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Genetic and morphologic variations in cultivated blue mussels (*Mytilus edulis* L.) in two Scottish sea lochs

İskoçya'da iki deniz gölünde yetiştirilen mavi midyelerde (*Mytilus edulis* L.) genetik ve morfolojik değişimler

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Abstract

Three years old raft cultivated mussels, *Mytilus edulis L.* were collected from commercial mussel raft systems, and the external and internal shell characteristics measured and electrophoresis technique was applied for Loch Etive (LE) and Loch Kishorn (LK) mussel populations on the west coast of Scotland. The external shell characteristics results showed that LE mussels had higher and wider shell length than LK mussels ($P<0.05$). The hinge plate : shell length, anterior adductor mussel scar : shell length ($P<0.001$) and posterior adductor muscle-ventral margin : shell length ($P<0.05$) ratios were higher in LE mussels than LK mussels. However the number of teeth on the hinge plate ($P<0.001$), ligament margin : shell length ratio were higher in LK mussels than LE mussels ($P<0.05$). In contrast, posterior adductor mussel scar : shell length and length of the byssal retractor muscle scar : shell length ratios were not significantly different between the lochs ($P>0.05$). Genetic identity was found as 0.69 and genetic distance 0.37 between LE and LK mussel populations.

Key words: Mytilus edulis, genetic, morphological variations

Introduction

Marine mussels belonging to the genus *Mytilus* are widely distributed and have proved to be important as model organisms for physiological, biochemical and genetic investigation. There is a significant genetic differentiation throughout the geographic range of *M. edulis*; this can be observed over distances from a few meters to many kilometers (Gosling, 1992). Genetic differentiation are very often statistically correlated with patterns of environmental variation (Gosling and Wilkins, 1981; Skibinski *et al.*, 1983). Morphological studies do not fully take into account that the environment can substantially influence the morphological characteristics of a given species. Therefore, systematic information that is relatively free of environmentally induced changes is highly desirable. For comparison of closely related species, electrophoresis has proved to be most efficacious technique. Electrophoresis has been extensively used to address question of geographic variation between population and species level systematics in bivalves (Skibinski *et al.*, 1983; Sarver and Foltz, 1993). The use of electrophoresis to characterise individual and population differences in genetic composition has assisted greatly in elucidating the systematic and taxonomic status of species (Varvio *et al.*, 1988; McDonald *et al.*, 1991). *Mytilus edulis* is present all around the Britain and Ireland but at low frequency in south west England. *Mytilus galloprovincialis* is present in south west England, south and west coast of Ireland and the north east Scotland (Skibinski *et al.* 1983). The two forms commonly inbreed and it is still controversial whether they should be regarded as separate species, subspecies or merely as "varieties" (Varvio *et al.*, 1988). While allozyme characters are the primary means of distinguishing among *Mytilus*, it would be useful to be able to identify the species using shell characteristics. Most of the comparisons of shell characters between *M. edulis* and *M. galloprovincialis* have concentrated on sites where both species and their hybrids co-occur (Seed, 1978; Ferson *et al.*, 1985, Beamount *et al.*, 1989); because shell characters of mussels are influenced by the environment (Seed, 1968). Sexual reproduction, genetic recombination and extremely large effective population size are all conducive to the maintenance of large amounts of genetic variation within mussel population. Over last two decades, there has been both geographically extensive and local intensive study of genetic differences among natural populations of *Mytilus* (Koehn *et al.*, 1976; Theisen, 1978; Goslind and Wilkins, 1981; Bulnheim and Gosling, 1988; Gartner-Kepkay *et al.*, 1983). Growth, condition index studies and cross-transplantation between Loch Etive and Loch Kishorn mussels showed that there were some reproductive and morphologic differences between two populations (Karayücel, 1996,1997; Karayücel and Karayücel, İ. 1997,1998). Therefore aim of the present study was to assess the genetic and morphologic variation between the Loch Etive and Loch Kishorn mussel populations.

