International Commission for the Northwest Atlantic Fisheries

Serial No. 5306

ICNAF Res. Doc. 78/XI/90

SPECIAL MEETING OF STACRES - NOVEMBER 1978

Genetic Variability in Northwest Atlantic Harp Seals, *Pagophilus groenlandicus*

by

D. M. Lavigne, J. P. Bogart, R. Danzman, W. W. Barchard, and M. Earle
Department of Zoology, University of Guelph
Guelph, Ontario, Canada

and

R. G. H. Downer
Department of Biology, University of Waterloo
Waterloo, Ontario, Canada

Abstract

Samples of heart, liver, kidney, and skeletal muscle were obtained from harp seal pups in the Gulf of St. Lawrence, and on the Front off the coast of Labrador, in March 1978. Of fifty-five presumptive gene loci which were resolved and assayed, only five were found to be polymorphic; the remaining fifty were monomorphic.

Northwest Atlantic harp seals were found to be among the least variable of vertebrate species examined to date. Frequency of polymorphic loci was 0.02 and estimated total heterozygosity was 0.01. All variable alleles segregated according to Hardy-Weinberg equilibrium and gene frequencies indicated no differences between Gulf and Front animals. Both Nei's index and Rogers' coefficient of genetic similarity between the two areas was >0.999.

For comparative purposes, some additional samples were obtained from harp seals born on the West Ice off Jan Mayen. Only five presumptive loci could be resolved and assayed from these tissue samples. Failure to resolve a larger number of loci is attributed to unavoidable differences in tissue collection techniques necessitated by logistics, and the use of 2-phenoxyethanol, a recommended protein preservative for tissue enzyme electrophoresis. Two of the five loci resolved were polymorphic, and gene frequencies within the Jan Mayen stock segregated in normal Mendelian ratios but differed from those of Northwest Atlantic harp seals.

Considering population sizes of harp seal stocks, available evidence suggests that low genetic variability is not a product of "bottlenecking" as has been suggested for elephant seals (Mirounga). Low heterozygosity in the harp seal may be related to specialization of the terrestrial mammalian plan for an aquatic existence. Coincidentally, low genetic variability may result from adaptation to non-random fluctuations in trophic resources as has been suggested for some marine invertebrates.

In conclusion, Gulf and Front harp seals were found to be genetically indistinguishable using tissue enzyme electrophoresis. There is no indication of reproductive isolation between Gulf and Front; on the contrary, the results suggest random interbreeding between the two areas reinforcing the hypothesis that northwest Atlantic harp seals comprise a single stock. On the basis of significantly different ($p<0.001$) frequencies for the MDH-1 isozymes, Jan Mayen harp seals represent a breeding unit in Hardy-Weinberg equilibrium which is at least partially isolated from Northwest Atlantic harp seals. Further samples will be required to adequately determine the magnitude of genetic difference between harp seals breeding in the Northwest Atlantic and on the West Ice.

From a management perspective, there would seem to be no difficulties with the present allocation of quotas or hunting effort between Gulf and Front. Not only are harp seals from these two areas genetically indistinguishable using present techniques, but extremely low heterozygosity indicates that these animals should be resilient to the effects of regulated exploitation. However, low genetic variability also suggests that harp seals may be extremely vulnerable to natural environmental changes and man-made perturbations in the marine ecosystem.

Introduction

More is known about Northwest Atlantic harp seals Pagophilus groenlandicus than about most exploited marine mammal stocks. Nevertheless, there remain numerous uncertainties and deficiencies in the basic biological data on which present management strategies are formulated. One of the most obvious questions is whether these seals comprise a randomly interbreeding stock or whether they represent two partially separate or entirely distinct breeding stocks.

Some data suggest that two stocks are present, one breeding in the Gulf of St. Lawrence, the other off the coast of Newfoundland in an area known as the Front. For example, Gulf harp seals whelp about one week earlier than Front seals (Sergeant 1965, 1976); Gulf and Front seals may mature at slightly different rates (Sergeant 1966, 1973a); and breeding females tend to show fidelity to their place of birth each spring, although young seals born in the Gulf seem to intermix with Front animals until they reach sexual maturity (Sergeant 1976, 1977).

