Morphometric Comparison of Dascyllus trimaculatus Populations from Bohol Sea, Philippines

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Abstract: Reef organisms are sporadically distributed in the marine environment. The survival of these populations depends on their connectivity. In the Bohol Sea, establishing connectivity among reef populations, particularly those in marine protected areas, is an important input to managers and environment planners. The most common approach to study connectivity in populations uses individual characters that may distinguish one population from another. These include morphological, morphometric and genetic markers. In this study, 16 morphometric characters of the ubiquitous damsel fish, Dascyllus trimaculatus, were measured using TPSDIG v.1 and compared among 199 samples in 7 sites to test for their sensitivity in detecting populations across sites and to determine patterns of connectivity in the Bohol Sea based on these characters. PCA and DCA implemented in STATISTICA v.12 indicate variability in shape among fish collected from Macajalar Bay on different parts of the bay, even though these populations are geographically closer to each other than the rest of the groups. With 89.22% variability explained by two principal components, 4 distinct clusters can be detected suggesting the Bohol Sea samples are not connected with the three Macajalar samples. Clusters are more likely to share and exchange resources demanding more integrated management schemes within them. It is interesting to note that the 2013 samples collected from 3 sites within the Macajalar Bay showed 93.44% likelihood of assigning individuals to their cluster, suggesting isolation among these sites and the need for local management for these sites within the Macajalar Bay and closer integration with each other.

Key Words: Bohol Sea;*Dascyllus trimaculatus*;Morphometrics

1. INTRODUCTION

The threespot damselfish, or as commonly known in the Philippines, Palata(Dascyllus trimaculatus), is a tropical fish found in reefs. Like all members of its family, D. trimaculatus has relatively short pelagic larval stages (Leray, M., Beldade, R., Holbrook, S.J., Schmitt, R.J., Planes, S., and Bernardi, G., 2009). During this larval stage, fishes are more likely to travel long distances than in the benthic adult stage because travel in the larval stage just involves being swept by ocean currents, while travel in the adult stage involves swimming from one place to another (Holbrook, S.J., and Schmitt, R.J., 1997). D. trimaculatus is found on many tropical countries in the Pacific and Indian Oceans. One such country is the Philippines.

Surveys in the Bohol Sea in the Philippines usually yield a large amount of benthic fauna. A single trawling has given way to multiple studies on different species of marine life (de Forges, B.R., Tan, S., Bouchet, P., Ng, P.K.L., and Saguil, N., 2009). Because of the diversity of the marine life in the Bohol Sea, and the pelagic larval stage of the D. trimaculatus, there is enough reason to believe that there are morphometric differences between D. trimaculatus populations. This study aims to determine whether the morphometric characteristics can produce distinct population signatures in the D. trimaculatus from the different sites, and to determine how many distinct groups can be resolved.

Molecular methods and morphometric methods have advantages and disadvantages. Microsatellite markers can be used to identify stock boundaries and inter-population exchange, but this involves delicate and complex reactions and is usually very expensive. However, morphometric data can be gathered cheaply, and relatively easily. It has also been widely used for determining the relationships of different groups of fish (Booke H.E., 1981).

Demographic connectivity can be estimated by differentiating populations and comparing it with the assigned source population of the samples. These data can be used to infer population structures of D. trimaculatus in the Bohol Sea. Knowing these can help government units improve the management of the protected areas in Bohol Sea for conservation.

2. METHODOLOGY

2.1. Fish Collection

Dascyllus trimaculatus were collected in five different sites within the Bohol Sea. These were in Camiguin Island, Apo Island, Selinog Island, Balicasag Isalnd, and Macajalar Bay. A map of the sites is shown in Figure 1. In Macajalar Bay, three additional sets of samples were collected. A map of these sites is shown in Figure 2. These samples were placed in plastic zipper bags, and were preserved in ethanol. These were frozen and shipped with an ice pack for transportation to the laboratory.

2.2. Morphometrics

2.2.1. Photography

Fish were thawed for five minutes after being taken out of storage from the freezer. Fish were laid on the dissecting pan, with the left side up. Fins were spread to make sure that the landmarks can be easily detected when photographed. A ruler was placed on the dissecting pan for scaling. Tags were placed on the pan alongside the fish for identification. Fish were photographed with the camera parallel to the plane of the dissecting pan, with the ruler and the tag in the field of view of the digital camera.

2.2.2. Measurement

The digital photographs were opened in the tpsdig program. Using this program, the following homologous landmarks were identified for each fish specimen:

(1)Anterior tip
(2)Base of the anterior end of dorsal fin
(3)Base of the posterior end of dorsal fin
(4)Dorsal junction of the tail and the tail fin
(5) Ventral junction of the tail and the tail fin
(6) Base of the posterior end of the caudal fin (7) Base of the anterior end of the caudal fin
(8) Base of the anterior end of the pelvic fin
(9) Posteriormost junction of tail and tail fin.

