fishes

MDPI

Article

Identification of "Bathed" Chinese Mitten Crabs (*Eriocheir sinensis*) Using Geometric Morphological Analysis of the Carapace

Yiqian Xu [1], Junren Xue [2], Hongbo Liu [1,2,*], Tao Jiang [2], Xiubao Chen [2] and Jian Yang [1,2,*]

1 Wuxi Fisheries College, Nanjing Agricultural University, Wuxi 214081, China; 2022813042@stu.njau.edu.cn
2 Laboratory of Fishery Microchemistry, Freshwater Fishery Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, China; xuejunren@ffrc.cn (J.X.); jiangt@ffrc.cn (T.J.); chenxb@ffrc.cn (X.C.)
* Correspondence: liuhb@ffrc.cn (H.L.); jiany@ffrc.cn (J.Y.); Tel./Fax: +86-510-8555-7823 (J.Y.)

Abstract: To confirm whether Chinese mitten crabs (*Eriocheir sinensis*), commonly known as hairy crabs or river crabs, in non-Yangcheng Lake areas undergo morphological convergence with the original crabs in the Yangcheng Lake purse seine and high-standard modified aquaculture ponds after being "introduced" or "bathed"-cultured, we employed a geometric morphometrics approach. This approach allowed us to compare and analyze the dynamic changes in the carapace morphology of both the original and "introduced" crabs in Yangcheng Lake and high-standard ponds in Kunshan City at 0, 7, 14, and 30 days after "bathing" culture. The geometric morphological analysis of the carapace was conducted using a system of 35 established landmarks. The stepwise discriminant analysis of the relative distortion score revealed morphological differences between "introduced" and resident original crabs in the Yangcheng Lake area and in high-standard modified ponds after 7, 14, and 30 days of bathing culture. The accuracy of the discriminant analysis was 100%. The results of the geometric morphological visualization demonstrated that the carapaces of the bathed crabs underwent adaptive changes in the water. However, even after one month of bathing culture, the "introduced" crabs in lakes and ponds could not reach the morphological characteristics of the original crabs, i.e., their carapaces did not exhibit the characteristics of the original crabs.

Keywords: *Eriocheir sinensis*; landmark; geometric morphometrics; "introduced"/"bathed" crab; discriminant analysis; aquaculture

Key Contribution: The carapaces of "bathed" crabs underwent adaptive changes in the water. The accuracy of the discrimination between bathed and original crabs was 100% using geometric morphometrics.

Citation: Xu, Y.; Xue, J.; Liu, H.; Jiang, T.; Chen, X.; Yang, J. Identification of "Bathed" Chinese Mitten Crabs (*Eriocheir sinensis*) Using Geometric Morphological Analysis of the Carapace. *Fishes* **2024**, *9*, 6. https://doi.org/10.3390/fishes9010006

Academic Editor: Alberto Teodorico Correia

Received: 11 November 2023
Revised: 13 December 2023
Accepted: 19 December 2023
Published: 21 December 2023

1. Introduction

Chinese mitten crabs (*Eriocheir sinensis*, H. Milne-Edwards, 1853), commonly known as river crabs or hairy crabs, belong to the genera Crustacean, Decapoda, Grapsidae, and *Eriocheir*. They are widely distributed in the eastern coastal provinces of China and represent a famous, high-quality, and important aquatic product [1]. Chinese mitten crabs are preferred by consumers owing to their delicious taste and rich nutrients, such as amino acids, vitamins, and minerals, which are easily digested and absorbed by the human body [2]. The Yangcheng Lake crabs, which are known for their distinctive features, including "green backs, white bellies, and golden claws with yellow hair", are considered the most prestigious specimens.

In the early 1990s, with the breakthrough in artificial breeding technology for Chinese mitten crabs and the support of local governments, purse seine and pond cultures of Chinese mitten crabs in Yangcheng Lake experienced unprecedented growth [3]. In 2005, the General Administration of Quality Supervision, Inspection, and Quarantine of China and the State Standardization Administration formally promulgated the "Geographical Identified Product Yangcheng Lake Chinese Mitten Crabs" and defined the associated protected area and special designation. Therefore, the Yangcheng Lake Chinese mitten crabs expanded both in scale and economic importance. In 2020, the Ministry of Agriculture and Rural Affairs issued Announcement No. 290, and "Yangcheng Lake Chinese mitten crabs" were granted the Geographical Identified Registration Certificate of Agricultural Products of the People's Republic of China. This certificate also covers crabs that are cultivated in high-standard, modified ponds. These ponds have continuous centralized transformation, separate irrigation and drainage facilities, and are equipped with three-level purification facilities, located around Yangcheng Lake. According to the Bureau of Agriculture and Rural Affairs of Suzhou City, Chinese mitten crab production in 2022 is expected to reach approximately 1500 tons in the Yangcheng Lake purse seine culture area and around 7900 tons in high-standard ponds. However, the annual sales volume of Chinese mitten crabs from Yangcheng Lake exceeds 10,000 tons due to the prevalence of illegal traders passing off regular crabs as Yangcheng Lake Chinese mitten crabs to maximize profits [4]. This deceptive practice harms the interests of consumers and farmers, disrupts the market, and severely tarnishes the reputation of the Yangcheng Lake crab's geographical identity.

Geographical identity plays a pivotal role in the development of agricultural products. They help distinguish similar products, enhance product competitiveness, increase the value of agricultural products, increase farmers' incomes, expand the market share of high-quality agricultural products, and promote trade development [5,6]. Therefore, it is essential to combat the fraud of the Yangcheng Lake crab and protect its geographical identity. Although legal measures related to geographically identified protection have improved in recent years and physical anti-counterfeiting marks have been continuously upgraded, counterfeiting remains prevalent, with various methods in use. The most common counterfeiting method on the market involves "introduced" crabs, also known as "bathed" crabs [7]. "Introduced"/"Bathed" crabs (hereinafter referred to as "bathed" crabs) refer to the transportation of foreign crabs to Yangcheng Lake, where they are cultured for a few days before being sold as resident genuine Yangcheng Lake crabs. The morphology of aquatic organisms is subject to environmental factors, including the water environment (e.g., temperature, depth) and the nutritional environment in which they live. Various studies [8,9] have confirmed this. Introducing Chinese mitten crabs to different water environments for "bathing" has the potential to alter the morphology of their carapace. Xue et al. [7] successfully differentiated "bathed" crabs from original crabs using geometric morphology, achieving a discrimination accuracy of 100%. However, there has been limited research on the dynamic changes in carapace morphology during the "bathing" process, as well as the characteristics of "bathed" crabs in high-standard modified ponds.

Traditional morphometric methods can describe the morphology of a sample by measuring the full length, body length, and other parts of the organism. However, this process may oversimplify an individual's shape and not provide a complete picture of changes in individual morphology. In contrast, geometric morphometrics is a quantitative method that can analyze morphological differences in organisms [10]. The analysis of objects through landmarks allows for the effective extraction of morphological information, resulting in a greater reduction in information loss and a more complete representation of the structure's geometric shape. Furthermore, this method enables the quantification and visualization of morphology, providing a better reflection of morphological changes in species [11,12]. For example, the landmark method has been applied to differentiate Chinese mitten crabs from various water systems [13,14] and to identify morphological similarities and differences between "bathed" and original crabs [7]. These studies have consistently achieved accurate discriminating results. In our study, we used the landmark method to compare the carapace morphology of original crabs from Yangcheng Lake and high-standard modified ponds with that of "bathed" crabs transported from the non-Yangcheng Lake area to two locations for "bathing". Our aim was to confirm that after a month of "bathing", the "bathed" crabs from Yangcheng Lake and high-standard modified ponds do not acquire the same morphological characteristics as the original crabs. An accurate understanding of the dynamic changes in carapace morphology during the "bathing" process offers crucial theoretical support for the identification of crabs of Yangcheng Lake origin and the protection of geographically distinct products.