Present harp seal management strategy is based primarily on the assumption that Northwest Atlantic harp seals belong to a single interbreeding stock (Lett and Benjaminsen 1977). Thus, in recent years quota allocations have not been equally distributed throughout the entire stock. The greatest proportion of hunting effort occurs on the Front and consequently the bulk of the catch is taken in this region. If Gulf and Front stocks are indeed separate, such a management strategy should logically lead to overexploitation of the Front stock. For management purposes, it is thus of some immediate importance to delineate harp seal stocks in the Northwest Atlantic.

A variety of techniques have been used in previous attempts to delineate distinct harp seal stocks. Morphological studies of skull and body dimensions indicated that northwest Atlantic harp seals (including both Gulf and Front) are more distinct in comparison to the two eastern stocks (the Jan Mayen stock off east Greenland, and the White Sea stock) than the latter two are from each other (Khuzin 1967, Yablokov and Sergeant 1963). Studies of erythrocyte antigens (Moller et al. 1966), haemoglobin electrophoresis (Naevdal 1966b) and serum immunoelectrophoresis using agar diffusion techniques (Borisov 1966) were unable to detect differences between Northwest Atlantic, Jan Mayen, and White Sea harp seals. Significant differences in frequencies of two of three alleles hypothesized to control synthesis of transferrin groups, and nearly significant differences in distribution of transferrin groups between Newfoundland seals and the two eastern stocks (Naevdal 1966a) were not confirmed in a subsequent study (Naevdal 1971). No "marked difference" in distribution of transferrin groups or frequencies of genes controlling them was found between harp seals from the Gulf and Front (Naevdal 1969).

In the present study, genetic differences between harp seals born in the Gulf of St. Lawrence and on the Front off Newfoundland were examined using starch-gel electrophoresis. This technique has been identified as the most appropriate for studying phylogenetic relationships between closely related taxa (Dobzhansky et al. 1977). It has proven to be a valuable tool for population geneticists, permitting quantitative examination of genetic variability within populations and species, and geographic variation within populations, and for systematic evaluation of races or species in a wide variety of living organisms from protozoa to man (Selander and Johnson 1973, Powell 1975, Dobzhansky et al. 1977). Interbreeding populations share a common gene pool and, barring localized environmental selection pressures, should possess similar gene frequencies. If a population is partially or completely isolated, this should be reflected in an alteration of gene frequencies for reasons of genetic drift, inbreeding, or founder effects and thus isolated or even partially isolated populations should become genetically distinctive.

In order to obtain a statistically valid estimate of gene frequencies it is necessary to obtain a large and representative sample of the population or stock in question. It is also important to sample a large number of gene products in order to determine relative variability and to circumvent the possibility that certain enzymes may be of selective value under very localized conditions. Also, it has been demonstrated that certain proteins may be "conservative" in that they are infrequently polymorphic whereas others are often polymorphic (Selander and Johnson 1973, Powell 1975, Ward 1977). The problem of delineating harp seal stocks would appear to be ideally suited to genetic analysis using electrophoretic techniques. The stocks are exploited annually in large numbers and tissue samples required for analysis can be readily obtained from pups whose birthplace is known.

In addition to the problem of delineating distinct stocks for management purposes, and of considerable interest, is the level of genetic variability present in the harp seal. Electrophoretic analyses of genetic variation in pinnipeds are scant, being available only for elephant seals. The southern elephant seal, Mirounga leonina has an estimated genetic

heterozygosity of only 3 per cent (McDermid et al. 1972) and the northern elephant seal M. angustirostris demonstrated no genetic variation (Bonnell and Selander 1974). In contrast, mean heterozygosity for most vertebrate species examined to date ranges from 4 to 10 per cent (Selander and Johnson 1973, Powell 1975, Selander 1976, Nevo 1978). The very low values in elephant seals are thought to be a consequence of fixation brought about by the decimation of these species by sealers in the last century (FAO, 1976; Bonnell & Selander 1974). Present stocks are thus descended from a relatively small founding population. Unlike elephant seals, the harp seal has maintained sizeable population numbers despite exploitation and the "bottleneck" phenomenon (Nei et al. 1975) should not have reduced genetic heterozygosity in this species. The harp seal thus provides an opportunity to examine further the suggestion that marine mammals exhibit lower levels of genetic heterozygosity than terrestrial mammals (Powell 1975).

