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Comparative analysis of morphological characteristics of clam (*Marcia opima*) in mannar coastal belt, Sri Lanka

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Abstract
The present study was focused on assessment of morphological variations of clam in Mannar lagoon, Sri Lanka. A total of 150 individual clams (fifty individuals per each site; Naruvilikkulam, Achchankulam and Mutharipputhurai) were collected from June to November 2017 and subjected to Discriminant Function Analysis (DFA) using morphometric parameters to identify intra-specific morphological variation. Length-weight relationship and condition factor were assessed for 3 Clam groups. Average shell length, shell height, length and width of hinge plate, and total weight of clams ranged at 47.30-52.40, 39.60-43.92, 23.41-26.37, 8.83-10.34 mm and 35.80-51.20 g respectively. Predicted model of DFA derived 99.8% and 0.2% of variances for function 1 and 2 respectively. All 3 Clam groups had negative allometric growth pattern (b= <3). Average condition factor (K) calculated for this species was 0.036 indicating unfavorable factors in living habitats. Basic morphological information of Clams is useful in aquaculture and fisheries sector of Sri Lanka.

Keywords: Coastal bivalves, morphological variation, discriminant function analysis, condition factor, allometric growth

1. Introduction

A bivalve mollusc particularly scallops, oysters, clams and mussels are one of the highly popular, nutritious seafood source. Global bivalve production has been gradually increased over the past 20 years due to reflection of strong demand [9]. Coastal and marine mollusks significantly contribute for 75.5% of marine aquaculture sector, with 5% of rapid increment of bivalve production [6]. Sri Lanka as a tropical island is rich with economically valuable coastal bivalve resources such as mussels (*Perna viridis, Perna perna*), clams (*Meretrix casta, Marcia opima*), cockles (*Anadora granosa*, *Gafrarium tumidum*), oysters (*Crassostrea madrasensis, Saccostrea cucullata*), and pearl oysters (*Pinctada maragaitifera*, *Pinctada vulgaris*) in coastal zones of Negombo, Chilaw, Kalpitiya, Mannar and Jaffna [31]. Fishing communities along coastal belt exploit bivalve resources from the natural habitats particularly during monsoon and tourism season [14, 31].

Marcia opima is one of the highly abundant bivalve species as an edible, delicious seafood source in Northern and North western coastal belt of Sri Lanka and it has also an incredible export value in the world market [28]. *M. opima* is primarily marine and found burrowing in sand and mud in quite shallow water bodies [22]. The combined production of cockle (*G. tumidum*) and clams (*M. opima* and *M. hiantina*) have shown a significant bivalve production in Sri Lanka since 1987. Accordingly, molluscs including clams are contributing for 4% of fish and fishery products in the exports market of Sri Lanka [19]. The availability of commercially important bivalve resource is probably reach the overexploitation level in future, if the harvesting of shellfish is still carried out continuously from natural habitats in coastal zone without implementing sustainable aquaculture and fishery management practices.

Although bivalve culture is still in the experimental stage in Sri Lanka, favorable conditions and factors such as optimum culture medium, low cost culture materials, novel technology, government support and high quality brood stock population are available for expansion and development of bivalve culture sector [21, 31]. Currently *M. opima* is gaining considerable attention in seeking potential of bivalve culture in local aquaculture farms through involvement of National Aquatic Resources Agency (NARA) with the partial support of

International Development Research Centre (IDRC). Additionally, current trends toward the sophisticated fishing methods and measures of sustainable utilization of mollusc resources become more significant with the available population dynamic information on shellfish resources. However, limited information is available on population structure, morphological characters of *M. opima* in Northern coastal belt of Sri Lanka. Therefore this study focused on addressing above gap by comprehensively investigation on population dynamic parameters and morphological variations of *M. opima* bivalves in different populations along Mannar coastal region to identify the unique morphological characteristics of Clams in Northern peninsula.