2. Materials and Methods

2.1. Sample Collection

On 10 October 2022, Day-0 samples of original Chinese mitten crabs were collected from high-standard modified ponds (crabs from Kunshan ponds) and the Yangcheng Lake purse seine culture area (crabs from Yangcheng Lake) in Kunshan City (Figure 1) and then transported to the laboratory for morphometric measurements. Simultaneously, Chinese mitten crabs from culture ponds in Jianhu Country (crabs from Jianhu ponds) were collected as "bathed" crabs on day 0 (Figure 1). A portion of these samples, along with some previously collected samples, were sent back to the laboratory for morphometric measurements. The remaining individuals were released to Kunshan ponds and the Yangcheng Lake purse seine culture area for continued "bathing". During bathing, the feeding modes of foreign crabs are identical to those of the native crabs with natural and exogenous food sources. On the 7th, 14th, and 30th days of "bathing" culture, corresponding to October 17th, 24th, and November 8th, four sets of crab samples were collected and sent to the laboratory for cryopreservation. This resulted in 15 sample groups: original crabs from Kunshan ponds, "bathed" crabs from Kunshan ponds, original crabs from Yangcheng Lake, and "bathed" crabs from Yangcheng Lake. For each of these 15 sample groups, 10 Chinese mitten crab samples (comprising 5 females and 5 males) with similar specifications were selected for morphological comparison (Table 1). Ten Chinese mitten crabs with similar specifications were selected for the experiment to minimize errors, and an equal number of male and female crabs were selected to avoid inaccurate results due to gender imbalance. It is worth noting that before the "bathing" culture, adult Chinese mitten crabs were used in this study, which were not affected by the molt.

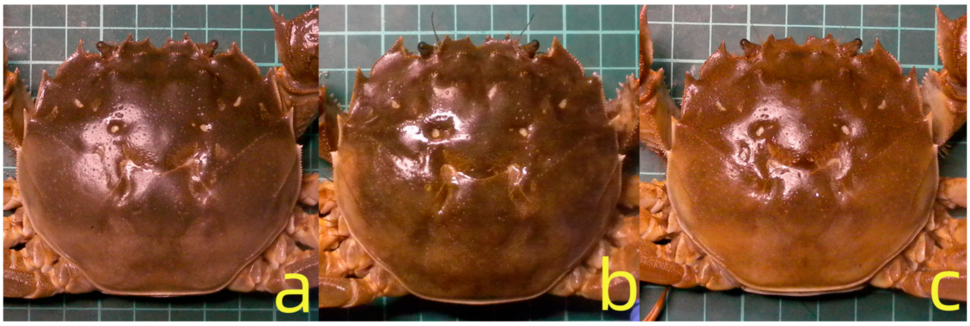

Figure 1. Chinese mitten crabs (*Eriocheir sinensis*) from three culture habitats. Notes: (**a**) crabs from Jianhu ponds; (**b**) crabs from Kunshan ponds; and (**c**) crabs from Yangcheng Lake.

2.2. Establishment and Extraction of a Landmark

Landmarks are points with distinctive and easily recognizable features [15]. The carapace of Chinese mitten crabs possesses such characteristics, making it an ideal subject for our study. To ensure landmark standardization, we maintained a fixed shooting height and focal length when capturing images of crab carapaces using a Nikon COOLPIX P6000 digital camera (Nikon, Tokyo, Japan). Images, once captured and processed, were imported into tpsDig2 (version 2.31) (Rohlf, 2015). Following landmark classification principles, we selected 35 landmarks on the crab carapace (Figure 2 and Table 2). The X and Y coordinate values of these landmarks were recorded to create a landmark data file.

Table 1. Morphometric information about the groups of Chinese mitten crabs used in this study.

Group Code	Sampling Date	Body Weight (g)	Carapace Length (mm)	Carapace Width (mm)	Body Height (mm)
JH	10 October 2022	166.23 ± 23.96	65.89 ± 1.85	72.75 ± 3.30	36.19 ± 1.13
KS		165.83 ± 38.97	64.79 ± 3.60	70.48 ± 5.15	35.46 ± 1.76
YC		121.80 ± 16.43	59.21 ± 2.17	64.74 ± 1.92	32.41 ± 1.84
KS	17 October 2022	157.17 ± 28.84	64.39 ± 2.73	70.28 ± 3.41	35.55 ± 1.17
KSBC		171.64 ± 29.96	64.75 ± 2.25	71.77 ± 2.89	37.00 ± 0.85
YC		139.81 ± 25.26	61.70 ± 2.51	67.93 ± 2.84	34.34 ± 1.79
YCBC		168.42 ± 28.53	64.35 ± 1.48	71.25 ± 2.30	36.46 ± 1.44
KS	24 October 2022	173.31 ± 29.59	65.26 ± 1.76	71.09 ± 2.92	36.92 ± 1.28
KSBC		164.97 ± 30.70	63.72 ± 2.11	71.58 ± 2.90	35.63 ± 1.15
YC		131.51 ± 17.79	60.21 ± 3.01	66.23 ± 2.70	33.83 ± 2.09
YCBC		165.76 ± 28.65	64.08 ± 1.73	71.11 ± 2.65	35.62 ± 1.03
KS	8 November 2022	176.92 ± 26.12	65.85 ± 1.75	72.53 ± 2.13	37.36 ± 1.06
KSBC		172.67 ± 24.45	68.20 ± 2.56	75.99 ± 2.88	41.39 ± 1.29
YC		135.47 ± 22.59	59.81 ± 3.02	66.60 ± 3.02	33.77 ± 1.21
YCBC		165.17 ± 25.75	62.98 ± 2.16	70.86 ± 2.71	36.37 ± 1.65

Note: Data are represented as the mean ± standard deviation (n = 10). Values in the same row with different letters are significantly different ($p < 0.05$). Note: JH: crabs of Jianhu pond origin; KS: crabs from Kunshan ponds; YC: crabs from Yangcheng Lake; KSBC: "bathed" crabs from Kunshan ponds; and YCBC: "bathed" crabs from Yangcheng Lake.

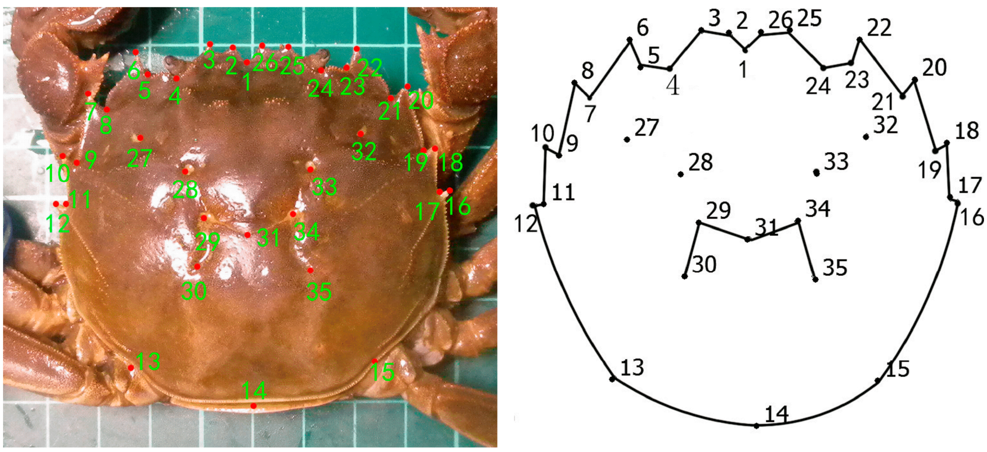

Figure 2. Location of carapace landmarks of Chinese mitten crabs (*Eriocheir sinensis*).

