MORPHOLOGICAL VARIATION AND DISTINCTION IN CULTURED AND WILD POPULATIONS OF *PSEUDOSCIAENA CROCEA* IN THE EAST CHINA SEA

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Abstract. The large yellow croaker (*Pseudosciaena crocea*), once a vital fishery resource in the East China Sea, has witnessed a substantial decline in its wild population towing to overfishing. The current resource circumstances mainly relies on artificial breeding, with limited contributions from wild populations. However, discriminating between wild and cultured populations of the large yellow croaker in market trade and parent fish selection remains largely dependent on experiential judgment, with precise and efficient discrimination methods lacking. In this study, the framework method was used to quantify the external morphological characteristics of wild and cultured populations. The differences in external morphology between the two populations were visualized using principal component analysis, and they were classified through stepwise discriminant analysis. Results revealed significant differences in the standardized morphological parameters among most large yellow croakers from these two populations. Additionally, their distributions on principal components 2 and 3 were dissimilar. Furthermore, significant sex-based differences were observed in the cultured population, whereas in the wild population, sex differences were comparatively minor. Cross-validation results revealed that the classification accuracy of distinguishing between different populations exceeded that for separating cultured or wild populations according to different sexes. These findings suggest that the framework method is effective for discriminating between cultured and wild populations of large yellow croaker, although it may not be as successful in distinguishing between individuals of different sexes in these populations. The outcomes of this research offer valuable support for large yellow croaker breeding initiatives.
Keywords: *large yellow croaker, discriminant analysis, principal components analysis, sex differences, species identification*

Introduction

The large yellow croaker (*Pseudosciaena crocea*), belonging to the Sciaenidae family and *Larimichthys* genus, is widely distributed in offshore waters up to 80 m in depth around China, with concentrated populations found in the southern Yellow Sea and the coastal waters adjacent to river estuaries in the East China Sea (Liu, 2013). These fish inhabit waters with a salinity range of 17.0–34.5, an optimal temperature of 18℃–25℃, dissolved oxygen levels typically exceeding 4 mg/L, and an optimal pH range of 7.85–8.35 (Zhao and Lin, 1991; Zhou and Li, 2018). This warm-temperate migratory fish migrates offshore to spawn during the breeding season from April to June each year (Xu and Chen, 2011). Sexual maturity is reached at 2–3 years of age,

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and their spawning behavior involves batch spawning with 2–3 episodes (Liu, 2013). The dietary composition of the species is notably diverse, changing as the fish develops (Liu, 2013). Feeding behavior is heavily influenced by water temperature, with lower temperatures resulting in reduced feeding (Zhao and Lin, 1991). The yellow croaker mainly exists in three geographical populations: the Daiqu, the Min-Yuedong, and the Naozhou populations. These populations exhibit variations in morphology, ecology, and molecular biology, as well as having nonoverlapping spawning grounds. The Daiqu population, characterized by a longer lifespan and delayed sexual maturity, is the primary geographical population in the East China Sea (Huang et al., 2012; Jiang et al., 2015). Before the 1970s, the yellow croaker played a unique and important role in China's saltwater economy, with distinct fishing grounds and fishing periods (Yu et al., 2022). The catch of this species in the East China Sea accounted for more than 90% of the national catch, with an annual yield of approximately 12×10^4 tons, making it the second-highest yielding species in the East China Sea economy after *Trichiurus japonicus* (Liu and Han, 2011).

Despite its historical significance, the large yellow croaker population has markedly decreased due to escalating fishing intensity and offshore habitat degradation. Since the mid-1980s, yields have been consistently lower than those of *Larimichthys polyactis*, *T. japonicus*, and other species (FMBMARA, 1956–2020). Following the introduction of the summer break system in the East China Sea in 1995, the fishing catch briefly recovered to nearly 4000 tons before continuing to decline, ultimately reaching a minimum annual yield of only 100 tons. From 1987 onward; however, advances in artificial breeding technology boosted aquaculture production of large yellow croaker, making it the highest-yield marine fish in China (FMBMARA, 1956–2020). Consequently, the primary composition of the large yellow croaker resource consists of cultured populations, with supplementation from wild populations.