2. Materials and Methods

2.1 Study area and Sample collection

The present study was carried out for three groups of clam, *Marcia opima* in Naruvilikkulam (8.860662 N, 79.92844 E), Achchankulam (8.82373 N, 79.92497 E) and Mutharipputhurai (8.794765 N, 79.92738 E) (Figure 1) from coastal area in Mannar, Sri Lanka from June to November 2017. A total number of fifty samples were randomly collected from each groups by hand picking. All collected samples were packed in polythene bags separately and then transferred into Meat Processing and Research Laboratory, Uva Wellassa University of Sri Lanka, Badulla, Sri Lanka with ice in a cooler box. After transportation, samples were stored at -20˚C in a freezer until the measurements were taken.

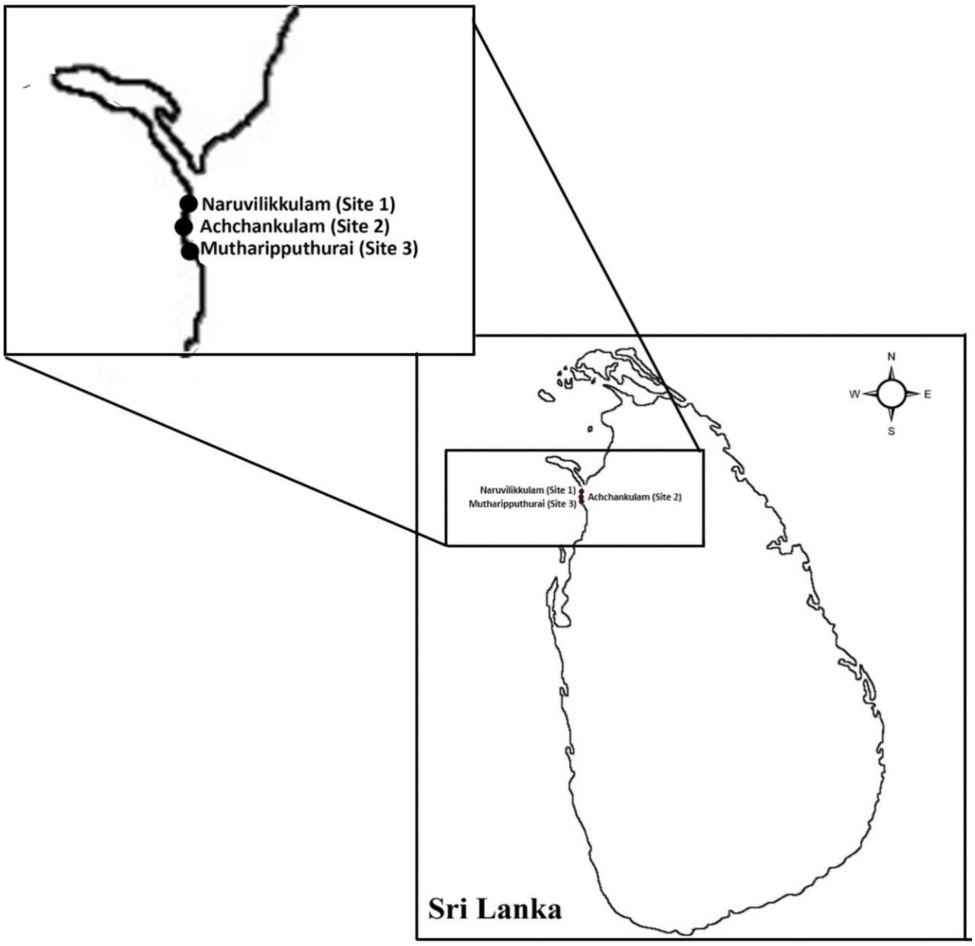

Fig 1: Location of Sampling Sites of Marcia opima

2.2 Morphometric Analysis

Four morphometric characters of *M. opima* bivalve samples were measured to the nearest value of 0.1 mm using digital Vernier caliper (GENERAL®, USA) (Table 1 and Fig 2) and measured parameters of *M. opima* were 1) VL: shell length 2) VH: shell height 3) HL: length of hinge plate 4) HW: width of hinge plate. Body weights (WW) were weighed to the nearest 0.01 g using an electronic balance (OHAUS®, USA). Sexes were not determined.

