

Image analysis for estimating soft body mass from shell morphology in *Crassostrea gigas*

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Abstract: Oyster farming is one of the oldest forms of aquaculture and one of the largest markets in the world. Oysters are sessile organisms whose shell morphologies are highly variable, and strongly adhere on host substrates. The relationship between shell morphology and soft body mass in oysters is poorly understood because of the difficulty in the morphological analysis of complex-shaped shells. The “ideal” shell shape for aquaculture production has not been determined for more than 2,000 years.

In this study, we tried to develop a new analytical approach to estimate the soft body mass from the shell morphology in Pacific oyster, *Crassostrea gigas*. We assumed that the whole shell morphology should be translated into numerical values that characterize the soft body mass, rather than traditional indices comprised of a limited number of linear distances such as shell length and width. We first collected the overhead photographic data from the shells. These data were transformed into numerical values that represent the morphological components using elliptic Fourier analysis. Then, we screened the morphological components that could affect the relative soft-body mass using a generalized linear model. Using these analytical results, we developed a numerical model suitable for evaluation of the oyster meat production by the shell morphology. Our results will help establish a new criterion for assessing the quality of oysters as seafood and promote oyster farming.

Key words: allometry, aquaculture, elliptic Fourier analysis, oyster farming

Introduction

Oyster farming is one of the oldest forms of aquaculture and believed that it had begun in ancient Rome (Laing and Bopp 2018). Global aquaculture production in oyster farming has continuously been increasing over the past 70 years, and the total production had increased 1.5-fold from 4 million tons in 2009 to 6 million tons in 2019 (FAO 2024). The major species used in oyster farming is Pacific oyster, *Crassostrea gigas*. With the increase in the market value of farmed oysters, the

production value had doubled from 3 billion USD to 6 billion USD during those 10 years. In addition to the east Asian countries, culture of eating oysters, as represented by oyster bars in USA and Europe, has become popular. Oyster farming is now one of the central parts of the world’s fisheries industry and has already been took root in our food culture.

Oysters are sessile organisms whose shell morphologies are highly variable, and strongly adhere on host substrates. These variations affect the size of the edible part of oysters; thus, shell morphology is an important factor in evaluating the oyster

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quality (Brake *et al.* 2003; Mizuta and Wikfors 2019). Notably, the ratio of shell height/shell length (D/L) or shell width/shell length (W/L) could be useful indices for determining the oyster quality (Mizuta and Wikfors 2019). For example, desirable shell traits have been suggested to have D/L of 0.33 or $D/L > 0.25$ and W/L of 0.67 or $W/L > 0.63$ (Brake *et al.* 2003; Mizuta and Wikfors 2019). However, practical use of these proposed ratios for quality classification seems to be limited because the validity of them is still under discussion.

Based on the current situation, we expected that the difficulty in morphological analysis for estimating the oyster quality could be caused by the complex-shaped shell, which disturbs universal measurement of linear distances. We further considered that whole shell morphology should be translated into numerical values that characterize the soft body mass, rather than traditional simple linear indices. In this study, we developed a novel analytical approach to estimate the soft body mass from shell morphology in Pacific oyster.

Materials and Methods

Data collection

The experimental animals were collected from a local oyster farm in Uramura (34°26'N, 136°53'E; Toba, Mie, Japan). After measuring the total and soft body weights, the upper surface of the shell was photographed. To collect stable photographic data, the target oyster shell was placed in a transparent acrylic box (20 × 20 × 20 cm), and photographs were taken by fixing a digital camera above the upper side. Subsequently, the contour of the shell was manually traced using a tracing paper, and the scanned data were used for elliptic Fourier analysis. The contour at the upper side of the target was converted into a Fourier series using SHAPE ver. 1.3 (Iwata and Ukai 2002). The Fourier series was used for principal component analysis to calculate the variables that indicated independent morphological changes. The effective number of principal components (PCs) was selected in accordance with the Kaiser criterion (Jackson 1993).

