

Effects of Genetics and Rearing Environments on Morphometric Variation in Hatchery Stocks of Asian Sea Bass, *Lates calcarifer* (Bloch, 1790)

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ABSTRACT

The effects of genetics and rearing environments on morphometric variation among hatchery stocks of Asian sea bass (300 days post-hatch) were investigated by multivariate analyses of 21 truss measurements. Ninety-day-old fingerlings from four stocks, including Ang Sila, Bang Pakong, Meklong and Paknam, were stocked in the same earthen pond and reared for 270 days. These stocks were genetically different from each other. Principal component analysis (PCA) of body measurements suggested very little body shape difference among populations. Subsequently, discriminant function analysis (DFA) was used to assign individuals to their stock of origin. The accuracy of assignment was highest for the Ang Sila stock (77.7%) followed by Bang Pakong (72.4%), Meklong (66.1%) and Pak Nam (65.5%). Analysis of the Ang Sila stock reared in two locations, Chantaburi and Prachuab Kirikhan provinces, suggested that salinity is an important factor affecting body shape differentiation. DFA identified the two most discriminating truss measures, both of which were correlated with head size. The classification functions assigned individuals to groups with 100% accuracy. The results demonstrated that multivariate morphometrics is an effective tool for detecting variation among stocks in Asian sea bass aquaculture.

Keywords: Barramundi, Discriminant function, Multivariate analysis, Principal component analysis, Truss morphometric

INTRODUCTION

Asian sea bass, or barramundi, *Lates calcarifer* (Bloch, 1790), are widely distributed throughout coastal areas of the Indian and the western Pacific oceans, from India to southeast Asia, China, Taiwan and southward from Indonesia, Papua New Guinea to northern Australia (www.fao.org/fishery/culturedspecies). Sea bass are euryhaline, and spend most of their lives in freshwater environments. As adults (3–4 years), they migrate to more saline waters to spawn. The species is known for its rapid growth rate, attaining a size of

3–5 kg within 2–3 years, and for its high tolerance to environmental fluctuations (Kungvankij *et al.*, 1984). These characteristics make sea bass suitable for coastal aquaculture, where the fish can grow in sea cages or in brackish or freshwater ponds, with salinity varying from 0 to 35‰ (Kungvankij *et al.*, 1984).

It is well documented that rearing environments have significant effects on fish growth and body form (Cibert *et al.*, 1999). Although genetic variation and growth performance among sea bass populations in Thailand have been

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investigated (Senanan *et al.*, 2015; Joerakate *et al.*, in press), there are no published studies concerning morphological variation. Variation in fish body shape is influenced by the underlying genetic architecture of an individual, environmental factors, or a combination of both. This phenomenon can be observed among individuals both within and between fish populations.

Genetic variation reflects the ability of a population to respond to natural or artificial selection, as well as the potential to adapt to changing environments over longer time scales (Allendorf and Luikart, 2007). Furthermore, fish are found to exhibit phenotypic plasticity, a change in the phenotypic expression of a given genotype in different environments. Plasticity in body shape is common among fishes in the families Cichlidae and Salmonidae, and is found to be highly adaptive (Winans, 1984; Meyer, 1990; Wimberger, 1992; Husemann *et al.*, 2017).

In this study, we used a truss morphometric approach to investigate the effects of genetics and environmental factors on body shape of farmed Asian sea bass. Truss morphometrics have been shown to be an effective tool to differentiate individuals of similar stocks or from potentially different populations of the same fish species (Corti *et al.*, 1988; Pannusa *et al.*, 2015; Siddik *et al.*, 2016; Zhang *et al.*, 2016). Body shape measurements have been used to identify the best parental strains for hybrid striped bass production to improve fillet yield (Bosworth *et al.*, 1998). Fillet yield is an important trait for the Asian sea bass processing industry. However, this trait is difficult to measure and cannot be directly used for selection of broodstock. Demonstration of genetic determination of morphometric characters in the present study would pave the way toward selection to improve fillet yield in Asian sea bass.

