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Physico-chemical Analysis and Morphometric Variability Within and Between Populations of *Pitar frizzelli* in Panguil Bay, Philippines

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Abstract

The study was conducted to determine variation within and between populations of a marine bivalve, Pitar frizzelli, based on fourteen significant biological characters of the shell by descriptive statistics, analysis of variance (ANOVA) and Tukey's post hoc test. The results revealed that there is significant difference within and between populations which may be phenotypical plasticity in response to different environmental and/or ecological conditions existing in the three areas. The physico-chemical parameters which included sediment salinity, pH, temperature and type of substrate, showed significant relationship with the morphological characters.

Keywords: physical and chemical parameters and mollusk

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1. Introduction

Individual variation occurs in populations of all species of sexually reproducing organisms. However, not all variations observed in a population are heritable. Variation in size, shape, coloration, behavior, physiology, or biochemistry may be a product of current environmental differences between the sites at which individuals are living, a product of genotypic differences between individuals from different sites, or a combination of both. The morphological characteristics of each species have been useful for examining patterns of variation. This paper reports on the differences of morphological characters observed in three different areas.

2. Methodology

2.1 Study Area

The study was carried out in Panguil Bay, Philippines which is nearly 14.5 kilometers in a NE-SW direction and 6.4 kilometers wide. It is bordered mostly by nipa palms backed by wide stretches of mangroves. The bay has an estimated total area of 186.59 km^2 with an average depth of 15.4 meters. It has an estimated volume of about 3.35 km^3 at Mean Lower Low Water (MLLW). The southern portion from Tubod-Silanga segment and southwards has an estimated area of 84.35 km^2 and an average depth of 7 meters. The outer portion of the bay from Tubod-Silanga segment and northwards has an estimated area of 102.24 km^2 with average depth of 22.3 at MLLW (Gorospe, 1996).

Figure 1 shows the three sampling stations, namely: Montay, Kolambugan, Lanao del Norte (location 1); Raw-an Pt., Baroy, Lanao del Norte (location 2); and Pacita, Lala, Lanao del Norte (location 3) selected along the intertidal zone of the study area. Three 25 meter transect lines in each of the three stations were designated as sampling area. The three line transects were established 15 meters away from each other and approximately 30 meters perpendicular to the shoreline.

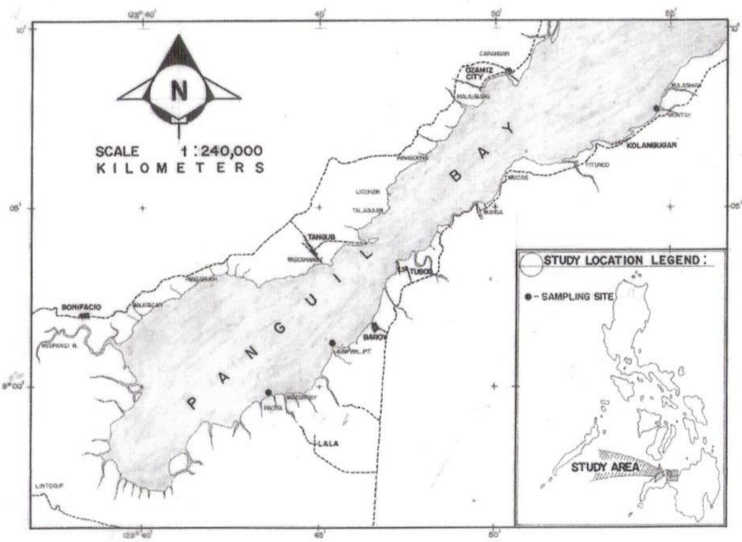

Figure 1. Map of Panguil Bay showing the locations (•) of the study area.

2.2 Collection of Samples

Samples were collected during low tide from the three transect lines in each station. They were collected by hand with the help of gleaners. The specimens were then transported live to the MSU-IIT, Iligan City laboratory for identification and processing. Thirty-two samples were collected from each station. A total of ninety-six samples were used in the biochemical and morphological analyses. Figure 2 shows the identification of the species based on their distinguishing characters as described in the Guide to Philippine Flora and Fauna (Garcia, 1986) and Shells of the Philippines (Springsteen and Leobrera, 1986).

