NAFO Sci. Coun. Studies, 6: 73-78

Genetic Structure of Mussel Populations in Eastern Canadian Waters*

L. M. Dickie
Marine Ecology Laboratory, Bedford Institute of Oceanography
P. O. Box 1006, Dartmouth, Nova Scotia, Canada B2Y 4A2

Abstract

Recent observations on the common blue mussel, *Mytilus edulis*, make it possible to discern for a marine species features of the genetic structure of local populations and the relationship between this genetic structure and important production parameters. The results provide an instance in which the population differences can be related to both growth and mortality rates under natural conditions, and thus indicate the potential impact of genetic selection on productivity.

Genetics of the Mussel Populations

Introduction and results

Recent studies of the genetic constitution of blue mussel populations in eastern Canadian waters have involved both macrogeographic and microgeographic observations. Sampling locations are illustrated in Fig. 1. In the macrogeographic study, mussels were sampled from near or below the low-water level at four localities. Two of these were on the Atlantic coast of Nova Scotia in Bedford Basin (BB) and at a reference station near Luke Island (LI) in St. Margaret's Bay (SMB), the third was located near the head of the Bay of Fundy (BF), and the fourth at Ellerslie on Prince Edward Island (PEI) in the Gulf of St. Lawrence. The sites were chosen to provide a contrast between the relatively stable environments along the Atlantic coast of Nova Scotia and the more variable and seasonably extreme environments of the Bay of Fundy and southern Gulf of St. Lawrence regions.

The microgeographic study involved sampling at six sites within St. Margaret's Bay. In addition to the reference station (LI), "open water" stations were located at Shut-In-Island (SII) and Northwest Cove (NWC), and "head-water" stations at Potato Island (PI), Todd Island (TI), and Frenchman's Point (FP). The openwater stations were chosen as instances of population sites in relatively stable oceanic environments, whereas the head-water stations were near tidal flats in areas of short-term and seasonal extremes of both temperature and salinity conditions. The head-water sites were intended to be examples of variable environments in parallel with the macrosampling sites in the Bay of Fundy and at Prince Edward Island.

The isoenzyme technique was adopted to characterize genetic constitutions of the mussel populatons. Horizontal starch gel electrophoresis, using the discontinuous Paulik buffer systems of Singh and Zouros (1978), was carried out on samples of 100 specimens chosen from collections of 200-300 medium-sized mussels taken at each of the sampling sites. After preliminary experiments, ability to record multiple alleles at four gene loci was verified for the macrogeographic collections (Gartner-Kepkay *et al.*, 1980): leucine aminopeptidase (LAP-1), peptidase 2 (PEP-2), phosphoglucose isomerase (PGI), and phosphoglucose mutase (PGM). Subsequently, aminopeptidase (AP) was added as the fifth locus (Gartner-Kepkay *et al.*, 1983) which was used in the micrographic study. The general results can be illustrated by the data for gene loci LAP-1 and PGM (Fig. 2), the upper and lower

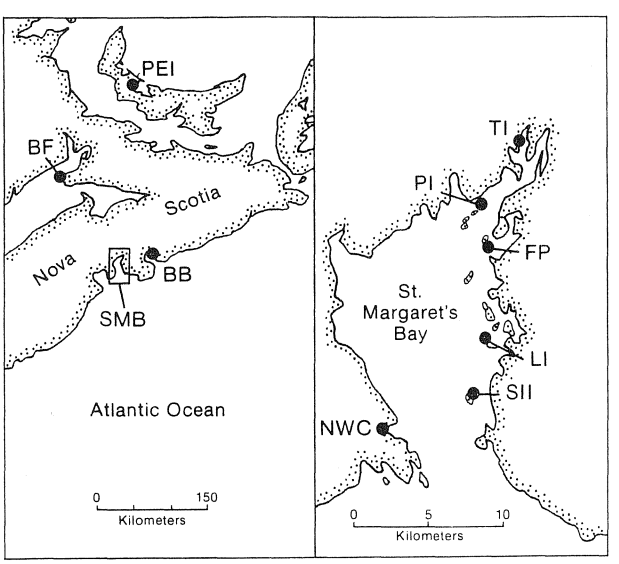

Fig. 1. Collection sites for the macrogeographic (left) and microgeographic (right) studies on blue mussel populations in eastern Canadian waters. (BB = Bedford Basin, BF = Bay of Fundy, FP = Frenchman's Point, LI = Luke Island, NWC = Northwest Cove, PEI = Prince Edward Island, PI = Potato Island, SII = Shut-In-Island, SMB = St. Margaret's Bay, and TI = Todd Island.)

