In this study, the heritabilities of six meat composition traits were studied using near-infrared reflectance spectroscopy (NIRS) in the golden shell strain of Pacific oyster. All 30 families used in this study were produced by nested mating. In total, progeny from crosses among 10 sires and 30 dams were communally reared and pedigree was reconstructed with six microsatellite markers. A total of 608 offspring were harvested at 16 months of age and unambiguously assigned to their respective parents. We estimated the heritability at 0.41 ± 0.19 for glycogen content, 0.51 ± 0.09 for total protein content, 0.89 ± 0.18 for total fat content, 0.40 ± 0.11 for zinc content, 0.87 ± 0.06 for selenium content and 0.65 ± 0.13 for ash content. Remarkably, there were moderate negative genetic correlations between protein and both glycogen (−0.65 ± 0.11) and fat content (−0.40 ± 0.09) as well as between shell height and glycogen content (−0.68 ± 0.11). Overall, this study demonstrated that in meat composition traits could be improved through selective breeding under commercial conditions. The correlations among composition traits and growth traits should be taken into consideration.

1. Introduction

In the past few decades, tremendous progress has been achieved in increasing the productivity of important aquaculture species, through selective breeding. Results of many published studies are highly encouraging with the potential for genetic improvement in aquatic productions, in particular for traits such as growth rate and disease resistance (Gjedrem and Rye, 2016). Among the desired traits, the characteristics of meat composition are important to consumers (Cochet et al., 2015). The characteristics of meat quality are complicated due to the contribution of many factors (Shahidi, 1998). The fat content trait is an important consideration because of the consumer's preference for the beneficial effects of good fat on human health (Laghari et al., 2014). Meanwhile, a fatty and reproductively ripe oyster may not be liked by some consumers in the West.

Consequently, increasing attentions is being focused on improving the traits of meat composition of aquatic animals. Up to now, a relatively small number of studies have been carried out on the genetic parameter estimation of composition traits, which were mainly focusing on the fat and protein contents of fish. For instance, in Atlantic salmon, rainbow trout and Nile tilapia, the estimated heritability of fat content was generally moderate to high and the genetic correlations with phenotypes varied dramatically among composition traits and yield traits (Rye and Gjerde, 1996; Gjedrem, 2017; Garcia et al., 2017). It is noteworthy that farmed fish are fed on defined diets that are different from wild algae that farmed molluscs filter.

The Pacific oyster *Crassostrea gigas* is the most widely farmed oyster species in the world. In consideration of the importance, selective breeding programs have long been performed for the improvement of economically important traits. In order to provide valuable information for breeding programs, the narrow-sense heritability (h^2), representing the relative magnitude of additive genetic variation, has been estimated to predict the feasibility of improving targeted traits in terms of growth-related traits, by analyzing full-sib families, Evans and Langdon (2006) demonstrated the heritability of body weight to vary from 0.471 to 0.569. With one-generation mass selection in three brood stocks of *C. gigas*, Li et al. (2011) reported heritability of shell height to be 0.149 to 0.402. Kong et al. (2015) reported the heritability estimates of growth-

* Corresponding author. Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, 5 Yushan Road, Qingdao, 266003, China.
E-mail address: qili66@ouc.edu.cn (Q. Li).

https://doi.org/10.1016/j.aquaculture.2019.734532
Received 27 June 2019; Received in revised form 20 September 2019; Accepted 21 September 2019
Available online 02 November 2019
0044-8486/

Table 1
Number of progeny assigned to each of the 30 full-sib families based on microsatellite genotyping after the exclusion of outlier composition data using the 95% confidence level.

Sire	1			2			3			4			5			6			7			8			9			10			total
Dam	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	608
Number	27	43	33	36	24	11	41	42	55	4	16	18	16	20	22	9	14	23	15	13	7	4	10	3	37	14	5	28	14	4	

related traits in *C. gigas* varied from 0.35 to 0.49 with a pedigree inferred by microsatellite markers. de Melo et al. (2016) showed that heritability estimates for field traits at harvest were from 0.12 to 0.58 over five generations and positive medium-to-high genetic correlations existed among the harvest traits. Xu et al. (2017) estimated the heritabilities of growth traits to be 0.10 to 42 in a black shell color strain of *C. gigas*, which resulted from 22 full-sib families in a nested mating design. Considering the mass mortality caused by bacteria and viruses, genetic parameters of resistence to pathogens in *C. gigas* were also estimated. Degremont et al. (2015) showed the narrow-sense heritability for the ostreid herpesvirus 1 (OsHV-1) ranged from 0.49 to 0.61 in spat *C. gigas*. Using the controlled infectious challenges of OsHV-1 and *Vibrio aestuarianus* to *C. gigas* at three different life stages, Azema et al. (2017) confirmed a strong genetic basis of resistance to OsHV-1. With respect to shell pigmentation, we have used the computer vision system (CVS) and *L*a*b** color system, to estimate the genetic parameters of shell color-related traits in the golden shell strain of *C. gigas* and demonstrated a moderate heritability (Wan et al., 2017).