Figure 2. Photograph of *P. fritzzelli* showing its ventral and dorsal view.

2.3 Determination of Physical Parameters

Determination of temperature for each station was done using a field mercury thermometer. Sediment temperature was obtained by inserting the thermometer to the substrate up to a depth of about 2-3 cm for two minutes. Substrate type was determined employing a series of sieves and classified based on Wentworth Scale (English *et al*., 1994).

2.4 Determination of Chemical Parameters

Chemical parameters were determined in each station per sampling. Interstitial salinity was measured using a refractometer. A pH meter was used for pH determination.

2.5 Morphometric Analysis

Figures 3 and 4 displays the fourteen morphometric shell characters used to determine variation within and between populations of *Pitar frizzelli.* Shell characters were as follows: (1) width; (2-3) depth of each valve; (4) length of hinge; (5) ligament; (6) lunule; (7) escutcheon; (8) anterior adductor muscle scar; (9) distance between pallial line and ventral shell margin; (10) distance between ventral edge of posterior adductor muscle scar and ventral shell margin; (11) distance between anterior edge of posterior adductor muscle scar and posterior shell margin; (12) distance between umbo and posterior end of ligament; and (13-14) weight of each valve. Measurements were made on the left valve using a vernier caliper. The ratio of each character to the total length was measured so as to remove the effect of age and the results were arcsine-transformed to normalize the data. The ratio of each valve's weight to the total weight was also determined.

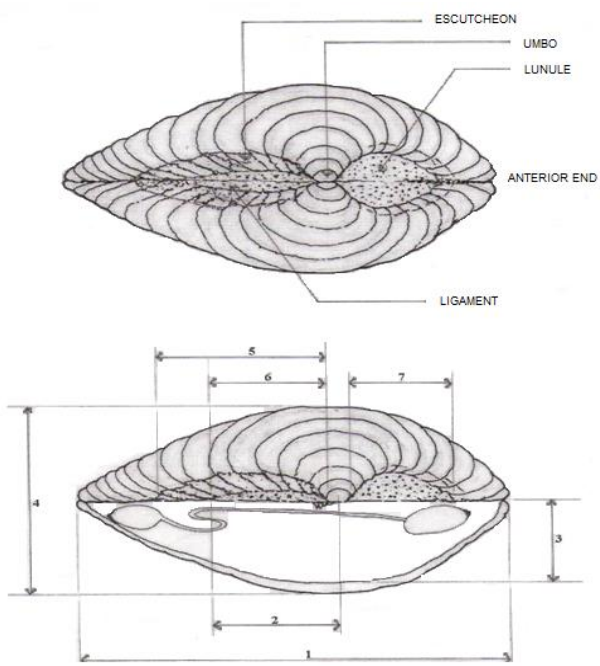

Figure 3. Shell anatomy of *Pitar fritzzelli* showing the morphological characters: (1) shell length, (2) distance between umbo & posterior end of ligament, (3) depth of left valve, (4) shell breadth, (5) escutcheon length, (6) ligament length, (7) lunule length.