* Presented at the Special Session on "Stock Discrimination in Marine Fishes and Invertebrates of the Northwest Atlantic", held at the Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada, during 8-10 September 1982.

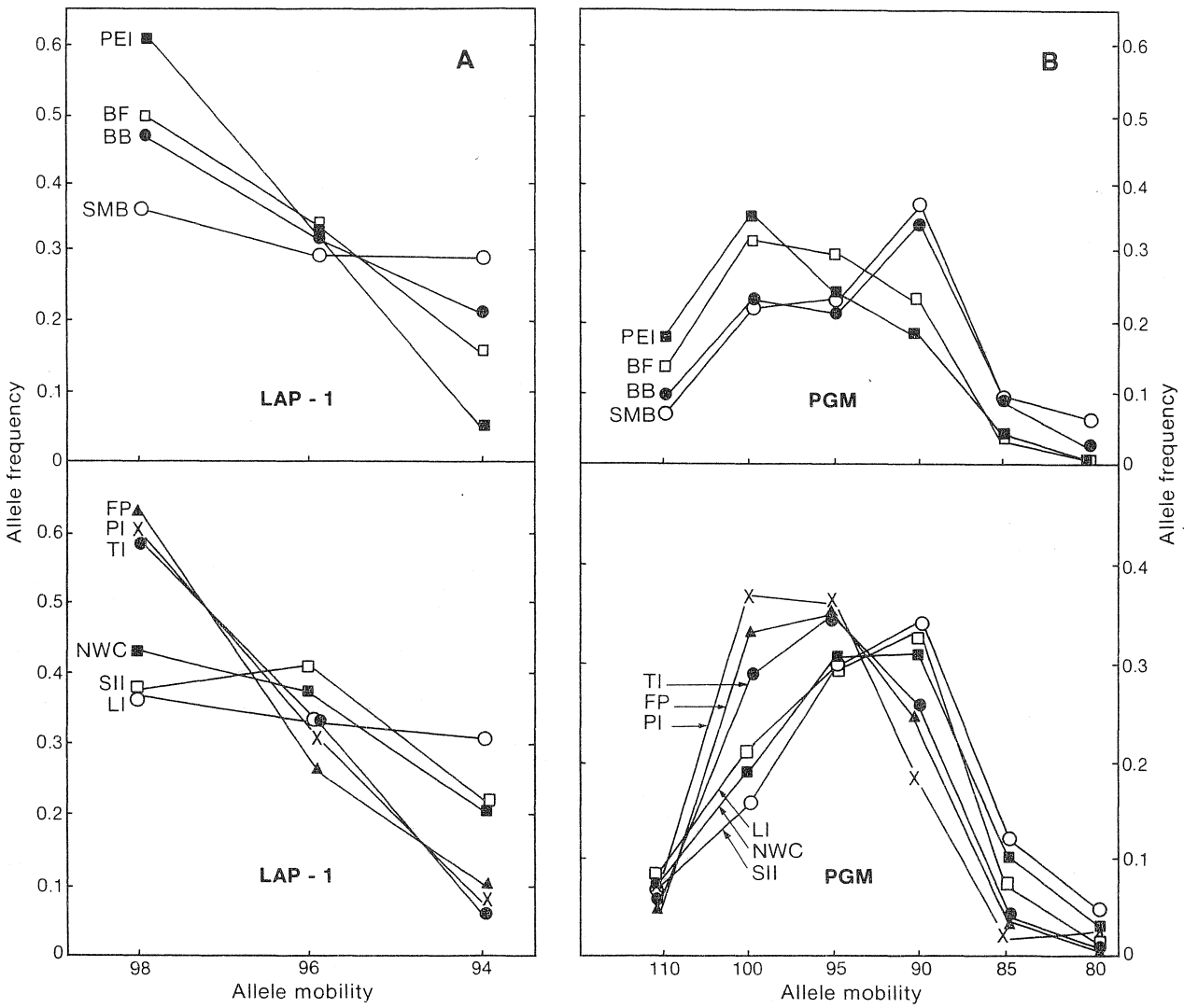

Fig. 2. Allele frequency distributions for the isoenzymes LAP-1 (A) and PGM (B) from electrophetic analyses of blue mussels in macrogeographic (upper panels) and microgeographic (lower panels) studies at sites identified in Fig. 1.