Table 2. Types and definitions of landmarks.

Landmark Type	Landmark Number	Definition
Type II	2, 3, 25, 26	Forehead margin
	4, 24	Concave point between the frontal margin and the first lateral tooth
	5, 23	Inner vertex of the first side tooth
	6, 22	Outer apex of the first lateral tooth
	7, 21	Inner vertex of the second side tooth
	8, 20	The outer apex of the second lateral tooth
	9, 19	Inner vertex of the third side tooth
	10, 18	Lateral apex of the third lateral tooth
	11, 17	Inner vertex of the fourth side tooth
	12, 16	Outer apex of the fourth lateral tooth
	13, 15	Concave point of the posterior edge
	27, 32	Concave point between the two holes in the middle parotid region and the second side tooth
	28, 33	Two holes in the middle parotid region
	29, 34	M-shaped pattern on the vertex
	30, 35	Lower vertex of the M-shaped pattern
	31	M-shaped pattern center
Type III	1	Frontal margin center
	14	Lower vertex of the shell length

2.3. Mean Shape and Thin-Plate Spline Analysis

Generalized Procrustes Analysis (GPA) was performed using tpsRelw software (version 1.70) to eliminate non-morphological variations, such as location, direction, and proportion, when selecting landmark points [16], obtain relative distortion scores, and obtain the mean shape of the samples (Figure 3) [17]. To visualize the variation in carapace shape between samples, we drew grid deformation plots for 15 crab carapaces and then compared the differences using thin plate spline analysis from the tpsRegr software (version 1.45). Changing landmarks could be readily visualized using Adobe Photoshop CS6 (Adobe Systems, San Jose, CA, USA) to alter the presentation of the deformation plot groups.

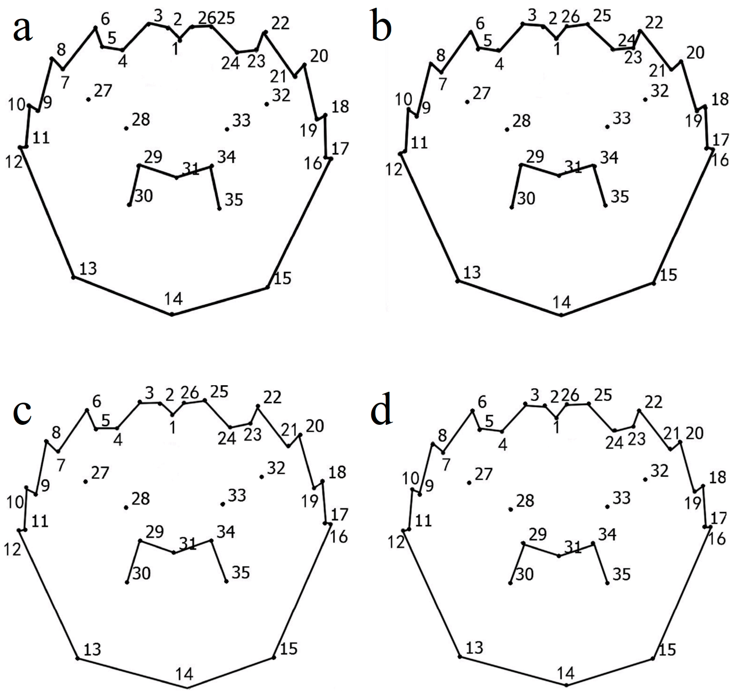

Figure 3. Overall mean shape and 35 landmarks of carapace of four groups of *Eriocheir sinensis* on day 30 of "bathing". Notes: (**a**) crabs from Kunshan ponds; (**b**) "bathed" crabs from Kunshan ponds; (**c**) crabs from Yangcheng Lake; and (**d**) "bathed" crabs from Yangcheng Lake.

2.4. Discriminant Analysis

Stepwise discriminant analysis was performed using SPSS 27.0 (IBM Corp., Armonk, NY, USA). This analysis was based on the relative distortion scores of the Chinese mitten crabs obtained from tpsRelw software, and it used the Bayes method.

3. Results

3.1. Morphological Visualization Analysis of Carapaces

The mean shape of the carapace morphology for all Chinese mitten crab samples was plotted using tpsRelw software (Figure 3).

When analyzing original and "bathed" Chinese mitten crabs from Kunshan ponds and the Yangcheng Lake, we observed that landmarks 11, 12, 16, and 17 had the highest contribution rates for both groups, explaining 66.815% of the morphological differences in Kunshan pond samples and 64.092% of the differences in Yangcheng Lake samples. The fourth lateral tooth contributed significantly to distinguishing the morphological differences between the carapaces of original crabs and "bathed" crabs during different periods.

The visualization of morphological differences in the carapaces of pond Chinese mitten crabs from the pond during different periods (Figure 4) revealed distinctive variations. Vectors of displacement on the grid were obtained with grid deformation plots. In the grid deformation plots, the arrows and short lines represent change vectors at each landmark

(Figure 4a). In Kunshan pond crabs, the main differences were concentrated in the frontal margin (landmarks 2, 3, 25, and 26), the first side teeth (landmarks 5, 6, 22, and 23), the second side teeth (landmarks 7, 8, 20, and 21), the third side teeth (landmarks 9, 10, 18, and 19), the fourth side teeth (landmarks 11, 12, 16, and 17), and M-shaped patterns (Figure 4b). When comparing carapaces from days 7, 14, and 30 with samples from day 0, we observed shifts in the frontal margin upward, slight upward shifts in the upper left vertex of the M-shaped pattern, upward shifts in the first, second, third, and fourth lateral teeth, and an increase in carapace length.

