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## Interpopulation differences in shell forms of the pearl oyster, *Pinctada imbricata radiata* (Bivalvia: Pterioidea), in the northern Persian Gulf inferred from principal component analysis and elliptic Fourier analysis

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**Abstract:** Metapopulation characterization and discrimination of the pearl oyster *Pinctada imbricata radiata* in the northern part of the Persian Gulf is unknown. This study was conducted to examine differences in shell morphology of *P. imbricata radiata* from the Hendourabi and Lavan islands using principal component analysis (PCA) and elliptic Fourier analysis (EFA). Both EFA and PCA differentiated the shell form of the 2 populations significantly. PCA indicated that the pearl oysters from Hendourabi tended to be longer, while those from Lavan were wider and greater in thickness (width). Based on EFA, the specimens of Hendourabi Island were more fusiform than those from Lavan. In addition, the Lavan specimens were rounder than those from Hendourabi. The Lavan specimens had more diversity in form than those from Hendourabi. In conclusion, EFA and PCA approaches are clear-cut tools to identify and separate populations based on morphological characteristics, and EFA is a faster method compared with PCA in the discrimination of form in *P. imbricata radiata*.

**Key words:** Elliptic Fourier analysis, Hendourabi, Lavan, principal component analysis, *Pinctada imbricata radiata*

### 1. Introduction

Identification of separate unit stocks is an essential requirement for management of fisheries' stocks. Genetic and morphometric techniques have been widely used to identify stocks of aquatic organisms (Palmer et al., 2004; Aguirre et al., 2006; Konan et al., 2010; Marquez et al., 2010; Tlig-Zouari et al., 2010; Garcia-Rodriguez et al., 2011). Allozyme (Apte and Gardner, 2001), isozyme (Hofstra et al., 1995), simple sequence repeat (Benzie and Smith-Keune, 2006; Lind et al., 2007; Yu et al., 2008), restriction fragment length polymorphism (Itoi et al., 2011), mitochondrial DNA (Cheng et al., 2011; Iwamoto et al., 2012), and random amplification of polymorphic DNA (Kim et al., 2008) are among the genetic techniques used. There are various morphometric approaches for detecting differences in the form of organisms (Cadrin and Friedland, 1999), e.g., landmark-based analyses (James Rohlf and Marcus, 1993; García-Rodríguez et al., 2011) and elliptic Fourier analysis (EFA) (Laurie et al., 1997; Palmer et al., 2004; Neto et al., 2006; Andrade et al., 2010; de Aranzamendi et al., 2010; Marquez et al., 2010). In addition to modern techniques, traditional methods such as principal component analysis (PCA) are still in

use to analyze intraspecific morphometric variation along a geographical range (Konan et al., 2010; Tlig-Zouari et al., 2010; Hahn et al., 2011; Fouquet et al., 2012; Zapata et al., 2012).

Natural selection influences the form of the organisms (Cadrin, 2000). However, morphological variation within species is often not caused by genetic differences (Hoffman et al., 2010; Zieritz et al., 2010), since body form changes during growth and development (Tripp-Valdez et al., 2012). During development, some features will disappear while others may remain, which reflects the life history of a species and phenotypic plasticity (Cadrin, 2000; Hoffman et al., 2010). Although phenotypic plasticity may not be as important as genetic variability, it is still influential in the evolution of traits (Stearns, 1992). Genetic techniques are expensive, labor-intensive, and time-consuming, and they require expertise. On the other hand, morphometric studies do not require sophisticated facilities, are simple for collecting data, and are inexpensive, which makes them ideal in differentiating populations.

The pearl oyster (*Pinctada imbricata radiata* Leach, 1814) is a benthic species that lives on sand banks and coral rock (Strack, 2008). The distribution of *P. imbricata radiata*

