Stock identification of short mackerel (*Rastrelliger brachysoma*) in Thai waters based on truss network system

Thotsagotphairee, C., Kanloung, T., Viboonkit, K., Tavitchasri, P. and Thongpradub, W.[*]

King Mongkut's Institute of Technology Ladkrabang Prince of Chumphon Campus, Pathiu, Chumphon, 86160, Thailand.

Abstract The results of discriminant function analysis (DFA) indicated the first two discriminant function (DF1 and DF2) accounted for 42.6% and 25.3%, respectively. The result of the stepwise discriminant function analysis revealed 16 characters from truss network system. Different characters of the DF1 were observed in head-related characters (1-17), anterior part of the body (2-3), posterior part of body (4-5, 15-16), and caudal trait (9-12). The DF2 showed the different characters in anterior part of the body (3-16), posterior part of body (5-16), and caudal trait (6-15, 6-14). According to the discriminant function analysis, 53.0% of the original groups were correct classification into their own populations. The plotting discriminant function exhibited the intermingling in all demographic groups. This morphometric study of *Rastrelliger brachysoma* indicated that short mackerel in Thai waters: the Gulf of Thailand and the Andaman Sea, had a common ancestor's origin.

Keywords: Marine fish, Population structure, Short mackerel, Truss network system

Introduction

The short mackerel (*Rastrelliger brachysoma*) are marine epipelagic fish, which are widely distributed in the Indo-Pacific region from the area of Taiwan, Malaysia, Indonesia, Philippines, Solomon Island, New Britain Island, Fiji, and India (Collette and Nauen, 1983). They are also found in both the Gulf of Thailand and the Andaman Sea (Puntuleng and Nasuchon, 2005). Short mackerel has a slender body. Its silvery-green upper body appears black dots aligned along with the lateral line. The ventral body is silver and the caudal fin is also yellowish (Collette and Nauen, 1983). The short mackerel is fed on a variety of food categories, with phytoplankton being the most dominant proportion of the foods (Collette and Nauen, 1983; Aye, 2020). The short mackerel is a schooling fish which inhabits near the coastal areas (Collette and

[*] **Corresponding Author:** Thongpradub, W.; **Email:** walaiporn.ma@kmitl.ac.th

Nauen, 1983; Kongseng *et al*., 2020). Their migration pattern in the Gulf of Thailand was found in the western coast and the eastern coast (Munprasit *et al*., 2020). The short mackerel resources have been subjected to overfishing and rapidly declined. The capture amounts of this species in Thailand are continuously increased. Reports from Thailand Department of Fisheries revealed that the quantity catches of short mackerel were 8,003.91 tons in 2019, which increased to 12,594.56 tons in 2021 (Department of Fisheries, 2020, 2022).

Information of population structure in any fish species is essential for management of fish stock in aquatic ecosystem. Fish stock identification has been reported by determining with the morphometric analysis. Truss network measurement, one of the morphometric analyses, is a method based on the measurement of distances among anatomical landmarks on the fish's body (Strauss and Bookstein, 1982; Rawat *et al*., 2017). Several previous studies related with the identification of fish populations based on truss network measurement approach were reported in many fish species; *Rastrelliger kanaguarta* Cuvier, 1816) (Sajina *et al*., 2011; Hakim *et al*., 2020), *Trichomycterus areolatus* Valenciennes, 1846 (Colihueque *et al*., 2017), *Cyclocheilichthys apogon* (Kenthao and Jearranaiprepame, 2018), *Macrognathus pancalus* (Mahfuj *et al*., 2019), *Chanda nama* (Azad *et al*., 2020) and *Upeneus vittatus* (Forsskal, 1775) (Nama *et al*., 2022). The information of fish stocks is an important database for the management of the fishery resources. Nowadays, the short mackerel has been declining due to overfishing and unregulated fishing operations in several countries including Thailand (SEAFDEC, 2021). Moreover, there are a few reports of the morphometric study of the short mackerel population structure in Thailand. Therefore, the objective was to determine the population structure of *R. brachysoma* distributed in the Thai waters by using truss network system.