To restore large yellow croaker resources in the sea, China has released a substantial number of juveniles into coastal waters, particularly in the East China Sea (Ding and He, 2011). Although the resource status in natural waters has improved, the population structure faces challenges due to heavy fishing pressure, with a tendency toward younger and smaller individuals (Yu et al., 2022). Germplasm identification plays a vital role in fish resource management, aquaculture, and genetic breeding. Although large yellow croaker cultivation has enhanced fish yields, it has also brought about various issues such as inbreeding, inadequate parent fish selection, and biological characteristic degradation due to high-intensity farming. In a previous study of large yellow croaker cultured in Xiangshan, the fat content of the cultured population was 6.4-fold higher than that of the natural population, whereas amino acid content was markedly lower (Li et al., 2001). Thus, protecting wild large yellow croaker resources, enhancing the species' genetic diversity, and improving the quality of cultured populations through the selection of superior parent fish have become pressing concerns for the sustainable development of the large yellow croaker industry (Zhou and Li, 2018). Due to the scarcity of wild large yellow croaker and the associated high fishing costs, the price of wild fish is significantly higher than that of cultured. However, in coastal cities along the East China Sea, market regulators cannot mandate sellers to provide provenance information of fish, raising the possibility that consumers may purchase cultured fish that labeled as wild. Accurate identification methods are crucial to ensure consumers' ability to distinguish between wild and cultured fish, thereby avoiding misrepresentation or fraud and protecting both consumer rights and market integrity.

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Morphological indicators are commonly used for species identification, with previous studies exploring differences between the geographical populations (Xu et al., 1962, 2022), spawning populations (Zhang et al., 2005), and males and females (Chen et al., 2014) of large yellow croakers using morphological parameters. Other studies have investigated the morphological differences between cultured and wild large yellow croakers (Chen et al., 2014; Wang et al., 2016). Nevertheless, distinguishing between wild and cultured populations through visual observation requires specialized knowledge, thus posing a challenge for untrained individuals. Currently, a precise method for discriminating between these populations based on morphology is lacking. Therefore, in the present study, the framework method was used to analyze the morphological characteristics of wild and cultured large yellow croakers, and discriminant analysis was applied to determine the accuracy of population discrimination. The objective was to develop an effective method for distinguishing between these two populations, laying the foundation for the protection and utilization of large yellow croaker germplasm resources in the East China Sea.

Materials and methods

Sampling

The wild *P. crocea* population investigated in this study was collected by the trawler Zhepuyu 57962 in the East China Sea between August and October 2022. The collection area was located at 123°00′–126°30′ E and 27°30′–30°00′ N, with specimens carefully preserved under ice-fresh conditions. Cultured yellow croaker were obtained from a yellow croaker aquaculture farm in Shengsi, Zhoushan, Zhejiang during the same period. After sampling, the specimens were transported to the laboratory for biological measurements, including body length, body weight, and other conventional biological data. The specimens were then dissected and observed, referencing Yin (1995) to determine their sex and maturity stage. To comprehensively encompass the body length distributions of both cultured and wild large yellow croaker populations, a meticulous sampling approach was adopted. Specifically, 78 individuals were carefully chosen to represent the cultured population, which typically exhibits a relatively narrow range of body lengths spanning from 185 to 314 mm. Conversely, to accurately reflect the more extensive variability observed in wild populations, 98 wild specimens were selected, encompassing a broader spectrum of body lengths ranging from 126 to 523 mm. This deliberate selection ensures a robust representation of both population subsets, facilitating a comprehensive analysis of their respective morphological characteristics. Morphological measurements and analysis were performed on individuals with complete external morphology, involving 98 individuals from the wild population and 78 individuals from the cultured population. Specific population numbers and body lengths of both populations are detailed in *Table 1*.

Table 1. *Sample composition of wild and cultured Pseudosciaena crocea*

Species	Number	Range of body length (minimum~maximum) /mm	Mean body length (mean ± SD) /mm
Wild	98	126~523	189 ± 55
Cultured	78	185~314	244 ± 33

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Body shape measurements

This study followed previous research (Liu et al., 2011; Arechavala-Lopez et al., 2012; Wang et al., 2020), and employed the frame measurement method to select 10 anatomical coordinate points (*Fig. 1*). Furthermore, using measurement software, the straight-line distances between different coordinate points were determined, resulting in 45 measurable traits related to frame structure. We utilize the notation 'px-py' to represent the distance between the coordinate points 'x' and 'y'.