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is limited to the western Atlantic regions and between the Tropic of Capricorn and Tropic of Cancer in the Indo-Pacific (Wada and Tëmkin, 2008). As with many benthic marine invertebrate populations, the pearl oyster of the Persian Gulf is structured as a metapopulation, emphasizing the need for understanding the spatial structure of *P. imbricata radiata* (Orensanz et al., 2006). Since the spatial structure of organisms encompasses important concepts for understanding population dynamics, an understanding of it is essential in the planning of management strategies (Orensanz et al., 2006). Lavan and Hendourabi are 2 islands located in the northern territory of *P. imbricata radiata* in the Persian Gulf. The metapopulation of the pearl oyster is composed of aggregated high-density individuals in discrete areas (Lavan and Hendourabi). These populations experience diverse environmental conditions in terms of coastal topography, heavy metal pollution, nutrient availability, and predation. In addition to different environmental conditions, the geographical barriers between populations of *P. imbricata radiata* may lead to morphometric differentiation. *Pinctada imbricata radiata* in the Persian Gulf are facing anthropogenic pressures, including overfishing and water pollution, leading to thinning of the population. To save these stocks, artificial insemination and release of spats to the wild seems necessary. However, the number of remaining stocks is quite low and mixing bloodstocks may lower the variability. Therefore, the present study aimed to characterize and discriminate the metapopulation of the pearl oyster *P. imbricata radiata* in the northern part of the Persian Gulf using PCA (the traditional method) and EFA. While a landmark-based technique can be used, placing landmarks on curvy objects such as shells is difficult and may be rather subjective. Therefore, we also used an outline-based geometric method, i.e. EFA. This study may be useful for fishery managers; however, morphological

studies may address taxonomical differences that might be of interest for taxonomists, as well.

## 2. Materials and methods

### 2.1. Sampling

Eighty specimens, 40 from Lavan (ranging 39.30–77.90 mm in shell height) and 40 from Hendourabi (ranging 42.38–79.12 mm in shell height), were randomly collected using SCUBA at depths of 6–8 m in the coastal waters of the Hendourabi and Lavan islands (Figure 1). Care was taken to collect individuals of the same size range to avoid size-specific variation in shell form. The specimens were scrubbed and preserved in a refrigerator. After removal of the soft parts, the left valves were digitized (concave side upwards) with a digital camera.

### 2.2. PCA

Six variables (SH: shell height; SL: shell length; SW: shell width; HL: hinge length; Hpn: height of the nacreous part; Lpn: length of the nacreous part) were measured to the nearest 0.01 mm using digital calipers (Guanglu™) (Figure 2). All measurements were done on the left valve (Huang and Okutani, 2003; Hwang et al., 2007; Tlig-Zouari et al., 2010). All morphological variables were transformed through dividing by the average (A) of SL, SH, and SW to remove the effects of size. To find significant differences in shape of the specimens from the 2 islands, PCA was performed on the 6 transformed morphological variables (i.e. SH/A, SL/A, SW/A, HL/A, Hpn/A, and Lpn/A), followed by an independent sample t-test on principal component (PC) scores. PCA was based on a correlation matrix, as variables had very different variances (Quinn and Keough, 2002).

### 2.3. EFA

The overall shape was studied by EFA (Kuhl and Giardina, 1982) using the contour coordinates (Lestrel, 1997; Hammer

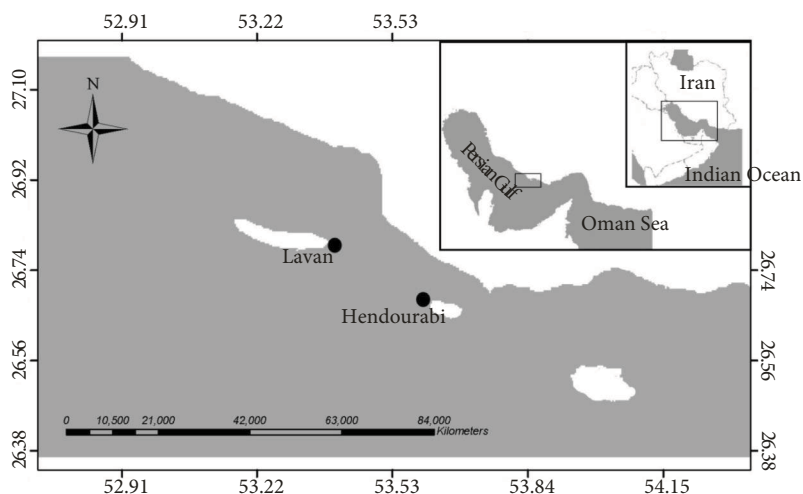
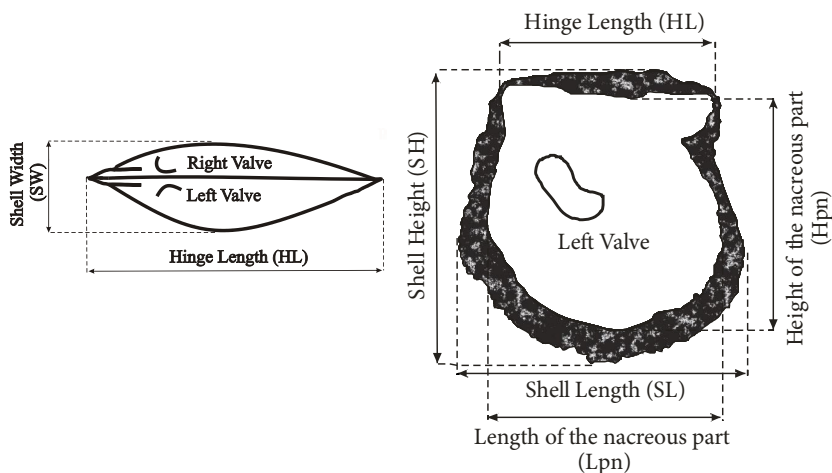