Materials and methods

Sample collection

Samples of *R. brachysoma* were collected in a total of 869 specimens from six sites of coastal areas in Thailand as shown in Figure 1, four provinces on the Gulf of Thailand, i.e. Chonburi (CB, N=150), Samut Songkhram (SMK, N=150), Chumphon (CP, N=150), Songkhla (SK, N=135) and two provinces on the Andaman Sea, i.e. Ranong (RN, N=134) and Krabi (KB, N=150). The samples were collected within the period of February to October 2021. The collected samples from each location were preserved in an ice box and

transported to laboratory for the measurement of morphometrics. *R. brachysoma* by using morphological identification based on the description of Collette and Nauen (1983). The animal study protocol has been reviewed and approved by The Animal Care and Use Committee of King Mongkut's Institute of Technology Ladkrabang, Thailand (ACUC-KMITL-RES/2021/009).
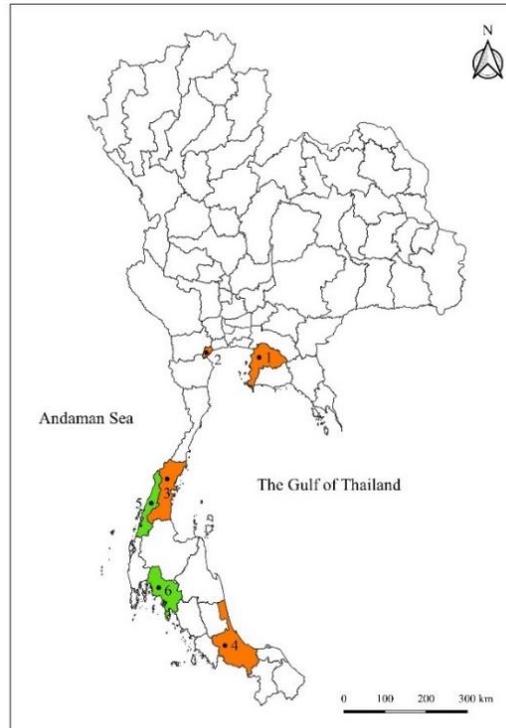
Figure 1. Map of Thailand showed six sampling sites for collection (*R. brachysoma*): 1) Chonburi (CB), 2) Samut Songkhram (SMK), 3) Chumphon (CP), 4) Songkhla (SK), 5) Ranong (RN), and 6) Krabi (KB)

Morphometric measurement

According to Sajina *et al*. (2011), morphometric measurement was performed by truss network system. The homologous point was determined on the left side of the body for 17 landmarks. A truss network of 38 lines was produced by connecting these landmarks, as shown in Figure 2. The measurement of the 38 distances was carried out by using digital calipers.

Figure 2. Location of landmarks used for morphometric truss network measurement of *R. brachysoma*
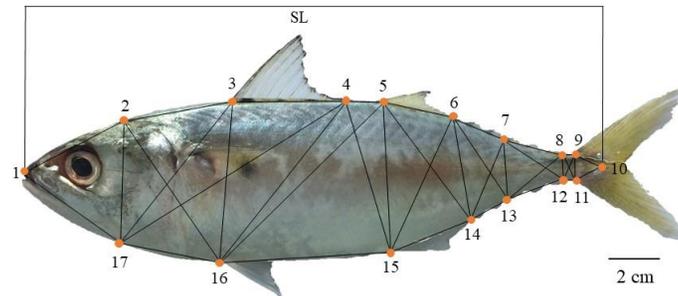
1	=	Tip of the snout	10 =	Last point of caudal
2	=	Posterior of bone supra occipital	11 =	Ventral origin of the caudal fin
3	=	Origin of the first dorsal fin	12 =	Insertion point of the last anal finlet
4	=	Insertion point of the first dorsal fin	13 =	Origin of the third anal finlet from anterior end
5	=	Origin of the second dorsal fin	14 =	Insertion point of the anal fin
6	=	Insertion point of the second dorsal fin	15 =	Origin of the anal fin
7	=	Origin of the third dorsal finlet from anterior end	16 =	Origin of the pelvic fin
8	=	Insertion point of last dorsal finlet	17 =	Intersection of preopercle and operculum on the ventral side of the body
9	=	Dorsal origin of the caudal fin	SL =	Standard length

Statistical analysis

The effect of size was eliminated from truss-based morphometrics data by using the following equation (Elliott *et al*., 1995):

$$M_{adj} = M(L_s/L_0)^b$$

where M_{adj} = a size-adjusted measurement, M = the original measurement, L_s = overall mean of the standard length for all fish from all samples, L_0 = standard length of fish in each sample, and parameter b was estimated for each character from the observed data as the slope of the regression of log M on log L_0.

