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Shell Morphometrics and Some Biochemical Aspects of the Pearl Oyster *Pinctada radiata* (Leach 1814) in Relation to Different Salinity Levels Around Bahrain

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ABSTRACT. Shell morphology of the pearl oyster *Pinctada radiata* (Leach 1814) collected from three different geographical areas on the north, east and west coast of Bahrain was analyzed. Muscle protein profiles and isozymes, using PAGE and SDS-PAGE, characteristics for these oysters were also studied. Substantial variation in shell morphometry was obtained with predominance of large-sized individuals (65-66, 75-80 mm) at both the north and the east coast, respectively, where the salinity levels were 40-42‰. Consistently smaller pearl oysters (35-40 mm) prevailed on the west coast of Bahrain where salinity levels ranged from 50 to 60‰. Some variations were observed among the phenotypic groups, only when PAGE and isozyme analyses were applied. In general, both approaches gave initially identical results with regard to the phylogenetic relationship of pearl oysters living in areas of differing salinities. SDS-PAGE gave no detectable differences among oysters from different sites.

The Arabian Gulf, located in a subtropical arid zone, is a highly stressfull environment due to excessive evaporation rate, shallow depth (ca. 100m.), and little river input. The Gulf is almost land-locked, and the only connection to the open ocean water is through the Straits of Hormuz. The turnover and flushing time have been estimated to be in the range of 3-5 years (Emery 1956, Reynolds 1993). This may explain the high salinity regime in the Gulf in comparison with other seas such as the Red Sea. Jones *et al.* (1978) attributed the reduced diversity of wide ranges of

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marine organisms to high salinity inside a lagoon along the Saudi Arabian coast. Similarly, Al-Sayed (1981) reported reduced littoral diversity in areas of high salinity in Abu Dhabi. Working on distribution patterns of coral reef communities around Bahrain, Sheppard (1985, 1988) reported low coral diversity with only 28 species of corals recorded. Staghorn coral (*Acropora* sp.) dominates the least saline and least turbid areas in the northern parts of Bahrain. He attributed the disappearance of *Porites* sp. communities to the greater salinity prevailing around Bahrain.

Despite the extreme environmental conditions of the Gulf, several marine organisms such as the pearl oyster *Pinctada radiata* are well established in offshore and nearshore areas around Bahrain (Rumaidh 1987). Natural populations of pearl oyster around Bahrain show considerable spatial differences in their sizes, with a predominance of large and small – sized individuals on east and west coasts of Bahrain, respectively. Environmental conditions, especially salinity, vary from one place to another around Bahrain. At the north and east coasts of Bahrain salinity ranges from 40-42‰, whereas it measures 50-60‰ on the western coast (Vousden 1988).

The present investigation was initiated to assess the variation in morphometric characteristics of pearl oysters collected from several locations of contrasting salinity around Bahrain, and to characterise and compare the muscale protein profiles of pearl oysters using some electrophoretic techniques such as PAGE, SDS-PAGE and isozyme characteristcs.

Materials and Methods

Sample collection:

Pearl oysters were collected randomly by SCUBA diving from three nearshore areas around Bahrain, namely, Saiyah (SY), Ras Hayan (RH) and Zallaq (ZQ) representing the north, east and west coasts, respectively (Fig. 1). Between 49 and 78 specimens were collected from each site, depending on the abundance of animals. The oysters were cleaned of extraneous encrustation on board ship. In the laboratory each shell was numbered and the shell length (SL), shell width (SW), hinge length (HL), hinge width (HW) and shell depth (SD) were recorded to the nearest 1mm using a plastic vernier caliper (Fig. 2).