In this study, a total of 440 adult fish (300 days old) from different genetic backgrounds were measured for 21 truss characters. Multivariate statistical analyses were utilized to determine morphometric differentiation among populations.

MATERIALS AND METHODS

Fish culture and experimental conditions

We assessed the effects of genetic background on Asian sea bass (Joerakate *et al.*, in press). Sea bass were produced from different hatchery stocks, including Ang Sila (Chon Buri Province), Bang Pakong (Chachoengsao Province), Meklong (Samut Songkram Province) and Paknam (Chumphon Province). Briefly, fingerlings (90 days post hatch) of these four populations were stocked separately in four cages ($6 \times 6 \times 1.2 \text{ m}^3$) set up within the same earthen pond ($16 \text{ m} \times 40 \text{ m} \times 1 \text{ m}$ or 640 m^3) at the Kasetsart University Fisheries Research Station in Samut Songkram Province. Each cage was stocked with 100 fish. Average size at stocking ranged from 9.48 ± 0.12 to $9.74 \pm 0.16 \text{ g}$ and from 8.13 ± 0.80 to $9.04 \pm 0.80 \text{ cm}$ total length. There was no water exchange during grow-out, but the ponds were topped off regularly to compensate for evaporation, maintaining the water depth within the net at 80–90 cm and the salinity within a range of 15–20 parts per thousand (‰). Aeration was provided through polyethylene pipes on the bottom of the ponds below the cages. Fish were fed to apparent satiation with a 40% protein commercial floating diet (Betagro Feed Mills, Bangkok, Thailand) twice daily at 07:00 and 17:00 hours. Samples were collected at 300 days of age for assessment of morphometric variation.

To determine the effects of rearing environment, 90-day-post hatch fingerlings of the Ang Sila stock were raised in two locations: Laem Sing farm (Chantaburi Province) and Sam Roi Yod farm (Prachuab Kirikhan Province). Water salinity ranged from 5 to 15 ppt in Sam Roi Yod farm and from 30 to 38 ppt in Laem Sing farm. One hundred fish were stocked in each of four stationary cages ($6 \times 6 \times 1.5 \text{ m}^3$) in a 1600 m^2 pond at Laem Sing farm, whereas 100 fingerlings were stocked in each of four stationary cages ($6 \times 6 \times 1.5 \text{ m}^3$) in a 3200 m^2 pond at Sam Roi Yod farm. Both groups were fed 40% protein commercial floating diet (CP Feed Mills, Bangkok, Thailand) twice daily at 07:00 and 17:00 hours. At both sites, aeration

was provided by paddle wheels and there was no water exchange. Water quality was monitored daily for dissolved oxygen and temperature. Twenty-five fish from each stationary cage were sampled for growth every four weeks and measured for body weight (BW), standard length (SL) and total length (TL). Means of body measurements from 30 to 300 days post-hatch fish were used to develop a length-weight relationship (LWR) model.

Morphometric analysis

After 210 days of rearing, fish were harvested at the three grow-out sites. They were lightly anesthetized with clove oil (1 mL/2.5 L of water) and placed on a light-colored background on their left side. Fish were photographed using a Nikon D40X digital camera (Nikon; Tokyo, Japan) mounted on a 190XPROB tripod (Manfrotto; Cassola, Italy). The truss network method (Bookstein *et al.*, 1985) was used to characterize whole-body shape from digital images. Sample sizes and mean body lengths are presented in Table 1.

Ten morphological landmarks were identified and 21 distance measurements were analyzed. Figure 1 displays locations of the landmarks and distances that were plotted on the images using SigmaScan Pro 4.0 (www.sigmaplot.com).