These are illustrated in Figure 3.

Using the rulers in the photographs, a ratio of the pixels to centimeters was identified for every photograph. Along with the landmarks, this generated a .tps file with the coordinates of the landmarks in the photograph. From the landmark coordinates and the pixels to centimeters ratio, lengths of the parameters were computed. The following factors were measured from the length between the landmarks:

(1) Landmarks 1 and 2
(2) Landmarks 1 and 8
(3) Landmarks 2 and 8
(4) Landmarks 2 and 7
(5) Landmarks 3 and 8

(6) Landmarks 2 and 3
(7) Landmarks 7 and 8
(8) Landmarks 3 and 7
(9) Landmarks 3 and 5
(10) Landmarks 6 and 4
(11) Landmarks 3 and 4
(12) Landmarks 5 and 6
(13) Landmarks 3 and 6
(14) Landmarks 4 and 5
(15) Landmarks 6 and 7
(16) Landmarks 1 and 9

These are illustrated in Figure 4.

2.3. Statistical Analysis

The data was analyzed with STATISTICA v.12. Using this software, Principal Component Analysis and Discriminant Analysis were performed.

Table 1. Sites and Samples

Site	Number of Samples
Balicasag	78
Apo	30
Selinog	28
Camiguin	86
Jasaan	50
Laguindingan	40
El Salvador	32

Figure 1. Collection sites in the Bohol Sea: Balicasag Island-Red, Apo Island-Green, Selinog Island-Yellow, Camiguin Island-Purple

Figure 2. A. Collection sites in Macajalar Bay: Jasaan-Pink, Laguindingan-Brown, El Salvador-Yellow

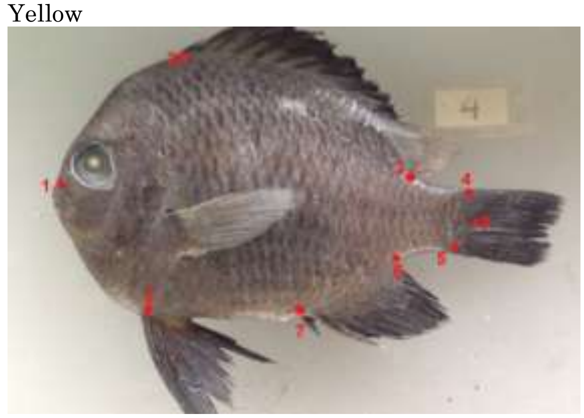

Figure 3. Landmarks in *Dascyllus trimaculatus* specimen

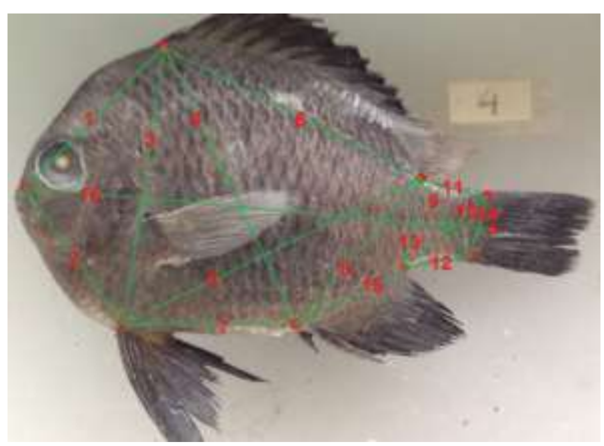

Figure 4. Parameters measured

3. RESULTS AND DISCUSSION

3.1. Statistical Analysis of Macajalar Bay Samples

3.1.1. Principal Component Analysis

Two of the characters (1 and 2) already constitute 98.07% of the variation in the Macajalar Bay (Figure 5). Instead of using all the characters, these two factors were used for the Principal Component Analysis. There are five distinct clusters formed in the scatterplot (Figure 6). Three are composed exclusively of El Salvador, Laguindingan, and Jasaan samples. The remaining two clusters are almost exclusively composed of Jasaan samples or Laguindingan samples only, with a few El Salvador samples in both clusters. There are no Laguindingan samples or Jasaan samples in the groups composed mostly of the other group. Individuals are grouped to their cluster with 93.44% accuracy.

3.1.2. Discriminant Analysis

The same two characters were used for the Discriminant Analysis. Three different clusters are formed, each composed mostly of samples from each of the three sampling sites (Figure 7). A few El Salvador samples can be found in the clusters predominated by samples from the two other groups, like in the PCA. Similarly, there are no samples from Jasaan or Laguindingan in the clusters predominated by the other group. There are some Jasaan samples in the cluster predominated by El Salvador samples. Individuals are grouped with the same accuracy as the PCA. There are fewer clusters in the DA than in the PCA, but if the clusters that are composed of Laguindingan samples and Jasaan samples only are lumped together with the other clusters that mostly contain samples from the respective locations, the clustering is exactly the same as the DA results.