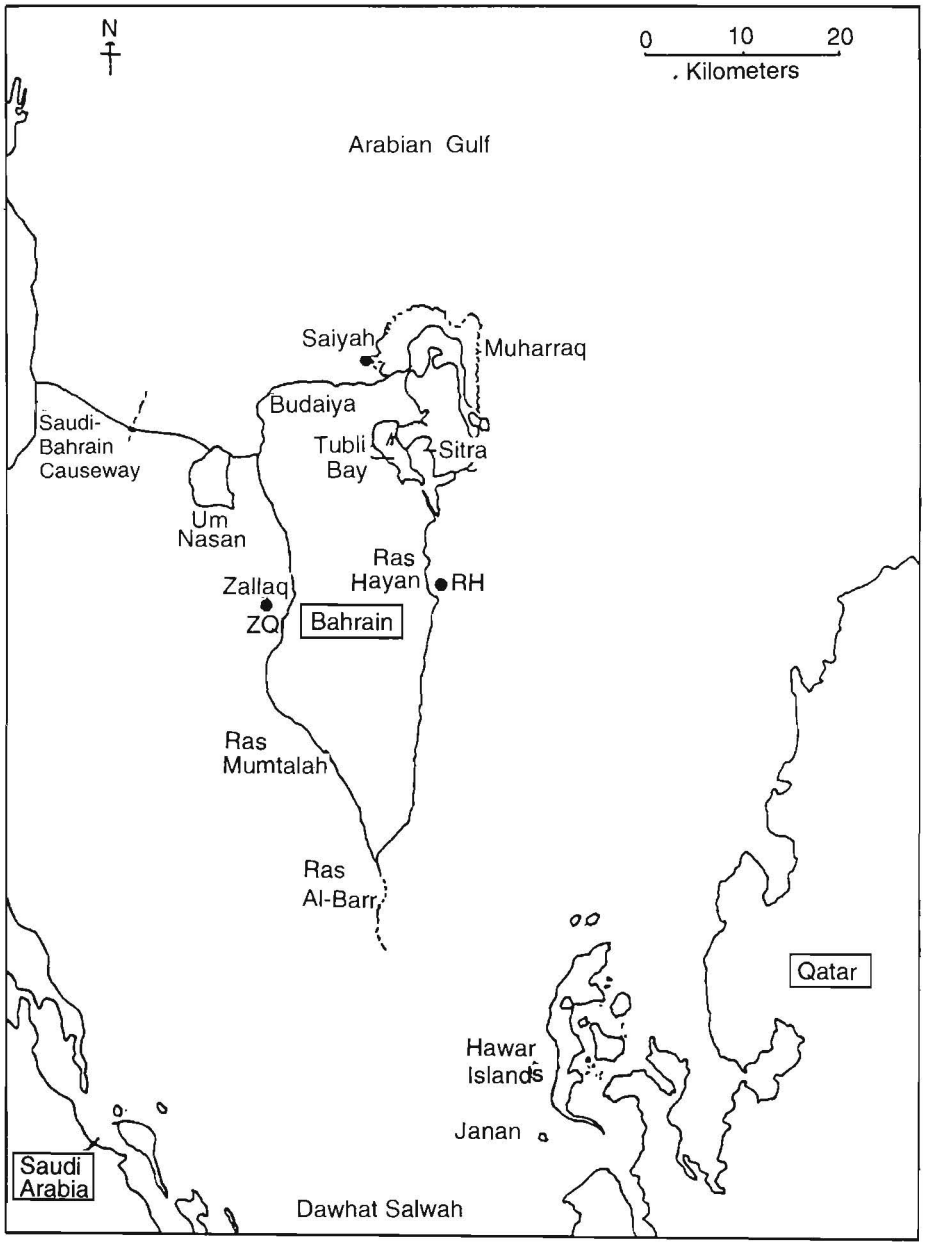

Fig. 1. Map of Bahrain showing sampling sites of *P. radiata* at east (RH), north (SY) and west (ZQ) coasts.