Two multivariate statistical analyses, namely, principal component and discriminant function analyses were performed to assess morphometric differentiation among populations. Principal component analysis (PCA) was used to describe body shape independent of fish size and to delineate groups of samples using the Paleontological Statistics software (PAST) (Hammer *et al.*, 2015). To eliminate the effect of fish size on the first principal component (Bookstein *et al.*, 1985), morphometric data were standardized to fish standard length (SL) as described by Elliot *et al.* (1995). Key truss measures that contributed to variation in body shape among populations were determined from the PCA loadings. Discriminant function analysis (DFA) was used to assign individuals into the stocks to which they most likely belonged on a morphometric basis in a three-step process. Firstly, canonical discriminant functions were created as a linear combination of discriminating variables (truss measurements) for which the first function provides the most overall discrimination among groups using SPSS Statistics 17.0 (SPSS, Inc. Released 2008, Chicago, USA). Secondly, the mean discriminant function coefficient or centroid was calculated for each group, and Wilks' lambda test was used to detect significant differences among group centroids. Lastly, using discriminant functions, fish were assigned to the group in which they had the highest classification scores.

Table 1. Populations of Asian sea bass, number of individuals sampled and average standard length at 300 days old.

Grow-out site/Population	N	Standard length (mean±SD) cm
Samut Songkram Province		
Ang Sila	58	37.47±2.79
Bang Pakong	58	37.05±2.87
Meklong	59	37.29±2.21
Paknam	61	37.52±3.57
Chantaburi Province		
Ang Sila	100	34.72±2.45
Prachuab Kirikhan Province		
Ang Sila	100	38.96±2.61

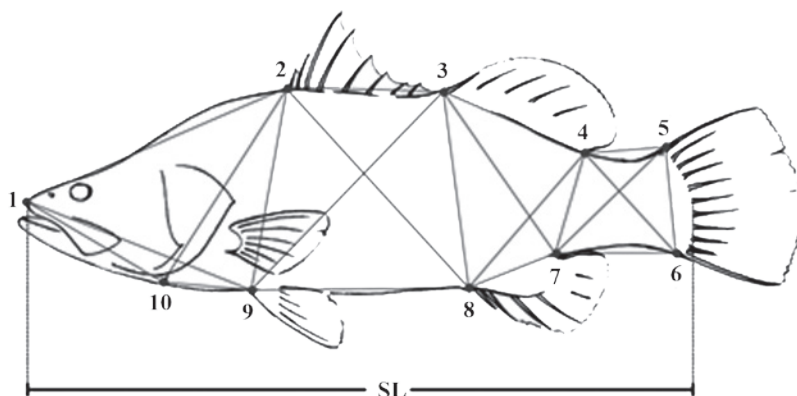


Figure 1. Locations and distances between landmarks for 21 truss variables (M). The measures include: M1 (1–2) tip of snout–anterior 1st dorsal fin, M2 (2–3) anterior 1st dorsal–anterior 2nd dorsal, M3 (3–4) anterior 2nd dorsal–posterior dorsal, M4 (4–5) posterior dorsal–anterior dorsal membrane from caudal fin, M5 (5–6) dorsal–ventral membrane from caudal, M6 (6–7) ventral membrane from caudal–posterior anal fin, M7 (7–8) posterior anal–anterior anal, M8 (8–9) anterior anal–anterior pelvic fin, M9 (9–10) anterior pelvic–posterior maxillary, M10 (1–10) tip of snout–posterior maxillary M11 (1–9) tip of snout–anterior pectoral fin, M12 (2–9) anterior 1st dorsal–anterior pectoral, M13 (2–10) anterior 1st dorsal–posterior maxillary, M14 (2–8) anterior 1st dorsal–anterior anal, M15 (3–8) anterior 2nd dorsal–anterior anal, M16 (3–9) anterior 2nd dorsal–anterior pelvic, M17 (3–7) anterior 2nd dorsal–posterior anal, M18 (4–7) posterior 2nd dorsal–posterior anal, M19 (D–H) posterior 2nd dorsal–anterior anal, M20 (4–6) posterior 2nd dorsal–anterior ventral membrane from caudal, and M21 (5–7) anterior dorsal membrane from caudal–posterior anal. SL = standard length

RESULTS AND DISCUSSION

Effects of stock on body shape variation

We conducted principal component analysis of morphometric data from sea bass from four populations reared in a common garden experiment. The first principal component accounted for 44% of the variability in fish body shape, whereas PC2 explained 16.8% of the remaining variability (Table 2). Subsequent principal components which explained < 10% were not considered. The first principal component was a measure of abdominal shape and size as indicated by positively signed coefficients for three characters, including M8, M12 and M16. The second principal component was correlated with head size (M1), length of the first dorsal fin (M2) and distance from anterior insertion of the dorsal fin to the anterior insertion of the anal fin (M14).