Statistical analysis

The correlation between the soft body mass ratio (wet soft body weight/total body weight) and morphological indices was analyzed using a generalized linear model (GLM) with log-transformation and Gaussian distribution. Total body weight, PCs, and distance from the means of the PCs were used as explanatory variables (Table 1). To evaluate the validity of the conventional method and the method used in this study, D/L

and W/L were also included as explanatory variables. The best model was selected using stepwise model selection in accordance with Akaike's information criterion (AIC). The significance of each parameter was tested using a likelihood ratio test. All statistical analyses were conducted using R software version 4.0.2 (R Core Team 2023).

Table 1 Variables used for the GLM analysis targeting the ratio of the soft body mass in the total body weight

Classification	Variable
Body size	Total body weight
Conventional indices for shell morphology	D/L (shell height / shell length)
	W/L (shell width / shell length)
Principal component scores by elliptic Fourier analysis targeting the top view of the shell	PC1
	PC2
	PC3
	PC4
	PC5
	PC6
	PC7
	PC8
	PC9
Distance from the means of the principal component scores above	dPC1
	dPC2
	dPC3
	dPC4
	dPC5
	dPC6
	dPC7
	dPC8
	dPC9
Cross terms of the body size and the indices characterizing the shell morphology	Total body weight × D/L
	Total body weight × W/L
	Total body weight × PC1
	Total body weight × PC2
	Total body weight × PC3
	Total body weight × PC4
	Total body weight × PC5
	Total body weight × PC6
	Total body weight × PC7
	Total body weight × PC8
	Total body weight × PC9
	Total body weight × dPC1
	Total body weight × dPC2
	Total body weight × dPC3
	Total body weight × dPC4
	Total body weight × dPC5
	Total body weight × dPC6
	Total body weight × dPC7
Total body weight × dPC8	
Total body weight × dPC9	

Results and Discussion

Elliptic Fourier analysis was used to convert the shell morphology data obtained from the overhead view into nine PCs (Fig. 1). PC1, which represented the highest proportion, indicated the morphological changes represented by W/L. PC2 was considered to indicate the direction of asymmetry in shell morphology. The morphological changes that could not be quantified by the conventional distance measurements were detected, as in the other PCs. The correlation of the scores in these PCs and the relative soft body mass was examined by GLM analysis with stepwise model selection to determine which morphological variation changes the soft body size. The actual relative soft-body mass and that estimated by the best model obtained in that analysis showed a high correlation (Fig. 2; $R^2 = 0.4859$), indicating that the model obtained in this study could be useful for estimating the soft-body size in the target oyster population.

Simple indices, such as D/L or W/L, have often been used for oyster quality classification (Mizuta and Wikfors 2019), but the result of our GLM analysis implies that these conventional indices might not be useful for estimating the oyster quality. The three models used in our analysis did not include any conventional morphological indices. Instead, PC2 and PC9 were included in the model (Table 2). Only the coefficients in PC9 were significant in all the models ($n = 33$; likelihood ratio test; $P < 0.05$). This indicates the importance of PC9 in determining the soft-body mass estimated from the shell morphology, although PC9 represented the lowest proportion in explaining the variations in the shell morphology. Since the PCs that represented the major morphological variations were not significant or eliminated from the models, the use of major morphological variations could mislead us in estimating the oyster quality. The effect of PC9 on the soft-body size changes was classified into two categories. The relative soft-body size decreased as the distance between the PC9 score and the mean increased. This suggests that the mean shape is the best one representing the highest relative soft body mass. Secondly, the effect of PC9 described above decreased as the body size increased. In other words, body size is considered a negative factor for the effect of PC9.