El Salvador is geographically located between the Jasaan and Laguindingan. Both Laguindingan and Jasaan are situated at the opposite ends of Macajalar Bay. It is possible that there may have been exchange between El Salvador and the either of the two other sites, but there seems to be no exchange between Laguindingan and Jasaan.

3.2. Statistical Analysis of Groups Collected in the Bohol Sea

3.2.1. Principal Component Analysis

Two of the characters (1 and 2) already constitute 80.93% of the variation in the Bohol Sea (Figure 8). Like in the Macajalar Bay samples, these two factors were used in the PCA. Only two distinct clusters are created (Figure 9). One cluster contains some of the Balicasag samples, and only those. The rest of the samples are clustered together in one group. Individuals were grouped to their clusters with 10.81% accuracy.

3.2.2. Discriminant Analysis

The same characters were used for the DA as in the Macajalar Bay samples. No distinct clusters were formed (Figure 10). However, samples converge toward one big cluster. Even though the same Balicasag samples that constitute the separate cluster in the PCA were far from the center of the cluster, they are still not distinctly separated.

There is no distinction in the populations from the different sampling sites. The Bohol Jet and the Iligan Eddy cycles the seawater around the Bohol Sea (Figure 14) (Smith & Sandwell, 1997). It is likely that exchange between populations occur through these currents. Citations should be in this format, APA style (Adamo, 1980; Chen and Hwang, 1992; Tan et al., 2005). They should be listed at the end of the paper in alphabetical order.

3.3. Statistical Analysis of All Groups

3.3.1. Principal Component Analysis

Two of the characters (1 and 2) already constitute 89.22% of the variation for all groups (Figure 11). Again, these are the factors used in the PCA. There are four distinct clusters formed in the scatterplot (Figure 12). Three of the clusters are each mostly composed of samples from one of the three sampling sites in Macajalar Bay. One cluster contains all the samples from the sites in the Bohol Sea.

3.3.2. Discriminant Analysis

Like in the other analysis, the same characters were used for the DA. Two clusters were formed (Figure 13). One cluster contains all Macajalar samples, and the other contains all the other samples from the rest of the Bohol Sea.

There is a big difference between the Bohol Sea samples and the Macajalar samples. This may be explained by the currents in the Bohol Sea. Even though the Bohol Jet and the Iligan Eddy allows the exchange of individuals between the different sites in the Bohol Sea, these mechanisms are not present in Macajalar Bay. This can be a factor in having different characteristics between locations found in the same bay.

4. CONCLUSIONS

Overall, the data suggests that the two characters can produce distinct population signatures in Macajalar Bay, but not within the rest of the Bohol Sea. There is a distinct difference between the samples from Macajalar Bay samples and samples from the rest of the Bohol Sea. In Macajalar Bay, there are three distinct clusters resolved, but there are no distinct clusters resolved in the rest of the Bohol Sea.

5. ACKNOWLEDGMENTS

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Table 2. Parameter means according to site

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
El Salvador	1.35	1.21	1.78	2.10	2.03	1.78	1.08	1.28	0.73	0.64	0.36	0.42	0.65	0.50	0.74	8.71
Jasaan	3.65	3.31	5.23	5.98	5.80	5.23	3.06	3.60	1.96	1.66	0.93	1.02	1.74	1.36	2.16	5.85
Laguindingan	1.97	1.67	2.64	2.99	2.88	2.64	1.54	1.73	1.04	0.89	0.55	0.64	0.86	0.69	1.01	3.19
Apo	4.27	3.55	5.95	6.86	6.93	6.06	3.55	4.29	2.12	2.19	1.98	1.81	9.31	1.01	1.07	2.63
Selinog	3.36	3.45	4.93	5.80	5.28	5.15	2.63	3.45	1.75	1.79	1.66	1.48	7.74	0.83	0.87	2.07
Balicasag	3.66	3.34	4.19	5.01	5.55	4.98	2.81	3.34	1.72	1.76	1.54	1.34	8.32	0.88	0.93	2.28
Camiguin	3.34	2.97	4.77	5.29	5.32	4.79	2.43	3.49	1.72	1.81	1.68	1.43	7.22	0.76	0.94	2.17