Materials and Methods

Fresh samples of heart, kidney, liver and skeletal muscle tissue were obtained from Northwest Atlantic harp seal pups killed in the 1978 seal hunt in the Gulf of St. Lawrence and on the Front, off the coast of Newfoundland. Tissues were excised from a similar part of the organ of each pup with care being taken to ensure that the sample was small enough to permit rapid and complete freezing. Individual tissue samples were placed in plastic bags and frozen at ambient environmental temperatures below $0^{o}C$. If ambient temperature was above freezing, tissue samples were quickly frozen by immersion in a freezing solution of acetone and dry ice. All frozen, bagged tissues from each pup were placed together in a cloth bag with an identification tag. Tissues were then maintained frozen on dry ice on a sealing vessel until they were shipped on dry ice to Guelph where they were stored, either on dry ice or in $-80^{o}C$ ultra-cold freezers. Complete samples were collected from 472 seals in the Gulf of St. Lawrence from 2 March to 15 March (Fig. 1) and from 501 seals on the Front from 13 March to 19 March (Fig. 2).

For comparison, additional samples were obtained from harp seal pups born on the West Ice between Jan Mayen and Svalbard. These tissues were

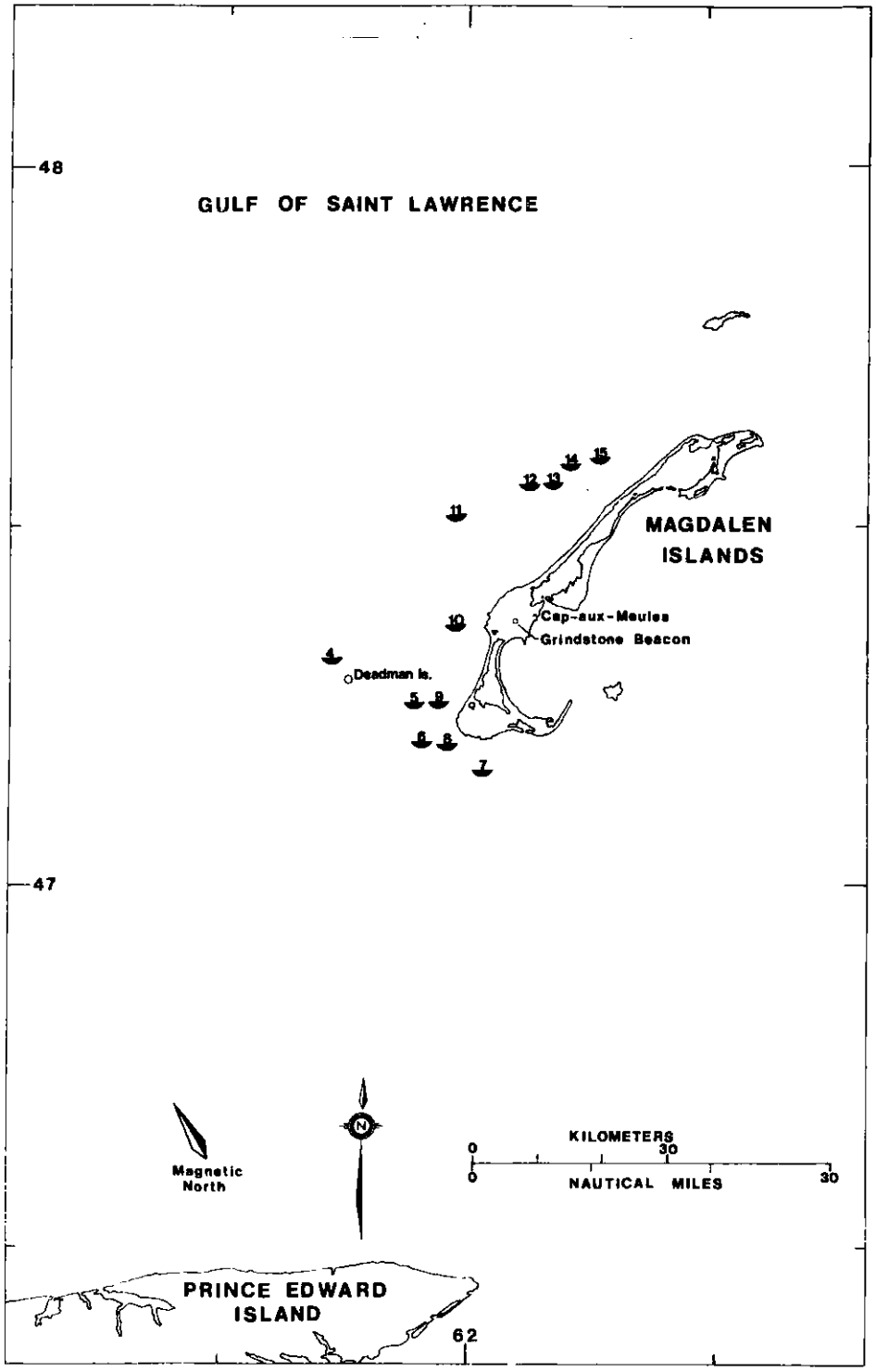

Fig. 1. Daily position of ships from which harp seal tissues were sampled for enzyme electrophoresis in the Gulf of St. Lawrence during March 1978.