Discriminant function analysis (DFA) was used to demonstrate the variations among short mackerel by classified respective groups based on truss network. The Wilks' Lambda was used to compare the difference between all group. Additionally, the population centroids were used to visualize the relationships between the groups of short mackerel and predict the percentage of specimens classified in each group from the original group using DFA. All above statistical analysis were done using SPSS version 28.

Results

A total of 869 specimens of *R. brachysoma* were collected from six sampling sites (Chonburi, Samut Songkhram, Chumphon, Songkhla, Ranong, and Krabi provinces) using measurements with the truss network method. There was no significant correlation between the standard length of the fish and transformed truss morphometric measurement of *R. brachysoma*. The non-significant result indicated that the effect of size was removed with allometric transformation.

In the discriminant function analysis (DFA) as shown in Table 1, of five discriminant functions (DFs) obtained, DF1 accounted for 42.6% of the variance, followed by DF2 (25.3%), DF3 (17.0%), DF4 (9.7%), and DF5 (5.5%). Eigenvalue less than 1 shows that the difference between groups is less than the difference within group in all the functions. In the canonical correlation analysis, the discriminant function respectively accounted for 0.616 (DF1), 0.516 (DF2), 0.443 (DF3), 0.349 (DF4), and 0.271 (DF5).

Wilk's lambda test indicates a significant difference in group centroids for discriminant scores of all five functions (Wilk's lambda < 1; *P* value <0.001) (Table 2).

The result of stepwise discriminant analysis revealed 16 characters of truss network from 38 characters (viz. 6-15, 4-5, 9-10, 2-3, 9-12, 3-16, 1-17, 1-2, 2-16, 15-16, 2-17, 5-16, 14-15, 6-14, 8-12, and 7-13). The characteristics mentioned above include the four characters from head, three characters from anterior of body, four characters from posterior of body, and five characters from caudal trait. The summary is shown in Table 3.

Table 1. Eigenvalue, percentage of the variance, percentage of cumulative and canonical correlation for 5 functions in *R. brachysoma* morphometrics measurement

Function	Eigenvalue	% of Variance	Canonical Correlation
DF1	0.612[a]	42.6	0.616
DF2	0.363[a]	25.3	0.516
DF3	0.244[a]	17.0	0.443
DF4	0.139[a]	9.7	0.349
DF5	0.079[a]	5.5	0.271

Note: a: First 5 canonical discriminant functions were used in the analysis

Table 2. Result of Wilk's lambda test (functions 1 through 5) for verifying differences among six populations of *R. brachysoma* with morphometric measurement characters using discriminant function analysis

Function(s)	Wilks' Lambda	Chi-square	df	*P* value
1 through 5	0.298	1037.664	85	0.000
2 through 5	0.480	628.601	64	0.000
3 through 5	0.654	363.427	45	0.000
4 through 5	0.814	176.374	28	0.000
5	0.927	65.144	13	0.000

Table 3. Summary of stepwise discriminant analysis of *R. brachysoma*

Distance	Wilk's lambda	F-statistics	Sig.
6-15	0.595	51.135	0.000
4-5	0.542	39.343	0.000
9-10	0.499	33.248	0.000
2-3	0.462	29.481	0.000
9-12	0.436	26.376	0.000
3-16	0.414	24.036	0.000
1-17	0.394	22.235	0.000
1-2	0.378	20.657	0.000
2-16	0.364	19.344	0.000
15-16	0.352	18.183	0.000
2-17	0.342	17.148	0.000
5-16	0.331	16.318	0.000
14-15	0.321	15.593	0.000
6-14	0.312	14.927	0.000
8-12	0.305	14.294	0.000
7-13	0.298	13.735	0.000

Note: Character descriptions were given as shown in Figure 2

The DFA revealed that the five discriminant functions could be used to classify the samples into their original groups as shown in Table 4. The first two discriminant functions were meaningful for DFA. The DF1 highly correlated with the head related characters (1-17), anterior part of the body (2-3), posterior part of body (4-5, 15-16), and caudal trait (9-12). The anterior part of the body (3-16), posterior part of body (5-16), and caudal trait (6-15, 6-14) highly contributed to the DF2.