Although the first and second components accounted for 60.8% of total morphometric variation, the PCA method was not effective in separating sea bass stocks in our study. Despite significant pair-wise genetic differentiation for five of the six comparisons, e.g., 0.079 between Ang Sila and Paknam (Joerakate *et al.*, in press), the PCA identified clusters of all four stock samples which showed high degrees of overlap among populations. The PCA plot shows very little morphometric differentiation among these four populations (Figure 2). A previous study reported that the PCA approach was less effective if populations were genetically similar or if there was weak association between genetic differentiation and morphometric variation (Pannusa *et al.*, 2015).

To detect the maximum amount of body shape variability, morphometric differences among the samples were further assessed by discriminant

Table 2. Component loadings for the first two principal components (PC1 and PC2) of truss morphometric characters (M1–M21) from four hatchery stocks of Asian sea bass. Asterisks indicate maximum loadings of three measurements for PC1 and PC2.

Measurement	PC 1	PC 2
M1	0.1376	0.5299*
M2	0.0098	-0.4943*
M3	0.0767	0.0212
M4	0.0238	0.0744
M5	0.0532	0.0097
M6	-0.0596	0.0456
M7	0.0376	0.0566
M8	0.3586*	-0.3084
M9	-0.0548	0.1190
M10	-0.1109	0.0321
M11	-0.1469	0.1536
M12	0.3780*	0.2302
M13	0.2755	0.3763
M14	0.2693	-0.3337*
M15	0.3300	-0.0054
M16	0.4495*	-0.1233
M17	0.2896	0.0180
M18	0.2178	0.0179
M19	0.2136	0.0332
M20	0.1474	0.0792
M21	0.0179	0.0208

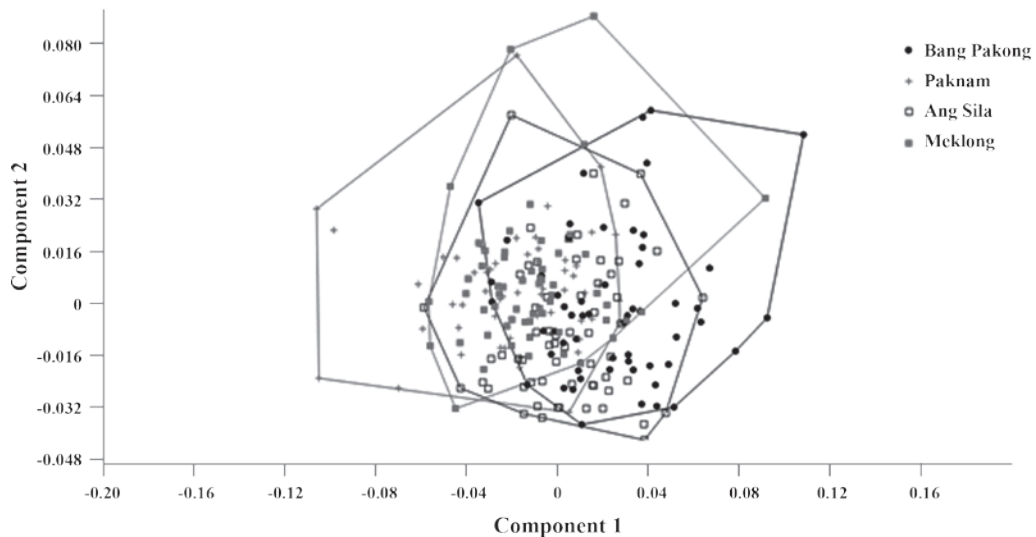


Figure 2. Results of the principal component analysis of truss variables for body shape differences among hatchery stocks of Asian sea bass. Principal component 1 and principal component 2 accounted for 44% and 16.8% of the total variation, respectively. Data for each stock are outlined with polygons.