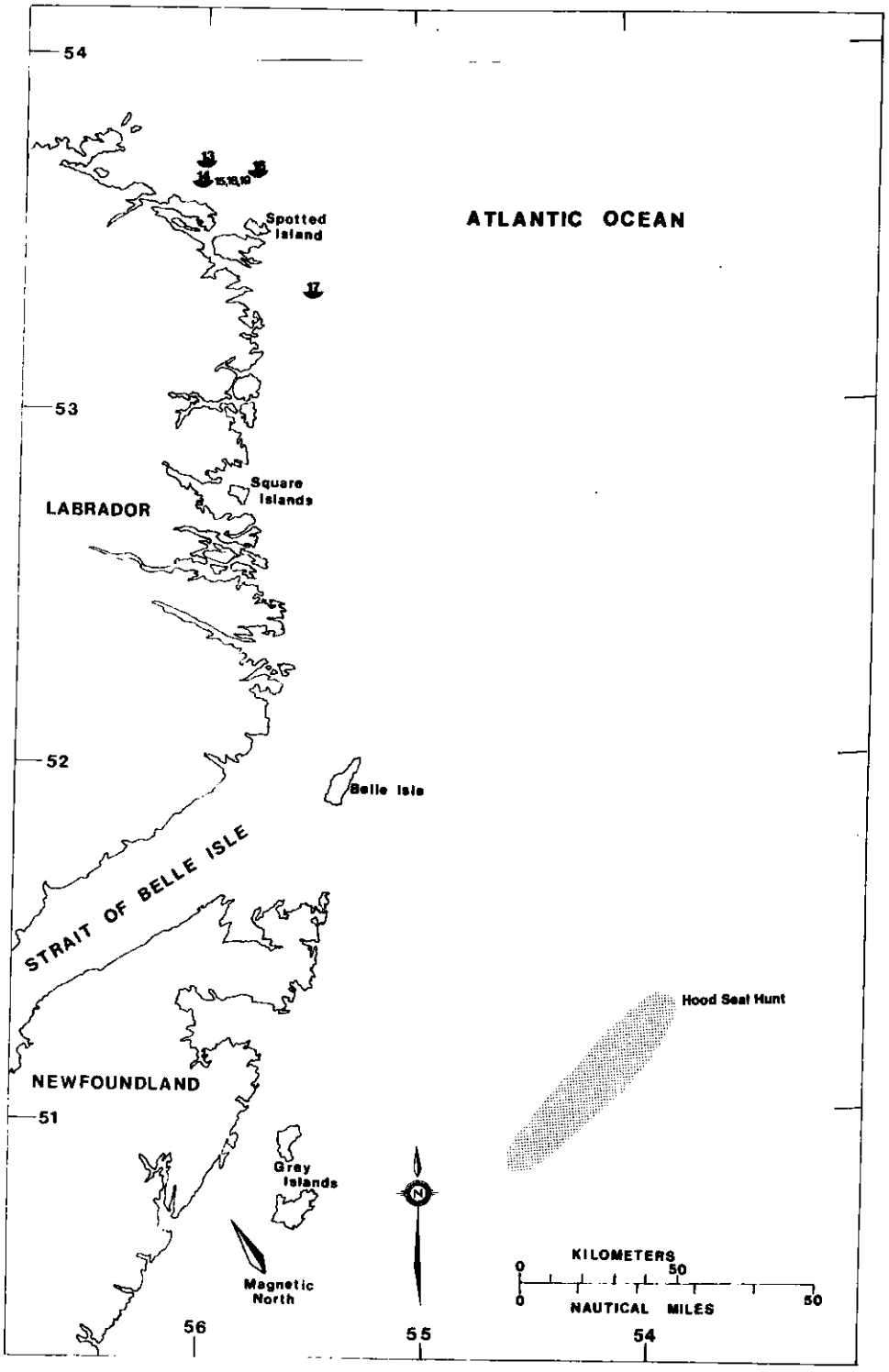

Fig. 2. Daily position of ships from which harp seal tissues were sampled for enzyme electrophoresis on the Front off the coast of Newfoundland during March 1978.

excised in a similar manner to those obtained in the Northwest Atlantic. Without facilities for making or storing dry ice, these samples were preserved in a 2 per cent solution of 2-phenoxyethanol and sea water (Nakanishi et al. 1969). Because of the cold ambient temperatures (-20°C) both phenoxyethanol and tissue samples were quickly frozen (Bryden, in Litt.). Tissues were stored in a sealing vessel's freezer until the ship returned to Bergen, Norway; they were later flown to Canada. However, on arrival at Toronto International Airport, samples from 27 of the 97 animals samples, were thawed. These tissues were placed on dry ice and transported to Guelph where they were stored with the Northwest Atlantic samples.

To control for the use of 2-phenoxyethanol on the West Ice, replicate samples were collected from 24 pups in the Gulf of St. Lawrence. One set of tissues was fast frozen, the other was treated with 2 per cent 2-phenoxyethanol and maintained at ambient temperature prior to being placed on dry ice on board a sealing vessel. An additional 24 adult females and their pups were sampled in conjunction with other research activities in the Gulf. Samples from these animals were placed in 2 per cent 2-phenoxyethanol and maintained at ambient temperatures for various lengths of time prior to freezing on dry ice.