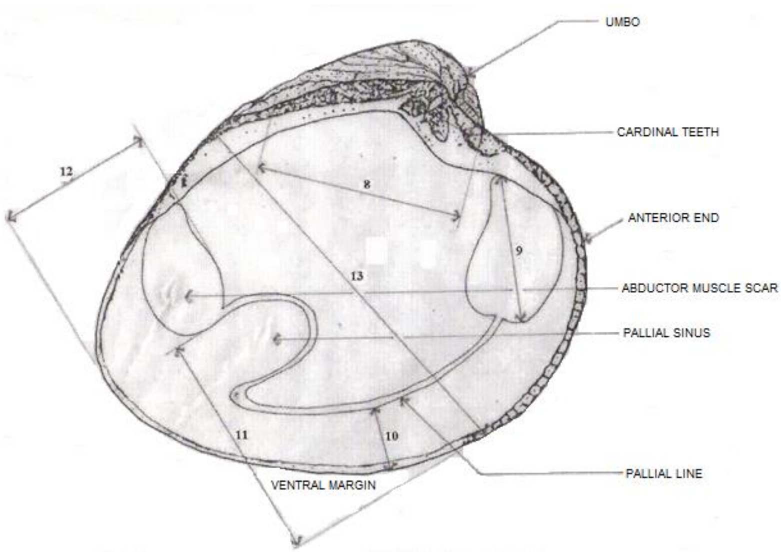

Figure 4. Shell anatomy of *Pitar fritzzelli* showing the morphological characters: (8) hinge length, (9) length of anterior adductor muscle scar, (10) distance between pallial line & ventral shell margin, (11) distance between ventral edge of posterior adductor muscle scar & ventral shell margin, (12) distance between anterior edge of posterior adductor muscle scar and posterior shell margin, (13) shell width.

2.6 Analysis of Data

The analysis of morphological data was performed using the Statistical Package for Social Sciences (SPSS). Prior to the analysis of variance (ANOVA), normality and homogeneity tests were conducted on the arcsine-transformed data. Normality test was performed using Wilk-Shapiro/Rankit Plot while homogeneity of the data was determined employing Levene's test. Non-homogeneous and homogeneous data were then subjected to analysis of variance and Kruskal-Wallis tests, respectively. The variation in characters within population was determined based on the coefficient of variation using descriptive statistics. The coefficient of variation for each character was computed employing the formula:

$$\text{Coefficient of variation} = \frac{\text{standard variation}}{\text{mean}} \times 100\%$$

Differences in character between populations were determined by ANOVA followed by Tukey's post hoc test, if significant. Based on the significant characters, the resemblance of *Pitar fritzzelli* within and between populations was measured employing relative chord distance along with the unweighted pair-group average method (UPGMA). The result of cluster analysis was presented in a dendrogram.

The relationship of each morphological character with the physical and chemical factors was examined using linear regression.

3. Results and Discussion

3.1 Physico-chemical Parameters

Table 1 below shows the percentage weight of the different size groups of sediments in each station. As depicted in the table below, the three localities contained a mixture of granules, very coarse, coarse, medium, fine, and very fine sands. Fine sand showed the highest mean percentage weight in three localities. Similarly, very coarse sand showed the least mean percentage weight in three localities. Jones *et al*. (1989) noted that the grain size of sediment vary between habitats and are not uniform between sites.

Table 1. Mean percentage weight of each grain type in three localities.

	Mean Percentage (%) Weight			
Mean size (mm)	Kolambugan	Baroy	Lala	Grain type
335	6.1	9.8	8	granules
170	3.1	5.5	5.1	very coarse sand
850	5.6	6.4	5.9	coarse sand
600	3.6	7.9	5.6	coarse sand
425	8.2	6	13.7	medium sand
300	13.6	12.4	19.4	medium sand
150	46.6	27.8	39.1	fine sand
75	15.9	9.75	14.6	very find sand

Table 2 displays the mean values of physico-chemical parameters. Baroy showed the highest substratum temperature at mean value of 32.2^0C. The pH reading, however, was observed highest in Kolambugan at mean value of 7.7. On the other hand, both Baroy and Lala showed similar salinity at mean value of 32.5 ppt.

Table 2. Mean values of the physical and chemical parameters in three localities.

Physico-chemical parameters	Mean (X)		
	Kolambugan	Baroy	Lala
Sediment Temperature (^{0}C)	30.9	32.2	31.8
Sediment Salinity ($^{0}/_{00}$)	32.5	28.2	27.2
Sediment pH	7.71	7.7	7.7

3.2 Morphometric Studies

There were 58 individuals of *Pitar frizzelli* collected from the three different areas, 14 from Kolambugan and 22 from both Raw-an Pt. and Lala. A total of fourteen quantitative biological characters of the shell were used in the analysis. The result showed that the depth of left valve, length of hinge, weight of left valve and depth of right valve are not normally distributed, while the rest of the parameters measured exhibited normal distribution.