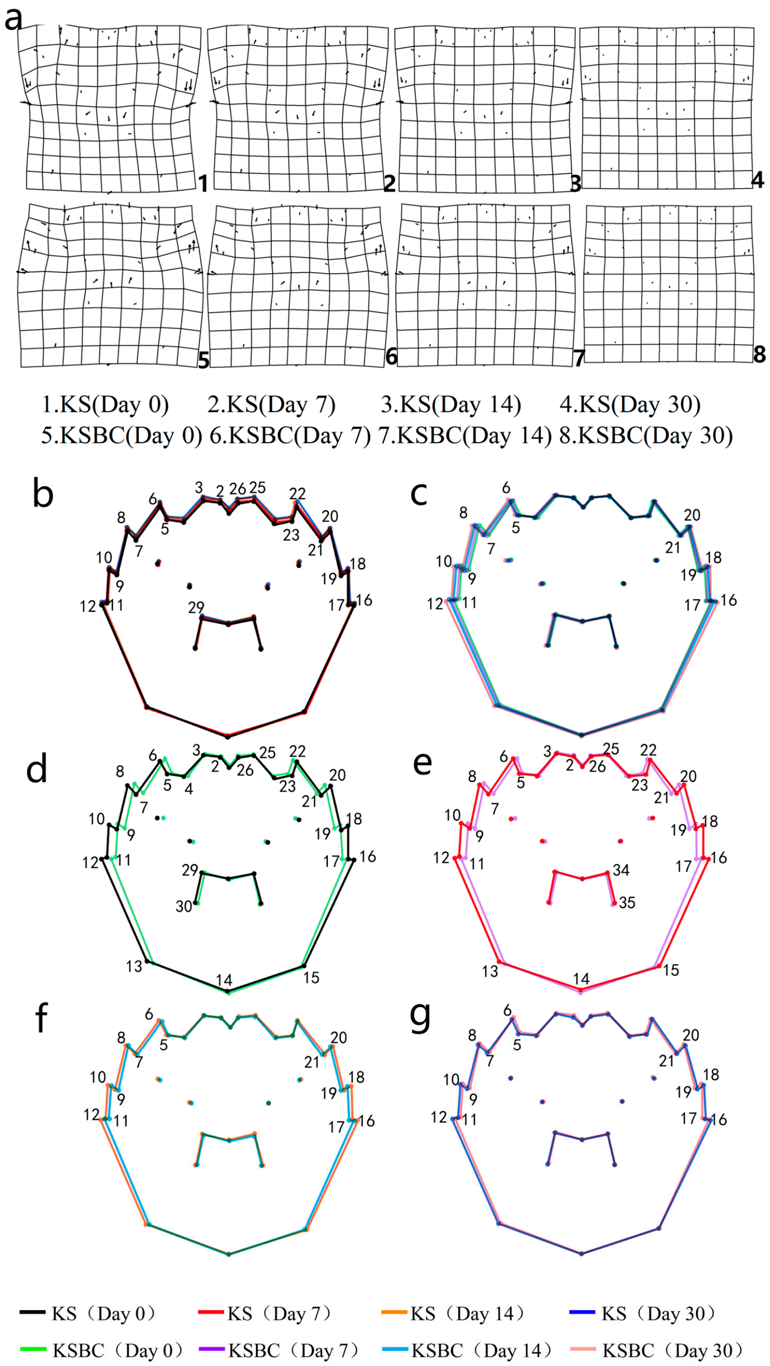

Figure 4. Visualization of morphological differences of *Eriocheir sinensis* carapaces in different periods for Kunshan ponds. Notes: KS: crabs from Kunshan ponds; KSBC: "bathed" crabs from Kunshan ponds. (**a**) The grid deformation plots of each group (The arrows and short lines represent change vectors at each landmark); (**b**) summary of original crabs; (**c**) summary of "bathed" crabs; (**d**) 0 days of bathing; (**e**) 7 days of bathing; (**f**) 14 days of bathing; and (**g**) 30 days of bathing.

Significant changes in the carapace morphology of Kunshan pond "bathed" crabs were primarily observed in the first lateral teeth (landmarks 5 and 6), second lateral teeth (landmarks 7, 8, 20, 21), third lateral teeth (landmarks 9, 10, 18, 19), and fourth lateral teeth (landmarks 11, 12, 16, 17). Other landmarks did not show significant alterations (Figure 4c). When comparing carapaces on days 7, 14, and 30 with day 0, we observed the first lateral tooth (landmarks 5 and 6) was moved upward to the left, and the second, third, and fourth lateral teeth expanded laterally, resulting in a wider carapace.

Comparison of carapaces between original and "bathed" crabs collected during the same period revealed differences concentrated in the frontal margin, lateral teeth, the posterior lateral margin of the carapace (landmarks 13, 14, and 15), and patterns of the M shape. However, the nature of these differences varied at different stages. The primary distinctions between "bathed" and original crabs were that "bathed" crabs had narrower and longer carapaces, all four lateral teeth contracted inward compared to original crabs, the frontal margin shifted upward, the posterior lateral margin shifted downward, and the left side of the M-shaped pattern shifted to the right on day 0 (Figure 4d). On day 7 (Figure 4e), except for the right side of the M-shaped pattern that changed to the left, the changes in other landmarks were consistent with those on day 0, albeit with a smaller range of change. On day 14 (Figure 4f), only the four lateral teeth showed differences, which were also smaller. On day 30 (Figure 4g), only the four lateral teeth exhibited slight inward contraction.

Visualization of the morphological differences of the carapaces between the resident and the "bathed" crab of different periods in Yangcheng Lake (Figure 5). The vectors of landmark displacement on the grid were obtained with grid deformation plots (Figure 5a). The results show that there are no significant differences between the crabs during the four periods, except for the downward shift of the posterior edge of the carapace (Figure 5b).

The changes in carapace shape were concentrated in the first and third lateral teeth and M-shaped patterns (Figure 5c). When comparing carapaces on days 7, 14, and 30 with day 0, we observed the first lateral tooth shifted upward, the third lateral tooth moving to the left, and a slight shift in the lower left vertex of the M-shaped pattern.

Primary differences in carapace morphology between original and "bathed" crabs from Yangcheng Lake, collected during the same period, were observed in the lateral teeth, the posterior margin of the carapace (landmarks 13, 14, and 15), and the M-shaped pattern. However, these differences varied at different stages. On day 0 (Figure 5d), all four lateral teeth of "bathed" crabs shrank inward compared to native crabs, and the posterior margin and M-shaped pattern shifted downward. On day 7 (Figure 5e), only the first, third, and fourth lateral teeth of "bathed" crabs contracted inward, and the downward offset of the posterior lateral margin and M-shaped pattern decreased. On day 14 (Figure 5f), the first and third lateral teeth slightly shifted upward, but the remaining landmarks did not show significant changes. On day 30 (Figure 5g), the second and third lateral teeth contracted slightly inward.

3.2. Discriminant Analysis of Morphological Differences of Carapaces

Comprehensive analyses were conducted on both the original and "bathed" crabs obtained from Kunshan ponds over four distinct periods (Figure 6 and Table 3). The scatter diagram resulting from discriminant analysis reveals significant changes in the carapace morphology of "bathed" crabs during the month-long "bathing" process. Morphological distinctions were observed on days 1, 7, 14, and 30, gradually approaching those of the original crabs. However, discernible morphological distinctions between the original and "bathed" crabs persisted. The discriminant analysis accuracy for "bathed" crabs remained at 100%.

Original and "bathed" crabs collected from Yangcheng Lake during four distinct periods were analyzed thoroughly, yielding discriminant results (Figure 7 and Table 4). The scatter plot displays substantial changes in the morphological characteristics of the carapace during the month of "bathing". Analogous to the observed trend in "bathed" pond crabs, "bathed" lake crabs also gradually converged toward the characteristics of the original crabs. However, the morphological distinctions between the original and "bathed" crabs persisted. The accuracy of the discriminant analysis for "bathed" lake crabs was consistently 100%.