Table 1: Morphometric parameters of *Marcia opima.*

Morphological Parameter	Abbreviations	Measurements
Total shell length	VL	The length from the tip of posterior end to the tip of anterior end
Shell height	VH	The height from the tip of umbo to the ventral margin
Length of hinge plate	HL	The distance from the posterior adductor scar to the anterior adductor scar
Width of hinge plate	HW	The distance from the tip of umbo to the bottom of hinge plate

Source: [8, 16].

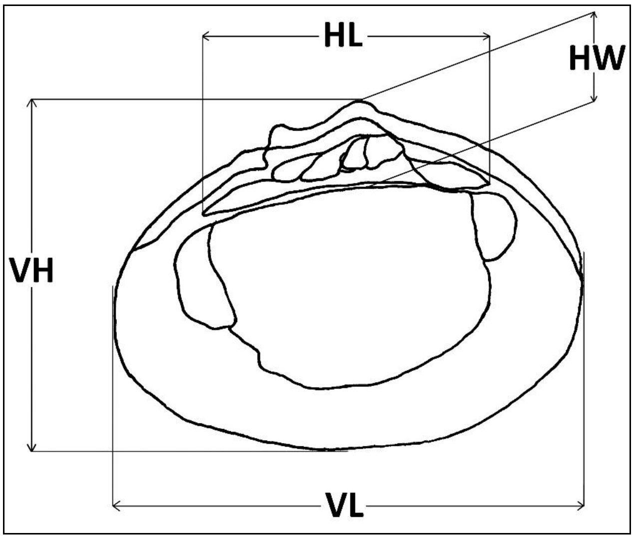

Fig 2: Morphometric measurements of *Marcia opima.* Source: [8, 16].

2.3 Statistical Analysis
Data were statistically analyzed using the SPPS statistical software version 20.0. Morphometric shell characteristics were subjected to size standardization by transforming each measurement into log_{10}values and expressed as a proportion between log_{10} transformed dependent variable and reference character. Shell length: log_{10}VL was used as the reference parameter in the current study [8, 16]. For a selected morphometric parameter (X) of a specimen, standardized data were expressed as log_{10}X (log_{10}VH, log_{10}HL and log_{10}HW) / log_{10}VL. Size standardization is an important in minimizing sizes and gender dependent variance of samples [16]. Discriminant Function Analysis (DFA) was performed to determine the morphological variations among shellfish populations in order to distinguish each population. Wilks' lambda was used as an indicator for the significance of the discriminant function and canonical correlation scores of derived functions were used to identify highly contributing factors important in discriminating populations. Scores of Standardized Canonical Discriminant Function Coefficients and Correlations were used to select morphometric characters which are highly important in discriminating the populations. Derived canonical scores for each function were used to employ the plot of Discriminant Function 1 against 2 for detection of similarities and deviations among each group of clams.

2.3 Length-weight relationship
Length-weight measurements were used to detect the relationship between total shell length and weight. Total shell length and weight compute the relationship as follows.
$W = aL^b$ [23]
W= body weight, L= total length, a= intercept on the length axis, b= slope or regression coefficient.
Log transformed length and weight parameters were used in linear regression.
Log W = Log a + b Log L
Log W= Log transformed body weight, Log L= Log transformed total length, b= regression coefficient or growth exponent, a= intercept
Analysis of variance (ANOVA) was applied to check whether the computed regression line is significant at significant level of 5% ($p<0.05$). The growth exponent (b) for each group was compared with 3 using student's t-test to identify the growth pattern [27]. The formula given below was used for this calculation.
$t_s = b - 3/ S_b$ [33]
t_s= Student's t-test, b=Slope, S_b= Standard error of the slope
The condition factor - K was calculated using the Fulton's index [7] of condition as given below.
$K = 100 \times W/L^b$ [20, 24, 29]
K= condition factor, W= body weight, L= total length