3.2.1 Variation in Shell Morphology within Population of Pitar fritzzelli

Figure 5 shows the variability of *P. fritzzelli* for each population measured based on the coefficients of variation of the characters. While Tables 3 to 5 show the descriptive statistics of *P. fritzzelli* from Kolambugan, Raw-an and Lala, Lanao del Norte, respectively. Among the fourteen morphological characters, the length of lunule is the least variable shell character in Kolambugan, the length of adductor muscle scar in Baroy, and the length of escutcheon in Lala. However, the weight of left valve is the most variable in both Kolambugan and Baroy and the weight of right valve is the most variable in Lala.

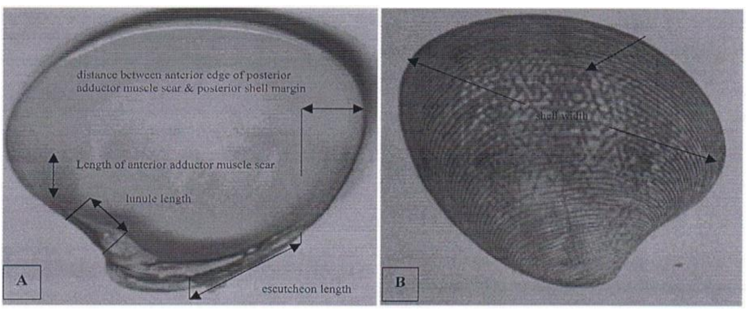

Figure 5. Photograph of the inner (A) and outer (B) shell showing characters with least and greatest coefficients of variation

Table 3. Descriptive statistics of the fourteen morphological characters of *P. fritzzelli* collected from Montay, Kolambugan, Lanaon del Norte.

Morphological Characters	N	Mean	Standard Deviation	Coefficients of Variation (%)
Weight of left valve	14	2.46	0.93	37.70
Weight of right valve	14	2.45	0.92	37.67
Distance between ventral edge of posterior adductor muscle scar & ventral shell margin	14	0.55	0.13	24.33
Distance between anterior edge of posterior adductor muscle scar & posterior shell margin	14	0.50	0.10	20.45
Length of anterior adductor muscle scar	14	0.23	0.04	17.21
Depth of right valve	14	8.83	1.10	12.40
Depth of left valve	14	8.78	1.00	11.40
Distance between pallial line & ventral shell margin	14	0.19	0.02	11.08
Length of escutcheon	14	18.93	1.95	10.28
Length of ligament	14	9.66	0.99	10.26
Distance between umbo & posterior end of ligament	14	0.73	0.08	10.20
Breadth of shell	14	27.55	2.45	8.88
Length of hinge	14	19.35	1.71	8.82
Length of lunule	14	10.24	0.82	8.00

3.2.2 Variation in Shell Morphology between Populations of P. fritzzelli

Table 6 and 7 statistical results present the comparison of the morphological characters of *P. fritzzelli* between populations done using analysis of variance. The depth of left & right valves, weight of left and right valves, distance between ventral & anterior edges of posterior adductor muscle scar, ventral and posterior shell margin, and distance between umbo and posterior end of ligament showed no variation among the three populations.

The breadth of shell, length of ligament, length of escutcheon, length of anterior adductor muscle scar, distance between pallial line and ventral shell margin, length of hinge and length of lunule, on the other hand, exhibited

Table 4. Descriptive statistics of the fourteen morphological characters of *P. fritzzelli* collected from Raw-an Pt., Baroy, Lanao del Norte.

Morphological Characters	N	Mean	Standard Deviation	Coefficients of Variation (%)
Weight of left valve	22	5.83	1.78	30.46
Weight of right valve	22	2.45	0.92	37.67
Distance between ventral edge of posterior adductor muscle scar & ventral shell margin	14	0.55	0.13	24.33
Distance between anterior edge of posterior adductor muscle scar & posterior shell margin	14	0.50	0.10	20.45
Length of anterior adductor muscle scar	14	0.23	0.04	17.21
Depth of right valve	14	8.84	1.10	12.39
Depth of left valve	14	8.78	1.00	11.40
Distance between pallial line & ventral shell margin	14	0.19	0.02	11.08
Length of escutcheon	14	18.93	1.95	10.28
Length of ligament	14	9.66	0.99	10.26
Distance between umbo & posterior end of ligament	14	0.75	0.08	10.20
Breadth of shell	14	27.55	2.45	8.88
Length of hinge	14	19.35	1.71	8.82
Length of lunule	14	10.24	0.82	8.00

significant variation between Kolambugan and Baroy populations and between Kolambugan and Lala populations.