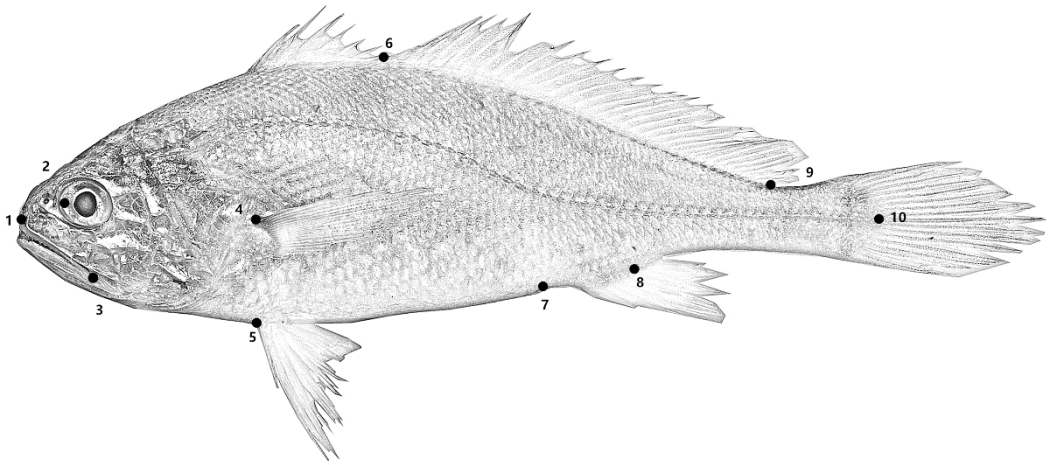

Figure 1. *Marked points for measuring Pseudosciaena crocea. Truss network system: 1. tip of premaxillary; 2. tip of eye; 3. anterior insertion of premaxillary; 4. dorsal insertion of pectoral fin; 5. anterior insertion of pelvic fin; 6. anterior insertion of dorsal fin; 7. anterior insertion of anal fin; 8. posterior insertion of anal fin; 9. posterior insertion of dorsal fin; 10. posterior extremity of lateral line*

To account for individual size variations in morphological traits, we standardized the data for each of the 45 measurable traits using the allometric method (Reist, 1985):

$$\log(M_{adj}) = \log(M) - \beta[\log(L) - \log(L_{mean})] \quad \text{(Eq.1)}$$

Here, M_{adj} represents the standardized data for measurable traits, M is the data for each measurable trait before standardization, L_{mean} is the average body length of the sample, L denotes the fork length of the individual, and β is the slope of log(M) against log(L).

Using the stepwise discriminant analysis (SDA) method in SPSS 23.0 software, a formula for discriminating between the two groups was established.

Results

Differences between cultured and wild populations

Out of the 45 standardized external morphological parameters, 23 displayed significant differences between the two groups ($P < 0.05$). Following standardization, principal component analysis was conducted on the 45 morphological parameters, where the first three main components contributed with 36.78%, 16.61%, and 12.16%

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respectively with the cumulative contribution rate being > 64.55% (*Table 2*). These components effectively represented the primary morphological characteristics of both groups. Remarkably, the wild population exhibited larger principal components 2 and 3 compared with the cultured population (*Fig. 2*), showing significant differences (FC2: $F = 37.094$, $P < 0.01$; FC3: $F = 5.146$, $P = 0.025$) (*Table 3*). Parameters with substantial FC2 score coefficients included p1-p4, p1-p6, p2-p4, p4-p7, p4-p8, and p5-p7, each showing significant differences between the two populations ($P < 0.05$). Similarly, parameters with notably large FC3 score coefficients included p3-p4, p3-p5, p3-p7, p4-p10, p6-p10, p7-p9, p7-p10, and p8-p10, which differed significantly between the populations ($P < 0.05$), except for p3-p5, p6-p10, and p8-p10.