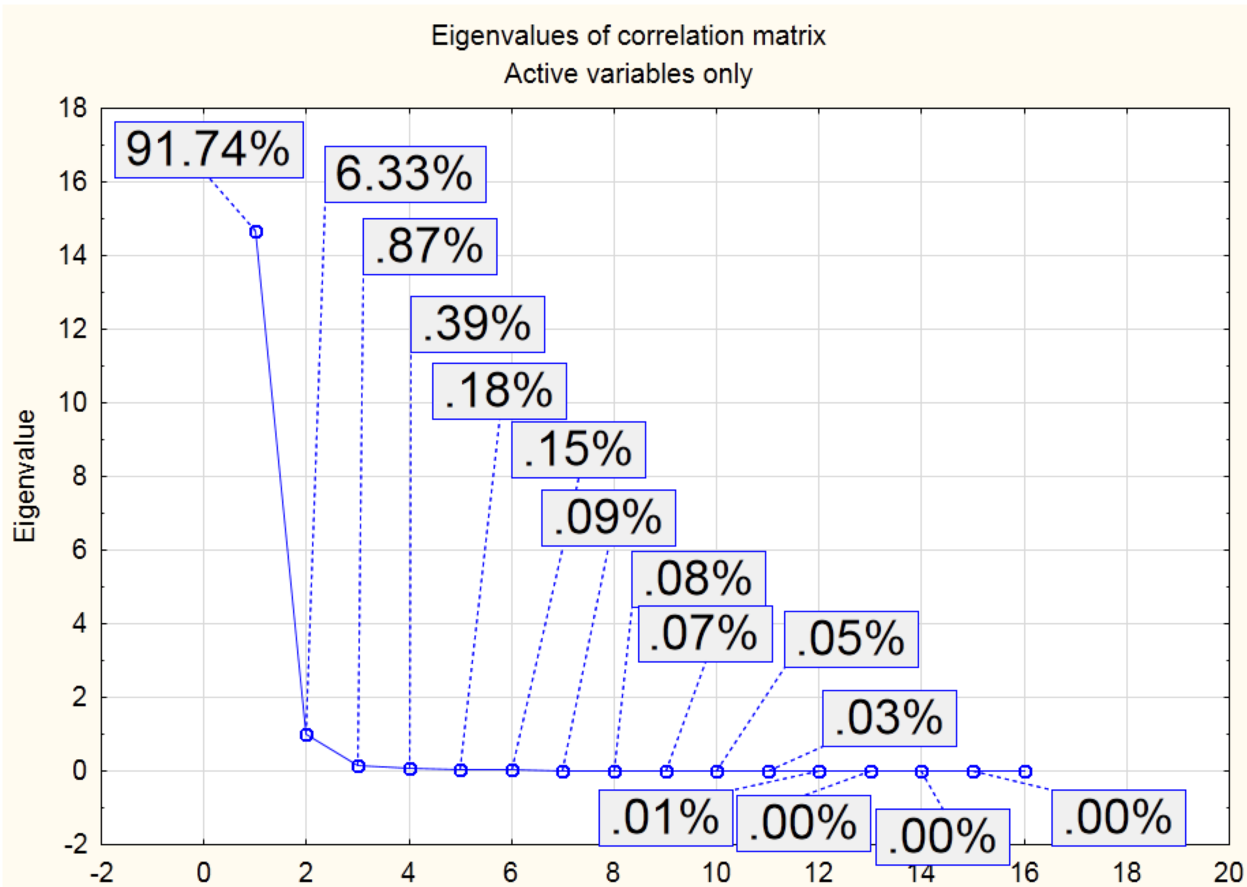

Figure 5. Scree plot of the eigenvalues of the principal components for all Macajalar samples