The chemical composition of oysters may vary greatly according to factors such as reproductive state, water temperature, salinity and diet of different seasons and regions (Hosoi et al., 2003; Pennarun et al., 2003; Dridi et al., 2007). Meanwhile, previous studies have demonstrated that glycogen content is at least partially controlled genetically (She et al., 2015; Liu et al., 2017a) in spite of its relation to gametogenesis (Dridi et al., 2007). Accordingly, it is beneficial to study the genetic basis of meat composition traits. However, the genetic parameters of meat composition traits have not been well studied in *C. gigas* with molecular pedigree.

Traditionally, characteristics of meat composition can be determined with chemical methods. For instance, glycogen content is often determined with the EnzyChrom glycogen assay kit (BioAssay Systems, Hayward, CA). Protein, fat, Zn, Se and ash contents are usually analyzed using Kjeldahl method (NSPRC, 2010b), Soxhlet extraction (NSPRC, 2008a), flame atomic absorption spectrometry (NSPRC, 2008b), atomic fluorescence spectrometry (NSPRC, 2010a), and incineration method (NSPRC, 2008c), respectively. But when it comes to selective breeding programs, it is necessary to use more efficient methods. Near Infrared Reflectance Spectroscopy (NIRS), as a time-saving and economical method to obtain compositional data, has been employed to determine quantitative parameters in oysters (Brown et al., 2012; Madigan et al., 2013; Wang et al., 2015). Recently, Guévélou and Allen. (2016) developed robust NIRS models predict the composition of eastern oyster *Crassostrea virginica* at different levels of ploidy.

In this study, we analyzed the traits of meat composition in the golden shell strain of *C. gigas*, using full-sib families constructed in our previous studies. We report here the estimated heritability of glycogen content (GC), total protein content (TPC), total fat content (TFC), zinc content (ZC), selenium content (SC) and ash content (AC). Data from this study lay a foundation for improvement of meat composition trait in *C. gigas* aquaculture.

2. Materials and methods

2.1. Family construction and pedigree reconstruction

A total of 30 full-sib families (10 sire half-sib families) were used in this study. Their molecular pedigree has been obtained in our previous studies (Wan et al., 2017). Briefly, 10 sires and 30 dams were used as parents for family construction. These individuals were taken from the F_4 generation of a selected population of *C. gigas* which had undergone four generations of artificial selection for golden shell color and growth traits. In order to perform a nested mating, sperm stripped from each sire were divided into three aliquots and used to fertilize with eggs collected from a corresponding dam. The progeny were communally reared from the D-shaped larval stage to harvest at 16 months of age.

The pedigree reconstruction was based on microsatellite parentage assignment. For DNA extraction (Li et al., 2006a), about 100 mg of the adductor muscle was collected from each parent and individual offspring. Then PCR amplification was performed with two sets of multiplex PCR markers (Set 1: Crgi3, ucdCg-146, and uscCgi-210; Set 2: ucdCg-120, ucdCg-198, and ucdCg-117) (Liu et al., 2017b). For genotyping, amplification products were resolved with ABI3130 DNA analyzer using a LIZ-500 internal size standard, the lengths of DNA fragments were assessed with the Gene Mapper v4.0 software. The parentage assignment was performed using CERVUS 3.0 (Kalinowski et al., 2007) with the likelihood-based approach. At last, 690 of 810 sampled individuals were unambiguously assigned to one pair of parents with the strict level of 95% confidence interval.

2.2. Trait measurements

Data for growth were collected from all the sampled offspring obtained in our previous work (Wan et al., 2017). The shell height, shell length, shell width, wet weight and shell weight were measured with electronic vernier callipers (0.01 mm accuracy) and an electronic balance (0.1 g accuracy). Then the dressing percentage (DP) was calculated (DP = (WT – SW)/WT * 100%). The soft body tissues of all the offspring were stored in a self-sealing plastic bags in a −80 °C refrigerator in preparation for lyophilization. The lyophilization was carried out with the vacuum freeze-drying equipment for about 12 h. The dry samples were ground into a fine powder that could be sieved through an 80-mesh (0.180 mm) screen.

In this study, the NIRS calibration model established by Wang et al. (2015) was applied to determine the chemical composition of oysters that were cultivated in the area of the Rushan sea and processed with the same preparation and analysis steps as described by Wang et al. (2015). Model-based predictions for GC and TPC were more accurate than that of TFC, ZC, SC and AC; however, with the correlation coefficients of calibration ranging from 0.9164 to 0.9927, they are precise enough to be used in genetic parameter estimation. Data beyond the limitations were eliminated with the assistant of the TQ Analyst software (Version 9.1.17, USA) by the built-in partial least squares (PLS) models. These outliers may randomly happen in oyster families due to the variations in instrument performance and meat composition (Bu et al., 2013). Protein, fat, glycogen, zinc, selenium and ash contents were estimated on a dry-weight basis. Data were collected through the procedure and calibration models for *C. gigas* developed by Wang et al. (2015). Briefly, the procedure includes the following steps: data acquisition through Fourier transform near-infrared reflectance spectrometer; capture of diffuse reflectance spectra with the RESULT software suite (Zang et al., 2012); analysis of spectral data using the spectrum analytic software TQ Analyst (Version 9.1.17, USA) with the applied the calibration model.