Table 2. *Characteristic factors and contributive proportions of principal components for wild and cultured P. crocea*

PCs	Eigenvalue	Cumulative%
1	16.551	36.780
2	7.024	52.389
3	5.471	64.548
4	3.909	73.234
5	2.480	78.745
6	2.002	83.193
7	1.631	86.818
8	1.286	89.676
9	1.006	91.911

Table 3. *Loading matrix of morphometric characteristic factors of the three principal components for wild and cultured P. crocea*

Variable	PC1	PC2	PC3	P-value	Variable	PC1	PC2	PC3	P-value
p1-p2	0.297	0.576	-0.15	0.011	p3-p10	0.854	-0.068	-0.063	0.253
p1-p3	0.114	0.286	0.151	0.856	p4-p5	0.33	-0.042	-0.032	0.078
p1-p4	0.511	0.76	-0.266	0.001	p4-p6	0.216	0.048	-0.045	0.076
p1-p5	0.594	0.526	-0.36	0.648	p4-p7	0.437	-0.798	-0.208	0.001
p1-p6	0.497	0.642	-0.106	0.012	p4-p8	0.615	-0.648	0.072	0.001
p1-p7	0.811	-0.112	-0.438	0.004	p4-p9	0.683	-0.409	0.393	0.421
p1-p8	0.878	0.211	-0.2	0.181	p4-p10	0.671	-0.439	0.507	0.001
p1-p9	0.887	0.342	0.097	0.002	p5-p6	0.565	0.144	-0.095	0.175
p1-p10	0.904	0.245	0.209	0.358	p5-p7	0.443	-0.756	-0.326	0.001
p2-p3	0.233	0.365	0.138	0.81	p5-p8	0.606	-0.496	-0.063	0.919
p2-p4	0.536	0.692	-0.305	0.001	p5-p9	0.737	-0.241	0.273	0.027
p2-p5	0.582	0.425	-0.267	0.948	p5-p10	0.748	-0.342	0.42	0.348
p2-p6	0.414	0.315	-0.172	0.001	p6-p7	0.627	-0.51	-0.117	0.001
p2-p7	0.783	-0.344	-0.394	0.001	p6-p8	0.738	-0.235	0.2	0.933
p2-p8	0.908	-0.011	-0.141	0.498	p6-p9	0.664	-0.088	0.464	0.036
p2-p9	0.883	0.136	0.169	0.001	p6-p10	0.673	-0.135	0.584	0.899
p2-p10	0.911	0.051	0.304	0.799	p7-p8	0.073	0.526	0.407	0.001
p3-p4	0.459	0.47	-0.495	0.019	p7-p9	0.289	0.513	0.604	0.001

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p3-p5	0.368	0.052	-0.531	0.151	p7-p10	0.306	0.41	0.77	0.001
p3-p6	0.38	0.386	-0.177	0.726	p8-p9	0.339	0.2	0.438	0.04
p3-p7	0.609	-0.373	-0.596	0.001	p8-p10	0.323	0.064	0.62	0.11
p3-p8	0.731	-0.137	-0.457	0.516	p9-p10	0.158	-0.128	0.342	0.001
p3-p9	0.804	0.02	-0.18	0.344					

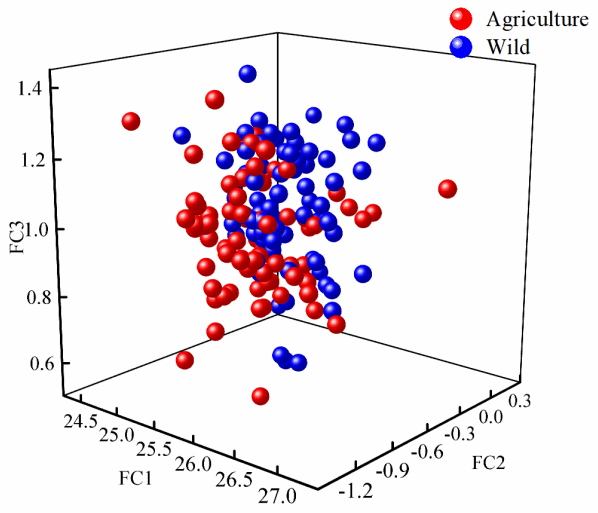

Figure 2. *Scatterplot scores of the three principal components for wild and cultured P. crocea*

Sex differences between wild and cultured populations

In the wild population, among the 45 standardized morphological parameters, only p1-p4 (0.76–0.75; $F = 4.969$, $P = 0.028$) and p4-p5 (0.39–0.42; $F = 6.452$, $P = 0.013$) exhibited significant differences between sexes. Principal component analysis of the 45 standardized morphological parameters yielded the first three main components contributing with 84.48%, 5.20%, and 3.45%, respectively, with a cumulative contribution exceeding 93.13%, representing the main morphological characteristics of the wild population. No significant differences were observed in these three principal components between sexes ($P > 0.05$).