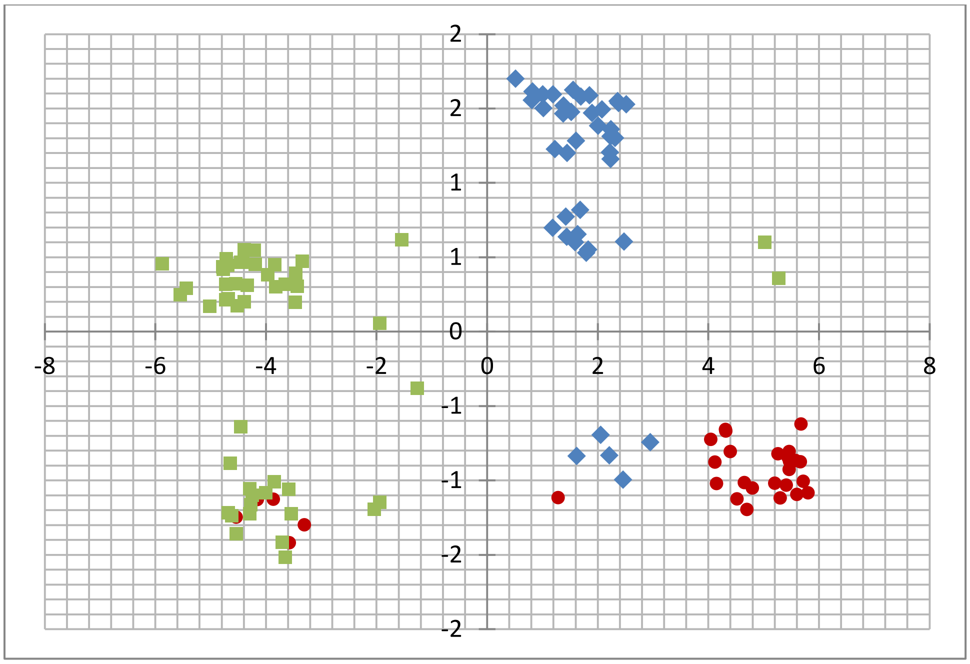

Figure 6. Case score plot (Root1 vs, Root 2): Red Circle-El Salvador, Green Square-Jasaan, Blue Diamond-Laguindingan

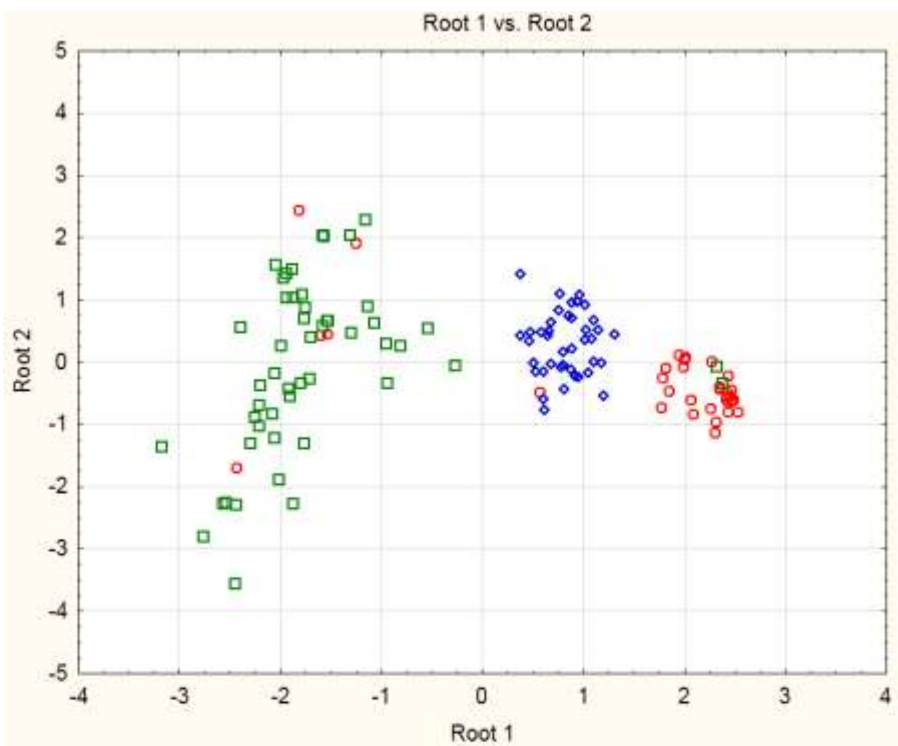

Figure 7. Discriminant Analysis. Red Circle-El Salvador, Green Square-Jasaan, Blue Diamond-Laguindingan