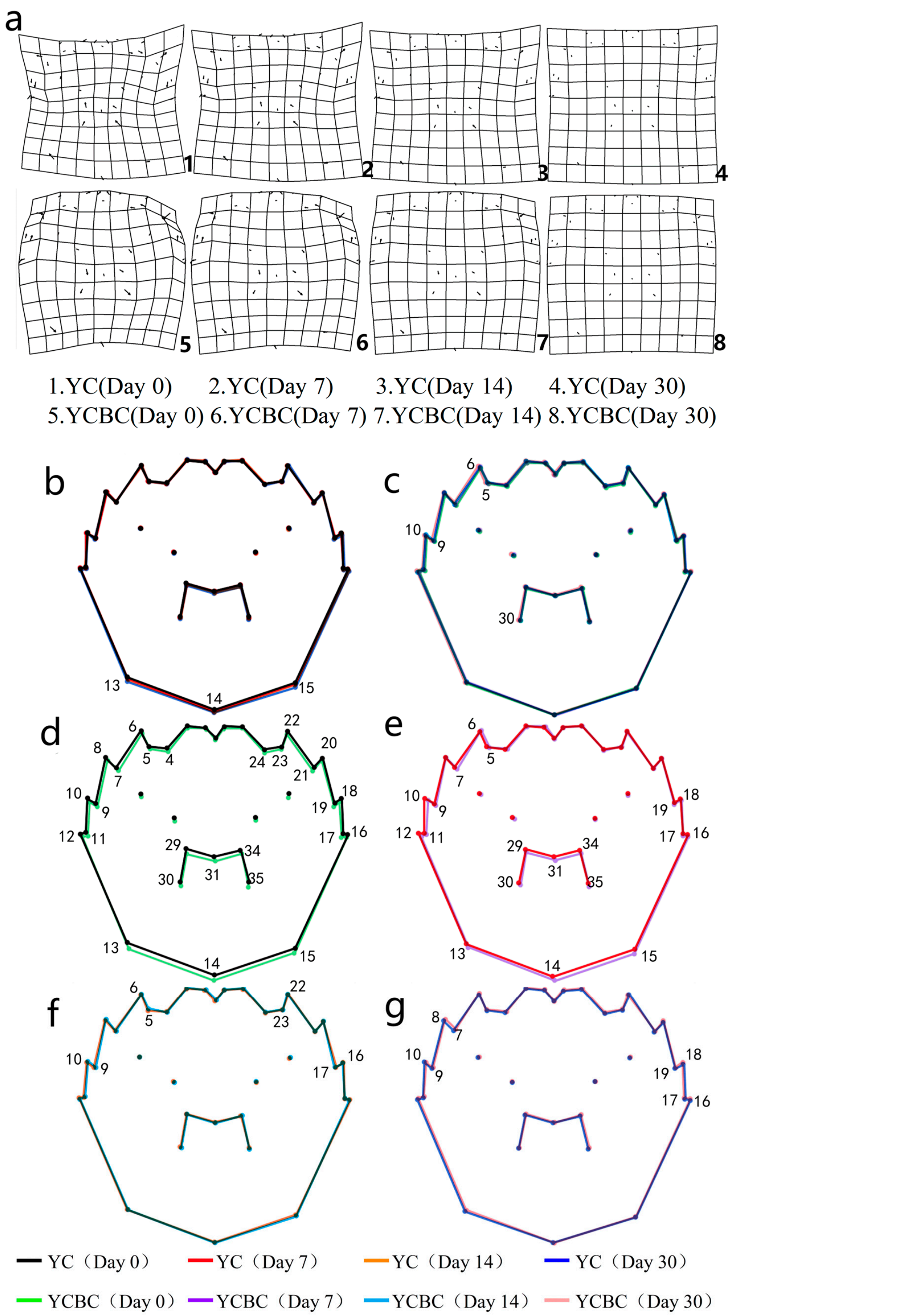

Figure 5. Visualization of morphological differences of *Eriocheir sinensis* carapaces in different periods for Yangcheng Lake. Notes: YC: crabs from Yangcheng Lake; YCBC: "bathed" crabs from Yangcheng Lake. (**a**) The grid deformation plots of each group (The arrows and short lines represent change vectors at each landmark); (**b**) summary of original crabs; (**c**) summary of "bathed" crabs; (**d**) 0 days of bathing; (**e**) 7 days of bathing; (**f**) 14 days of bathing; and (**g**) 30 days of bathing.

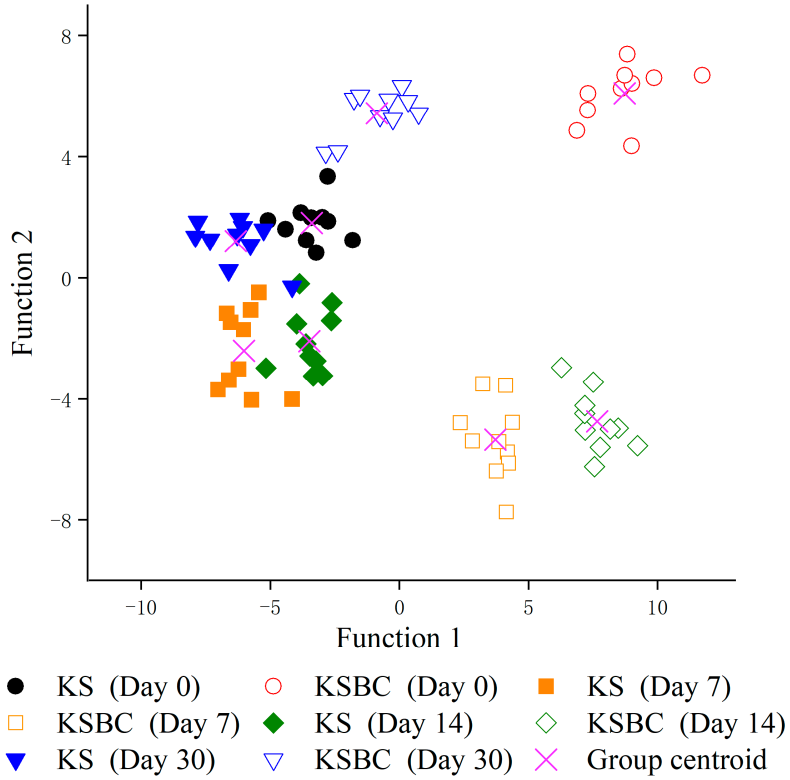

Figure 6. Discriminant analysis scatter plot of *Eriocheir sinensis* based on the carapace (original and "bathed" crabs from Kunshan ponds). Notes: KS: crabs from Kunshan ponds; KSBC: "bathed" crabs from Kunshan ponds.

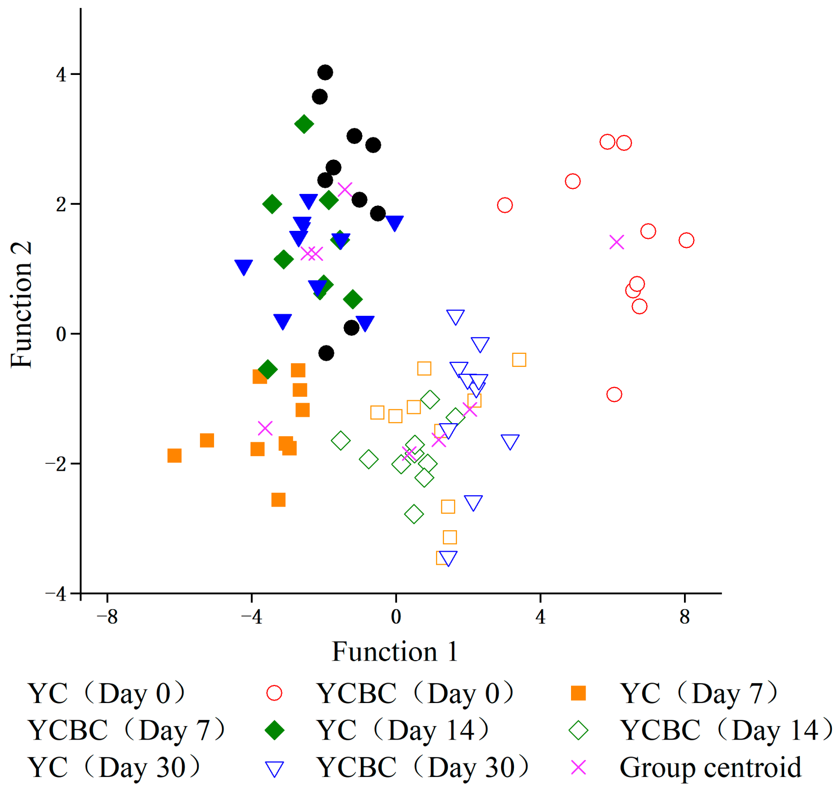

Figure 7. Discriminant analysis scatter plot of Chinese mitten crabs based on the carapace (original and "bathed" crabs from Yangcheng Lake). Notes: YC: crabs from Yangcheng Lake; YCBC: "bathed" crabs from Yangcheng Lake.

Table 3. Results of discriminant analysis for Chinese mitten crabs according to carapace (original crabs and "bathed" crabs from Kunshan ponds).