Conversely, in the cultured population, among the 45 standardized morphological parameters, female individuals showed significantly larger values for p1-p4 (0.795–0.752; $F = 6.198$, $P = 0.015$), p1-p6 (0.919–0.888; $F = 4.084$, $P = 0.047$), p2-p4 (0.719–0.684; $F = 5.167$, $P = 0.026$), p3-p4 (0.658–0.616; $F = 5.259$, $P = 0.025$), and p3-p6 (0.900–0.863; $F = 4.084$, $P = 0.047$) compared with males. Principal component analysis of the 45 standardized morphological parameters in the cultured population revealed that the first three main components contributed with 39.80%, 15.01%, and 12.48%, respectively, with a cumulative contribution rate of 67.29%, representing the main morphological characteristics of this population. Among the three principal components, only FC2 showed a significant difference between sexes ($P < 0.05$). Parameters with larger FC2 score coefficients included p1-p4, p2-p4, p3-p4, p3-p5, and p4-p8, of which p3-p5 and p4-p8 exhibited no significant difference between sexes ($P < 0.05$). These findings indicated a substantial difference between the sexes in the cultured population, whereas a minor difference was observed in the wild population (*Fig. 3*).

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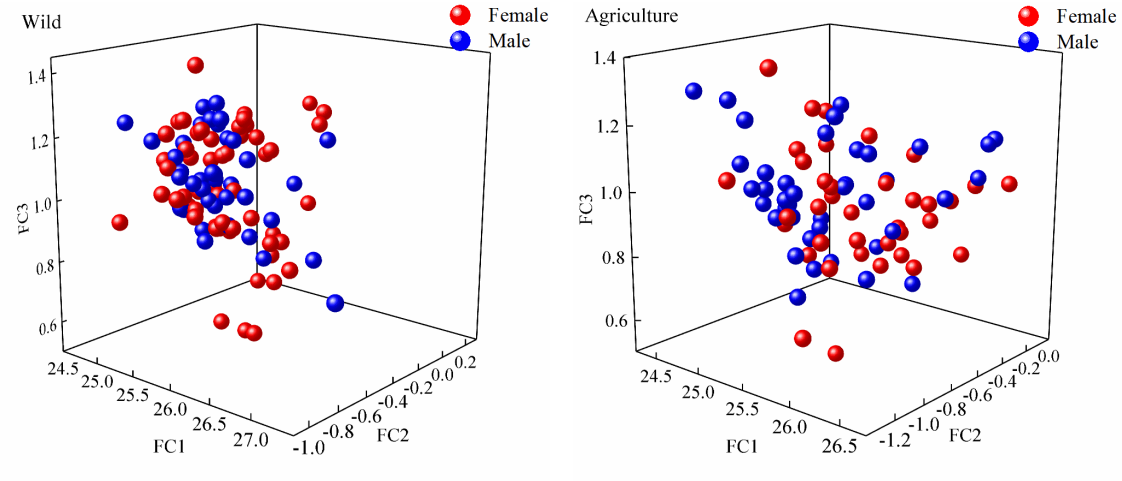

Figure 3. *Scatterplot scores of the three principal components for female and male individuals of wild and cultured P. crocea*

Discriminant analysis

Results of SDA between populations revealed that 12 standardized external morphological parameters, namely p1-p4, p1-p5, p1-p6, p1-p8, p2-p4, p2-p5, p2-p6, p2-p8, p4-p7, p5-p10, p6-p7, and p8-p9, could be used for population discrimination between the wild and cultured populations. These parameters, as indicated by typical discrimination coefficients and Wilk's λ analysis results, captured the majority of intergroup differences, with Wilk's λ values ranging from 0.706 to 0.189. Notably, the majority of individuals from the wild (95.9%) and cultured (96.2%) populations were correctly classified, resulting in an overall classification success rate of 96.0% (*Table 4*).