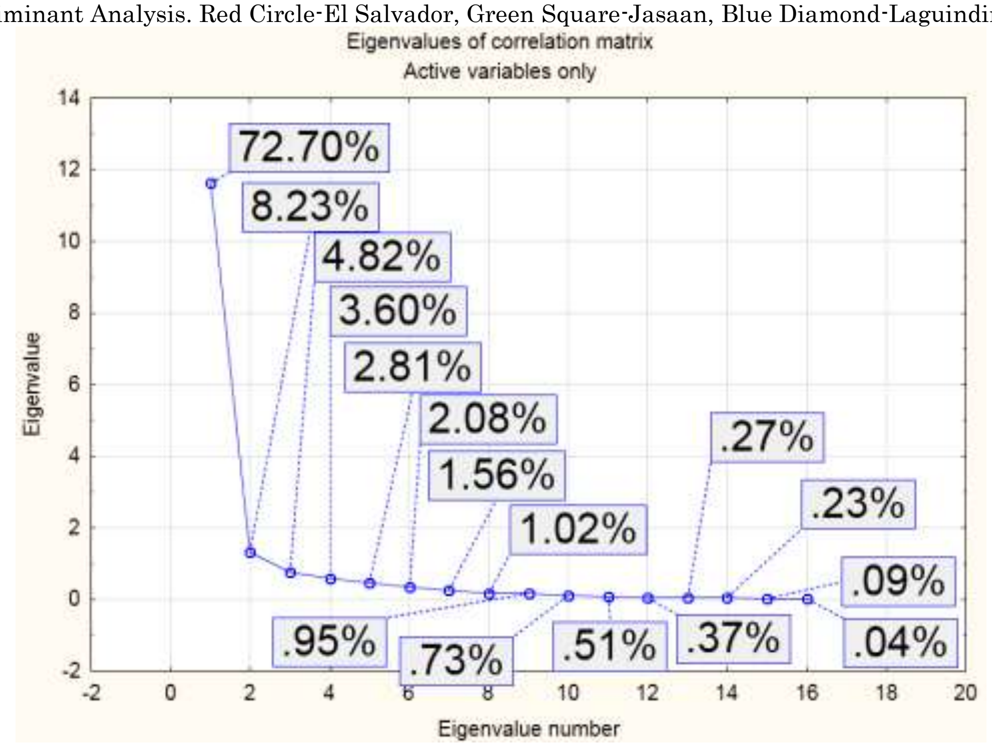

Figure 8. Scree plot of the eigenvalues of the principal components for early 2000s samples

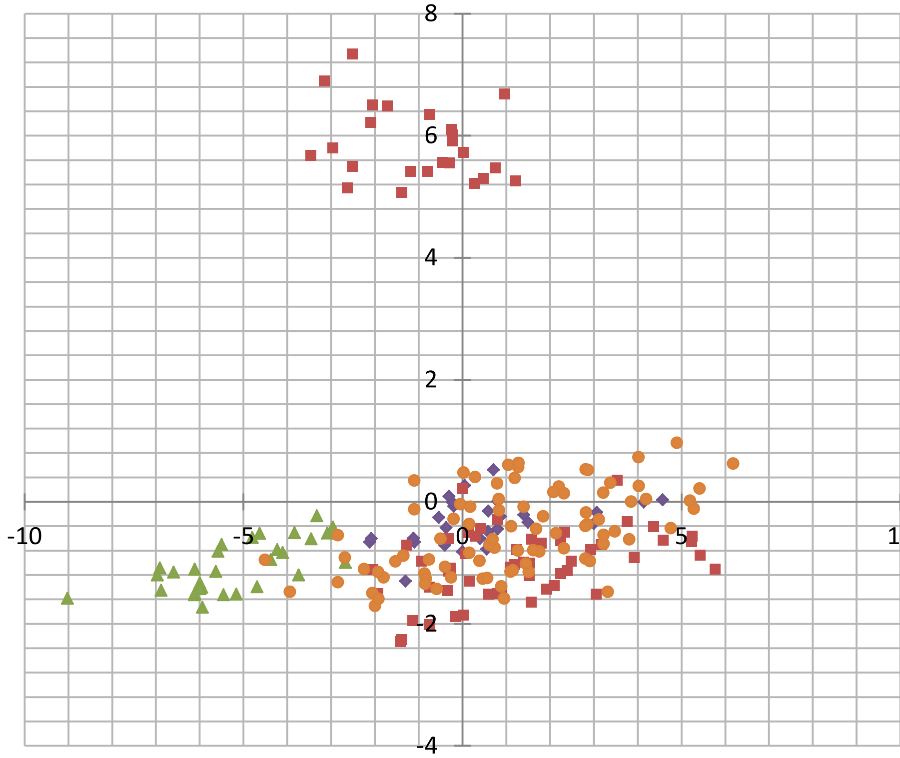

Figure 9. Case score plots. Label colors denote sampling sites. Green Triangle-Apo, Purple Diamond-Selinog, Red Square-Balicasag, Orange Circle-Camiguin.