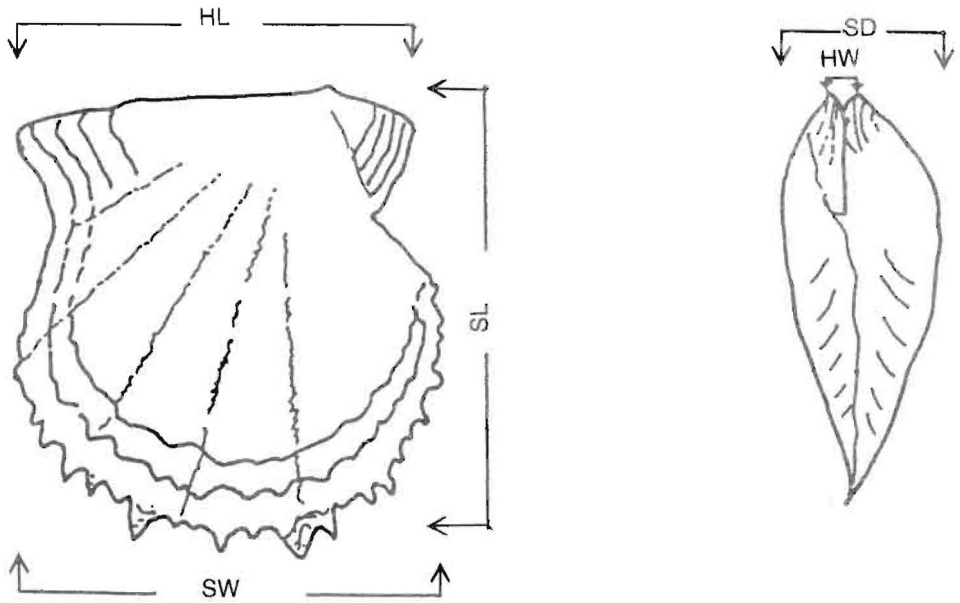

Fig. 2. Shell measurements of *P. radiata*, SL = Shell length SW = Shell width, HL = Hinge length, SD = Shell depth, HW = Hinge width.

Protein extraction:

One gram of each oyster adductor muscle was ground individually in 10 ml buffer (0.1M– Tris-HC1 + 1% SDS, pH 6.7) and then kept for one hour at 4° C. The homogenates was centrifuged (Beckman model J2-21, F1 USA) for 15 min. at 10000 rpm. Clear supernatants were collected and kept frozen until use. A number 10 individuals were analyzed from each site.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE):

Electrophoresis was carried out using a discontinuous buffer system (Laemeli 1970) and a sturdier slab gel electrophoresis unit (Mini-Protein II dual slab cell, Bio RAD, F1 USA). The protein concentrations of the samples were assayed using the technique of Lowry (Lowry *et al.* 1951). Protein samples containing 5% 2-mercapto-ethanol were boiled for 10 min. and uniform amounts of proteins were loaded directly on to 12% SDS-gels. Running conditions, staining and destaining were carried out according to standard procedures (Laemeli 1970). Molecular

weights of different bands were determined using an SDS protein mixture containing the following proteins: Phosphorylase b (94 KD), albumin (67 KD), ovalbumin (43 KD), carbonic anhydrase (30 KD), trypsin inhibitor (20 KD), α-lactalbumin (14 KD).

Extraction of native proteins for PAGE:

Buffer-soluble proteins were extracted by grinding 1 g of muscle from each oyster individual in 2 ml of phosphate buffer (pH 7.0) in a pestle and mortar and the mixture was kept overnight at 4° C. The homogenates were clarified separately by two successive centrifugations, firstly for 20 minutes at 4,000 rpm (Beckman CPR, refrigerated Centrifuge, U.K), and, secondly, in a high speed centrifuge (Beckman Model J2-21 refrigerated Centrifuge, F1, USA) at 15,000 rpm for 30 minutes. Protein was precipitated by the addition of an equal volume of cold 7.5% trichloracetic acid (TCA) to the supernatant. The solution was centrifuged at 10.000 rpm for 10 minutes to pellet the protein. The pellets were washed with cold acetone and then air dried. Finally, the pellets were resuspended in 200-300 µl buffer (0.1 M Tris-HC1, pH 6.7). Proteins were loaded on native gels as described in SDS-gels and the relative mobily values (Rf) were calculated.