In the laboratory, tissues were partially thawed, minced with a scalpel, and homogenized in an equal volume of deionized water. The homogenate was then frozen at -70°C, thawed and clarified by centrifugation at 14,500 G for 30-40 min at 4°C. The supernatant was stored at -70 to -90°C.

Starch-gel electrophoresis was carried out in a 4°C cold-room, or at room temperatures of about 22°C employing the methods of Selander et al. (1971). A series of experimental trials was performed in order to determine the most favourable buffer system, tissue type, and running time which produced the best resolution of the following soluble enzymes: Acid Phosphatase, Adenosine Deaminase, Alcohol Dehydrogenase (ADH), Adenylate Kinase (AK), Aldolase, Alkaline Phosphatase (Alk. Phos.), Glycerophosphate Dehydrogenase (GPDH), Creatine Kinase (CK), Esterase, Fumarase, Glyceraldehyde-3-Phosphate Dehydrogenase (G-3-PDH), Glucose-6-Phosphate (G-6-P), Glucuronidase, Glutamate Dehydrogenase (GDH), Glutamic Oxaloacetic Transaminase (GOT), Hexokinase, Isocitrate Dehydrogenase (IDH), Leucine Amino Peptidase (LAP), Indophenyl Oxidase (IPO), Lactate Dehydrogenase