3. Results
The mean total length of *M. opima* ranges between 47.30-52.41 mm for 3 groups of clams (Table 2). The highest mean value for shell length (VL), shell height (VH) and length of hinge plate (HL) was recorded for bivalve samples collected from Mutharipputhurai, while the greatest mean value for width of hinge plate (HW) was 10.34 ± 1.57 mm for samples in Naruvilikkulam (Table 2). However, lowest mean VL, VH and HL were for clam groups collected from Naruvilikkulam (Table 2). Total weight shellfish samples ranged at 35.80-51.19 g and highest shell weight was recorded for the samples collected from Mutharipputhurai (Table 2).

Table 2: Mean morphometric shell parameters of *M. opima* sampled from different populations.

Morphometric characteristics	Naruvilikkulam (Site 1)	Achchankulam (Site 2)	Mutharipputhurai (Site 3)
VL (mm)	47.30±3.02	49.08±3.86	52.41±2.69
VH (mm)	39.61±2.58	41.45±2.80	43.92±2.49
HL (mm)	23.41±2.33	25.46±2.54	26.37±2.33
HW (mm)	10.34±1.57	8.83±0.95	9.78±1.15
WW (g)	35.80±4.95	46.46±6.25	51.19±8.34

*VL: shell length, VH: shell height, HL: length of hinge plate, HW: width of hinge plate, WW: total weight

As revealed by results of DFA, 3 standardized measurements: Log_{10}HW/ Log_{10}VL, Log_{10}HL/ Log_{10}VL and Log_{10}VH/ Log_{10}VL were used as predictor variables. Ratio between log transformed width of hinge plate /shell length (Log_{10}HW/VL) and log transformed length of hinge plate /shell length (Log_{10}HL/VL) showed strong statistical difference among sampling sites ($p<0.05$). Log determinant values were relatively similar and equality of covariate matrices was violated based on Box's M score in the model. The predicted model by DFA derived 2 functions and Canonical variate analysis revealed that function 1 produced highest significant Canonical correlation (0.562), Wilks' lambda (0.684, $p<0.05$) (Table 3). Highest eigenvalues was recorded for discriminant functions 1 in the model. The first function of the model explained 99.8% of variance, while second function contributes for only 0.2% of variance (Table 3 and 4).

Table 3: Extracted Eigen values for the functions of DFA

Function	Eigenvalue	% of variance	Cumulative %	Canonical Correlation
1	0.461[a]	99.8	99.8	0.562
2	0.001[a]	0.2	100.0	0.030

*[a.] First 2 canonical discriminant functions were used in the analysis.

Table 4: Wilks' Lambda scores for the derived functions of DFA

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 2	0.684	55.523	6	0.000
2	0.999	0.134	2	0.935

The structure matrix table records correlation of each standardized measurements with each discriminant function (Table 5). Ratio between log transformed width of hinge plate / shell length ($Log_{10}HW/VL$) and log transformed length of hinge plate / shell length ($Log_{10}HL/VL$) were identified as the most strongest parameters contributing for the function 1, while log transformed shell height / shell length ($Log_{10}VH/VL$) was highly contributed for the second function in the predicted model (Table 5). The derived model suggested that 55.3% of cross-validated grouped cases are correctly classified. Highest positive group centroid value was recorded by Naruvilikkulam and Achchankulam for function 1 and 2 respectively (Table 6). Scores closer to the each centroid belongs to the particular site. The derived scores at the canonical variate axis plotted between Canonical discriminant function 1 and 2 were distributed at the range of -3 to +3. The particular scores for Naruvilikkulam, Achchankulam and Mutharipputhuraiwere within the range of -3 to +2, -3 to +3 and -1 to +3 correspondingly (Fig 3).

The three groups showed the significant overlapping on Canonical variate axis (Fig 3). The growth pattern of shellfish populations were determined by length-weight relationship. Derived linear relationship of logarithmic transformation of weight and length were computed for 3 groups as given below (F=35.39, 28.30, 28.70 for linear regression between total weight and shell length for Naruvilikkulam, Achchankulam and Mutharipputhurai populations respectively at $p<0.05$).