Figure 1. Map of the study area showing the fishing grounds.



**Figure 2.** Morphological measurements of the shell in *P. imbricata radiata* that were used in PCA.

and Harper, 2008). For each specimen, chain codes were recorded on the contour to calculate the elliptical Fourier descriptors (EFDs) of the first 20 harmonics. The normalized EFDs were calculated based on the first harmonic ellipse that corresponds to the first Fourier approximation of the contour information (Kuhl and Giardina, 1982). According to the elliptic Fourier transformation program (Chc2Nef), the size and orientation of the contour was standardized in accordance with the size and alignment of the major axis of the ellipse (Iwata, 2001). A PCA of the normalized EFDs was performed using a variance–covariance matrix of the coefficients (Iwata, 2001). The software Shape v. 1.3 was used for all of the analyses (Iwata and Ukai, 2002). An independent sample t-test was used to examine significant differences between the PCA scores of the 2 islands’ specimens using SPSS 17 (SPSS, Inc.).

### 3. Results

#### 3.1. PCA

Over 66% of variance was explained by PC1 and PC2. The PC1 coefficients were positive for all shell components except for SW and Lpn. PC2 coefficients for the shell width and shell length were negative (Table). SW, SL, and Hpn were mainly loaded on PC1. SH and Lpn were chiefly loaded on PC2. Therefore, greater PC1 scores would show larger SW, SL, and Hpn, while greater PC2 scores would show larger SH and Lpn.

A t-test on PC1 scores indicated that the oysters from Lavan had greater mean PC1 scores than those from Hendourabi; thus, SW, SL, and Hpn in Lavan specimens were larger than those from Hendourabi (df: 77, t: -12,  $P < 0.001$ ). A t-test on PC2 scores indicated that df: 77, t: -1.435,  $P = 0.155$ .

PC1 and PC2 scores from the pearl oysters from Hendourabi and Lavan are shown in Figure 3.

The SH and Hpn vectors had the same direction, which were in the opposite direction of the SW vector, indicating that oysters with larger SH had larger Hpn but smaller SW. The HL and SL were in the same direction, showing that SL and HL were positively correlated.