Group	Discrimination Accuracy							
	Original Crabs (Day 0)	"Bathed" Crabs (Day 0)	Original Crabs (Day 7)	"Bathed" Crabs (Day 7)	Original Crabs (Day 14)	"Bathed" Crabs (Day 14)	Original Crabs (Day 30)	"Bathed" Crabs (Day 30)
Original crabs (Day 0)	10 (100%) *	0	0	0	0	0	0	0
"Bathed" crabs (Day 0)	0	10 (100%)	0	0	0	0	0	0
Original crabs (Day 0)	0	0	10 (100%)	0	0	0	0	0
"Bathed" crabs (Day 0)	0	0	0	10 (100%)	0	0	0	0
Original crabs (Day 0)	0	0	0	0	10 (100%)	0	0	0
"Bathed" crabs (Day 0)	0	0	0	0	0	10 (100%)	0	0
Original crabs (Day 0)	0	0	0	0	0	0	10 (100%)	0
"Bathed" crabs (Day 0)	0	0	0	0	0	0	0	10 (100%)

Note: * Crab individuals in each group were accurately identified (discrimination accuracy %).

Table 4. Results of discriminant analysis for Chinese mitten crabs according to carapace (original crabs and "bathed" crabs in Yangcheng Lake).

Group	Discrimination Accuracy							
	Original Crabs (Day 0)	"Bathed" Crabs (Day 0)	Original Crabs (Day 7)	"Bathed" Crabs (Day 7)	Original Crabs (Day 14)	"Bathed" Crabs (Day 14)	Original Crabs (Day 30)	"Bathed" Crabs (Day 30)
Original crabs (Day 0)	10 (100%) *	0	0	0	0	0	0	0
"Bathed" crabs (Day 0)	0	10 (100%)	0	0	0	0	0	0
Original crabs (Day 0)	0	0	10 (100%)	0	0	0	0	0
"Bathed" crabs (Day 0)	0	0	0	10 (100%)	0	0	0	0
Original crabs (Day 0)	0	0	0	0	10 (100%)	0	0	0
"Bathed" crabs (Day 0)	0	0	0	0	0	10 (100%)	0	0
Original crabs (Day 0)	0	0	0	0	0	0	10 (100%)	0
"Bathed" crabs (Day 0)	0	0	0	0	0	0	0	10 (100%)

Note: * Crab individuals in each group were accurately identified (discrimination accuracy %).

4. Discussion

4.1. Rationality of Landmark Selection

Traditional morphometric techniques can use multivariate statistical analysis to study morphological variables (like linear distance, angle size, and proportion). However, these methods have limitations, such as non-homologous data, irreproducibility, and the inability to discuss size and shape separately [18]. Thanks to the advancements in statistics, graphics, surveying, and other fields, geometric morphometry has emerged as a rapidly developing field that overcomes these shortcomings. The landmark method, renowned for its high accuracy, is the dominant approach in geometric morphometry. It facilitates statistical analysis of geometric measurement data and offers insights into diverse morphological questions; such questions arise in fields including development [19], evolution [20], and ecology [21,22], among other related disciplines. Jiang and colleagues (2014) successfully differentiated wild Chinese mitten crabs from varying water systems by utilizing the landmark method, which resulted in higher discrimination accuracy as compared to their earlier comparative research that depended on traditional morphometric methods. In their study, they selected 30 landmarks, and the average discriminant accuracy reached 87.67%. However, Zheng et al. [13] used 35 landmarks to analyze the morphological distinctions between Chinese mitten crabs from various habitats, achieving a remarkable discriminant accuracy of 100%. Subsequent studies employing these same landmarks consistently reached this level of accuracy, underscoring the direct impact of landmark selection on the ability to discriminate morphological differences in Chinese mitten crabs. Therefore, in our study, we adopted the same set of landmarks to ensure the accuracy of our intraspecific analysis. We investigated two "bathing" environments, specifically the Kunshan ponds and Yangcheng Lake. The regression coefficients for the tangent space distance and the Platts distance of the carapace landmarks were 0.999771 and 0.999764, respectively, nearly equal to 1, indicating the effectiveness of our landmark selection.

4.2. Analysis of Morphological Differences between Original and "Bathed" Crabs

In this study, the geometric morphometrics method based on landmarks was used to identify the dynamic changes in morphological differences between the carapaces of original and "bathed" crabs from Yangcheng Lake. The results confirm that there are morphological differences between "bathed" and original crabs before and after "bathing", but "bathed" crabs showed adaptive changes during "bathing", gradually reducing the morphological differences to the original crabs.

Zheng et al. [13] selected different *E. sinensis* from Taihu Lake, Hongze Lake, Gaobao Lake, Changdang Lake, and Yangcheng Lake in the Yangtze River system and from the breeding waters of Bacheng and Xinghua "Honggao" and the natural waters of Chongming in the Yangtze River estuary and analyzed the differences in the morphology using the landmark method. The results showed that there are significant morphological differences between *E. sinensis* of different origins and that the precision of the origin discrimination of E. sinensis carapace based on the landmark method was 100%. In this study, 35 carapace landmarks were selected for original crabs from Jianhu ponds, Kunshan high-standard modified ponds, and Yangcheng Lake, and data were analyzed and discriminated. The morphology before "bathing" was also analyzed. The results indicate notable morphological differences among the three carapaces. The accuracy of the discriminant analysis was 100%, which is consistent with previous studies. It could be confirmed that the landmark method has high accuracy regarding the morphological analysis and origin identification of Chinese mitten crabs.

In the present study, two groups of Chinese mitten crabs, that is, "bathed" crabs from Kunshan ponds and Yangcheng Lake, were compared with original crabs from the same locations after one month of "bathing" culture. The results showed that morphological differences between original and "bathed" crabs remained after a month of "bathing", and the accuracy of the discrimination was 100%. "Bathed" crabs could not be "transformed" into Chinese mitten crabs originating from high-standard modified ponds in Kunshan and the Yangcheng Lake purse seine culture area. The landmarks with the highest contribution rates (>60%) in both sample groups were 11, 12, 16, and 17. The contribution rates for the other landmarks were less than 40%, indicating that the lateral teeth contributed the most to the morphological differences of Chinese mitten crabs. Davis et al. [23] suggested that the shift from longer to shorter spine lengths in blue crabs (*Callinectes sapidus*) raised in hatcheries, in contrast to their wild counterparts, may be due to environmental adaptation resulting from reduced exposure to predators rather than genetic drift caused by hatchery conditions. Although the precise biological significance of variations in fine carapace structures observed via landmarks cannot be determined at present, it is possible to assume that these changes could facilitate adaptation to biotic (e.g., predators) and abiotic (e.g., substrate type) factors in the Yangcheng Lake environment.

Xue et al. [24] used geometric morphometrics to assess shape variation in the carapace of Chinese mitten crabs in Yangcheng Lake throughout a year of cultivation. They noted that it takes approximately six months for the carapace morphology of native crabs to stabilize and accurately reflect their origin. Similarly, Xue et al. [7] introduced non-Yangcheng Lake crabs to the Yangcheng Lake area for one month and compared the morphological differences of the carapaces of original and "bathed" crabs before and after "bathing". The differences in carapace morphology between original crabs and "bathed" crabs were determined to be significant and 100% distinguishable after 1 month of "bathing" culture. The carapace morphology of original and "bathed" crabs did not converge after only one month, which also follows the results. In this study, the landmarks with the highest rate of contribution before and after "bathing" were 11, 12, 16, and 17 and 16, 17, 18, and 19, respectively, suggesting that the lateral teeth contributed the most to morphological differences in the carapaces of Chinese mitten crabs, which also follows the results of previous studies.