function analysis (DFA). Three discriminant functions were constructed from a combination of nine truss characters (M2, M6, M8, M11, M12, M13, M15, M17 and M20) for predicting group membership (Table 3). Results revealed that the first, second and third standardized canonical DF functions accounted for 50%, 39.8% and 10.2% of total variation, respectively. According to the standardized canonical discriminant function coefficients, the most discriminating variable for the first DF function was M13 (anterior insertion of the first dorsal fin–posterior margin of maxillary). For the second function, M17 (anterior insertion of the second dorsal fin–posterior insertion of anal fin) had the largest contribution to the discrimination among groups. For the third function, M2 (first dorsal fin length) was the most discriminating measurement.

Wilks’ lambda test indicated significant differences in group means or centroids for discriminant scores of all three functions ($\lambda < 1$; P value < 0.000) (Table 4).

The DFA method proved useful in separating and assigning sea bass individuals to their group of origin. Discrimination plots based on two mathematical functions displayed a separation of group centroids from four stocks in discriminant space (Figure 3). Group centroids of two populations, Paknam and Meklong were closer to each other than the other two, indicating that they shared more similarity in body shape. Meanwhile, Ang Sila and Bang Pakong samples were far apart on the plot. Results of the body shape variation among stocks were supported by the accuracy of assignment. As expected, higher percentages of correct assignment were obtained for the distinct stocks of Ang Sila (77.7%) and Bang Pakong (72.4%), whereas more samples from Meklong and Paknam were misclassified (Table 5).

In the present study, body shape variation was evident from the DFA, suggesting that the observed morphological differences in sea bass populations were primarily influenced by

Table 3. Standardized canonical discriminant function coefficients from discriminant analysis of morphometric traits of four Asian sea bass populations.

Measurement	Function 1	Function 2	Function 3
M2	0.639	0.604	0.769
M6	0.843	0.581	-0.276
M8	-0.641	-0.205	0.410
M11	0.205	-0.343	0.359
M12	-0.705	0.654	0.728
M13	1.382	0.316	0.276
M15	-0.519	-0.992	-1.051
M17	0.697	1.154	0.309
M20	-0.795	-0.148	-0.259

Table 4. Results of Wilks’ lambda test for differences in morphometric measurements among four populations of Asian sea bass.

Test of Function(s)	Wilks' lambda	Chi-square	df	P value
1 through 3	0.251	315.880	27	0.000
2 through 3	0.484	165.977	16	0.000
3	0.840	39.786	7	0.000

Table 5. Classification results for the discriminant function analyses of morphometric traits of Asian sea bass. Values in rows indicate the number of each sample group classified as a member of each predicted group. Percentage values of total sample size are given in parentheses.

Population	Ang Sila	Bang Pakong	Meklong	Paknam	Total
Ang Sila	47 (77.7)	6 (9.8)	6 (9.8)	2 (3.3)	61
Bang Pakong	6 (10.3)	42 (72.4)	6 (10.3)	4 (6.9)	58
Meklong	5 (8.5)	7 (11.9)	39 (66.1)	8 (13.6)	59
Paknam	5 (8.6)	6 (10.3)	9 (15.5)	38 (65.5)	58