panels containing the results of the macrogeographic and microgeographic studies respectively.

For the LAP-1 locus in the microgeographic study (Fig. 2A, lower panel), samples from the three open-water sites show approximately equal proportions of each of the three alleles recorded, whereas the samples from the head-water (variable environment) sites show a high proportion of fast-migrating alleles and a low proportion of slow-migrating alleles. A similar phenomenon is shown by the macrogeographic study (Fig. 2A, upper panel). Here the Bedford Basin sample is most like the open-water St. Margaret's Bay sample, the Prince Edward Island sample is similar to the head-water samples and the Bay of Fundy sample appears to be intermediate. In fact, results for the other alleles recorded in the macrogeographic study indicate that PEI-BF may be regarded as one pair and SMB-BB as another, even if in this instance the two pairs were close to overlapping.

A parallel difference between the open-water and head-water samples may be seen in data for the PGM locus (Fig. 2B). In this case, there were six alleles common to the nine sampling sites. The microgeographic sampling in St. Margaret's Bay (lower panel) again shows differentiation into two groups, and the macrogeographic samples show the Atlantic coast sites (BB, SMB) to be distinct from those (PEI, BF) where variable environmental conditions exist.

Results for the other two isoenzymes (PEP-2 and PGI) used in the macrographic study were similar to those in Fig. 2. Separation on the basis of environmental type was not so clear for the PGI data from St. Margaret's Bay, but statistical comparisons among successive pairs of samples from the nine populations reinforced the conclusions that these allele frequencies represent two population types. Accordingly, indices of genetic identity between sample locations were calculated (Nei, 1972) from the data for the four isoen-

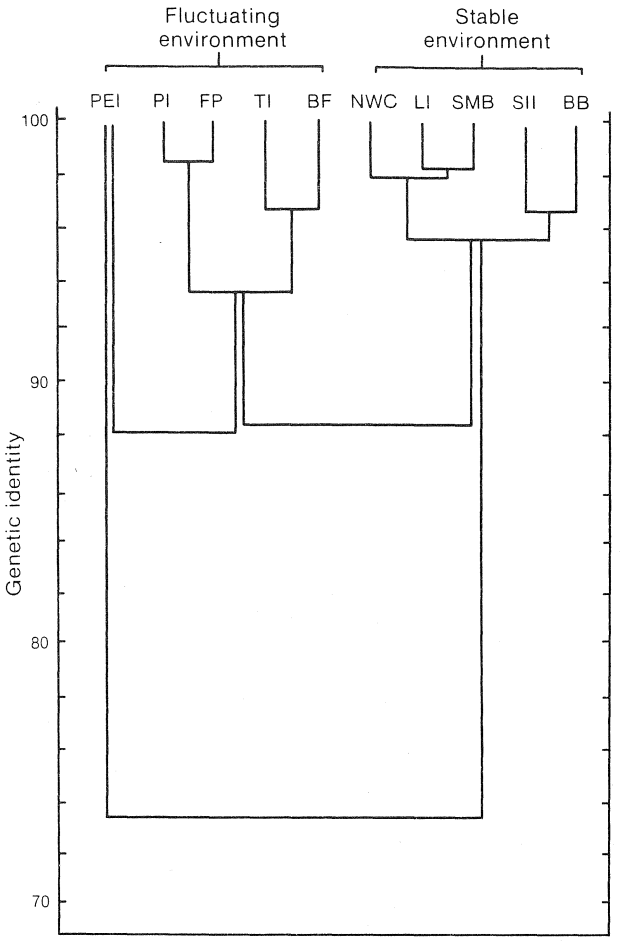

Fig. 3. Dendrogram of genetic identities of blue mussel populations based on frequency distributions of four isoenzymes for samples collected at sites identified in Fig. 1.

zymes. The results are summarized in a dendrogram (Fig. 3), which shows strong similarities among localities classed within the "fluctuating" or the "stable" environment but much weaker parallels between the two environmental types.