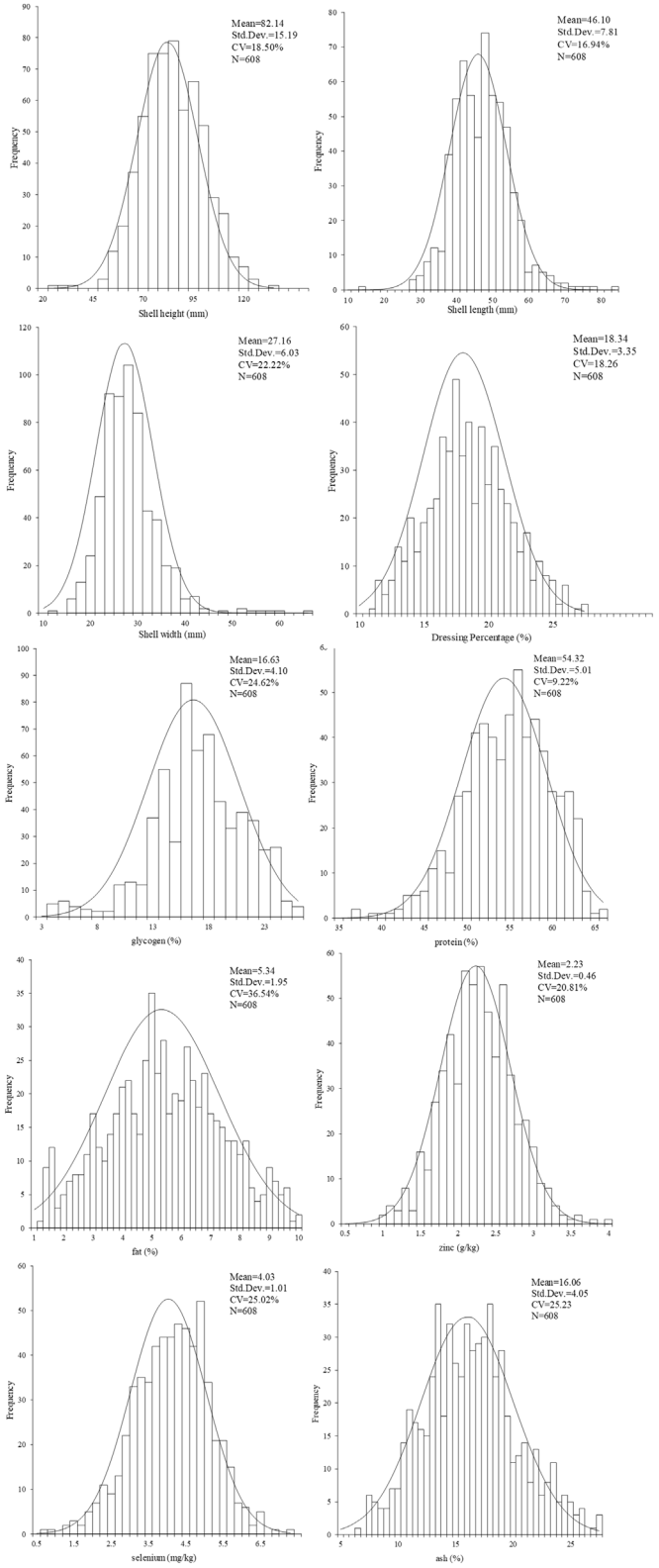

Fig. 1. Distribution of growth and composition traits among individuals (n = 608) remaining after parentage assignment and exclusion of outlier composition data presented on a dry-weight basis (%). Std. Dev is the standard deviation of all the individuals, CV is the coefficient of variation (SD/mean *100%), and N is the number of oysters.

2.3. Data analysis

The data were tested for normality and homogeneity of variances with SPSS 16.0. Based on determined pedigrees, we estimated heritabilities, genetic and phenotypic correlations as well as standard errors using the animal model implemented by ASREML 3.0 in the R Programming Language (Gilmour et al., 2009). Due to the simplification of the random common environment effect resulted from communal rearing as well as scheduled examination, separation and reloading of oysters in culturing lanterns, the model can be expressed as follows to estimate the variance components for each trait:

$$Y_{ijk} = \mu + \alpha_{ijk} + I_j + e_{ijk} \quad \text{(model 1)}$$

Observation Y from sire i, dam j and individual k, was predicted from variables on the right-hand side of the equation. The μ was the mean value of the trait, whereas α_{ijk} was the additive genetic effects for the ijkth animal. I_j was the random effects common to each full-sib family (including maternal, environmental and partially dominant effects). e_{ijk} was the residual error. Genetic and phenotypic correlations were calculated among composition traits and growth traits using the bivariate model (extension of the model 1 above) in ASREML 3.0.