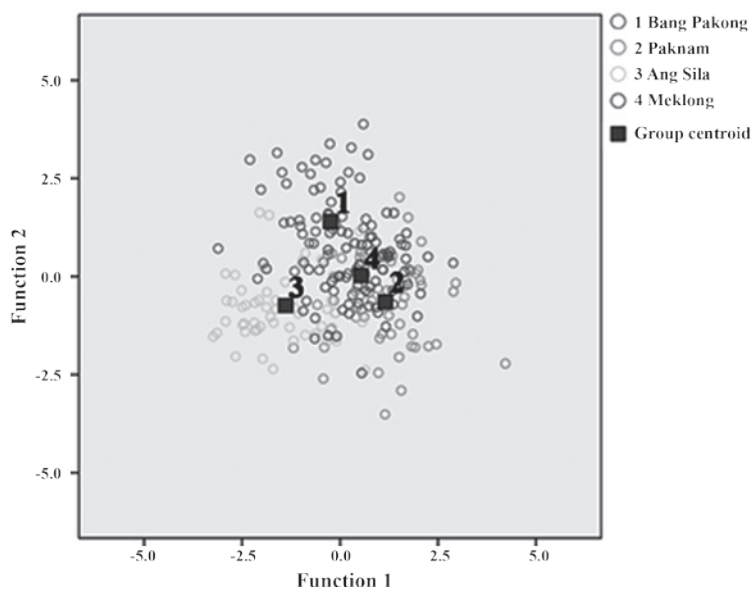


Figure 3. Scatter plot of discrimination functions 1 and 2 for body shape variation, with group centroids for four hatchery stocks of Asian sea bass.

genetic factors (stock origin). Factors related to environmental factors such as rearing practices or cage effect, may also have contributed to this variation, but was expected to be minimal. Genetic differentiation among stocks of Asian sea bass has been documented in previous studies (Senanan *et al.*, 2015; Joerakate *et al.*, in press). Founder populations of these stocks originated from different river systems in the upper Gulf of Thailand (Joerakate *et al.*, in press). The Bang Pakong stock originated from wild populations in the Bang Pakong River and has been domesticated for over 40 years. The Ang Sila stock was founded from

Asian sea bass stocks obtained from cage culture operations along the east coast of the Gulf of Thailand. The origin of the Paknam fish was the Chumphon River, which runs through the west coast of the Gulf of Thailand. The Meklong population was originally collected from cage culture in the Meklong River. Differences in morphometrics among populations may have been due to ancestral morphometric differentiation or to domestication and selection programs applied in each stock. Selection for growth over four generations resulted in body shape differentiation of Thai silver barb (*Barbonymus gonionotus*) broodstocks when

compared to those of the founder populations (Pannusa *et al.*, 2015). In common carp (*Cyprinus carpio*), morphometric distinction between aquaculture stocks was apparently due to their respective genetic constitutions (Corti *et al.*, 1998). However, relatively few differences in body shape were observed among eight founder stocks of Nile tilapia used for development of the GIFT (Genetically Improved Farmed Tilapia) strain (Eknath *et al.*, 1991).

Effects of rearing environment on fish body shape

The length-weight relationship (LWR) for Ang Sila sea bass can be described by the equations: $BW = 0.0099TL^{3.10}$, $R^2 = 0.9821$ for Prachuab KiriKhan, and $BW = 0.0106TL^{3.09}$, $R^2 = 0.9836$ for Chantaburi fish, where BW is the predicted individual fish weight in grams and TL is measured total length in centimeters (Figure 4). LWR models indicated isometric growth of fish at both sites. In general, LWR can be used to quantify the condition

of the fish, which in turn is correlated to body shape (Strange, 1996).

The principal component analysis of Ang Sila sea bass raised at Laem Sing and Sam Roi Yod farms revealed that PC1, PC2 and PC3 accounted for 29.5%, 19.95%, and 12.34% of the total morphometric variation, respectively. The variation in three measures, including M3 (soft dorsal fin length), M10 (tip of snout–posterior margin of maxillary) and M11 (tip of snout–anterior insertion of pelvic fin) contributed the maximum loadings of PC1. The measurements of M1 (tip of snout–anterior insertion of the first dorsal fin), M2 (first dorsal fin length) and M13 (anterior insertion of dorsal fin–posterior margin of maxillary) had the largest loadings for PC2 (Table 6).

A plot of the PC1 and PC2 scores revealed a high degree of separation between the two groups of Ang Sila samples, with only some overlap (Figure 5).