Enzyme staining:

The Rf values of various bands were calculated. Each band was assigned a given number starting from the most rapidly migrating one, which was given number 1 and followed by number 2 for the second band, *etc.* Initially, enzyme stains were evaluated for resolution and stainability using the tri-glycine HC1 buffer system. However, only 3 enzyme stains produced well-resolved bands, *i.e.* esterase, leucine aminopeptidase, and catalase. Enzyme assays were performed as described by Bosland and Williams (1986). Data of Rf values were averaged from at least 5 separate runs.

Evaluation:

All data were analysed for statistical significance by using one-way analysis of variance (Statgraf Program). Multiple comparisons among means were made with Duncan New Multiple Range Test (Puri and Mullen 1980) Statistical significance was determined by setting the aggregate type 1 error rate at 5% ($p < 0.5$) for each set of comparisons.

Qualitative differences among *P. radiata* were scored visually, taking into consideration the whole profile of each one. Comparisons were made using the similarity index (Jaccard 1928) adopted to electrophoretic patterns (Hadocova *et al.* 1980). Similarity index (fs) = (100 C)/(A+B–C). (A = number of bands present in

one isolate; B = number of bands present in the compared taxon; C = number of similar bands between the two taxa to be compared). Data, as shown in Tables 1 and 2, were averaged from five gels (10 individuals for each site).

Results

Shell Morphometry:

The different phenotypic characters of the natural pearl oyster *Pinctada radiata* populations at 3 coastal sites were statistically analyzed using One Way Anova, and mean shell characters were subsequently compared by the Duncan's test (Table 1). The analysis indicates basic differences in shell morphometry among the different sampling stations around Bahrain. Shell length, which is the most commonly used parameter was apparently higher at RH (east coast) than at SY (north coast). The statistical comparison revealed no appreciable differences in all morphological features (SL, SW, HL and SD) between both these stations, with the exception of shell hinge width. However, oyster morphometry at both these sites was significantly higher compared to those of station ZQ (west coast) in all aespects ($p \leq 0.05$). Shell hinge width was the only phenotypic character that varied markedly ($p < 0.05$) among the 3 sites (Table 1), being the highest (3.78 mm) at station SY and the lowest (1.37 mm) at station ZQ. The oyster population at station SY (north coast) was dominated by large (60-65 mm length group) and relatively older individuals

Table 1. Shell morphometry (Mean ± S.E.) of *P. radiata* populations at different study sites around Bahrain (SY: north; RH: east; ZQ: west). Figures in the same column sharing the same superscript are not significantly different at $p \leq 0.05$

Stations	Shell length (mm)	Shell width (mm)	Shell depth (mm)	Hinge length (mm)	Hinge width (mm)
SY	67.14 ± 0.80^a	64.24 ± 0.84^a	27.13 ± 0.49^a	53.11 ± 0.74^a	3.78 ± 0.17^a
RH	70.31 ± 1.24^a	64.37 ± 1.45^a	27.40 ± 1.04^a	53.46 ± 0.96^a	3.24 ± 0.19^a
ZQ	31.74 ± 1.17^b	30.64 ± 1.17^b	11.76 ± 0.50^b	28.59 ± 0.9^b	1.37 ± 0.16^b
ANOVA	273.02*	176.98*	83.46*	183.46*	83.46*

*Statistically significant at $p < 0.05$.

(3-4 mm hinge width group) than those at station RH despite the prevalence of larger individuals (75-80 mm) at station RH, as indicated from frequency histograms of shell length and shell hinge width for each site (Figs. 3 and 4).