To investigate the dynamic changes in the carapace morphology of "bathed" Chinese crabs, samples were taken on days 0, 7, 14, and 30 during "bathing", and a comparative study was performed. Based on the grid deformation diagram (Figures 4 and 5), the four lateral teeth of "bathed" crabs from Kunshan ponds expanded outward, and the carapace became wider, gradually reducing the morphological differences from the carapaces of the original crabs. The lateral teeth and M-shaped patterns of "bathed" crabs in the Yangcheng Lake changed, and the morphological differences to the original crabs gradually reduced, but the trend of change was not as notable as that of "bathed" crabs from the Kunshan ponds, while the morphological changes of original crabs from Kunshan ponds and those from Yangcheng Lake were smaller. Based on the results of the discriminant analysis, the landmark method can accurately distinguish Chinese mitten crabs with the same origin but from different periods. In summary, "bathed" crabs will undergo adaptive changes in the new aquaculture water environment, gradually reducing the morphological differences to the original crabs, and the effect of "bathed" crabs will be more notable for standardized "bathing" in high-standard modified ponds. However, after one month of "bathing" culture, morphological differences between "bathed" and original crabs remained, and the discrimination accuracy was 100%. Kim et al. [25] compared the morphological characteristics of *Platichthys stellatus* in cultured and wild environments and reported that the same source of *P. stellatus* produced morphological differences between specimens in captivity on farms and those released to the open coast for 3 to 36 months. The released populations were morphologically closer to wild populations of *P. stellatus* than to captive populations. Tulli et al. [26] evaluated the morphology of sea bass from aquaculture systems, that is, extensive offshore marine cages and inland basins, and reported that sea bass will undergo morphological changes under different aquaculture conditions. In summary, if aquatic animals are kept away from their original habitats, that is, in an unfamiliar environment, for a long time, their phenotypes will adapt according to the characteristics of the environment. To reduce costs and make profits, illegal traders will allow Chinese mitten crabs to be "bathed" for a shorter period. Therefore, it is even less likely that "bathed" crabs will be "whitewashed" into original crabs. What exact environmental factors that promote this morphological change are required in future studies?

4.3. Ecological Significance of Morphological Difference Analysis of Carapace

The evident morphological variations in aquatic organisms closely correspond to their specific environmental and nutritional conditions. Kishida et al. [8] indicated that juvenile *Trachurus japonicus* displays adaptability to its developmental surroundings: the streamlined form is well-suited for feeding on larval *Engraulis japonicus* in coastal waters, whereas the compressed morphology is advantageous for associating with jellyfish in offshore waters. Many studies have demonstrated differences in morphology among aquatic organisms in varying environments. The identification of adaptive changes in the "bath crab" examined in this study further solidifies the notion that the environment promotes morphological changes in organisms. These findings can serve as a foundation for scholars studying environmental adaptive changes.

4.4. Application Value of Carapace Morphology Discrimination of "Bathed" Crabs

As a well-known geographically identified production in China, the brand value of "Yangcheng Lake Chinese mitten crabs" cannot be underestimated, and the behavior of unscrupulous traders selling counterfeit Yangcheng Lake Chinese mitten crabs damages the reputation of this geographically significant product. Therefore, it is important to identify the origin of Chinese mitten crabs and trace the product. Previously, researchers have identified the origin of aquatic products via morphology [27], taste [28,29], organic components [30], elements [31,32], and DNA barcoding [33]. The discriminant results were relatively accurate, but these methods also have disadvantages due to their high cost and time-consuming and lethal nature. Because crabs are fresh products, the methods are not suitable for the rapid identification of 'bathed' crabs on the market because their freshness and accuracy of identification must be ensured simultaneously. The geometric morphometric method only requires the measurement of the external morphology of Chinese mitten crabs, which is low-cost, convenient, and non-lethal. The results of previous studies on the origin discrimination of Chinese mitten crab carapace confirmed the high accuracy of this method. In this study, 100% accuracy was achieved when identifying crabs at different stages of "bathing". The experimental results show that "bathed" crabs can be accurately identified if the geometric morphometric method is used, independent of the "bathing" duration. The landmark method is simple, fast, and accurate in identifying morphology and is of advantage for the protection of geographically identified products. The combination of this method with other disciplines will help establish a rapid identification system for counterfeit crabs.

5. Conclusions

Based on the geometric morphological analysis of Chinese mitten crab carapaces, crabs "bathed" for 7, 14, or 30 days could be accurately distinguished from the original crabs in Yangcheng Lake and high-standard ponds in this study. The identification accuracy was 100%. According to the grid visualization, the carapace of "bathed" crabs possibly underwent adaptive changes, gradually reducing the morphological differences compared to the original crabs. This study further confirms that even after a month of "bathing" culture, "bathed" crabs from the lake and ponds do not exhibit the morphological characteristics of the original crabs. These findings provide a theoretical basis and methodology for the effective identification of "bathed" crabs for up to 1 month and the protection of crabs of Yangcheng Lake origin. In the future, we will combine biometrics with geometric morphometrics to automatically identify and classify Chinese mitten crab carapaces in order to provide a reference for the protection of other aquatic products.

Author Contributions: Conceptualization, Y.X., H.L., J.X. and J.Y.; methodology, Y.X., J.X., H.L., T.J. and J.Y.; investigation, Y.X., J.X., H.L., X.C. and T.J.; resources, Y.X., H.L., J.X., T.J. and X.C.; funding acquisition, H.L.; writing—original draft, Y.X., J.X., H.L., T.J. and X, C.; writing—review and editing, H.L. and J.Y.; supervision, H.L. and J.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Project of Kunshan Yangcheng Lake Crab Industrial Research Institute for Industrial Development Needs in 2022 [no funding number] and the Project of Kunshan Yangcheng Lake Crab Industrial Research Institute [grant number HX2021509300].

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of the Freshwater Fisheries Research Center, Chinese Academy of Fisheries Sciences (protocol code 2011AA1004020012, 16 January 2011).

Data Availability Statement: Relevant information has been included in the article.

Acknowledgments: We thank Min Jiang and Zhongbin Chen for their support in sampling.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wu, H.; Ge, M.; Chen, H.; Jiang, S.; Lin, L.; Lu, J. Comparison between the nutritional qualities of wild-caught and rice-field male Chinese mitten crabs (*Eriocheir sinensis*). *LWT-Food Sci. Technol.* **2020**, *117*, 108663. [CrossRef]
2. Cheng, H.; Wu, H.; Liang, F.; Ge, M.; Jiang, S.; Lin, L.; Lu, J. Comparison of the nutritional quality of three edible tissues from precocious and normal adult female Chinese mitten crabs (*Eriocheir sinensis*). *J. Aquat. Food Prod. Technol.* **2021**, *30*, 49–61. [CrossRef]
3. Wang, X.; Zheng, R.X.; Zhang, D.M.; Lei, X.Y.; Wang, S.; Wan, J.W.; Liu, H.J.; Chen, Y.K.; Zhao, Y.L.; Wang, G.Q.; et al. Effects of different stocking densities on growth performance, nutritional quality and economic benefit of juvenile female Chinese mitten crabs (*Eriocheir sinensis*) in rice-crabs culture systems. *Aquaculture* **2022**, *553*, 738111. [CrossRef]
4. Zheng, C.C.; Jiang, T.; Luo, R.J.; Chen, X.B.; Liu, H.B.; Yang, J. Geometric morphometric analysis of the Chinese mitten crabs *Eriocheir sinensis*: A potential approach for geographical origin authentication. *N. Am. J. Fish. Manag.* **2021**, *41*, 891–903. [CrossRef]