Material and Methods

Sample collection

Shell morphometrics and genetics of raft cultivated mussels (*Mytilus edulis* L.) were studied in Loch Etive (LE) and Loch Kishorn (LK) on the west coast of Scotland (Fig. 1). Mussel samples (3 years old) were collected from commercial raft cultured ropes by hand and transported alive to Institute of Aquaculture laboratory in a cool box. 128 mussels from Loch Etive and 134 mussels from Loch Kishorn were used to measure shell characteristics and electrophoresis. The mussels were cleaned of fouling organisms (epibiotic growth). Shell length, height and width were measured by using calipers accurate to 0.1 mm while the other shell traits (length of posterior adductor muscle scar (pam), length of posterior retractor muscle scar (lbrs), distance between ventral edge of posterior adductor muscle scar and ventral margin of shell (pam-vm), length of anterior retractor muscle scar (arms), length of anterior adductor muscle scar (aams), length of hinge plate (hp) and ligament margin (lm)) were measured under a stereomicroscope (McDonald *et al.*, 1991) (Figure 2).

Horizontal starch gel electrophoresis technique was used to determine genotypic differentiation between Loch Etive and Loch Kishorn mussels. A small piece of digestive gland and adductor muscle tissue was sampled from each mussel using a scalpel and scissors and put in small plastic tubes (Eppendorf). Samples were stored separately at 70°C until further use.

For electrophoresis, tissues were taken from the deep freezer, thawed for a few minutes and then placed in ice. The samples were moistened with 0.5 ml of 0.1 molar buffer (mixture of tris: 30.25 g; EDTA: 29 g; boric acid: 7.3 g and $MgCl_2$ $6H_2O$: 5.08 g was dissolved in distilled water to make 2500 ml buffer (pH:8)) and purified sand was added to the tubes; the sample was then homogenized using a glass rod and centrifuged at 6.000 rpm for 15 min. Samples were absorbed onto 10 x 2 mm pieces of Whatman No.1 filter paper.

Preparation of Starch Gel

About 66 g starch (Sigma Ltd.) was mixed with 450 ml of distilled water and 50 ml 0.1 molar buffer solution in a Buchner flask. The mixture was heated with constant rotation of the flask to an almost translucent jelly state, quickly degassed using a vacuum water pump and then poured into 6 mm thick gel frames. The gels, covered with a glass plate, were allowed to set and cool overnight at room temperature, or for 1-2 hours at 4°C in a refrigerator. Then gel was taken out of the frame and a parallel cut was made 3 cm from the edge to create an origin. The samples (filter paper) were placed along this cut with about 25-30 samples per gel and one tracking dye (0.1 % phenol blue) at the each end of the gel to indicate mobility through the gel. When all samples were correctly arranged, the frame was placed back on the gel and a perspex spacer positioned

between the gel and frame to keep the sample slot closed (to keep the sample tight).

The gel was then placed in an electrophoretic bath with a buffer. A gauze wick soaked in the buffer was applied to either end of the gel to connect the gel and buffer. The gel was then covered with a plastic sheet to reduce evaporation and ice in a plastic bag was placed onto the plastic sheet to prevent heating of the gel. The bath tray was covered with a transparent lid and placed in a refrigerator at 4°C.

The gel was allowed to run for one hour with an electrical current of 45 mA, and then the filter papers were removed and the gel was run again overnight with a 30 mA current. The following morning, the gel was taken from the refrigerator and removed from the bath. It was then sliced horizontally into three slices and stained for glucose phosphate isomerase (GPI, 5.3.1.9) and phosphoglucomutasse (PGM, 2.7.5.1) (Beaumont *et al.*, 1989).