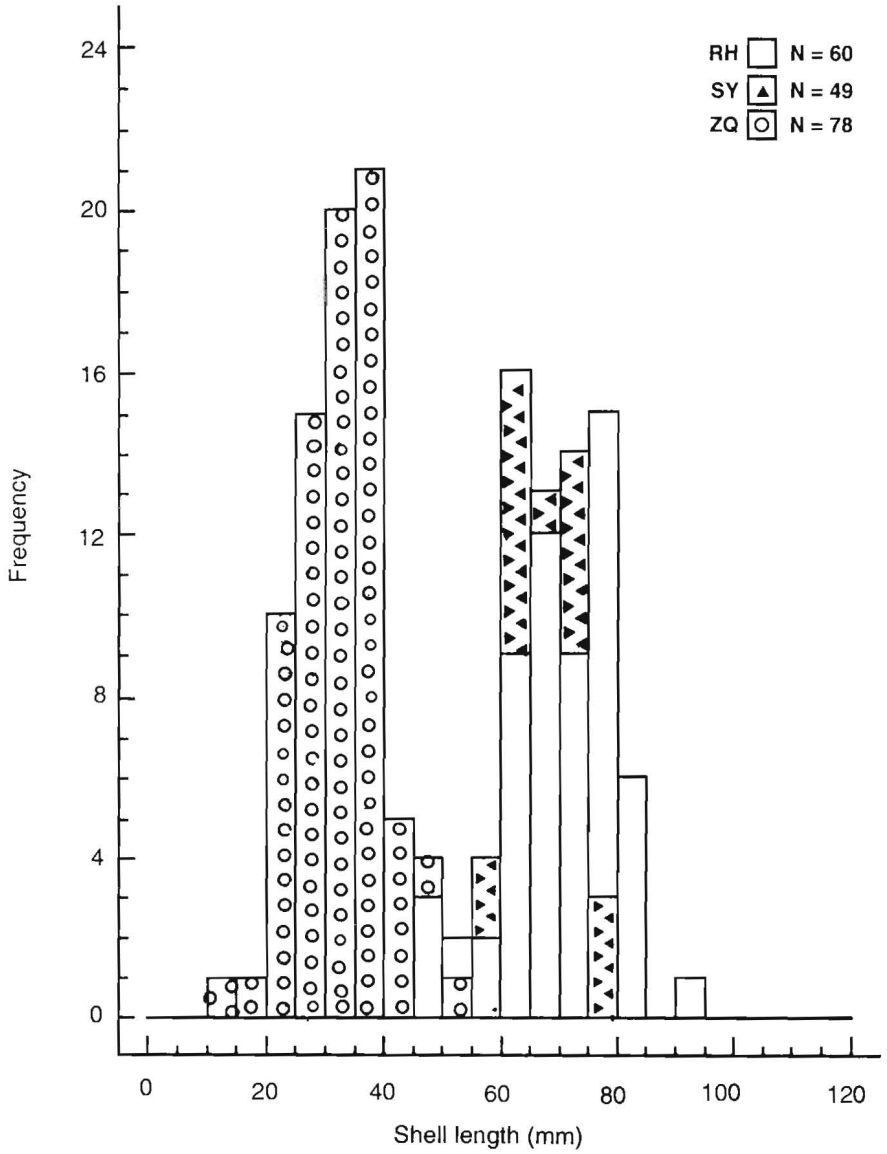

Fig. 3. Shell length frequency distribution of *P. radiata* at east (RH) north (SY) and West (ZQ) coasts.