By contrast with this generalization, the results from the additional gene locus (AP) in the microgeographic study show no differences among localities (Fig. 4). Four alleles were identified at this locus in populations at all sampling sites in St. Margaret's Bay, but only one locality showed a slight deviation from an apparently common frequency distribution among localities. This contrasting pattern is significant in interpreting the overall results.

One further set of data is useful in interpreting the evidence of genetic population segregation in the mussel. It is well-known from isoenzyme studies of bivalue mollusc populations that allele frequencies at the post-settlement stage tend to show a deficiency in the relative occurrence of heterozygotic animals in the population relative to the proportions that would be expected at genetic equilibrium (Levinton and Koehn,

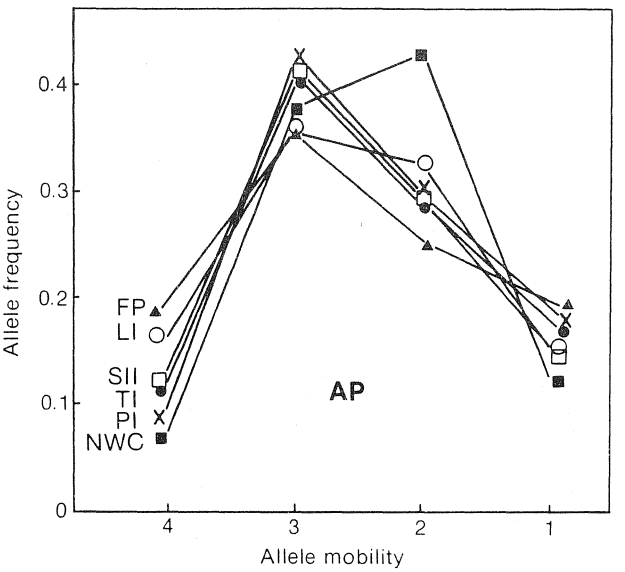

Fig. 4. Allele frequency distributions for isoenzyme AP from electrophoretic analysis of blue mussels at sites identified in Fig. 1.

1976; Zouros and Foltz, 1984). This characteristic was also evident in the data considered in this paper. The results of heterozygote deficiency for the five isoenzymes in samples from the six sites in St. Margaret's Bay relative to the frequency expected at Hardy-Winberg equilibrium are given in Table 1. Deficiencies range from 30 to 70% for the four gene loci which showed microhabitat separation but are distinctly less for the AP locus. There were statistically different proportions of heterozygotes among the gene loci within each sampling locality and distinctly different degrees of deficiency among localities for the LAP-1 and PEP-2 loci.

Conclusions from the isoenzyme study

The results show differences in the isoenzymes present in mussel populations at various localities in eastern Canada. From the macrogeographic data for the LAP-1 and PGI enzymes, one might conclude that this difference indexed genetic isolation of populations from the various sites. However, this interpretation becomes suspect from the evidence that the strong pairing of the PEI and BF samples is apparently based on a similarity of environmental pattern despite the wide geographical separation of sample locations. This relation of genetic population structure to microhabitat characteristics is confirmed by the difference between localities within St. Margaret's Bay, where the generally active water circulation and exchange within the bay give good reason to suppose that these microhabitat differences reflect selective survival from a common mixed population of zygotes. The hypothesis that there may be a common brood stock is supported by the evidence that the AP isoenzyme was uniformly distributed throughout the bay and showed a near equilibrium population of alleles. The indication that

TABLE 1. Heterozygote deficiency values for five isoenzymes at six localities in St. Margaret's Bay. (Significance levels from tests for homogeneity among loci and localities are indicated by NS for P>0.05, * for P<0.05, ** for P<0.01 and *** for P<0.001.)