Heritabilities (h^2) was calculated with the following equation:

$$h^2 = \frac{\sigma_\alpha^2}{\sigma_\alpha^2 + \sigma_i^2 + \sigma_e^2} \quad (2)$$

where σ_α^2 was the additive genetic variance, σ_i^2 (the random effect variance) and σ_e^2 (the residual variance) were the non-additive variance. The genetic and phenotypic correlations among composition traits and growth traits were calculated as follows:

$$r = \frac{\sigma_{XY}}{\sqrt{\sigma_X^2 \sigma_Y^2}} \quad (3)$$

To calculate genetic correlations, σ_{XY} was the estimated additive genetic covariance between trait X and trait Y, whereas σ_X^2 and σ_Y^2 were the additive genetic variances of the two traits. When it comes to phenotypic correlations, σ_{XY} was the phenotypic covariance, and σ_X^2 and σ_Y^2 were the phenotypic variances. Statistical analysis was performed with t-test to determine the significance of heritability, genetic and phenotypic correlations as well as the difference between genetic and phenotypic correlations. $t = \frac{h^2}{\sigma_{h^2}}$ and $t = \frac{R_A(x,y)}{\sigma_{R_A}(x,y)}$ were used to test The significance of heritabilities and genetic correlations (Liu et al., 2005).

3. Results

3.1. Descriptive statistics and phenotypic correlations

After parentage assignment and exclusion of outlier on the composition data, a total of 608 individuals for the 30 full-sib families were analyzed (Table 1). The distributions of their growth and composition traits were showed in Fig. 1. In the composition traits, the coefficient of variation (CV) for fat content was highest (36.54%), while the variation of protein content was lowest (CV 9.22%).

The values of phenotypic correlations were all low-to-moderate with the highest but non-significant positive correlation between SC and shell height (0.40 ± 0.27) and the highest but non-significant negative between SL and GC (−0.15 ± 0.14) as well as between DP and TFC (−0.15 ± 0.13).

3.2. Heritability and genetic correlations

The heritabilities of GC, TPC, TFC, ZC, SC and AC, their phenotypic and genetic correlations were listed in Table 2. Among all the six traits analyzed, TFC showed the highest heritability (0.89 ± 0.18; $P < 0.01$) and ZC was the lowest (0.40 ± 0.11; $P < 0.01$). There were medium but significant positive correlations between TFC and ZC

Table 2
Genetic correlations (above diagonal), phenotypic (below diagonal) correlations and heritabilities (in the diagonal) of composition traits. GC, TPC, TFC, ZC, SC and AC represent glycogen, protein, fat, zinc, selenium and ash content. Values after the plus/minus are standard errors. Significance: **p < 0.01, *p < 0.05.

Parameter	GC	TPC	TFC	ZC	SC	AC
GC	0.41 ± 0.19*	−0.65 ± 0.11**	0.23 ± 0.15	0.10 ± 0.11	−0.80 ± 0.27**	−0.66 ± 0.12**
TPC	−0.74 ± 0.05**	0.51 ± 0.09**	−0.40 ± 0.09**	0.30 ± 0.25	0.34 ± 0.15*	0.27 ± 0.14
TFC	0.33 ± 0.13*	−0.30 ± 0.09**	0.89 ± 0.18**	0.81 ± 0.13**	0.27 ± 0.15	−0.35 ± 0.15*
ZC	−0.05 ± 0.08	0.21 ± 0.12	−0.55 ± 0.12**	0.40 ± 0.11**	−0.33 ± 0.18	0.25 ± 0.22
SC	−0.47 ± 0.05**	0.40 ± 0.27	0.14 ± 0.11	−0.20 ± 0.11	0.87 ± 0.06**	0.70 ± 0.24**
AC	−0.52 ± 0.19**	0.35 ± 0.12**	−0.45 ± 0.10**	0.21 ± 0.13	0.69 ± 0.21	0.65 ± 0.13**

(0.81 ± 0.13; $P < 0.01$), as well as between SC and AC (0.70 ± 0.24; $P < 0.01$). In contrast, medium but significant negative correlations were found between GC and TPC (−0.65 ± 0.11; $P < 0.01$), SC (−0.80 ± 0.27; $P < 0.01$), AC (−0.66 ± 0.12; $P < 0.01$). In terms of the phenotypic correlations, there were medium but significant ($P < 0.01$) negative values between GC and TPC (−0.74 ± 0.05; $P < 0.01$), GC and AC (−0.52 ± 0.19; $P < 0.01$), TFC and ZC (−0.55 ± 0.12; $P < 0.01$), as well as medium but non-significant positive value between SC and AC (0.69 ± 0.21).