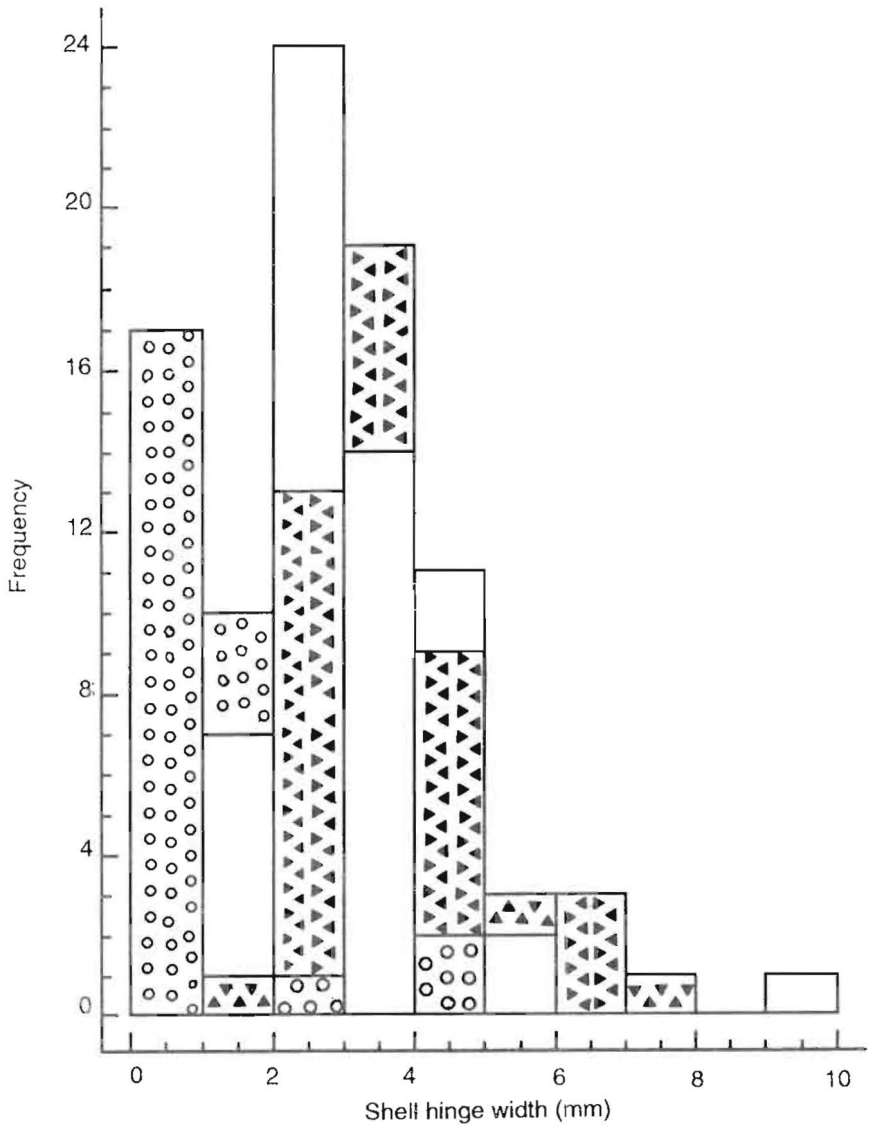

Fig. 4. Frequency distribution of shell hinge width of *P. radiata* at east (RH), north (SY) and west (ZQ) coasts.

Native Gels (PAGE):

Analysis of native proteins of different oyster individuals collected from several sites, using PAGE revealed heterogeneity in location and intensity of protein bands.

The difference in protein banding patterns was enough to allow quantitative and qualitative comparisons between different individuals. Qualitative assessment was carried out by visual inspection (Table 2) whereas quantitative comparison involved determination of similarity coefficients (Table 3). Analysis showed that oysters of different sites have four bands in common *i.e.* band no 2 (Rf 0.13), band no. 5 (Rf

Table 2. Protein banding patterns and Rf values of native proteins prepared from oysters of different sites. Each reading represents the average from 5 PAGE, (+): Clear band, (–): No band

Band No.	RH	SY	ZQ	Rf*
1	+	+	–	0.11
2	+	+	+	0.13
3	+	+	–	0.30
4	–	+	+	0.33
5	+	+	+	0.37
6	–	–	+	0.48
7	–	–	+	0.54
8	+	+	+	0.74
9	+	+	+	0.93

$$*Rf = \frac{\text{Distance of band migration (cm)}}{\text{Total migration distance (cm)}}$$

Table 3. Similarity indices (fs), using Jaccard index (Jaccard 1928), of protein banding pattern between *P. radiata* of different sites

	RH	SY	ZQ
RH	100	85.7	50
SY		100	62.7
ZQ			100

0.37), band no. 8 (Rf 0.74) and band no. 9 (Rf 0.93). Profiles of ZQ consistently featured bands no. 6 (Rf 0.48) and 7 (Rf 0.54) which were absent in oysters of other sites. Profiles of RH and SY were characterised by the presence of bands no. 1 and 3 (Rf 0.11 and Rf 0.30, respectively). Band 4 (Rf 0.33) was found only in profiles of SY and ZQ.