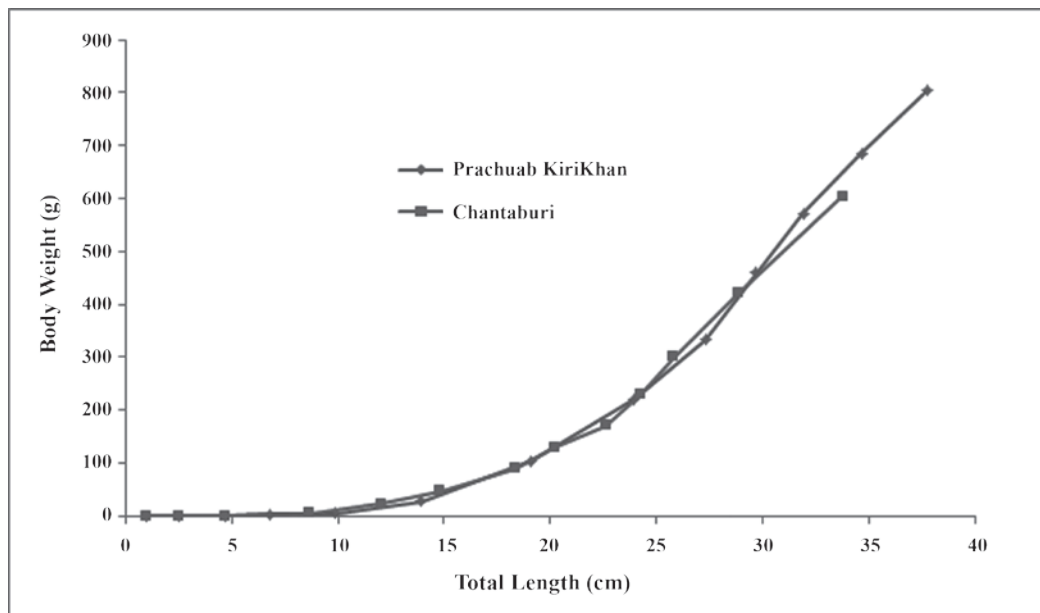


Figure 4. Length-weight relationships for Ang Sila sea bass reared at Prachuab KiriKhan and Chantaburi provinces.

Table 6. Component loadings for the principal components (PC1 and PC2) of truss morphometric characters (M1–M21) of Ang Sila stock samples of Asian sea bass reared at Chantaburi and Prachuab KiriKhan Province. Asterisks indicate maximum loadings of three measurements for PC1 and PC2.

Measurement	PCA loading 1	PCA loading 2
M1	0.09	0.55*
M2	0.26	-0.55*
M3	-0.34*	-0.05
M4	0.24	0.11
M5	0.04	0.00
M6	0.18	0.04
M7	-0.08	0.00
M8	-0.30	-0.15
M9	-0.12	0.09
M10	0.53*	0.02
M11	0.40*	0.11
M12	-0.01	0.17
M13	-0.10	0.39*
M14	0.12	-0.36
M15	0.08	0.02
M16	-0.03	-0.07
M17	-0.04	0.03
M18	0.20	0.03
M19	0.06	0.02
M20	0.20	0.06
M21	0.21	0.05

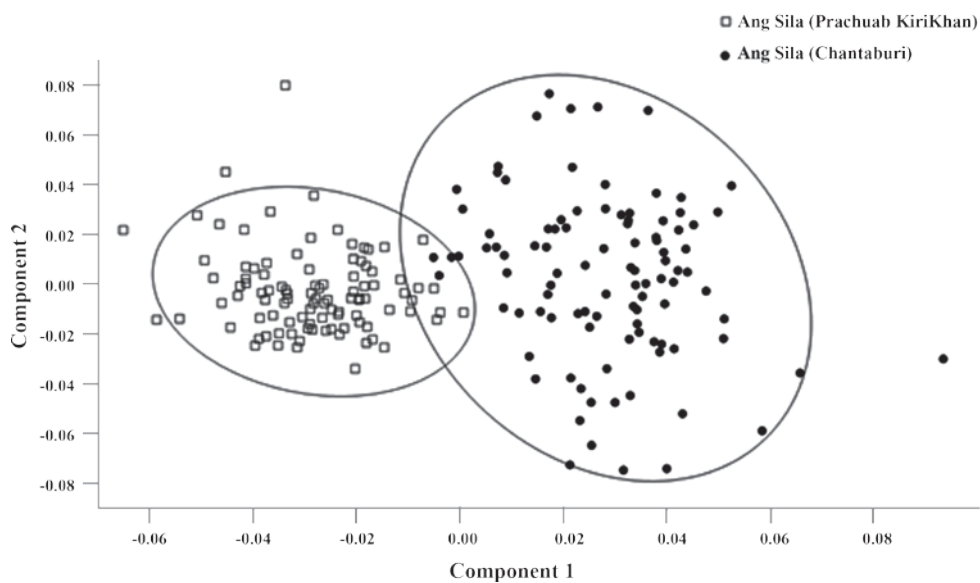


Figure 5. Principal component analysis of morphometric characters of the Ang Sila stock of Asian sea bass reared in two locations