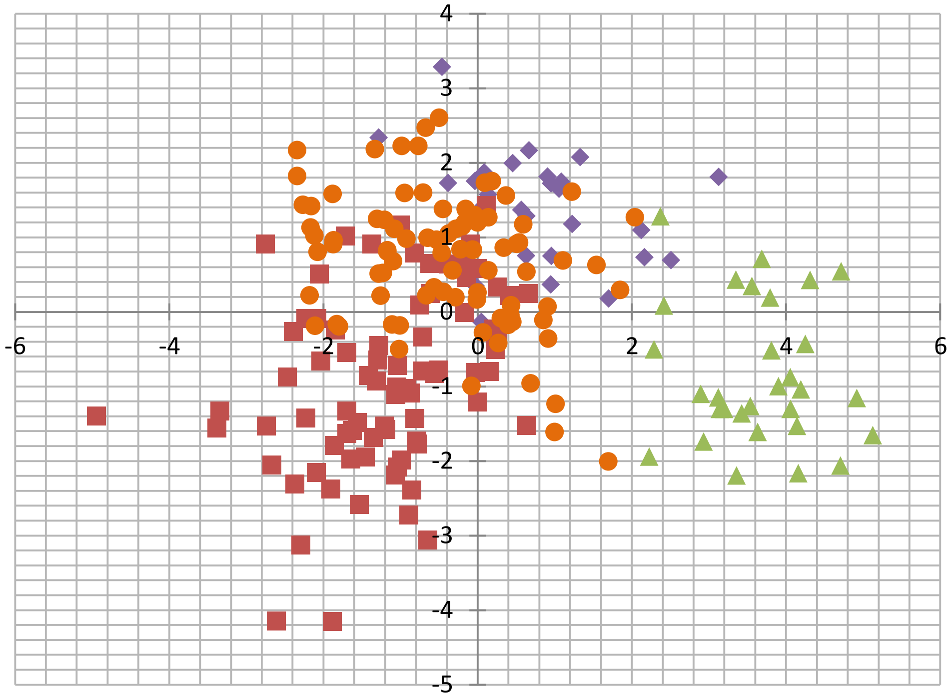

Figure 10. Discriminant Analysis plot (Root 1 vs, Root 2). Label colors denote sampling sites. Green Triangle-Apo, Purple Diamond-Selinog, Red Square-Balicasag, Orange Circle-Camiguin.

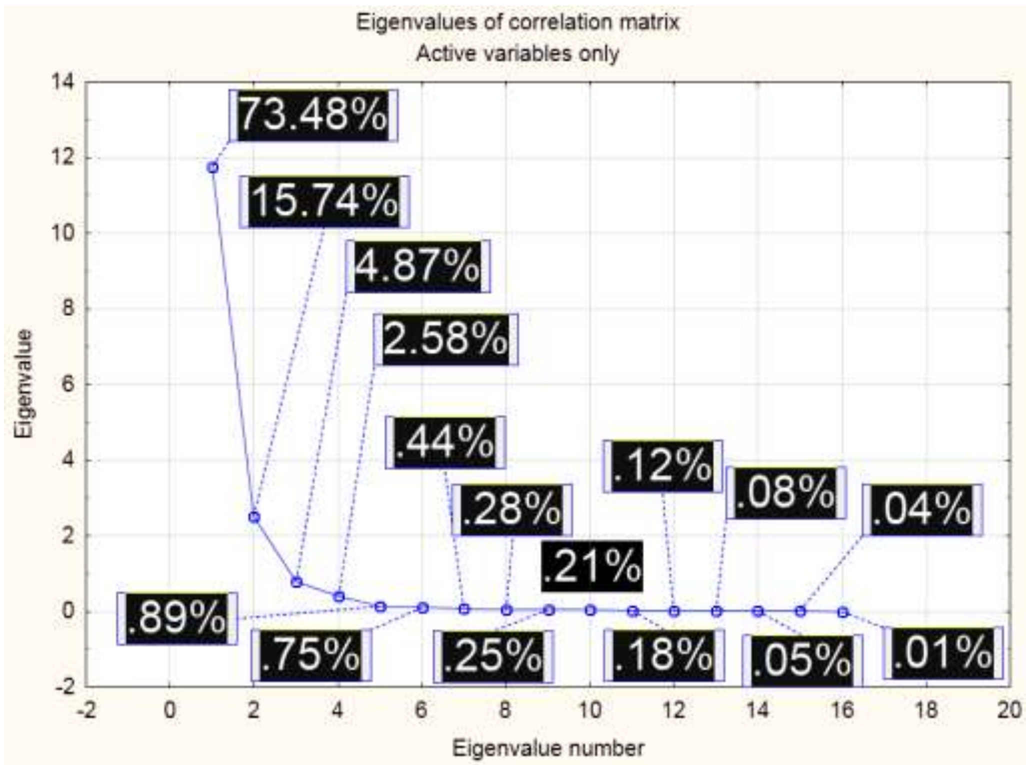

Figure 11. Scree plot of the eigenvalues of the principal components for all samples