The observed variation in protein banding pattern among oysters of different sites was used to elucidate the degree of similarity among them. Similarity coefficient values for each banding pattern, were calculated and compared (Table 3). The data showed that profiles of RH and SY were closely related (fs 85.7). However, pairwise comparison indicated relatively distant relationship between RH and ZQ (fs 50), as well as between SY and ZQ (fs 62.7).

Isozyme Analysis:

The results of polyacralamyde gel of leucine aminopeptidase for about 10 individuals in each station are shown diagramatically in Fig. 5. A maximum of 3

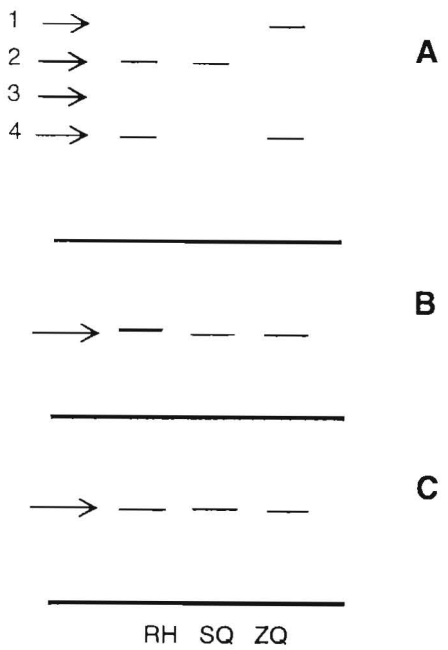

Fig. 5. Computer generated zymogram of three isozymes of pearl oyster collected from RH, SY and ZQ. Isozyme systems are **A** = *Leucine aminopeptidase,* **B** = *Catalase* and **C** = *Esterase.*

different bands was recorded among the profiles of each group. Variation included not only the number of bands but also the intensity of the bands. Accordingly, each profile has its own characteristic leucine aminopeptidase banding pattern. The three bands were polymorphic *i.e.* present in some individuals and absent in others. Complete similarity in banding pattern was not observed among zygmograms of all individuals. The profile of individuals belonging to SY showed only one band indicating homozygosity of monomeric protein. On the contrary, the other two stations (RH and ZQ) showed two bands-pattern reflecting a possible heterozygous nature of protein.

All individuals in the three sites showed only one monomorphic band for catalase and estrase isozymes. This monomorphic band is more dense in the catalase zymogram of RH individuals, when compared to individuals from the other two sites.

Discussion

In the present work, pearl oyster *Pinctada radiata* inhabiting coastal areas of contrasting salinities, exhibited considerable variation in gross shell morphology. In general, the pearl oyster *Pinctada radiata* inhabiting relatively low salinity, north and east, coastal habitats had shells which tended to be externally large, wide and deep, but internally appeared to be thick, lustrous and opaque. In areas of high salinity on the west coast, the shells exhibit greatly reduced dimensions when compared to the other two sites. The significant differences in the morphological characteristics recorded among the studied population could be attributed to the abundance and availability of food in low saline areas (Price *et al.* 1984). Moreover, hypersalinity is known to modify the osmoregulatory capacity of the membrane permeability system so that permeability to water decreases, while that to the ions increases. This would influence uptake of nutrients, accumulation of catabolites, and even slow metabolism. It seems that, although the pearl oyster is tolerant to high salinity, individuals in these areas had greatly retarded growth producing stunted forms. Such forms look identical externally to the oysters living in lower salinity areas. In this respect, the pearl oyster behaves in the same way as reported for two asteroid species, living under a high salinity regime. These two species demonstrated dwarfism as indicated by Price (1982).

In the present study electrophoresis has been used to characterize muscle protein profiles in individuals with a given geographical or temporal pattern. However, estimates of the genetic distance based on genetic polymorphism (allelic variation) between different oyster population were not the objective of this study.