The appropriate stains for the enzyme system to be examined were weighed and mixed with staining buffer solution as above and 2 % agar (at approximately 50-60 °C). This mixture was poured over the slice allowed to set and then incubated at 37°C until the banding patterns became visible. These enzymes were chosen for their staining properties and stability in *Mytilus edulis* (Beaumont *et al.*, 1988). The electropherograms were then analyzed and scored for the respective genotypes and when necessary they were preserved in gel fixative solution (mixture of 4 units ethyl alcohol, 1 unit distilled water and 5 units acetic acid). Finally, they were dried to seal onto filter paper for storage.

Shell trait differences between the lochs were tested by one-way ANOVA. Allele frequencies, heterozygosity and Hardy-Weinberg distribution were performed according to Ferguson (1980), while genetic identity and distance were calculated according to Nei (1972).

Results

Visual observation of shell colour and shape between the Loch Etive (LE) and Loch Kishorn (LK) mussels showed distinguishable differences between the sites. Mussel from LE had a very dark bluish-black color compared to the brownish or brownish-black colour of LK mussels. Mussels from LE had a higher height : length ratio and width : length ratio i.e they had a broader and wider body shape than LK mussels ($P<0.05$). Retractor muscle scar (lbrs) and ligament margin (lm) were higher in LK than LE ($P<0.05$). The teeth frequency is shown in Figure 3. The average tooth number was found to be higher in LK (7.71±0.41) than LE (2.56±0.36) ($P<0.001$). The number of teeth was found 4.0±0.21 on the right valve and 3.71±0.21 on the left valve in LK mussels while it was 1.56±0.24 on the right valve and 1.0±0.15 on the left valve in LE mussels.

Mean ratios of shell characteristics also showed significant difference between the sites. The mean (± SE), minimum and maximum ratios of shell traits are given in Table 1. The hinge : plate shell length, anterior adductor mussel scar : shell length ($P<0.001$) and posterior adductor muscle-ventral margin : shell length ($P<0.05$) ratios were higher in LE mussels than LK mussels. However ligament margin : shell length ratio was higher in LK mussels than LE mussels ($P<0.05$). In contrast, posterior adductor scar : shell length and length of the byssal retractor muscle scar : shell length ratios were not significantly different between the sites ($P>0.05$).

Allele frequencies, at each of two loci examined for LE and LK mussels are presented in Table 2. Allele frequencies are given in order of decreasing anodal mobility; 110 is the fastest and 75 is the slowest. The most common allele was GPI^{100} (Glucose phosphate isomerase) with 60 % occurrence in LE and 33 % in LK. Whereas the most common alleles were PGM^{90} (Phosphoglucomutase) and PGM^{95} with a 33 % occurrence in LE, while PGM^{90} was the most common allele with a 42 % in LK.

The calculated heterozygosity was found to be 0.50 for PGM in LE and LK while it was o.47 and 0.50 for GPI loci in LE and LK respectively. The observed heterozygosity was 0.33 in LK and 0.40 in LE for PGM loci. However for GPI loci, observed heterozygosity was 0.38 in LK and 0.45 in LE. The Hardy-Weinberg distribution for frequencies of genotypes is given in Table 3. The similarity of observed and expected values support the hypothesis that the populations are in Hardy-Weinberg equilibrium. Nei's index for genetic identity was used the similarity among the two populations and was found as 0.69 while genetic distance was 0.37.