Locality	LAP-1	PEP-2	PGM	PGI	AP	Signif.
Northwest Cove	-0.31	-0.67	-0.33	-0.58	-0.10	***
Shut-In-Island	-0.57	-0.53	-0.40	-0.23	-0.33	*
Luke Island	-0.52	-0.46	-0.44	-0.35	-0.12	*
Potato Island	-0.72	-0.30	-0.44	-0.30	-0.00	***
Frenchman's Point	-0.60	-0.40	-0.44	-0.30	-0.09	***
Todd Island	-0.68	-0.57	-0.43	-0.47	-0.17	***
Probability	**	*	NS	NS	NS	

the alleles showing the strongest differences among localities also showed the strongest heterozygote deficiences seems to confirm that these distributions must be the result of active microhabitat selection pressure.

The evidence for differences among the isoenzymes indicates further that the observed allele frequencies have been independently determined by different environmental selection forces. On this basis, the AP isoenzyme appears to be related to some basic characteristic of the species *M. edulis*, whereas the highly variable LAP-1 may be associated with the physiological mechanisms that permit osmotic adaptation to environmental changes in salinity (Koehn, 1978). The physiological functions of the other enzymes are not known.

Growth and Mortality Studies

Introduction and results

The production studies described here began before the genetic differences outlined above became known. However, in an early experiment (Freeman and Dickie, 1979), the growth patterns of individual mussels indicated that at least the growth part of the production process may have a genetic basis. These subsequent studies have involved a triple-transfer experiment conducted over a 5-year period at three localities, which were later involved in the isoenzyme study, namely, Bedford Basin and St. Margaret's Bay near Halifax, Nova Scotia, and Ellerslie on Prince Edward Island (Fig. 1). In each of three successive years, a sample of about 200 healthy mussels was collected from natural beds in each of these localities. After cleaning and holding them for a short period to ensure good condition, 25 mussels of specified size-classes were withdrawn, individually numbered, and suspended in a plastic cage from rafts moored at each of the three sites. Initially, two size-classes (40 ± 2.5 mm and 50 ± 2.5 mm shell length) of 25 animals each from each of the three stocks (total of 150 animals) were held at each site. The first transfers were made in late spring of 1976, and the second was conducted in the same way in 1977. Because there were no apparent differences in growth between the two previously established size-groups, the 1978 transfers involved 50 mussels of the 40 mm size-class at each site. Additionally 50 mussels of 15 ± 2.5 mm shell length were transferred in 1978. The mussels in each sample at each site were measured once a month, mortalities were noted, and the cages were cleaned and replaced. Observations on each sample were continued for approximately 24 months, providing data on growth and mortality of identified individuals for four successive 12-month periods.

For analysis of the production (growth and mortality) which varied with time during the year, the monthly data were compiled over successive 3-month seasons, because preliminary analysis indicated that seasonal periods of this length provided the most satisfactory homogeneous sample units. Although size, as indexed by shell length, is an important predictor of growth rate, preliminary analysis showed that relative growth is not linear with size. Accordingly, a measure of length and an index of weight ($L^{2.65}$) were adopted as covariates in the analysis. Both growth and mortality data normalized in this fashion were examined by the classical 4-way analysis of covariance (Tables 2 and 3). The tables show how the total variance is divided among the main classifications of stock, site, season and year, together with their two-way interactions. The overall analysis provides an explanation for about 80% of the observed total variance in growth and 50% of the total variance in mortality.

In these analyses, the variance explained by the "stock" term may be considered as an index of the relative importance of genetic constitution on the production parameters of the mussel, because the samples compared were taken at the same time from genetically different populations and reared together at each site during the experimental period. By the same logic, the variance explained by the "site" classification gives a measure of the relative importance of what is generally referred to as "environment". The "seasonal" term is only of limited interest in this examination of the data, but the results were needed in relaton to a further study of the actual production of mussels. However, the "year" term can, in a sense, be considered as a scaling factor by which to judge the significance of the experimental results and is thus of

TABLE 2. Analysis of covariance of seasonal growth in mussel populations. (Significance levels are indicated by ** for P <0.05 and by *** P<0.01.)