The genetic correlations between the traits of composition and shell dimension at harvest were weak (Table 3) except that there were medium but significant positive correlations between SC, shell height (0.71 ± 0.09; $P < 0.01$) and shell width (0.52 ± 0.16; $P < 0.01$) as well as between AC and shell width (0.50 ± 0.22; $P < 0.05$). On the contrary, medium but significant negative correlation was obtained between GC and Shell height (−0.68 ± 0.11, $P < 0.01$). With respect to the genetic correlations between composition and growth traits, there were significant differences between them ($P < 0.01$; Table 3). Notably, non-significant differences were only found between TFC, shell width, and total weight, as well as between ZC, shell height, shell length and shell width.

4. Discussion

4.1. Heritabilities of meat composition traits

The heritability estimates were significantly high for GC, TPC, TFC, ZC, SC and AC, and ranged from 0.40 ± 0.11 to 0.87 ± 0.06, which suggested that these meat composition traits of the breeding population of the golden shell strain of *C. gigas* possess the potential to be candidate traits for selective breeding programmes under the commercial rearing condition. In view of a lack of similar study in molluscs, it is useful to compare *C. gigas* with fish species. Specifically, heritabilities of fat content ranged from 0.00 ± 0.23 to 0.68 ± 0.19 in different tissues of nine fish species and that of protein content ranged from 0.02 ± 0.04 to 0.19 ± 0.10 in rainbow trout (Gjedrem, 2017), where low, moderate and high heritabilities were all observed. Thus, the high heritability estimates for meat composition traits in this study were at least supported by part of previous studies. It is noteworthy that the high heritability estimates in our study for ZC, SC and AC, meaning that metal content was strongly affected by genetics, was supported by the moderate heritabilities of metal contents in *C. gigas* reported by Camara et al. (2005) but conflict with the low heritability in Sydney rock oyster *Saccostrea glomerata* reported by Lee et al. (2015). Considering that the common environment effects were eliminated by their communally rearing from D-shaped larvae stage in this study, the higher estimates may reflect the enhanced capacity to parse out genetic effects because of the absence of common environment effects, and the differences in biology and resource allocation between species, which warrants further validation with heritability estimation of other aquaculture shellfish. Indeed, heritability estimates for similar traits can change along with species, culture duration and environmental factors (Visscher et al., 2008). Hence, the specific conditions of oysters used in our study should be emphasized, that is, they were cultured for 16 months in Rushan sea area and were harvested in October, when the gametogenic cycle was in the resting phase (Li et al., 2006b).

4.2. Correlations among meat composition and growth-related traits

Comprehensive analyses showed that the phenotypes and genetic correlations were low to moderate among the six meat component traits. It should be noted that there were moderate negative genetic correlations between TPC and both GC and TFC (−0.65 ± 0.11 and −0.40 ± 0.09, respectively), and low positive genetic correlation between GC and TFC (0.23 ± 0.15), meaning that these three meat composition traits are connected by some physiological metabolic processes and related genes (Ernande et al., 2004). Actually, it has been well established that the metabolism of protein, glycogen and fat was linked by the corresponding enzymes (e.g, enzymes related to tricarboxylic acid cycle) (Park et al. (1998); Fromentin et al. (2011); Li et al. (2017) and was closely linked with energy metabolism (Goward and Nicholls (1994); Attwood (1995).

The phenotypic and genetic correlations between TPC and metal contents (ZC, SC and AC) in our study were positive, which were consistent with the general consensus that one of the primary roles of protein is the homeostasis of essential metals. These positive correlations were in accordance with the generally negative correlations between GC and metal contents, because GC was strongly correlated with TPC. In addition, we also found the genetic correlation between TFC and ZC to be high and positive, which is supported by the knowledge that zinc status is linked to fatness on a genetic basis (Tepaamorndech et al., 2014). The positive correlations between AC and both ZC and SC are consistent with the fact that the mount of the ash content was strongly linked with the metal contents.

We also found noteworthy correlations between growth-related traits and both SC and AC to be positive, indicating the linkage between growth performance and the ability of metal accumulation. On the other hand, there were no significant correlations between DP and meat composition traits in spite of the moderate genetic correlation between DP and ZC, meaning their weak relationships. Negative genetic correlations were observed between GC and growth-related traits, which may reflect trade-offs between glycogen accumulation and growth, similar to trade-offs between reproductive effort and both survival and growth (Ernande et al., 2004).