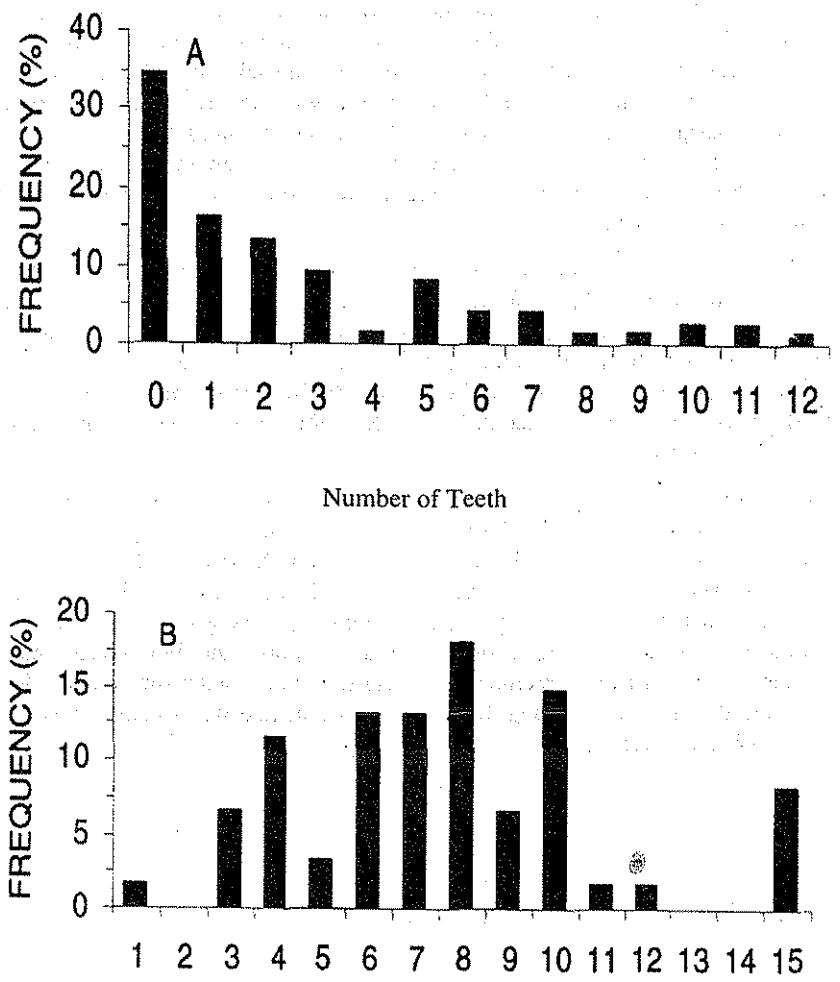

Figure 3. Frequency of hinge teeh in Loch Etive (A) and Loch Kishorn (B) mussel populations.

Table 1. Mean, standard error (SE), minimum and maximum ratios of shell characteristics of Loch Etive and Loch Kishorn mussels measured in this study. L: shell length; hp: hinge plate; arms: length of anterior retractor muscle scar; aams: anterior adductor muscle scar; pam: posterior adductor muscle scar; lbrs: length of byssal retractor muscle scar; pam-vm: distance between ventral edge of posterior adductor muscle scar and ventral margin of shell; lm: ligament margin; W: shell width; H: shell height. LE: Loch Etive; LK: Loch Kishorn. Common superscripts in the same column are not significant.

Site		hp: L	arms: L	aams: L	pam: L	lbrs: L	pam-vm: L	lm: L	W:L	H:L	W:H
LE	Mean	0.0108^{b}	0.0077^{a}	0.0108^{b}	0.0148^{a}	0.0285^{a}	0.0287^{b}	0.0424^{a}	0.396^{b}	0.525^{b}	0.756^{a}
	SE	0.0002	0.0002	0.0002	0.0002	0.0004	0.0003	0.0006	0.0004	0.001	0.002
	Min	0.0084	0.0037	0.0081	0.011	0.0208	0.0230	0.0252	0.34	0.47	0.63
	Max	0.0142	0.0133	0.0137	0.0192	0.0383	0.0369	0.0562	0.47	0.59	0.92
LK	Mean	0.0095^{a}	0.0081^{a}	0.0090^{a}	0.0143^{a}	0.0288^{a}	0.0267^{a}	0.0453^{b}	0.369^{a}	0.492^{a}	0.752^{a}
	SE	0.0001	0.0002	0.0001	0.0002	0.0004	0.0003	0.0004	0.004	0.0001	0.001
	Min	0.0079	0.0059	0.0071	0.0120	0.0226	0.0225	0.0378	0.32	0.44	0.63
	Max	0.0125	0.0136	0.0115	0.0181	0.0359	0.0316	0.0537	0.43	0.53	0.90

Table 2. Allele frequencies of mussel (*Mytilus edulis*) from Loch Etive (LE) and Loch Kishorn (LK) populations. GPI: Gluco phosphate isomerase, PGM: Posphoglucomutase.