Analysis of muscle proteins using SDS gels prepared from large and small-sized individuals collected from the east and west coast, respectively, revealed complete homogeneity in both band location and intensity. This can be attributed to the insensitivity of this technique to resolve minor differences in SDS-dissociated proteins. Furthermore, PAGE was used in this study, to characterize different phenotypic groups (large and small-sized) using water-extracted muscle proteins. Reproducible results were obtained by applying this technique to all members of the same oyster group. However, distinctive variation in native protein banding patterns (Table 2), and in leucine aminopeptidase (*Lap*) zymograms (Fig. 5) were clear and could be used to differentiate between the two different oyster sizes. The *Lap* profile for SY individuals characteristically showed one-banded pattern. On the contrary, the *Lap* profiles of the RH and ZQ individuals each showed uniquely two-banded pattern. Several studies (Gosling 1984, Bulnheim and Gosling 1988) have successfully used three partially diagnostic enzymes, namely octopine dehydrogenase (*Odh*) esterase-D (*Est-D*) and leucine aminopepitidase-1 (*Lap-1*), in differentiating two forms of mussels namely *Mytilus edulis* and *Mytilus galloprovincialis* based on allele frequency differences.

Our data, based on similarity index showed a close relationship in protein profiles between oyster individuals of station SY and station RH (fs = 85.7). On the contrary, less similarity was recorded when comparing profiles of station ZQ to station SY and RH profiles (fs = 50 and 62.5, respectively). Thus, the data are consistent with those obtained using the morphometric approach in differentiating between different sized oysters.

The loci of catalase and esterase (Fig. 5, B and C) were monomorphic in all tested individuals of the three sites. Accordingly, the electrophoretic results of these two isozymes were of insignificant importance.

In this study, the *Lap* locus clearly differs among the three populations. This conclusion is derived from analyzing 5^{-10} individuals at each station. However, future investigation of the *Lap* locus may reveal its significance as a diagnostic feature in ecological and systematic studies of oysters.

In conclusion, our study suggests that true identification of closely related pearl oyster forms, could not be achieved based on shell morphology alone, because morphology changes greatly in response to the prevailing environmental conditions. Accordingly, polyacrylamide gel electrophoresis and isozyme techniques, in conjunction with shell morphometry, could provide more reliable results with regard

to phylogenetic relationship among different forms of pearl oysters (Nei 1972, Avis 1975, and Ayala 1975). However, knowledge of physiological, immunological and cytological characteristics are commonly regarded essential for speciation among siblings (Gosling 1984).

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دراسة مورفولوجية وبيوكيميائية لمحار اللؤلؤ المجمع من مناطق بحرية مختلفة الملوحة حول البحرين

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في هذا البحث تم تجميع محار اللؤلؤ *Pinctada radiata* من مناطق بحرية مختلفة في شدة ملوحتها حول البحرين . وقد لوحظ اختلافات في حجم الأصداف المجمعة طبقاً للإختلاف في درجة ملوحة المنطقة . في المناطق الشمالية والشرقية حيث درجة الملوحة عالية (٤٠-٤٢ جزء في الألف) تم تجميع محار كبير الحجم نسبياً (٦٥-٦٦ ، ٧٥-٨٠ ملليمتر على الترتيب) . بينما المناطق الغربية ذات الملوحة المرتفعة نسبياً (٥٠-٦٠ جزء في الألف) صغيرة الحجم (٣٥-٤٠ مم في الطول) . هذا التباين في حجم الأصداف طبقاً للإختلاف في درجة ملوحة الماء في المناطق المختلفة تمت محاولة ربطه بإختلافات على مستوى كيمياء البروتين . ولهذا أجري طرق التفريد الكهربي المختلفة (PAGE, SDS- PAGE, Isozymes) لبروتينات العضلات لتقييم الإختلافات في حجم الأصداف ومدى إنعكاسها على الأختلافات في نوعية البروتين .

وهذا البحث يمثل الفصل الأول فقط في برنامج بحثي طويل الأمد نحاول الأجابة فيه عن تساؤل محدد وهو : هل الأختلافات الشكلية والحجمية بين محار اللؤلؤ المجمع من مناطق مختلفة في درجة ملوحتها ترجع فقط إلى الظروف البيئية أم الوراثية أم كليها معاً .