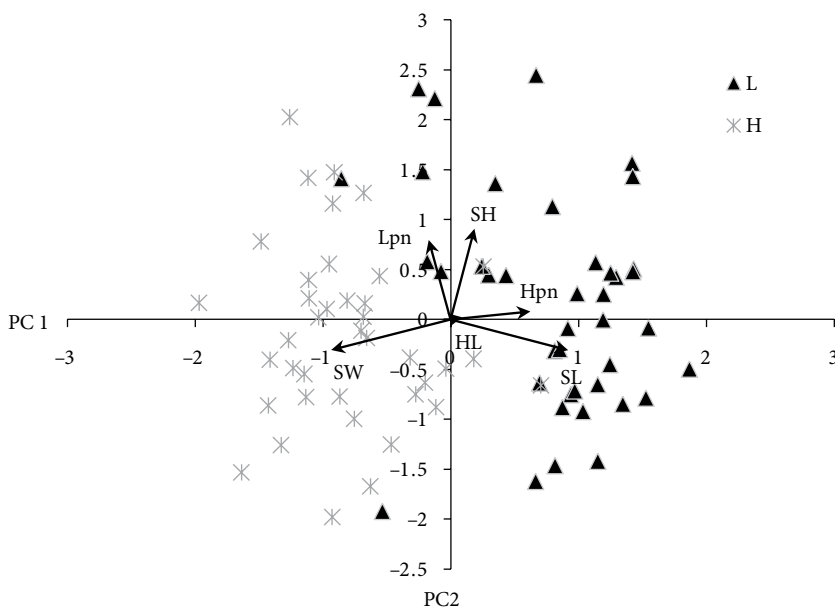
#### 3.2. EFA

Figure 4 shows PC1 and PC2 of PCA performed on coefficients of EFDs in 2 populations of *P. imbricata radiata* from the Lavan and Hendourabi islands. About 95% of shape difference in the samples in the 2 populations was explained by PC1 and PC2. The diversity of shape in specimens from Lavan was greater than that of those from Hendourabi. The t-test on PC scores detected significant differences between specimens of the 2 islands, indicating that shapes of the oysters from Lavan and Hendourabi islands were significantly different (PC1: df: 65.6, t: 7.18,  $P < 0.001$ ; PC2: df: 76, t: -4.1,  $P < 0.001$ ).

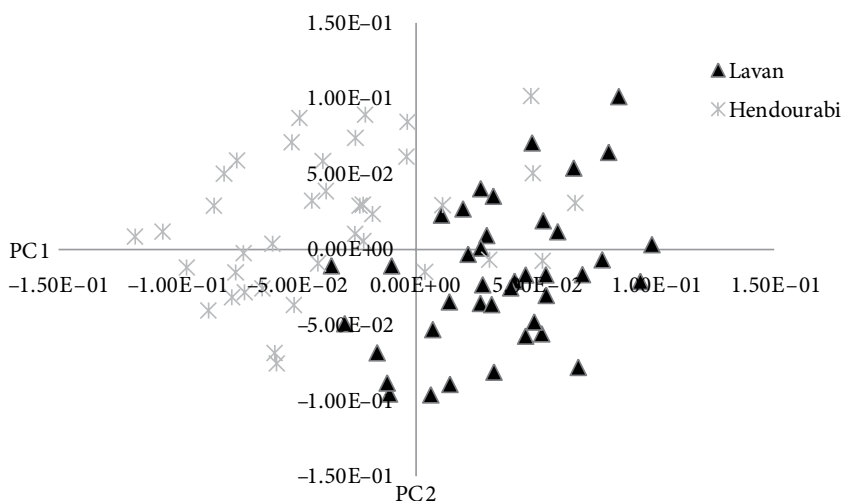
There was a major difference between specimens of the Lavan and Hendourabi islands in the dorsal lip (in the

**Table.** Principal component coefficients generated by PCA for *P. imbricata radiata* from Hendourabi and Lavan islands.

Shell component	PC1	PC2
SW	-0.934	-0.305
HL	0.090	0.022
SH	0.187	0.908
SL	0.920	-0.307
Hpn	0.625	0.080
Lpn	-0.171	0.796
Eigenvalue	2.343	1.646
% of variance	39.056	27.44



**Figure 3.** The first and second principal components scores of *P. imbricata radiata* from Hendourabi (X) and Lavan (▲) islands generated by PCA.



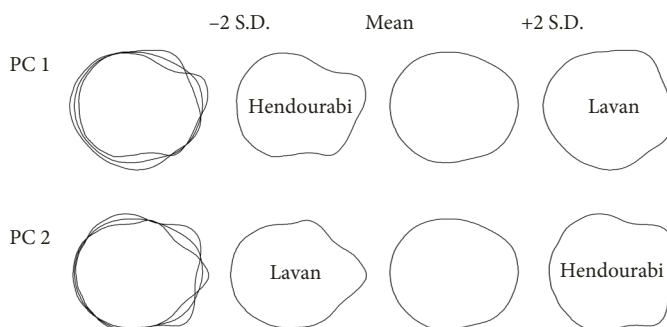
**Figure 4.** The first and second principal components scores of *P. imbricata radiata* from Hendourabi (X) and Lavan (▲) islands calculated by PCA performed on the normalized EFDs.