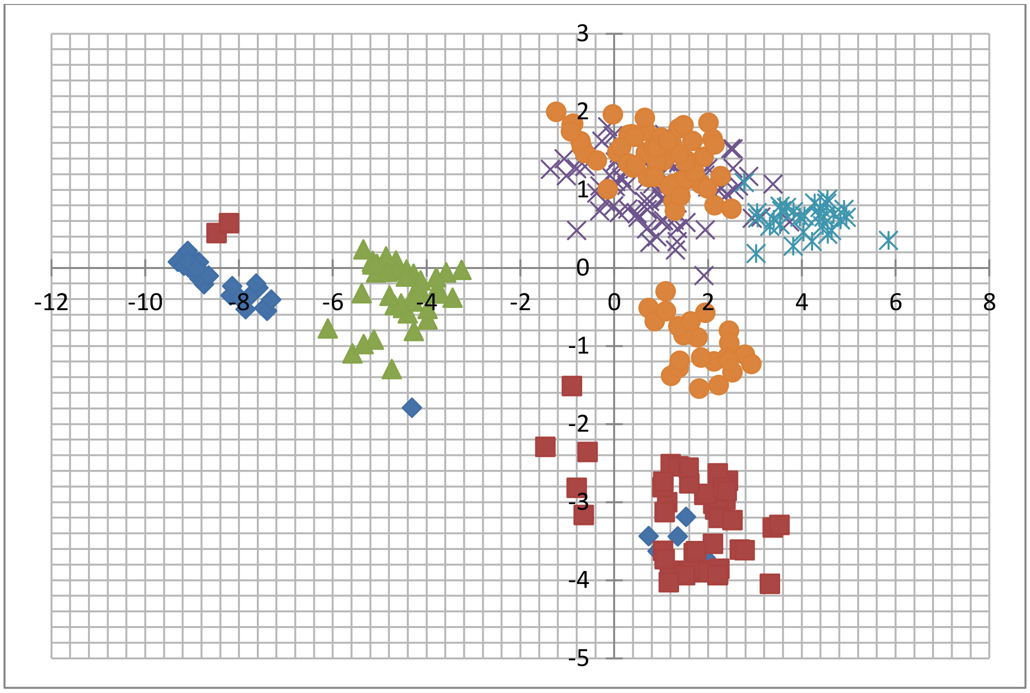

Figure 12. Case score plot (Root1 vs, Root 2): Blue diamond-El Salvador, Red Square-Jasaan, Green Triangle-Laguindingan, Purple Cross-Camiguin, Blue Asterisk-Apo, Orange Circle-Balicasag

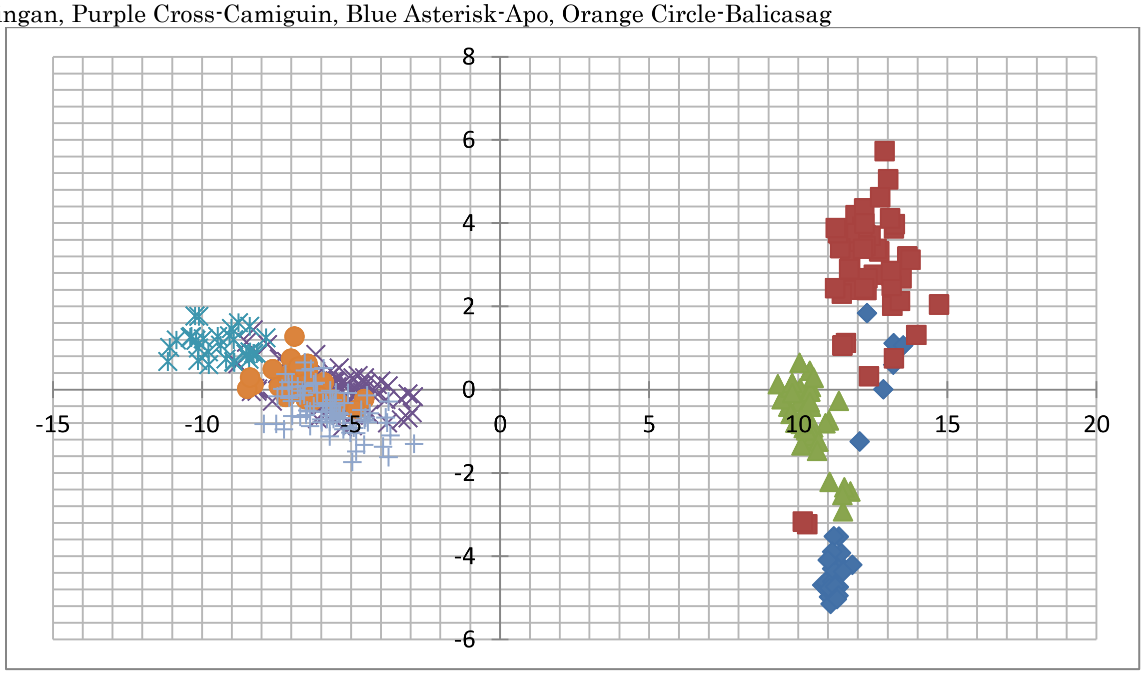

Figure 13. Discriminant Analysis plot (Root 1 vs, Root 2): Blue diamond-El Salvador, Red Square-Jasaan, Green Triangle-Laguindingan, Purple Cross-Camiguin, Blue Asterisk-Apo, Orange Circle-Balicasag

Figure 14. Bohol Jet and Iligan Bay Eddy in the Bohol Sea

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