Locus	Site	110	105	100	95	90	85	80	75
GPI	LE	0.00	0.02	0.60	0.02	0.32	0.02	0.02	0.00
	LK	0.06	0.12	0.33	0.28	0.17	0.06	0.00	0.00
PGM	LE	0.00	0.056	0.139	0.334	0.334	0.083	0.056	0.00
	LK	0.00	0.00	0.073	0.122	0.415	0.146	0.146	0.098

Table3. Observed distribution and expected Hardy-Weinberg equilibrium distribution of genotypes for posphoglucomutase (PGM) and glucose posphate isomerase (GPI) locus in Loch Eitive (LE) and Loch Kishorn (LK) mussel populations.

			GENOTYPES				
Loci	Site		AA	AB	BB	$\chi 2$	p
PGM	LE	Observed	30.55	44.44	25.0	0.727	0.695
		Expected	27.86	49.84	22.3		
PGM	LK	Observed	26.47	41.18	32.35	1.485	0.476
		Expected	22.15	49.83	28.03		
GPI	LE	Observed	45.71	42.86	11.43	0.222	0.989
		Expected	45.02	44.18	10.79		
GPI	LK	Observed	35.00	32.50	32.50	6.226	0.046
		Expected	26.26	49.96	23.77		

Discussion

The electrophoretic technique makes it possible to compare allele frequencies and levels of genetic variability within and between different populations of a species and between different species. Allele frequencies were shown to be different between the sites and loci. Ferguson (1980) reported that samples of species taken from different areas may differ significantly in their allelic frequencies. This could be due to selection for different homozygotes under varying environmental conditions or to genetic drift in isolated populations. Murdock *et al.* (1975) declared that two populations only 100 m apart had quite different allelic frequencies, while some widely (350 km) separated populations had almost identical allelic frequencies. In this case a significant correlation was found between allelic frequencies and the relative amount of wave action (exposure) at the site investigated. The microgeographic differentiation described by Gartner-Kepkay *et al.* (1983) between sites and led them to suggest that "environmental selection is the most likely explanation for the genetic differences apparent among population facing extensive gene flow. However Koehn *et al.* (1984) reported that differentiation of two populations could be attributed solely to population genetic mechanism, rather than systematic differentiation (i.e. reproductive isolation). Ferguson (1980) suggested that in a widespread panmictic population, selection might produce differential survival in different regions and result in allelic frequency variation. In Ireland and U.K., geographic variation has been interpreted as resulting from the mixing of *M. edulis* and *M. galloprovincialis* (Gosling and Wilkins, 1977, 1981; Skibinski and Beardmore, 1979). However some areas e.g. north west Europe and east coast of U.S.A., south of Cape Cod, where only pure population of *Mytilus edulis* have been analysed, allele frequencies within each region are remarkably homogeneous over large geographic distances (Gosling and Wilkins, 1981; Skibinski *et al.*, 1983; Koehn *et al.*, 1984; McDonald *et al.*, 1991).