Thus, this case study indicated the unreliability of conventional simple indices of shell morphology in determining the oyster quality and proposed a novel morphological factor to estimate the quality. The biological function that relates to the morphological changes determined by PC9 is not clear in this study; therefore, we are currently working on this issue. In the

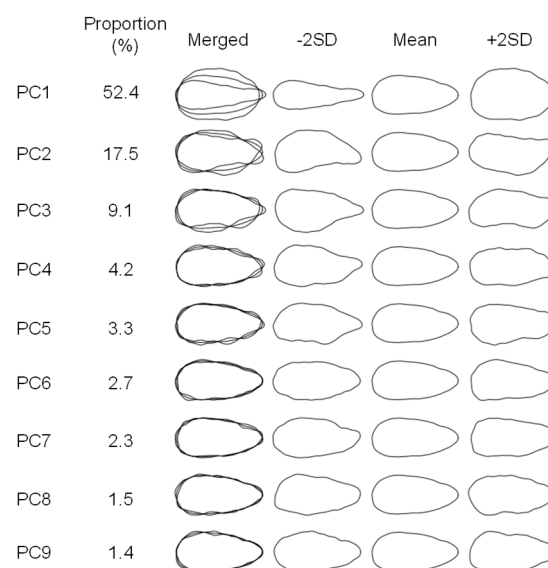


Fig. 1 The results of the elliptic Fourier analysis and subsequent PC analysis

The respective figures at mean, -2SD and +2SD indicate the shape changes when each PC score took those mean, -2SD and +2SD values.

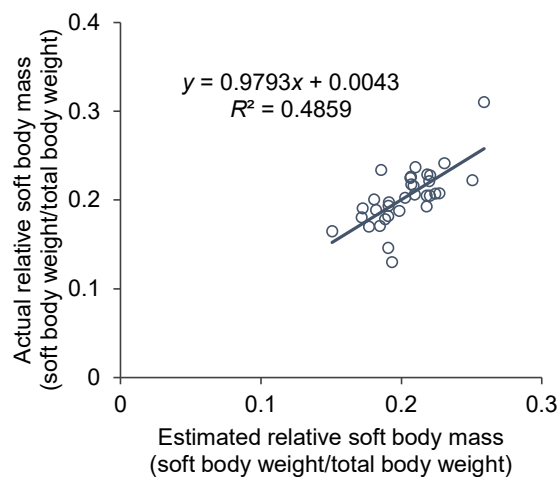


Fig. 2 The correlation between the actual relative soft body mass and that estimated by the model obtained by the GLM analysis

present study, it was suggested that the PCs that determine the relative soft body size seem to change depending on the environmental factors. Therefore, PC9 cannot always be used as a reliable morphological index to estimate the oyster quality. Reliable morphological indices that could practically be used for evaluation of the oyster quality have not been found for decades, since the morphology of oyster shells is highly variable. Further studies will help to establish a more efficient marketing strategy for oyster farming.

Table 2 The results of the GLM analysis targeting the ratio of the soft body mass in the total body weight

	Model			
	1 (best)	2	3	Null
AIC	-142.22	-142.05	-142.04	-130.28
Intercept	-1.067 (0.122)	-0.9737 (0.1395)	-1.074 (0.123)	-1.593 (0.027)
Total body weight	-0.007567 (0.002022)**	-0.008506 (0.002082)**	-0.007145 (0.002020)**	
Estimated coefficient (SE)	PC2	-0.6472 (0.4406)	-0.8544 (0.4610)	-0.6165 (0.4505)
	dPC5		-1.914 (1.569)	
	dPC9	-35.07 (11.23)***	-40.52 (11.86)***	-0.3078 (10.87)***
	dPC7	2.561 (1.841)	2.810 (1.861)	
Total body weight × dPC9	0.454 (0.1972)*	0.5307 (0.2034)*	0.4105 (0.1964)*	

The three models that represented lower AIC values and the null model were shown.

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ ($n = 33$; likelihood-ratio test)

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Annotated Bibliography of Key Works

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In this study, the authors developed a software that transforms the morphologies of target objects from photographic data using elliptic Fourier analysis. This software enables an easy analysis using elliptical Fourier descriptors.