As moderate-to-high levels of heritabilities were found in various composition traits including GC, TPC, TFC, ZC, SC and AC in the strain of golden shell *C. gigas*, these results indicate a potential for genetic improvement of predictable effects through artificial selection. Furthermore, it is necessary to emphasize that Genotype × environment interactions are common in cultured shellfish. For example, Langdon et al. (2003) found G × E interactions to affect yields of Pacific oyster families significantly ($P < 0.001$). Evans and Langdon (2006) reported significant G × E interactions and these interactions were not large enough to counteract favourable gains in different environments. Even though Genotype × environment interactions on meat composition traits in oysters are not detected, it is reasonable to assume these interactions to exist. But the studied oyster families of the present study were reared in a single site, where G × E

Table 3
Genetic and phenotypic correlations between the composition and growth traits of 16-month-old golden shell color *C. gigas*. GC, TPC, TFC, ZC, SC and AC represent glycogen, protein, fat, zinc, selenium and ash content. r_g and r_p are genetic and phenotypic correlation, respectively. Significance: $^{**}p < 0.01$, $^{*}p < 0.05$. Values after the plus/minus are standard errors.

Trait	GC		TPC		TFC		ZC		SC		AC	
	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p
Shell height (mm)	−0.68 ± 0.11**	−0.20 ± 0.22	0.20 ± 0.12	0.01 ± 0.11	0.18 ± 0.11	0.11 ± 0.13	−0.20 ± 0.15	−0.10 ± 0.09	0.71 ± 0.09**	0.40 ± 0.27	0.45 ± 0.15**	0.20 ± 0.08*
Shell length (mm)	−0.31 ± 0.15*	−0.15 ± 0.14	−0.02 ± 0.06	0.04 ± 0.15	0.22 ± 0.17	0.15 ± 0.23	−0.27 ± 0.22	−0.08 ± 0.12	0.44 ± 0.12**	0.15 ± 0.08	0.33 ± 0.21	0.11 ± 0.13
Shell width (mm)	−0.23 ± 0.13	0.01 ± 0.09	0.05 ± 0.13	−0.03 ± 0.08	0.16 ± 0.16	0.18 ± 0.22	−0.01 ± 0.20	0.02 ± 0.11	0.52 ± 0.16**	0.33 ± 0.06**	0.50 ± 0.22*	0.18 ± 0.12
Dressing Percentage (%)	0.29 ± 0.27	0.21 ± 0.11	0.03 ± 0.29	−0.09 ± 0.11	−0.32 ± 0.27	−0.15 ± 0.13	0.44 ± 0.27	0.15 ± 0.10	−0.23 ± 0.26	−0.10 ± 0.12	−0.23 ± 0.27	−0.10 ± 0.12

interactions cannot be evaluated. Whether our results are applicable should then be reassessed before their application to different environments. Meanwhile, when aiming to improve composition traits, it is important to consider the correlations among composition traits and growth traits and their positive or negative effects on each other.

In conclusion, we measured the various traits of meat composition in oyster with NIRS technology. The heritability estimates of GC, TPC, TFC, ZC, SC and AC were high and correlations were moderate among some of the composition traits and growth traits, which offered the basis for the further improvement of the golden shell strain of *C. gigas*.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported by grants from National Natural Science Foundation of China (31772843 and 31741122), the Fundamental Research Funds for the Central Universities (201762014), Shandong Province (2017LZGC009), and research project of the Ocean University of China-Auburn University Joint Research Center for Aquaculture and Environmental Science.

References

Attwood, P.V., 1995. The structure and the mechanism of action of pyruvate carboxylase. Int. J. Biochem. Cell Biol. 27 (3), 231–249.

Azema, P., Lamy, J.B., Boudry, P., Renault, T., Travers, M.A., Degremont, L., 2017. Genetic parameters of resistance to Vibrio aestuarianus, and OsHV-1 infections in the Pacific oyster, *Crassostrea gigas*, at three different life stages. Genet. Sel. Evol. 49 (1), 23.

Brown, M.R., Kube, P.D., O'Connor, S., Cunningham, M., King, H., 2012. Application of near-infrared reflectance spectroscopy for the rapid chemical analysis of Sydney rock oyster (*Saccostrea glomerata*) and Pacific oyster (*Crassostrea gigas*). J. Shellfish Res. 31, 1051–1060.

Bu, D., Wan, B., McGeorge, G., 2013. A discussion on the use of prediction uncertainty estimation of NIR data in partial least squares for quantitative pharmaceutical tablet assay methods. Chemometr. Intell. Lab. 120, 84–91.

Camara, M.D., Griffith, S.M., Evans, S., 2005. Can selective breeding reduce the heavy metals content of pacific oysters (*Crassostrea gigas*), and are there trade-offs with growth or survival? J. Shellfish Res. 24, 979–986.

Cochet, M., Brown, M., Kube, P., Elliott, N., Delahunty, C., 2015. Understanding the impact of growing conditions on oysters: a study of their sensory and biochemical characteristics. Aquacult. Res. 46, 637–646.

de Melo, C.M.R., Durland, E., Langdon, C., 2016. Improvements in desirable traits of the Pacific oyster, *Crassostrea gigas*, as a result of five generations of selection on the west coast USA. Aquaculture 460, 105–115.