At the GPI locus, six alleles were observed in *Mytilus edulis* in Loch Etive and Kishorn and two most common alleles were dominant. In contrast, at the GPI locus up to nine alleles have been observed in populations of *Mytilus edulis* on the east coast of North America. In the present study, genetic identity was 0.69 and genetic distance 0.37. These results show that there is similarity between the populations at the two sites, but genetic distance between the sites show that the populations are not pure. There are some polymorphism and heterozygosity for two populations. Unfortunately there is no published data to compare on the genetic of mussels in two sites on the west coast of Scotland. However cross-transplantation and growth experiments (Karayücel, 1996) showed that there are some significant differences in shell morphology and spawning periods (Karayücel and Karayücel, 1997) in cultivated mussels of two populations. Present study showed that mussels from Loch Etive have higher and wider shell than Loch Kishorn mussels. There were also some significant differences in internal shell characteristics (Table 1). Similar shell characteristics were reported

by Kautsky *et al.* (1990) from the Baltic sea mussels having a more narrow and elongated shape than North sea mussels. Gosling (1992) reported that in South-West England, hybridization, but little integration, was occurring between *M. edulis* and *M. galloprovincialis*, but at other localities e.g. east and north east parts of Scotland, North-East England at the exposed sites on the Atlantic coast of Ireland, integration between this species is extensive (Skibinski and Beardmore, 1979; Gosling and Wilkins, 1981). Overall shell shape in *Mytilus* is so variable, both within and between the two forms of mussels, that it has little if any value in taxonomic studies. The differences between the two forms of mussels are greater than between geographically isolated populations of most species, they are hardly large enough to justify *M. galloprovincialis* being considered a distinct species. Gosling concluded (1984) that *M. galloprovincialis* could not be regarded as more than a race or subspecies of *M. edulis*. Identification on shell characters alone is difficult even impossible. Seed (1972), in a detailed morphological survey of mussels from sixteen locations on the French coasts, points out that over 30% of all the mussels examined during the investigation would have been misidentified on external characters alone. Similar problems of identification have been encountered in south west England. In a survey of Irish mussel populations, Seed (1974) reported that gross shell morphology is completely unreliable in separating the two forms; mussels of every conceivable shape were encountered from one locality to another. The shell of *M. galloprovincialis* tends to be higher and flatter than in *M. edulis*, giving distinctly different transverse profiles in the two forms. The anterior adductor scar and hinge plate size have generally been regarded as more reliable in separating two forms (Seed, 1978) while the mean adductor scar ratios (adductor scar length/shell length) vary from one locality to another the values tend to be consistently lower in *M. galloprovincialis* than in *M. edulis*. In the present study, mussels from Loch Etive had higher and wider shell length ($P<0.05$) than Loch Kishorn mussels. The ratio of adductor scar length : shell length ratio and hinge plate : shell length ratio is found significantly higher in Loch Etive than Loch Kishorn mussels. Unfortunately there is not any similar experiment on the experimental site or pure *M. galloprovincialis* site to compare with our findings. Karayücel and Karayücel (1997) declared that salinity in LK was significantly higher than LE. All these results show that Loch Etive and Loch Kishorn mussel populations differ in genetic structure and shell characteristics as a result of environmental characters i.e. salinity. This result is in agreement with several authors who reported significant affect of salinity and temperature on allele frequencies (Koehn *et al.*, 1976; Levinton and Suchanek, 1978).