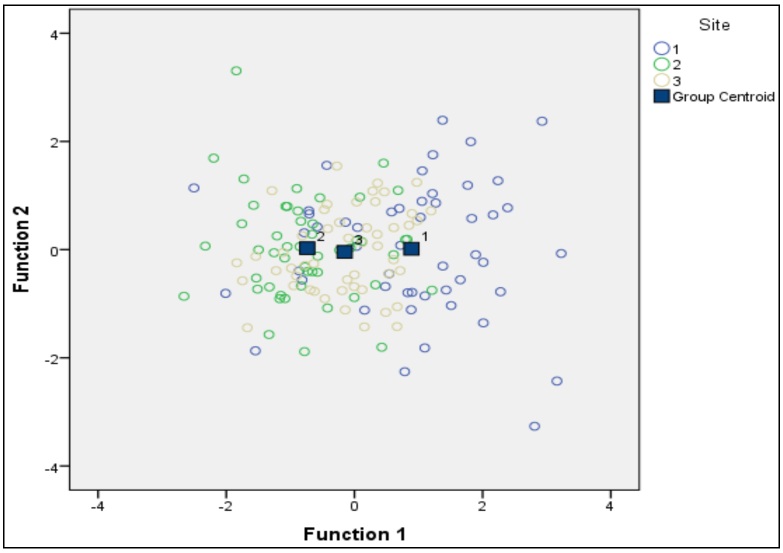

Fig 3: Clustered Plot between function 1 and 2 derived by Discriminant Function Analysis DFA)

Table 5: Structure Matrix for correlations between discriminating variables and standardized canonical discriminant functions

	Function	
	1	2
$Log_{10}HW/VL$	**0.841***	**0.483**
$Log_{10}HL/VL$	-0.371*	0.330
$Log_{10}VH/VL$	-0.159	0.965*

*Largest absolute correlation between each variable and any discriminant function.

Table 6: Group Centroids for each function for the sampling sites

Site	Function	
	1	2
Naruvilikkulam	0.889	0.015
Achchankulam	-0.736	0.027
Mutharipputhurai	-0.153	-0.042

*Unstandardized canonical discriminant functions evaluated at group means

LogWeight = 1.473 LogLength - 0.916 (Site: Clams in Naruvilikkulam)

LogWeight = 1.030 LogLength -0.077 (Site: Clams in Achchankulam)

LogWeight= 1.916 LogLength - 1.590 (Site: Clams in Mutharipputhurai)

The relationship between total weight and shell length recorded a positive correlation coefficient (r =0.652, 0.609, 0.612 for Naruvilikkulam, Achchankulam and Mutharipputhurai populations respectively) for all the 3 Clam groups ($p<0.05$) (Figure 4, 5, 6). The growth patterns for all 3 groups reflect the exponential growth of total weight with shell length. Intercept was negative for all the regression relationships recorded for 3 Clam populations (Fig 4, 5, 6). Growth exponent, b was less than 3 for all 3 groups (1.473, 1.030 and 1.916 for clam samples collected from Naruvilikkulam, Achchankulam and Mutharipputhurai respectively). Further t-test indicated that there was a significant difference between 3 and recorded b value for all 3 groups (Calculated t value = 43.17, 71.80, 21.41 for Naruvilikkulam, Achchankulam and Mutharipputhurai groups respectively, $p<0.05$). Further mean condition factor (K) for Clam groups from Naruvilikkulam, Achchankulam and Mutharipputhurai was recorded as 0.034, 0.039 and 0.036 respectively.