5. Duvaleix, S.; Emlinger, C.; Gaigné, C.; Latouche, K. Geographical indications and trade: Firm-level evidence from the French cheese industry. *Food Policy* **2021**, *102*, 102118. [CrossRef]
6. Ye, H.P.; Yang, J.; Xiao, G.S.; Zhao, Y.; Li, Z.M.; Bai, W.D.; Zeng, X.F.; Dong, H. A comprehensive overview of emerging techniques and chemometrics for authenticity and traceability of animal-derived food. *Food Chem.* **2023**, *402*, 134216. [CrossRef] [PubMed]
7. Xue, J.R.; Jiang, T.; Chen, X.B.; Liu, H.B.; Yang, J. Geometric morphometric analysis on the carapace difference between 'bathed' and Yangcheng Lake originated *Eriocheir sinensis*. *Acta Hydrobiol. Sin.* **2020**, *44*, 587–594. (In Chinese) [CrossRef]
8. Kishida, M.; Kanaji, Y.; Xie, S.; Watanabe, Y.; Kawamura, T.; Masuda, R.; Yamashita, Y. Ecomorphological dimorphism of juvenile *Trachurus japonicus* in Wakasa Bay, Japan. *Environ. Biol. Fishes* **2011**, *90*, 301–315. [CrossRef]
9. Kenthao, A.; Jearranaiprepame, P. Ecomorphological diversification of some barbs and carps (*Cyprininae*, *Cyprinidae*) in the Lower Mekong Basin of Thailand. *Zoology* **2020**, *143*, 125830. [CrossRef] [PubMed]
10. Fox, N.S.; Veneracion, J.J.; Blois, J.L. Are geometric morphometric analyses replicable? Evaluating landmark measurement error and its impact on extant and fossil Microtus classification. *Ecol. Evol.* **2020**, *10*, 3260–3275. [CrossRef] [PubMed]
11. Zelditch, M.; Bookstein, F.L. Morphometric Tools for Landmark Data: Geometry and Biology. *BioScience* **1998**, *48*, 855–858. [CrossRef]
12. Braga, R.; Crespi-Abril, A.C.; Van der Molen, S.; Bainy, M.C.R.S.; Ortiz, N. Analysis of the morphological variation of *Doryteuthis sanpaulensis* (Cephalopoda: Loliginidae) in Argentinian and Brazilian coastal waters using geometric morphometrics techniques. *Mar. Biodivers.* **2017**, *47*, 755–762. [CrossRef]
13. Zheng, C.C.; Jiang, T.; Luo, R.J.; Chen, X.B.; Liu, H.B.; Yang, J. Landmark-based morphometric identification of different geographical origins for the Chinese mitten crabs (*Eriocheir sinensis*). *J. Fish. China* **2017**, *41*, 1896–1907. (In Chinese) [CrossRef]
14. Jiang, X.D.; Cheng, Y.X.; Pan, J.L.; Li, X.D.; Wu, X.G. Landmark-based morphometric identification of wild *Eriocheir sinensis* with geographically different origins. *J. Fish. Sci. China* **2019**, *26*, 1116–1125. (In Chinese) [CrossRef]
15. Bookstein, F.L. Landmarks. In *Morphometric Tools for Landmark Data: Geometry and Biology*; Cambridge University Press: Cambridge, UK, 1992; pp. 55–87.
16. Farre, M.; Lombarte, A.; Tuset, V.M.; Abello, P. Shape matters: Relevance of carapace for brachyuran crab invaders. *Biol. Invasions* **2021**, *23*, 461–475. [CrossRef]
17. Rohlf, F.J. Bias and error in estimates of mean shape in geometric morphometric. *J. Hum. Evol.* **2003**, *44*, 665–683. [CrossRef]
18. Lishchenko, F.; Jones, J.B. Application of shape analyses to recording structures of marine organisms for stock discrimination and taxonomic purpose. *Front. Mar. Sci.* **2021**, *8*, 667183. [CrossRef]
19. Baranov, S.G. Use of a geometric morphometric method to determine the developmental stability of *Betula pendula* Roth. *Biol. Bull.* **2017**, *44*, 547–551. [CrossRef]
20. Da Silva, F.O.; Fabre, A.-C.; Savriama, Y.; Ollonen, J.; Mahlow, K.; Herrel, A.; Mueller, J.; Di-Poi, N. The ecological origins of snakes as revealed by skull evolution. *Nat. Commun.* **2018**, *9*, 376. [CrossRef]
21. Adrian, B.; Smith, H.F.; Hutchison, J.H.; Townsend, K.E.B. Geometric morphometrics and anatomical network analyses reveal ecospace partitioning among geoemydid turtles from the Uinta Formation, Utah. *Anat. Rec.* **2022**, *305*, 1359–1393. [CrossRef]
22. Bazzi, M.; Campione, N.E.; Kear, B.P.; Pimiento, C.; Ahlberg, P.E. Feeding ecology has shaped the evolution of modern sharks. *Curr. Biol.* **2021**, *31*, 5138. [CrossRef] [PubMed]
23. Davis, J.L.D.; Young-Williams, A.C.; Aguilar, R.; Carswell, B.L.; Goodison, M.R.; Hines, A.H.; Kramer, M.A.; Zohar, Y.; Zmora, O. Differences between hatchery-raised and wild blue crabs: Implications for stock enhancement potential. *Trans. Am. Fish. Soc.* **2004**, *133*, 1–14. [CrossRef]
24. Xue, J.R.; Jiang, T.; Chen, X.B.; Liu, H.B.; Yang, J. Shape variation in the carapace of Chinese mitten crabs (*Eriocheir sinensis* H. Milne Edwards, 1853) in Yangcheng Lake during the year-long culture period. *Eur. Zool. J.* **2022**, *89*, 210–221. [CrossRef]
25. Kim, S.K.; Yoon, S.C.; Youn, S.H.; Park, S.U.; Corpus, L.S.; Jang, I.K. Morphometric changes in the cultured starry flounder, *Platichthys stellatus*, in open marine ranching areas. *J. Environ. Biol.* **2013**, *34*, 197–204. [PubMed]
26. Tulli, F.; Balenovic, I.; Messina, M.; Tibaldi, E. Biometry traits and geometric morphometrics in sea bass (*Dicentrarchus labrax*) from different farming system. *Ital. J. Anim. Sci.* **2009**, *8*, 881–883. [CrossRef]
27. Ibáñez, A.L. Fish scale shape variation by year and by geographic location, could scales be useful to trace fish? A case study on the Gulf of Mexico. *Fish. Res.* **2014**, *156*, 34–38. [CrossRef]
28. Ding, L.; Guo, Z.; Hou, Y.; Zhu, P. KECA for identifying the habitats of Chinese mitten crabs (*Eriocheir sinensis*) based on aroma analysis. In Proceedings of the 7th International Conference on Control Automation and Information Sciences (ICCAIS), Hangzhou, China, 24–27 October 2018; pp. 401–404.
29. Wang, S.; Wu, X.G.; Tao, N.P.; Wang, X.C.; Zheng, Y. Nutritional and flavor quality analysis of Liu Yuehuang (*Eriocheir sinensis*). *J. Chin. Inst. Food Sci. Technol.* **2017**, *17*, 219–227. (In Chinese) [CrossRef]
30. Fonseca, V.F.; Duarte, I.A.; Matos, A.R.; Reis-Santos, P.; Duarte, B. Fatty acid profiles as natural tracers of provenance and lipid quality indicators in illegally sourced fish and bivalves. *Food Control* **2022**, *134*, 108735. [CrossRef]
31. Zhang, X.; Cheng, J.; Han, D.; Zhao, X.; Chen, X.; Liu, Y. Geographical origin traceability and species identification of three scallops (*Patinopecten yessoensis*, *Chlamys farreri*, and *Argopecten irradians*) using stable isotope analysis. *Food Chem.* **2019**, *299*, 125107. [CrossRef]

32. Xu, Y.J.; Peng, K.X.; Jiang, F.; Cui, Y.M.; Han, D.F.; Liu, H.; Hong, H.Y.; Tian, X.H. Geographical discrimination of swimming crabs (*Portunus trituberculatus*) using stable isotope and multi-element analyse. *J. Food Compos. Anal.* **2022**, *105*, 104251. [CrossRef]
33. Haye, P.A.; Segovia, N.I.; Vera, R.; Gallardo, M.d.l.Á.; Gallardo-Escárate, C. Authentication of commercialized crabs-meat in Chile using DNA Barcoding. *Food Control* **2012**, *25*, 239–244. [CrossRef]