There is one discriminant function for a two-group discriminant analysis. The function used all 21 truss measures to identify both groups of Ang Sila origin (Table 7). Results indicated that the two most discriminating variables for the DF function were M10 (tip of snout–posterior margin of maxillary) and M11 (tip of snout–anterior insertion of pectoral fin). Both measures were correlated with head size.

A test of the discriminant function indicated significant differences in group centroids between the two groups of Ang Sila stock (Wilks' lambda = 0.121; P value < 0.0001), suggesting that reliable classification was possible. Discriminant function analysis was very effective for the Ang Sila data, for which individuals were assigned to groups with 100% accuracy. Differences in morphology of the two groups appeared to be the result of environmentally induced differences in growth rates that led to shape differences. Another factor, negative allometric growth in head length (i.e.,

larger fish having smaller head) may also have contributed to body shape variation. Meyer (1990) reported negative allometry in growth of pharyngeal jaws between morphs of neotropical cichlid fish *Cichlasoma citrinellum*. The different water salinities at the two farms (5–15‰ at Sam Roi Yod and 30–38‰ at Laem Sing) probably affected fish shape, although there were several other potential causes related to rearing factors. Partridge and Lymbery (2008) reported that barramundi grew faster in less saline habitats than in more saline habitats. Specific growth rates ranged from –0.38 to 0.7%/day at 45‰, 1.86 to 2.18%/day at 15‰, and from 2.30 to 2.62%/day at 5‰. In addition, they found that fish required more potassium at hyperosmotic salinity (45‰) than in near-isosmotic (15‰) or hyposmotic (5‰) salinities. In our study, Prachuab KiriKhan fish, which grew faster, had smaller heads than those of Chantaburi. Plasticity in body-shape development during smoltification was reported in a stock of Chinook salmon (*Oncorhynchus tshawytscha*) raised at two

Table 7. Standardized discriminant coefficients from discriminant analysis of morphometric traits of Asian sea bass from the Ang Sila stock reared at two locations.

Measurement	Function 1
M1	0.106
M2	0.134
M3	-0.222
M4	0.152
M5	0.055
M6	0.263
M7	-0.087
M8	-0.307
M9	-0.109
M10	0.586
M11	0.516
M12	-0.021
M13	-0.059
M14	0.049
M15	0.062
M16	-0.069
M17	-0.055
M18	0.259
M19	0.078
M20	0.150
M21	0.328

locations with different water temperatures (Winans, 1984). Shape variability in the early development of Chinook salmon was found to be associated with the caudal region. Wimberger (1992) found that young cichlid fishes (*Geophagus* sp.) displayed body shape plasticity in response to different diets. Fitzgerald *et al.*, (2002) reported that yellow perch (*Perca flavescens*) receiving different ration sizes of the same commercial pellet exhibited changes in body shape. In striped bass (*Morone* spp.), body shape variation was found to be correlated with fillet weight, providing useful information for the development of a model to predict this trait in live fish (Bosworth *et al.*, 1998).

CONCLUSION

The present study demonstrated the contributions of both genetic and environmental influences on morphometrics of Asian sea bass. The greatest differences among four genetically distinct hatchery populations reared under semi-controlled pond environments were found in the shape of the head and in body depth. Moreover, the Asian sea bass exhibited morphometric differences, apparently as a result of different environmental conditions at each location. This result indicates the ability of sea bass to respond to environmental changes in the short term. The variation in body shape offers the potential for selective breeding to improve traits correlated with body shape such as fillet weight and yield in Asian sea bass.

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