Source of variation	Sum of squares	df	Mean square	F	Signif. of F
Covariates	9.671	2	4.835	439.54	***
Length	2.922	1	2.922	265.58	***
Weight	1.003	1	1.003	91.14	***
Main effects	4.877	11	0.443	40.30	***
Stock	0.732	2	0.366	33.26	***
Site	2.136	2	1.068	97.09	***
Season	0.946	3	0.315	28.66	***
Year	0.725	4	0.181	16.47	***
2-way interactions	2.032	41	0.050	4.50	***
Stock-site	0.204	4	0.051	4.63	***
Stock-season	0.610	6	0.102	9.24	***
Stock-year	0.098	8	0.012	1.11	0.356
Site-season	0.077	6	0.013	1.16	0.327
Site-year	0.241	8	0.030	2.74	**
Season-year	0.716	9	0.080	7.23	***
Explained	16.559	54	0.307	27.91	***
Residual	3.663	333	0.011		
Total	20.243	387	0.052		

TABLE 3. Analysis of covariance of seasonal mortality in mussel populations. (Significance levels are indicated by * for P<0.05, ** for P<0.01 and *** for P <0.001.)

Source of variation	Sum of squares	df	Mean square	F	Signif. of F
Covariates	10.217	2	5.108	4.41	*
Length	9.934	1	9.934	8.58	**
Weight	10.115	1	10.115	8.74	**
Main effects	116.223	9	12.914	11.16	***
Stock	30.997	2	15.449	13.39	***
Site	17.845	2	8.922	7.71	***
Season	72.404	3	24.135	20.85	***
Year	2.112	2	1.056	0.91	0.403
2-way interactions	153.334	29	5.253	4.54	***
Stock-site	7.297	4	1.824	1.58	0.181
Stock-season	48.843	6	8.141	7.03	***
Stock-year	8.903	4	2.226	1.92	0.107
Site-season	70.143	6	11.690	10.10	***
Site-year	13.783	4	3.446	2.98	*
Season-year	12.280	5	2.456	2.12	0.064
Explained	278.775	40	6.969	6.02	***
Residual	276.595	239	1.157		
Total	555.370	279	1.991		

more interest. There is no simple *a priori* criterion for judging how different were the various sites chosen for the experiments in relation to the possible range of variation in production of mussels in the areas. However, some common knowledge is available on the magnitude of year-to-year variation normally expected in a given environment. The evidence that the F-ratios for "site" are more than five times the F-ratios for "year" provides some assurance that the sites chosen for the comparison are different, in a significant way, relative to normal environmental vicissitudes.

Using parallel arguments, the F-ratios for "stock" and "site" (Tables 2 and 3) provide an index of the relative importance of genetics and environment on the growth and mortality of the blue mussel. It is particularly interesting to note that the results for growth and mortality are quite different. In the case of growth (Table 2), "site" accounts for about three times the variance accounted for by "stock". In the case of mortality (Table 3), the reverse is evident, with "stock" accounting for about twice the variance accounted for by "site". This simple interpretation is slightly complicated by the fact that, in the growth data, there is also a statistically significant interaction of "stock" and "site", i.e. the stocks themselves performed quite differently in the various sites. The concept of "site" and "stock" as primary variables is therefore somewhat naive, but the interaction is small relative to the main effects and attention need not be diverted from them. The interaction of "stock" and "site" is not statistically signficant in the analysis of mortality data.

Conclusions from production study

Growth and mortality comprise the most important production processes which affect a given cohort of animals and are widely used in fisheries management calculations. However, it is common experience that variations in growth are evident in population samples, whereas mortality is usually difficult to measure.

In common with experience from studies of many marine animals, important variation in growth of mussels is associated with environmental conditions. The growth data also indicate that the genetic constitution of the stock contributes significantly to the characteristic stock growth and that this contribution is larger than normal year-to-year variation. However, growth appears to make a relatively small contribution to total variation likely to be encountered in stock production.