3.3 Cluster Analysis

3.3.1 Within Population

The results of the UPGMA clustering method on the fourteen morphological characters of *P. fritzelli* for each population are presented in the dendrograms in Figures 6, 7 and 8.

Table 5. Descriptive statistics of the fourteen morphological characters of *P. fritzzelli* collected from Pacita, Lala, Lanao del Norte.

Morphological Characters	N	Mean	Standard Deviation	Coefficients of Variation (%)
Weight of left valve	22	4.51	1.52	33.66
Weight of right valve	22	2.45	0.92	37.67
Distance between ventral edge of posterior adductor muscle scar & ventral shell margin	14	0.55	0.13	24.33
Distance between anterior edge of posterior adductor muscle scar & posterior shell margin	14	0.50	0.10	20.45
Length of anterior adductor muscle scar	14	0.23	0.04	17.21
Depth of right valve	14	8.84	1.10	12.39
Depth of left valve	14	8.78	1.00	11.40
Distance between pallial line & ventral shell margin	14	0.19	0.02	11.076
Length of escutcheon	14	18.93	1.95	10.28
Length of ligament	14	9.66	0.99	10.26
Distance between umbo & posterior end of ligament	14	0.75	0.08	10.20
Breadth of shell	14	27.55	2.45	8.88
Length of hinge	14	19.35	1.71	8.82
Length of lunule	14	10.24	0.82	8.00

3.3.2 Between Population

Figure 9 shows the relationship of the *P. frizzelli* among three populations. Based on the seven significant characters, the result shows that Baroy and Lala populations are closely related to each other and neither to the Kolambugan population.

3.4. Correlation Between Physico-chemical Parameters and the Morphological Characters

Table 8 shows the significant relationship of the physical and chemical parameters with the morphological characters. The value of coefficients

Table 6. Comparison of the twelve morphological characters of *P. fritzzelli* in the three localities.

Morphological Characters	Homogeneity Test		ANOVA		Homogeneous Subsets		
	Levene Statistic	Sig.	F value	Sig.	Location 1	Location 2	Location 3
Breadth of shell	1.847	0.167 ns	5.578	0.006**		____	____
Depth of left valve	0.867	0.426 ns	2.151	0.126 ns	____	____	____
Depth of left valve	0.171	0.843 ns	2.011	0.144 ns	____	____	____
Length of ligament	1.352	0.267 ns	11.795	0.000**		____	____
Length of escutcheon	1.890	0.161 ns	5.133	0.009**		____	____
Weight of left valve	2.883	0.064 ns	1.924	0.156 ns	____	____	____
Weight of right valve	2.811	0.069 ns	1.905	0.159 ns	____	____	____
Length of anterior adductor muscle scar	0.306	0.738 ns	22.104	0.000**		____	____
Distance between pallial line & ventral shell margin	0.989	0.378 ns	8.643	0.001**		____	____
Distance between ventral edge of posterior adductor muscle scar & ventral shell margin	0.888	0.417 ns	3.162	0.058*	____	____	____
Distance between anterior edge of posterior adductor muscle scar & posterior shell margin	0.284	0.754 ns	2.811	0.069ns	____	____	____
Distance between umbo & posterior end of ligament	0.946	0.754 ns	1.604	0.210ns	____	____	____

Legend: * significant at α = 0.05, ** highly significant at α = 0.05
ns not significant at α = 0.05
1 (Kolambugan); 2 (Raw-an); 3(Lala)
____ (Raw-an & Lala are homogeneous);
__________(Kolambugan, Raw-an & Lala are homogeneous)

Table 7. Comparison of the two morphological characters of *P. fritzzelli* in the three localities.