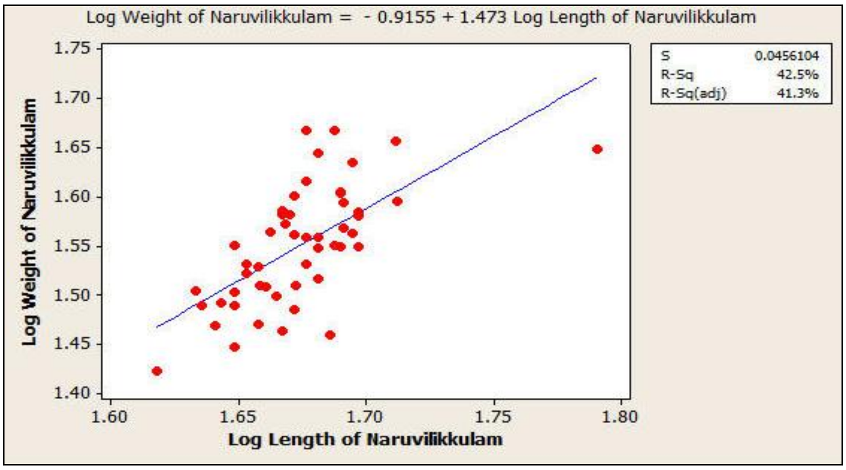

Fig 4: Fitted line plot of length-weight relationship for Clams in Naruvilikkulam

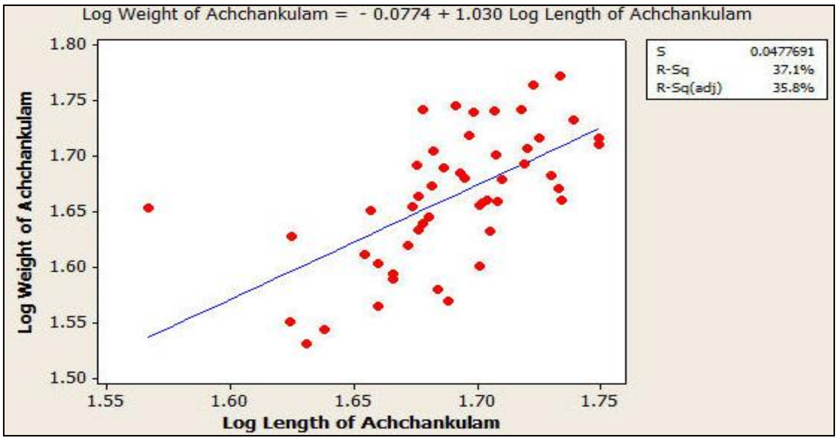

Fig 5: Fitted line plot of length-weight relationship for Clams in Achchankulam

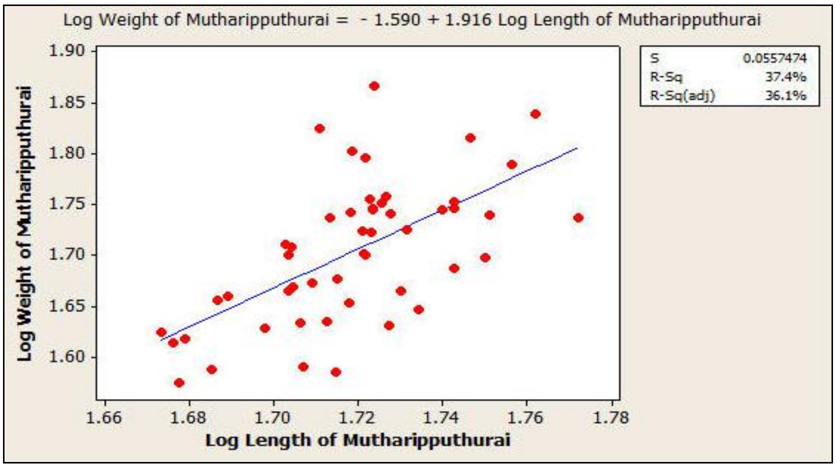

Fig 6: Fitted line plot of length-weight relationship for Clams in Mutharipputhurai