The results for mortality are quite different. After the strong seasonality of mortality has been taken into account, there is apparently relatively small variation in mortality rate with time. In fact, the "year" term for mortality was statistically insignificant as an explanation of the total variance. However, the total variation in mortality among the different samples was very high, and the results show that most of this variation is attributable to differences in the source of the stocks. The mussel samples drawn from three different source areas showed strikingly different patterns of mortality, and these differences persisted no matter where the samples were cultured. It is interesting to note that mortality was lowest among mussels taken from the Ellerslie site, which exhibits extremes in environmental variation and which, with short production periods, would be generally considered a "tough" environment. The highest mortality occurred in mussels from Bedford Basin, which has a comparatively mild climate and relatively high primary productivity. It is not known whether these coincidences represent cause-effect relations, but they are consistent with the experience

of shellfish breeders in other areas that "hardier" seed stock is found in varying environments.

The relative importance of growth and mortality as determinants of production is not readily generalizable from species to species. The results of the production study of mussels are not presented here because they are not very applicable to fish studies. Mussels are relatively short-lived animals and mortality of adults is corresponding high. What is perhaps of general interest from the mussel experiments is that variation in mortality was considerably greater than variation in growth, so that any judgment of the productive capacity of either a site or a mussel stock could be seriously in error if it were based on comparison of growth rates alone.

General Conclusions

Results of the growth and mortality studies appear to verify that genetic constitution of mussel populations may be an important (probably the most important) determinant of adult stock production. However, parallel studies of isoenzyme patterns in the mussel populations showed that genetic constitution is sensitive to microhabitat variation. In fact, the observed differences in genetic constitution from place to place were apparently caused by the effects of small environmental changes on a common breeding stock. However, because the experiments involved post-settlement stages from natural habitats, the observed differences may have been due to some additional phenotypic variation. In a sense, the mussel data may reflect environmental differences through genotype on a relatively short time-scale.

In addition, mussels may represent a class of organisms which, over time, has incorporated a relatively high proportion of its total adaptive capacity into the genotype (Gause, 1947). The relation between genotypic and phenotypic variation is a rather large area of genetic study. In the present context, if genotypic adaptation is an especially important factor in mussels, changes in isoenzyme frequencies may be more sensitive indices of stock differences than would be the case in fishes or other species in which the adults display versatile behaviorial and physiological patterns in relation to the environment.

Even with these limitations on comparing fishes and molluscs, the demonstration that differences in isoenzyme frequencies indexed important differences in population productivity is of general significance. In the case of mussels, production differences apparently characterize stocks in different localities even when they reflect short-term genetic effects in relation to microhabitat differences. The likelihood that this is generally true in the presence of genetic differences emphasizes the importance of defining breeding stock differentiation as an aspect of production studies and underlines the importance of making parallel observations on other species.

References

FREEMAN, K. R., and L. M. DICKIE. 1979. Growth and mortality of the blue mussel in relation to environmental indexing. *J. Fish. Res. Bd. Canada*, **36**: 1238-1249.

GARTNER-KEPKAY, K. E., L. M. DICKIE, K. R. FREEMAN, and E. ZOUROS. 1980. Genetic differences and environments of mussel populations in the Maritime Provinces. *Can. J. Fish. Aquat. Sci.*, **37**: 775-782.

GARTNER-KEPKAY, K. E., E. ZOUROS, L. M. DICKIE, and K. R. FREEMAN. 1983. Genetic differentiation in the face of gene flow: a study of mussel populations from a single Nova Scotia embayment. *Can. J. Fish. Aquat. Sci.*, **40**: 443-451.

GAUSE, G. F. 1947. Problems of evolution. *Trans. Conn. Acad. Arts Sci.*, **37**: 17-68.

KOEHN, R. K. 1978. Physiology and biochemistry of enzyme variation: the interface of ecology and population genetics. *In* Ecological genetics: the interface, P. Brussard (ed.), Springer-Verlag, N. Y., p. 51-72.

LEVINTON, J. S., and R. K. KOEHN. 1976. Population genetics. *In Marine mussels: their ecology and physiology, B. L. Bayne (ed.), Cambridge Univ. Press, N. Y., Chapter 10.*

NEI, M. 1972. Genetic distance between populations. *Amer. Nat.*, **106**: 283-292.

SINGH, S. M., AND E. ZOUROS. 1978. Genetic variation associated with growth rate in the American oyster (*Crassostrea virginica*). *Evolution*, **32**: 342-353.

ZOUROS, E., and D. W. FOLTZ. 1984. Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia*, (In press).