Degremont, L., Lamy, J.B., Pepin, J.F., Travers, M.A., Renault, T., 2015. New insight for the genetic evaluation of resistance to Ostreid Herpesvirus infection, a worldwide disease. In: Crassostrea gigas. PLoS One, vol. 10, e0127917.

Dridi, S., Romdhane, M.S., Elcafsi, M., 2007. Seasonal variation in weight and biochemical composition of the Pacific oyster, *Crassostrea gigas* in relation to the gametogenic cycle and environmental conditions of the Bizert Lagoon, Tunisia. Aquaculture 263, 238–248.

Ernande, B., Boudry, P., Clobert, J., Haure, J., 2004. Plasticity in resource allocation based life history traits in the Pacific oyster, *Crassostrea gigas*. I. Spatial variation in food abundance. J. Evolution Biol. 17, 342–356.

Evans, S., Langdon, C., 2006. Effects of genotype x environment interactions on the selection of broadly adapted Pacific oysters (*Crassostrea gigas*). Aquaculture 261, 522–534.

Fromentin, C., Azzout-Marniche, D., Tomé, D., Even, P., Luengo-Guyonnot, C., Piedcoq, J., Fromentin, G., Gaudichon, C., 2011. The postprandial use of dietary amino acids as an energy substrate is delayed after the deamination process in rats adapted for 2 weeks to a high protein diet. Amino Acids 40 (5), 1461–1472.

Garcia, A.L.S., de Oliveira, C.A.L., Karim, H.M., Sary, C., Todesco, H., Ribeiro, R.P., 2017. Genetic parameters for growth performance, fillet traits, and fat percentage of male Nile tilapia (*Oreochromis niloticus*). J. Appl. Genet. 4, 527–533.

Gilmour, A.R., Gogel, B.J., Cullis, B.R., Thompson, R., 2009. ASReml User Guide Release 3.0. VNS International Ltd., Hemel Hempstead, United Kingdom 1-398.

Gjedrem, T., 2017. Possibility for improving carcass composition and meat quality traits by selective breeding. Int. J. Cur. Res. Rev. 9, 11–18.

Gjedrem, T., Rye, M., 2016. Selection response in fish and shellfish: a review. Rev. Aquac. 10, 1–12.

Goward, C.R., Nicholls, D.J., 1994. Malate dehydrogenase: a model for structure, evolution, and catalysis. Protein Sci. 3 (10), 1883–1888.

Guévélou, E., Allen Jr., S.K., 2016. Use of Near Infrared Reflectance Spectroscopy (NIRS) for the rapid compositional analysis of di-, tri-, and tetraploid eastern oysters