4. Discussion

This study revealed the scientific information on *M. opima* along Mannar coastal region. As recorded by previous literature records, the mean value of shell length of *M. opima* is 45 mm sampled from Ashtamudi backwaters, Kerala, India [1], the average length of *Meretrix casta* (backwater hard clam) from Dutch canal of Sri Lanka ranges from 34 – 43 mm reported by Appukuttan *et al.* (1985) [13]. However the average shell length of *M. opima* collected from Mannar coastal belt was greater than 45 mm in the current study by revealing that the growth rates of *M. opima* in selected habitats were comparatively higher than previous findings. Average total weight of *M. opima* groups (44.48 g) along Mannar coastal belt was higher compared to the previous literature record of periwinkle: *T. fuscatus* (weight ranges at 1.16-5.87 g with mean of 2.54g ± 0.57) sampled from okrika estuary, Niger-delta during October 2015 to February 2016 [26]. The necessity of having thicker and heavier shells for mussel: *Mytilus edulis* inhabiting in intertidal, dry zones was noted [25]. Well-developed shells cause in increasing the shell mass or allowing for containing water. Significant portion of energy is used for growth of strong shells to survive under the extreme conditions and protection from predatory attack in intertidal coastal ecosystems [10]. Thus, shell based morphometric measurements of coastal bivalves are considered as indicative parameters for adaptation to the extreme environmental factors in their habitats.

Results of current study derived a model to identify intra-specific morphological variations of *M. opima* with significant canonical correlation for function 01 (r = 0.56) (p<0.05) with >99% of cumulative variance. Relatively similar results were recorded by past literatures. The predicted model to distinguish morphological differences between *Mytilus edulis* and *M. trossulus* has derived significant canonical correlation (r = 0.66) based on the length-standardized measurements (Wilks' lambda = 0.56, p < 0.05) was reported [11]. Further, derived discriminate functions have recorded the significant association between all the shell characteristics in the predicted model of current study. Previous study conducted by Mallet and Carver (1995) [15], have developed the higher correlation for the shell height (ranged at 39 - 43 mm of shell height) instead of shell length in the predicted model produced for identification of morphological differences of *Mytilus edulis* and *Mytilus trossulus* bivalve species from Nova Scotia, Atlantic Canada, revealing that shell height is an important parameter in the assessment of morphological characters of bivalves. Thus, shell based morphological parameters (shell length, width and height) act as effective tool in providing precise, accurate and reliable information to identify morphological difference of bivalve populations.

As reveled by the current study, the significant overlapping of plotted graph between 2 derived functions (Function 1 and 2) denoted that coastal bivalves, *M. opima* sampled from Mannar coastal lagoon do not succeed in distinguishing as geographically separated populations, even though group centroid scores were clearly separated in the plot (Fig 6). Thus, sampled bivalves represent a common stock of *M. opima* useful in stock identification and differentiation in capture fishery and fisheries management sector in Sri Lanka [2]. However, previous studies have recorded the intra-specific morphological variability of shellfish populations based on the sampling locations [4, 16, 32]. Shell height of *Mytilus* sp. is significantly different with sampling locations, due to the environmental differences in respective intertidal habitats (p<0.05) have identified [15].

As revealed by Gardener (1996) [8], the model developed by Canonical Discriminant Analysis (CDA) for predicting morphological variations of mussels has failed to distinguish morphometric differences even between *Mytilus edulis, Mytilus galloprovincialis* due to significant proportion of hybrid mussel varieties within the zone. Thus, hybridization is one of key factor affecting on morphological similarity between two species. Further *Mytilus edulis* and *M. trossulus* collected from east coast of New-found land denoted the consistent morphological characteristics [11]. Discriminant Function Analysis (DFA) failed to distinguish *M. rosenbergii* populations sampled from four geographical locations in Southern province in Sri Lanka [18]. Accordingly intra-specific morphological differences/similarity is depending on environmental and genetic factors [30]. Thus, sharing of similar morphological characteristics by *M. opima* in three locations ensure the mixing of individual bivalves by sea current and exposing to similar environmental conditions due to closer proximity among sampling sites along Mannar coastal belt. Further, local environmental and habitat conditions are not considered as most powerful factors in distinguishing bivalve populations of selected three sampling sites in the present study [16]. However, genetic studies are recommended to confirm the findings of morphological similarity of *M. opima* in the current study.