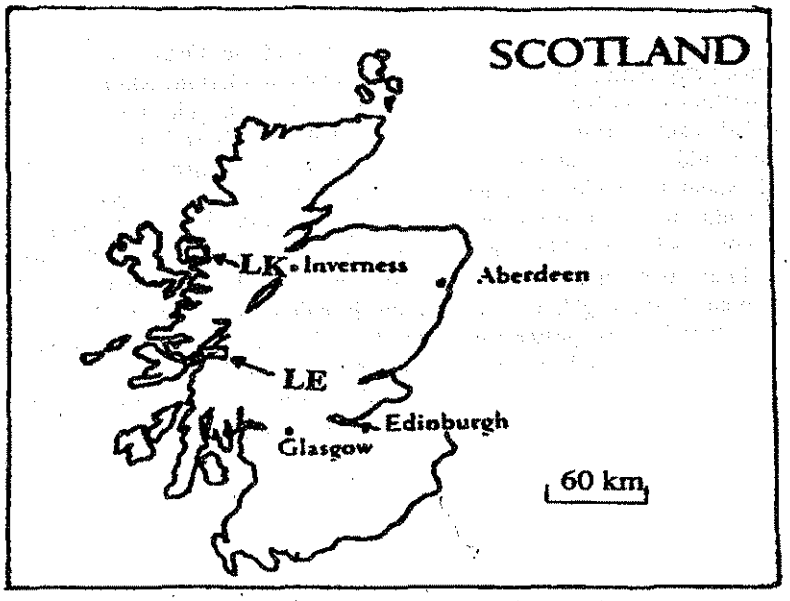

Figure 1. Map of Scotland shows experimental sites. LE: Loch Etive, LK: Loch Kishorn.

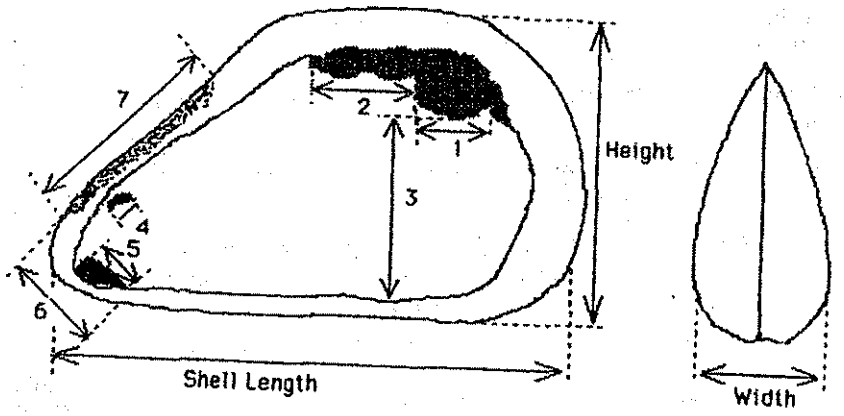

Figure 2. Shell terminology and paremeters measured in this study. 1: length of posterior adductor muscle scar (pam), 2: length of posterior retractor muscle scar (lbrs), 3: distance between ventral edge of posterior adductor muscle muscle scar and ventral margin of shell (pam-vm), 4: length of anterior retractor muscle scar (arms), 5: length of anterior adductor muscle scar (aams), 6: length of hinge plate (hp) and 7: ligament margin (lm).

Özet

İskoçyanin batısındaki Etive gölü ve Kishorn gölünde ticari olarak raft (sal) sisteminde yetiştiriciligi yapılan 3 yaşındaki midyelerin (*Mytilus edulis* L.) iç ve dış kabuk özellikleri ölçülmüş ve electrophoresis tekniği uygulanmıştır. Dış kabuk karakteristik özellikleri göstermiştir ki Etive gölündeki midyelerin kabukları Kishorn gölündeki midyelerin kabuklarından daha yüksek ve geniştir ($P<0.05$). Hinge plate'nin kabuk boyuna oranı ve anterior adductor kas izinin kabuk boyuna oranı ve posterior adductor kas ile ventral margin arasındaki mesafenin kabuk boyuna oranları Etive gölünde Kishorn gölüne oranla daha yüksek bulunmuştur ($P<0.05$). Bununla birlikte hinge plate üzerindeki diş sayısı ($P<0.001$), ligament margin'in kabuk boyuna oranı Kishorn gölünde Etive gölünden daha yüksek olarak elde edilmiştir ($P<0.05$). Buna karşılık, posterior adductor kas izinin kabuk boyuna oranı ve byssal retractor kas izi boyunun kabuk boyuna oranı iki göl arasında farklılık göstermemiştir ($P>0.05$). Etive gölü ve Kishorn gölü midye populasyonları arasındaki genetik benzerlik 0.69 ve genetik farklılık 0.37 olarak bulunmuştur.

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