Table 4. *The result of stepwise discriminant analyses for morphological traits of of wild and cultured P. crocea*

Group	Classification sample		Total	Accuracy (%)
	Wild	Cultured		
Wild	94	4	98	95.9
Cultured	3	75	78	96.2

Considering sexes, SDA identified three standardized morphological parameters for discriminating between males and females in wild and cultured populations. For the wild population, p1-p4, p2-p6, and p4-p5 could be used, whereas in the cultured population, p5-p6, p4-p9, and p5-p9 could be employed. Typical discrimination coefficients and Wilk's λ analysis results indicated minimal differences between the sexes in the wild population, with three standardized parameters yielding Wilk's λ vales of 0.937–0.847. Conversely, the cultured population exhibited Wilk's λ values between 0.899 and 0.676. In the wild population, only 62.1% of females and 52.5% of males were correctly classified, resulting in an overall classification success rate of 58.2% (*Table 5*). Among the cultured population, 75.7% of females and 68.3% of males were

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correctly classified, giving an overall classification success rate of 71.8% (*Table 5*). These results suggest that using SDA based on standardized external morphological parameters effectively distinguishes between wild and cultured populations, although distinguishing between sexes within these populations, particularly the wild population, remains challenging.

Table 5. *The result of stepwise discriminant analyses for morphological traits of female and male wild and cultured P. crocea*

Group	Sex	Classification sample		Total	Accuracy (%)
		Female	Male		
Wild	Female	36	22	58	62.1
	Male	19	21	40	52.5
Cultured	Female	28	9	37	75.7
	Male	13	28	41	68.3

Discussion

Differences between cultured and wild populations

Fish being particularly susceptible to environmentally induced morphological changes exhibit greater intrapopulation and interpopulation differences compared with other vertebrates (Wimberger, 1992). The wild population of large yellow croaker often faces challenges in securing an optimal energy supply due to their reliance of environmental factors for food and the high energy expenditures associated with activities such as hunting and predator avoidance. Consequently, their accumulation of body fat is slower than that of cultured populations, resulting in suboptimal growth. Our study examined 45 standardized morphological traits of cultured and wild *P. crocea* populations, and we ultimately identified 23 traits that differed significantly between the two groups. These differences mainly manifested in the head structure and parts controlling swimming, possibly arising due to differences in feeding behavior, physical activity, and reproductive migration between the two populations. Similar morphological differences were observed in studies comparing cultured and wild squid populations (Satjarak et al., 2022). Notably, the present study identified more morphological differences between the two populations compared with previous research, which had primarily focused on traditional morphological measurements of cultured and wild large yellow croaker populations, including head and tail shape measurements (Humphries et al., 1981). Traditional morphological measurements yield a limited number of parameters, making it challenging to comprehensively capture fish body shape characteristics and hindering effective species differentiation. In contrast, the framework measurement method more comprehensively uses fish body information, providing more accurate discrimination results. This method, involving the selection of representative coordinate points and their connection into lines, effectively divides the fish body into multiple parts, fully reflecting its external morphological characteristics (Han et al., 2020).

Moreover, previous studies have shown that cultured populations have a higher degree of obesity and a larger body width-to-length ratio compared with wild populations (Zhang et al., 2005, 2007). However, this phenomenon was not observed in the current study, which may be attributed to changes in cultivation methods. Relevant studies have also shown that the fat content in various parts of large yellow croaker,

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including the liver and muscle as well as the whole fish tends to increase with increasing fat content in their diet. Historically, yellow croaker breeding activities were primarily conducted in relatively small offshore cages. In these intensive breeding environments, substantial amounts of high-fat feed were used to accelerate the growth of cultured fish (Boujard et al., 2004; Li et al., 2012; Yan et al., 2015). Given the limited space within the cages, energy expenditure among cultured groups was minimal, leading to fat accumulation. Nowadays, with advancements in aquaculture technology, large yellow croaker cultivation predominantly involves deep-water net cages and relies on high-protein bait fish as the primary food source. Additionally, fish in these cages have more space for movement. These changes in cultivation practices have resulted in alterations in the body shape of cultured populations.