The DFA showed that 53.0% of correct classification could be classified into their own groups. The percentage of correctly classified specimens was the highest in the KB populations (66.0%), whereas the lowest percentage was found in the RN population (38.8%). In Table 5, the result revealed that all populations were not fully separated.

Table 4. Structure matrix of discriminant functions of *R. brachysoma* using truss morphometric characters

Distance	Function				
	DF1	**DF2**	**DF3**	**DF4**	**DF5**
SL	**0.784**	0.021	0.138	-0.176	-0.252
1-17	**0.588**	-0.137	0.261	-0.027	-0.168
15-16	**0.313**	0.198	-0.101	-0.100	0.026
14-15	0.264	0.262	-0.073	0.182	-0.023
6-15	0.085	**0.700**	**0.331**	0.068	0.235
6-14	0.057	**0.483**	0.141	-0.045	-0.083
3-16	0.097	**0.348**	**0.423**	-0.160	-0.029
9-12	**0.347**	-0.077	**0.416**	-0.124	0.217
2-16	0.281	0.254	0.408	-0.107	0.323
2-17	0.169	-0.053	0.392	0.136	0.188
4-5	**0.352**	0.174	**-0.379**	0.319	-0.117
8-12	0.202	0.014	0.305	-0.061	-0.166
2-3	**0.350**	0.010	-0.194	-0.633	0.137
1-2	0.211	-0.031	0.235	0.582	-0.109
5-16	0.230	**0.351**	0.166	-0.372	-0.006
7-13	0.145	0.148	-0.074	-0.226	-0.205
9-10	0.238	0.264	0.511	-0.144	-0.555

Note: Character descriptions were given as shown in Figure 2

Table 5. Percentage of specimens classified in each group from the original group using discriminant function analysis*

Population	Percentage of Predicted Group Membership						Total
	CB	**SMK**	**CP**	**SK**	**RN**	**KB**	
CB	**44.0**	22.7	9.3	7.3	14.0	2.7	100.0
SMK	11.3	**60.7**	9.3	4.7	3.3	10.7	100.0
CP	14.0	3.3	**48.0**	12.0	12.7	10.0	100.0
SK	11.1	3.0	20.0	**60.0**	5.9	0.0	100.0
RN	14.9	11.2	17.2	9.0	**38.8**	9.0	100.0
KB	4.0	4.7	8.0	10.7	6.7	**66.0**	100.0

Note: *53.0 % of original group case correctly classified

The result showed the discriminant analysis on the distribution plot using the truss network method. The samples were classified into six populations according to the collected sites, including 4 provinces located in the Gulf of Thailand (Chonburi, Samut Songkhram, Chumphon, and Songkhla) and 2 provinces located in the Andaman Sea (Ranong and Krabi). An intermingling of *R. brachysoma* was observed among in all demographic groups shown in Figure 3.
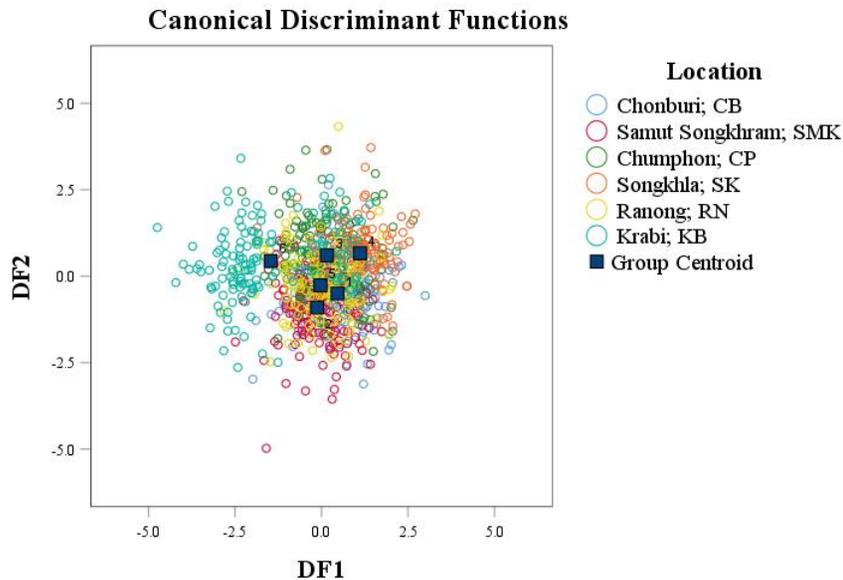
Figure 3. Discriminant analysis plot of *R. brachysoma* using truss network analysis in six populations