Morphological Characters	Homogeneity Test		Kruskal Wallis		Homogeneous Subsets		
					Location		
	Levene Statistic	Sig.	F value	P value	1	2	3
Length of hinge	6.357	0.003**	21.14	0.0000**		____	____
Length of lunule	5.845	0.005**	21.44	0.0000**		____	____

Legend: * significant at α = 0.05, ** highly significant at α = 0.05
ns not significant at α = 0.05
1 (Kolambugan); 2 (Raw-an); 3(Lala)
____ (Raw-an & Lala are homogeneous

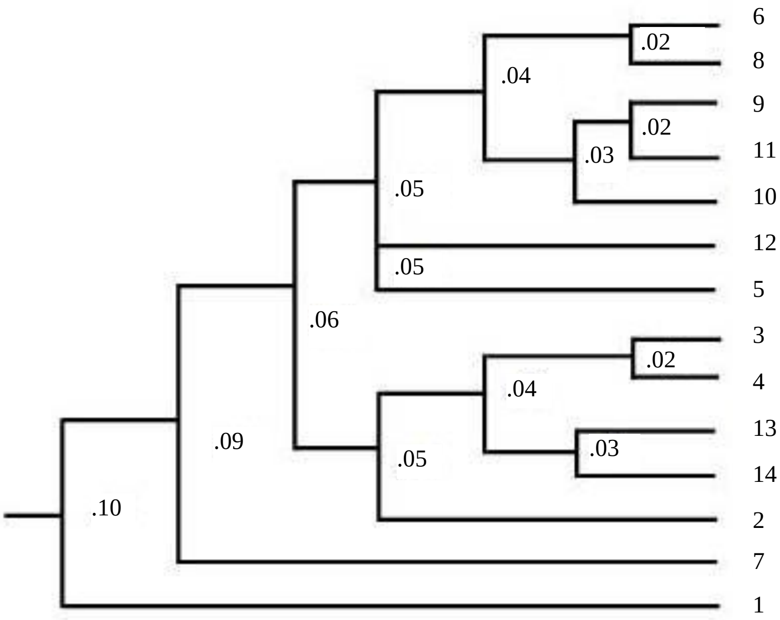

Figure 6. Dendrogram showing the relationships of *P. fritzzelli* samples collected from Montay, Kolambugan, Lanao del Norte based on the fourteen morphological characters.

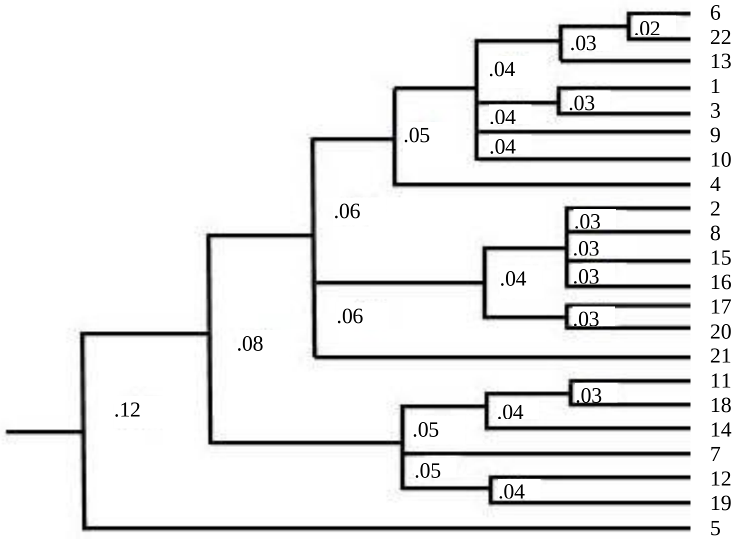

Figure 7. Dendrogram showing the relationships of *P. fritzzelli* samples collected from Raw-an Pt., Baroy, Lanao del Norte based on the fourteen morphological characters.