Also, the current study revealed the reliable, scientific information on growth pattern and condition factor of *M. opima* bivalves in Mannar coastal regions. Length-weight relationship is considered as an index of growth pattern of vertebrates and invertebrates (fins fishes and shell fishes) [26]. Intermediate scores of positive correlation coefficient were recorded for length-weight relationships of all the 3 Clam groups (p<0.05), implying possible prediction of weight for a specific length and proportionately increment of weight with increasing total length of bivalves. However, correlation coefficient (r=0.81) of length-weight relationship for *T. fuscatus* was significantly higher compared to the current findings [26]. When the calculated “b” value of length-weight relationship is 3.0, the growth pattern of considered populations is isometry. Further allometric growth pattern is recorded at b<or> 3.0, while “negative allometry” and “positive allometry is recorded when b<3.0 and b>3.0 in the linear regression respectively[3]. Based on the results of the current study, b value is significantly less than 3.0 by showing negative allometric growth pattern for *M. opima* samples in 3 sampling locations of Mannar coast. Negative allometry growth denotes that weight gaining rates are relatively higher than the length increment rate of coastal bivalves in Mannar [12]. Similar findings were recorded by the previous studies conducted for Periwinkle: *Tympanotonus fuscatus* populations (Okrika estuary Cross River, Bonny River, Lagos Lagoon in Niger delta, Nigeria) [12, 17, 26, 29]. Allometric growth relationship between shell length and weight can be used for monitoring and assessment of growth of these mollusks species under the natural conditions [10]. Similar growth pattern of Clams again reflects less morphological diversity confirming results of extracted model of DFA for 3 sampling sites in Mannar coastal belt. Condition factors (K) were <1 for all Clam groups in Mannar coast. Previous studies have noted the contradictory results compared to the current results. Higher K value is recorded for periwinkles; *T. fuscatus* from Okrika estuary (K=18.9) and Lagos Lagoon (K=8.0 & 16.1) in Niger Delta [17, 26]. However, Udo (2013) [29] had noted lower K (<1) for periwinkles in Cross River estuary. Fulton’s condition index for freshwater clam (*Egeria radiate)* was recorded as 1.13 [5]. Condition factor is used as an index of well-beingness and heaviness/size of organism. Condition factor of a particular species is depended on several factors such as food availability, diversity of food items, foraging behavior, dependence on stored food energy, reservation of food energy and breeding performance. Lower K value of *M. opima* is associated with food scarcity, reproductive performance and breeding. Thus, Clams exhibited reduced growth rate during sampling period. During implementation of culture practices for *M. opima*, optimum environmental conditions need to be maintained by controlling spawning/breeding performance within the culture environment for achieving highest growth rate. Since *M.*

opima has a higher culture potential in Sri Lanka, this kind of morphological data provide fundamental approach on identification of culture environment, growth pattern and phenotypic variations useful in aquaculture practices. As a nutritional rich, delicious sea food source, economic value of *M. opima* can be further increased through novel value added products in local and international market and ultimately that leads to phenomenal growth in fisheries and aquaculture industries in Sri Lanka.

5. Conclusion

The current study reveals the morphological aspects of clam (*M. opima*) in Naruvilikkulam, Achchankulam and Mutharipputhurai regions. Average morphometric shell measurements were relatively highest for *M. opima* samples collected from Mutharipputhurai (Site 3). However all 3 Clam groups share similar morphometric characteristics in all 3 sites and consider as one stock. Less morphological diversity among 3 Clam groups is associated with environmental and climatic factors. Clam groups exhibit negative allometric growth pattern ($b<3$). Condition factors of all Clam groups are <01 due to different biological processes (e.g.: Reproduction) and unfavorable conditions in the intertidal habitats during sampling period. Combined approach of morphological and genetic analysis for Clams is recommended for further verification of current results.

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