direction of the lateral hinge teeth), posterodorsal, and anterodorsal parts of the shell (Figure 5). Lavan specimens were more rounded than those from Hendourabi, which was due to the straightened edges of the posterodorsal and anterodorsal lips. The dorsal lip of the Lavan specimens was in the oblique position. Consequently, Lavan specimens had a more stretched perpendicular line on the ventral lip (drawn from the umbo) than the Hendourabi specimens. Due to this oblique mode of the dorsal edge, the Lavan samples were well-distinguished from Hendourabi specimens. In general, specimens from Hendourabi Island were more fusiform than those from Lavan.

#### 4. Discussion

Our results showed 2 separated populations with different shell shape from Hendourabi and Lavan. Both EFA and PCA successfully differentiated shell shape among populations and almost 95% of the assignments were correct, as reported previously. For example, Tlig-Zouari et al. (2010) studied *P. imbricata radiata* along the Tunisian coastline by using multivariate statistics analysis. Marquez et al. (2010) studied the striped clam *Ameghinomya antiqua* in North Patagonia, Argentina, using EFA and landmarks.

Based on PCA, specimens from Lavan had larger SH and Hpn and tended to be longer, while pearl oysters



**Figure 5.** Shape variation of EFA analysis in *P. imbricata radiata* from Lavan and Hendourabi islands.

from Hendourabi tended to be wider. In addition, the thickness (width) of pearl oysters from Lavan Island was greater than that of those from Hendourabi. Based on EFA, diversity of shape in Lavan specimens was greater than that of Hendourabi individuals, showing that Lavan specimens had shells that were more stretched than those from Hendourabi. While both methods detected morphological differences, EFA is a faster method than PCA in discrimination of form in *P. imbricata radiata*.

Some marine invertebrates with a long planktonic larval stage have no separated populations either morphologically or genetically, even over long distances, such as the gastropod *Littorina scutulata* (Kyle and Boulding, 2000) and the sea urchin *Echinothrix diadema* (Lessios et al., 1998). However, some benthic species with a long larval stage have differentiation either genetically or morphologically, even over a short geographical range, e.g., the gastropod *Littorina plena* (Kyle and Boulding, 2000) and the pearl oyster *Pinctada margaritifera* (Benzie and Ballment, 1994). *Pinctada imbricata radiata* releases a large number of sperm and eggs for external fertilization at the same time (O'Connor and Lawler, 2004; Saucedo and Southgate, 2008). Development of the larvae lasts 16–30 days, the length of which is influenced by environmental factors such as water temperature, nutrition, and the availability of an appropriate settlement substrate (Gervis and Sims, 1992). These reproductive characteristics are suitable for random fertilization, resulting in a large population size over a wide geographic area. Consequently, low genetic differentiation among *P. imbricata radiata* populations will be seen over long distances. Interpopulation differences in morphometric features have been explained by environmental conditions, namely outcome of the phenotypic

plasticity and genetic variability (Colgan and Ponder, 2002). Variations in morphometric characteristics of *P. imbricata radiata* have been assigned to environmental conditions, in particular exposure to wave action, salinity, and temperature (Beaumont and Khamdan, 1991; Tlig-Zouari et al., 2010). Therefore, our results may suggest that there are different environmental conditions and also geographical barriers between Hendourabi and Lavan that have led to morphometric differentiation.

In summary, PCA and EFA support the idea that the Lavan and Hendourabi pearl oysters are separated from each other. The EFA and PCA methods can be easily applied to other pearl oysters. These methods are cheap, the relevant software is user-friendly, and they are less time-consuming than classical genetic alternatives. Nevertheless, EFA is considered a better approach than the traditional one (PCA), particularly for discrimination between shapes of *P. imbricata radiata* when characterized solely by the shell outlines. Since selection of landmarks for the shape analysis is performed automatically, EFA is thus a faster method compared with the traditional PCA, which needs several measurements for each individual. Although the differences between Lavan and Hendourabi populations might be due to the difference in geographic locations, genetic variability may explain the differences, an issue that needs to be investigated in a further study.

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