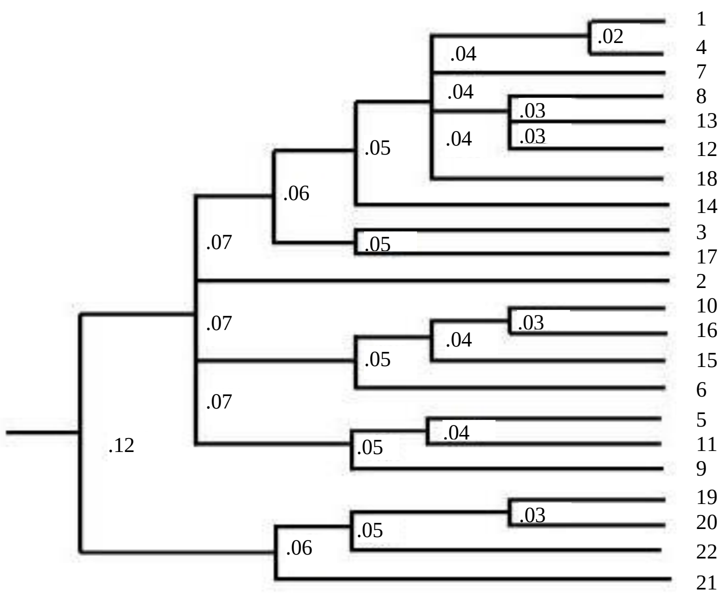

Figure 8. Dendrogram showing the relationships of *P. fritzzelli* samples collected from Pacita, Lala, Lanao del Norte based on the fourteen morphological characters.

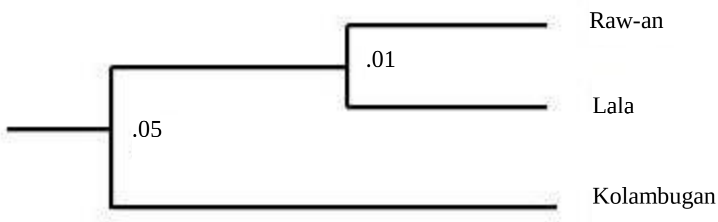

Figure 9. Dendrogram showing the relationships of *P. fritzzelli* among three populations based on the breadth of shell, length of ligament, length of escutcheon, length of anterior adductor muscle scar, distance between pallial line & ventral shell margin, length of hinge and length of lunule.

determines the degree of correlation of the morphological characters with the physical and chemical parameters. The positive coefficients indicate a linear relationship, whereas, the negative coefficients signify an inverse relationship. It is shown in the table that all of the predictors for each character showed significant relationship indicating that the observed variation in shell morphology within and between populations of *Pitar frizzelli* is influenced by the current environmental conditions of the area.

This study shows that there exist variations within and between populations**.** Variation within population is more evident only on the weight of the valves which could be attributed by exposure to common environmental conditions which, according to Innes and Bates (1995), may result in an increase in morphological similarity.

The differences in shell morphology observed between populations, however, could probably be due to clinal variation, which could either be due to environmental differences, genotypic differences, or both. In many cases, environmental variation can affect growth and differentiation patterns, leading to remarkable morphological variation within a species. As observed in Table 8, sediment salinity and temperature differ among the three populations while the sediment pH is the same.

In addition, there was a considerable difference in the percent weight of sediment type in three populations. This could probably account for the different mean sizes of samples observed among populations. Kolambugan has the smallest shell with an average shell length of 32.45 mm than with the samples from Raw-an with 39.44mm and from Lala with 36.85mm.

Table 8. Linear correlation between the morphological characters and physico-chemical parameters.