(*Crassostrea virginica*). Aquaculture 459, 203–209.
Hosoi, M., Kubota, S., Toyohara, M., Toyohara, H., Hayashi, I., 2003. Effect of salinity change on free amino acid content in Pacific oyster. Fish. Sci. 69, 395–400.
Kalinowski, S.T., Taper, M.L., Marshall, T.C., 2007. Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16, 1099–1106.
Kong, N., Li, Q., Yu, H., Kong, L., 2015. Heritability estimates for growth-related traits in the Pacific oyster (*Crassostrea gigas*) using a molecular pedigree. Aquacult. Res. 46, 499–508.
Laghari, M.Y., Lashariab, P., Zhanga, Y., Sun, X., 2014. Identification of quantitative trait loci (QTLs) in aquaculture species. Rev. Fish. Sci. Aquac. 22, 221–238.
Langdon, C., Evans, F., Jacobson, D., Blouin, M., 2003. Yields of cultured Pacific oysters *Crassostrea gigas* Thunberg improved after one generation of selection. Aquaculture 220, 227–244.
Lee, J.H., Birch, G.F., Cresswell, T., Johansen, M.P., Adams, M.S., Simpson, S.L., 2015. Dietary ingestion of fine sediments and microalgae represent the dominant route of exposure and metal accumulation for Sydney rock oyster (*Saccostrea glomerata*): a biokinetic model for zinc. Aquat. Toxicol. 167, 46–54.
Li, Q., Yu, H., Yu, R., 2006a. Genetic variability assessed by microsatellites in cultured populations of the Pacific oyster (Crassostrea gigas) in China. Aquaculture 259, 95–102.
Li, Q., Liu, W., Shirasu, K., Chen, W., Jiang, S., 2006b. Reproductive cycle and biochemical composition of the Zhe oyster *Crassostrea plicatula* Gmelin in an eastern coastal bay of China. Aquaculture 261, 752–759.
Li, Q., Wang, Q., Liu, S., Kong, L., 2011. Selection response and realized heritability for growth in three stocks of the Pacific oyster Crassostrea gigas. Fish. Sci. 77, 643–648.
Li, B.S., Song, K., Meng, J., Li, L., Zhang, G.F., 2017. Integrated application of transcriptomics and metabolomics provides insights into glycogen content regulation in the Pacific oyster *Crassostrea gigas*. BMC Genomics 18, 713.
Liu, X.L., Chang, Y.Q., Xiang, T.H., Cao, X.B., 2005. Estimates of genetic parameters for growth traits of the sea urchin, *Strongylocentrotus intermedius*. Aquaculture 243, 27–32.
Liu, S.W., Li, Q., Yu, H., Kong, L.F., 2017a. Relationship between single nucleotide polymorphism of glycogen synthase gene of Pacific oyster *Crassostrea gigas* and its glycogen content. J. Ocean Univ. China 16, 168–174.
Liu, T., Li, Q., Song, J., Yu, H., 2017b. Development of genomic microsatellite multiplex PCR using dye-labeled universal primer and its validation in pedigree analysis of Pacific oyster (*Crassostrea gigas*). J. Ocean Univ. China 16, 151–160.
Madigan, T., Kiermeier, A., Carragher, J., de Barros Lopes, M., Cozzolino, D., 2013. The use of rapid instrumental methods to assess freshness of half shell Pacific oysters, *Crassostrea gigas*: a feasibility study. Innov. Food Sci. Emerg. Technol. 19, 204–209.
NSPRC, National Standards of the People's Republic of China, 2008a. Determination of Crude Fat in Foods GB/T 14772-2008. Issued by Ministry of Health, People's Republic of China (in Chinese).
NSPRC, 2008b. Meat and Meat Products—Method for Determination of Zinc GB/T 9695.20-2008. Issued by Ministry of Health, People's Republic of China (in Chinese).
NSPRC, 2008c. Meat and Meat Products—Determination of Total Ash GB/T 9695.18-2008. Issued by Ministry of Health, People's Republic of China (in Chinese).
NSPRC, 2010a. National Food Safety Standard—Determination of Selenium in Foods GB 5009.93-2010. Issued by Ministry of Health, People's Republic of China (in Chinese).
NSPRC, 2010b. National Food Safety Standard—Determination of Protein in Foods GB 5009.5-2010. Issued by Ministry of Health, People's Republic of China (in Chinese).
Park, J.Y., Kim, C.H., Hong, S.K., Suh, K.I., Lee, K.U., 1998. Effects of FFA on insulin-stimulated glucose fluxes and muscle glycogen synthase activity in rats. Am. J. Phys. 275 (2 Pt 1), E338–E344.
Pennarun, A.-L., Prost, C., Jl, H., Demaimay, M., 2003. Comparison of two microalgal diets. 2. Influence on odorant composition and organoleptic qualities of raw oysters (*Crassostrea gigas*). J. Agric. Food Chem. 51, 2011–2018.
Rye, M., Gjerde, B., 1996. Phenotypic and genetic parameters of composition traits and flesh color in Atlantic salmon, *Salmo salar* L. Aquacult. Res. 27, 121–133.
Shahidi, F. (Ed.), 1998. Flavor of Meat, Meat Products and Seafoods, second ed. Blackie Academic & Professional, London.
She, Z.C., Li, L., Qi, H.G., Song, K., Que, H.Y., Zhang, G.F., 2015. Candidate gene polymorphisms and their association with glycogen content in the Pacific oyster *Crassostrea gigas*. PLoS One 10, e0124401.
Tepaamorndech, S., Kirschke, C.P., Huang, L., 2014. Linking cellular zinc status to body weight and fat mass: mapping quantitative trait loci in Znt7 knockout mice. Mamm. Genome 25 (7–8), 335–353.
Visscher, P.M., Hill, W.G., Wray, N.R., 2008. Heritability in the genomics era–concepts and misconceptions. Nat. Rev. Genet. 9, 255–266.
Wan, S., Li, Q., Liu, T., Yu, H., Kong, L., 2017. Heritability estimates for shell color-related traits in the golden shell strain of Pacific oyster (*Crassostrea gigas*) using a molecular pedigree. Aquaculture 476, 65–71.
Wang, W., Yang, J., Li, Q., Ji, R., Gong, X., Li, L., 2015. Development of calibration models for rapid determination of chemical composition of Pacific oyster (*Crassostrea gigas*) by near infrared reflectance spectroscopy. J. Shellfish Res. 34, 303–309.
Xu, L., Li, Q., Yu, H., Kong, L., 2017. Estimates of heritability for growth and shell color traits and their genetic correlations in the black shell strain of Pacific Oyster *Crassostrea gigas*. Mar. Biotechnol. 19, 421–429.
Zang, H., Li, L., Wang, F., Yi, Q., Dong, Q., Sun, C., Wang, J., 2012. A method for identifying the origin of chondroitin sulfate with near infrared spectroscopy. J. Pharm. Biomed. Anal. 61, 224–229.