Sexual dimorphism

Differentiating between female and male fish is a critical aspect of fish biology research and a key technique for the protection, artificial reproduction, and propagation of rare fish species. Fish allocate their energy resources to various tissues to support different life activities, with females and males emphasizing distinct activities at specific growth stages. This phenomenon is not unique to large yellow croaker and is observed in other fish species, with studies indicating that the morphological differences between male and female individuals vary at different growth stages in *Salmo salar* (Schaeffer et al., 2018), *Scatophagus argus* (Wu et al., 2014), and *Cynoglossus semilaevis* (Chen et al., 2008; Ji et al., 2011). The present study revealed that sexual dimorphism in large yellow croaker is not significant in both cultured and wild populations, with the differences being much smaller than those between the overall populations. This may be due to sexual dimorphism in large yellow croaker not being evident during their early life stages (Jia et al., 2012). Previous research suggests that morphological differences between genders may change as individuals grow. Early in life, individuals invest more energy in growth activities, and because both female and male individuals share similar lifestyles, including feeding and migration, their morphological differences are not substantial. However, as individuals reach maturity, morphological distinctions between males and females become increasingly evident. This shift is attributed to individuals allocating more energy to reproductive activities as they mature, with females investing relatively more in reproduction. This difference is most noticeable in the markedly higher gonad index of females compared with males. This phenomenon is also observed in other fish species. Studies have shown that sexual dimorphism varies across growth stages in *Pseudobagrus ussuriensis* (Jia et al., 2012), *Siniperca chuatsi* (Wang et al., 2006), and *Paramisgurnus dabryanus* (Wang et al., 2005).

In the present study, the discrimination accuracy between females and males in the cultured population was higher than that in the wild population. This may be attributed to the presence of more sexually mature individuals in the cultured population. Male fish mature earlier than female fish, and mature individuals are smaller in size, with decreased growth rates following sexual maturity. Conversely, female fish mature later, are larger in their mature state, and invest more in reproductive activities (e.g., a larger gonadal index), leading to greater morphological differences between sexes. Additionally, as large yellow croaker grow in size, the accuracy of discriminating between male and female individuals improves (Chen et al., 2014).

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Discriminant analysis

Morphological characteristics serve as crucial indicators for distinguishing between artificially cultured and wild populations. These characteristics enable the rapid and simple differentiation of the origin (wild vs. farmed) of various aquatic animals, including Atlantic cod (*Gadus morhua*) (Uglem et al., 2011) and broad-headed catfish (Whan-air et al., 2018). Although, in the current study, the discrimination accuracy rate was > 95% when distinguishing between wild and cultured populations, it is still unclear whether morphological methods could differentiate the cultured population from the wild population after the habitat environment of the cultured population changes. Sterns (Stearns, 1983) reported that fish adapt to environmental changes by altering their physiology and behavior, which ultimately leads to morphological changes. Consequently, the morphological characteristics of cultured large yellow croaker may also change after the modification of their habitat. Therefore, it remains uncertain whether morphological methods can effectively distinguish cultured large yellow croaker from the wild population after multiplication and release. In addition to morphological methods, other techniques are used in group discrimination. Short-term identification of released populations and the wild population can be achieved through methods such as pigment marking and fin cutting. Over the medium and long term, identification can be accomplished through otoliths, bone trace element marking, and molecular methods. Hence, the effectiveness of the morphological discrimination method under various growth stages and changing environmental conditions needs further validation when compared to other methods.

When employing morphological methods for sex discrimination in large yellow croakers in this study, limitations in accuracy were observed. To enhance the precision of gender identification, future research efforts could be redirected towards exploring alternative and more effective discriminatory approaches. Notably, the analysis and application of muscle nutritional composition and trace elements have demonstrated significant potential in sex discrimination, suggesting their potential as key factors for improving accuracy. This insight underscores the importance of incorporating these considerations into subsequent investigations, aiming to achieve more precise and reliable sex discrimination outcomes in studies of large yellow croakers.

Conclusions

Results of the present study provides an efficient and accurate method to distinguish cultured large yellow croaker from wild individuals by employing the framework method. By visualizing the differences in external morphology through principal component analysis and using stepwise discriminant analysis for classification, we found substantial variations in standardized external morphological parameters between wild and cultured populations. In terms of sex differences, our research indicates that variations are more prominent within the cultured population compared with the wild population. These results are of paramount importance for the sustainable development of the large yellow croaker industry in the East China Sea. They offer valuable insights into the protection and utilization of the germplasm resources of this species. Overall, our findings contribute to the broader goals of enhancing genetic diversity, improving the quality of cultured populations, and restoring the ecological balance in the East China Sea.

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