Morphological Characters	ANOVA		Estimates of Coefficients		
	F	Sig.	Predictors	Coefficients	Sig.
Breadth of shell	24.948	0.000	Temperature	5.253	0.000
			Coarse sand 1	0.601	0.030
			Salinity	-3.227	0.000
			Medium Sand 2	-0.867	0.020
			Granules	0.275	0.032
Depth of left valve	27.007	0.000	Temperature	2.114	0.000
			Coarse sand 1	0.297	0.010
			Salinity	-1.403	0.000
			Medium Sand 2	-0.297	0.000
Depth of right valve	22.884	0.000	Temperature	1.960	0.000
			Coarse sand 1	0.447	0.000
			Salinity	-1.095	0.000
			Medium Sand 2	-0.416	0.002
Length of ligament	42.561	0.000	Temperature	3.796	0.000
			Coarse sand 1	0.482	0.011
Length of lunule	11.342	0.000	Temperature	0.925	0.000
			Coarse sand 1	0.295	0.005
Length of hinge	23.124	0.000	Temperature	2.693	0.006
			Coarse sand 1	1.103	0.000
			Fine sand	-0.160	0.033
Length of escutcheon	15.753	0.000	Coarse sand 1	0.537	0.010
			Fine sand	-0.170	0.000
			pH	9.121	0.001
Color of the inner center of shell	25.996	0.000	Very fine sand	-0.05443	0.000
			Very coarse sand	-0.196	0.000
			Medium sand 2	-0.04375	0.006
Color under the umbones	12.084	0.000	Salinity	0.171	0.000
			pH	-1.174	0.000
			Very coarse sand	-0.08878	0.001
Markings on the periostracum	11.141	0.000	pH	-0.591	0.000
			Coarse sand 1	-0.02957	0.022
Length of anterior adductor muscle scar	42.547	0.000	Coarse sand 2	0.04664	0.000
			Fine sand	-0.005182	0.001
			Salinity	-0.06038	0.000
			Medium sand 1	-0.03982	0.000
Distance between pallial line and ventral shell margin	29.296	0.000	Coarse sand 2	0.02678	0.000
			Medium sand 2	0.01927	0.000
			Salinity	-0.02672	0.000
Distance between ventral edge of posterior adductor muscle scar and ventral shell margin	17.960	0.000	Coarse sand 2	0.04233	0.000
			Very coarse sand	0.04286	0.000
			Salinity	-0.04872	0.004
Distance between anterior edge of posterior adductor muscle scar and posterior shell margin	18.555	0.000	Coarse sand 2	0.02280	0.031
			Very coarse sand	0.04265	0.000
			Salinity	-0.04414	0.001
			Temperature	0.06477	0.040
Distance between umbo and posterior end of ligament	5.099	0.003	pH	-0.418	0.001
			Temperature	0.05206	0.021
			Coarse sand 1	0.02011	0.037

According to Nybakken (1982), the coarser the grain size, the greater the volume of interstitial space and hence, the larger the interstitial organisms that can inhabit the area. Conversely, the finer the grain size, the lesser the space available and the smaller the organisms must be to inhabit the area.

In addition, the observed variation in shell morphology may be one component of adaptive differences among the individuals of *Pitar fritzzelli*. Stebbins (1977) stated that phenotypic modification is often adaptive which enables an individual to adjust to its immediate environment to improve its chances of survival. A comparative studies on *Mytilus* species have shown that the organism exhibit several morphological adaptations to an epifaunal existence (Stanley 1970, 1972, 1983), such as a reduced anterior, flattened ventral surface combined with a well-developed abyssal system to attach the mussel to the substrate. An expanded posterior margin of the shell may also be an adaptation to maintain adequate current flow under crowded conditions (Stanley, 1972), although Seed (1968) found that crowding resulted in a more elongated shell and other studies (Seed, 1968; Gardner *et al*., 1993; Stirling and Okumus, 1994) have also found an association between slower growth rate and more elongated shells. Norberg and Tedengren (1995) observed that *M. edulis* with elongated shells were more prone to starfish predation than were mussels with a more eccentric shell shape. Shell shape appears to be involved in differential adaptation by *M. edulis* and *M. galloprovincialis* to wave exposure (Gardner and Skibinski, 1991; Willis and Skibinski, 1992).

4. Conclusion and Recommendation

Bivalve such as *P. frizzelli* within a population which are exposed to common environmental conditions may also exhibit variation on some morphological characters.

P. fritzelli between populations also exhibit variation on various morphological characters which could be attributed to phenotypical plasticity in response to different environmental and/or ecological conditions existing in the three areas.

Differences in shell morphology might also be due to genetic differences, it is therefore recommended that biochemical analysis be conducted and correlated with morphometric data so as to determine whether genetic characteristics had contributed to the differences in shell morphology.

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