Discussion

The truss characters of *R. brachysoma* including head (1-17), anterior part of the body (2-3, 3-16), posterior part of the body (4-5, 15-16, 5-16), and caudal trait (9-12, 6-14, 6-15) exhibited the significant variation within the populations. Differences in patterns of head-related characters and body features (anterior part of the body and posterior part of the body) were influenced by the difference in ecological conditions, such as food availability (Hyndes *et al*., 1997; Darlina *et al*., 2011). The difference in swimming pattern as well as water velocity and in response to hydrological conditions might have influenced on the variation in the caudal character (Sajina *et al*., 2011; Imre *et al*., 2020). The result was similar to the previous research based on truss analysis of *R. kanaguarta* (Darlina *et al*., 2011), *C. apogon* (Kenthao and Jearranaiprepame, 2018), *Engraulis encrasicolus* L. (Turan *et al*., 2004), which reported that the morphometric characters were significantly different in the head-related characters, anterior part of the body, posterior part of the body, and caudal trait. It is possible that these population undergo phenotypic changes due to environmental adaptation. Fish exhibit higher level of variation within and among populations than other vertebrates, and has high phenotypic plasticity in response to environmental changes (Hossain *et al*., 2010; Mir *et al*., 2013). Environmental factors have an influence on the morphometric variation

of fish such as water temperature, nutrition, salinity, food as well as water current (Shuai *et al*., 2018; Wardiatno *et al*., 2021). Moreover, ocean surface currents play a critical role in marine larval dispersal (Akib *et al*., 2015).

The DFA had been performed to distinguish different fish stocks of same species (Turan *et al*., 2004; Kenthao and Jearranaiprepame, 2018; Jiang *et al*., 2020). In this study, the result of DFA 53.0% indicated that the population of the short mackerel collected from six sampling sites exhibited an intermingling between groups. This result in line with previous research revealed that the fish stocks of *Labeo rohita* Hamilton, 1822) (Mir *et al*., 2013), *Macrognathus pancalus* (Mahfuj *et al*., 2019), and *Barilius bendelisis* (Kumar and Singh, 2019) were intermingled among each stock, which had the average percentage of originally grouped cases correctly 62.3%, 72%, and 83.8%, respectively. The sampling sites of this study as well as all previous studies mentioned above were geographically separated. It may imply that all stocks of short mackerel in Thai waters, both in the Gulf of Thailand and the Andaman Sea, had a common ancestor's origin. Character variation among population may be influenced by environmental adaptation.

The plot graphs of the DF1 and DF2 (Figure 3) also suggested that all populations of *R. brachysoma* in Thai waters were intermingling from each population in the discriminant space. Short mackerel have migratory behavior of the species between the stocks in the inner and the eastern part of the Gulf of Thailand, and short mackerel populations can have their unique migratory behavior and route, affecting gene flow among populations (Kongseng *et al*., 2020). Pattern migration of short mackerel in the Andaman Sea divided into 3 groups therefore may cause a mixed stock of short mackerel populations in the Andaman Sea. Moreover, the short mackerel may share a stock between neighboring countries (Bhatiyasevi, 1997). The stocks of *Sarda sarda* identified by using truss network system were found no morphological differences between the geographically separated populations, suggesting all population were overlapped (Turan *et al*., 2016). The previous study of *Phycis phycis* showed a separation in the sampling areas although some overlapping was observed (Vieira *et al*., 2016). However, the previous research on *Pomatomus saltatrix* revealed that from plot graphs, the sample stocks were well separated from each other (Bal *et al*., 2021).

In conclusion, stock identification of *R. brachysoma* in Thai waters in this study was studied by using measurement truss network system. All sampling populations were high intermingling between groups. *R. brachysoma* are a migratory fish species that clearly dominate migrations along the coast of the Gulf of Thailand and the Andaman Sea. Truss network system is an effective technique for identifying different fish stocks, leading to the development of

fisheries management. However, the further research on the combination of molecular genetic methods and the truss morphometrics is beneficial for the identification population of fish to provide more accurate and complete information.

Acknowledgements

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