(LDH), Malic Enzyme (ME), Malate Dehydrogenase (MDH), Mannose Phosphate Isomerase (MPI), Octanol Dehydrogenase (ODH), Peptidase, 6-Phosphogluconate Dehydrogenase (6-PGD), Phosphoglucose Isomerase (PGI), Phosphoglucomutase (PGM), Sorbitol Dehydrogenase (SDH), and Xanthine Dehydrogenase (XDH). The buffer systems utilized for the initial screening were: Tris-citrate pH 6.7, Tris-citrate pH 8.0, Trismaleic EDTA pH 7.4, PGI phosphate pH 6.7, Tris-versene borate pH 8.0, Lithium hydroxide pH 8.25, Tris-HCI pH 8.5. If good resolution of an enzyme system was encountered in an early trial, further trials for the particular enzyme were not carried out.

Consistently demonstrable electromorphic loci for each enzyme system were scored from cathode to anode; the least anodally migrating locus being designated 1. Allelic variant isozymes were designated relative to the farthest anodally migrating isozyme which was given a value of 100. Cathodally migrating isozymes were treated in a similar manner but were given negative values.

Enzymes were classified as monomeric, dimeric, or tetrameric according to their behaviour in the gel and from information obtained from other studies involving the same enzyme systems in a variety of other vertebrate species. Monomeric isozymes demonstrate two bands in a heterozygote, representing the products of two variant alleles; homozygotes demonstrate only one of the two bands. Heterozygotes for dimeric isozymes produce a three-banded pattern consisting of the two homodimeric bands on either side of a heterodimeric band which is usually more intensely stained. The homodimeric band represents the products of two variant alleles and the heterodimeric band represents the combination or hybrid product from these same alleles. Tetrameric isozymes represent those enzymes which are coded by two different genes and each allele produces a product which is identifiable electrophoretically. Homozygotes for tetrameric isozymes demonstrate a five-banded pattern consisting of two homotetrameric bands, and three heterotetrameric bands which are made up of a combination of the coded gene products. The above phenotypes are shown in Fig. 3.

Genetic distances between harp seals sampled in the Gulf of St. Lawrence and on the Front were computed using the formulations of Rogers (1972) and Nei (1972). Genetic heterozygosity estimates were based on actual counts of presumed heterozygous genotypes (phenotypes) (Selander and Johnson 1973). Mean heterozygosity ($\bar{H}$) is defined as the number of

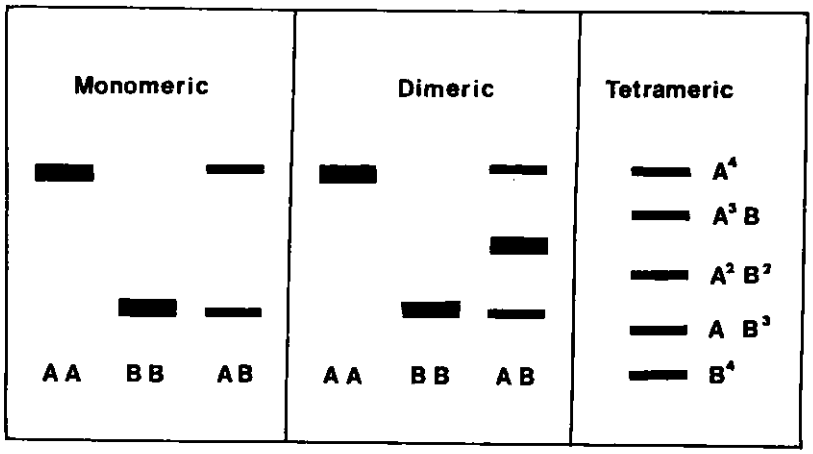

Fig. 3. Phenotype designations for isozymes

presumed heterozygotic genotypes divided by the product of the number of individuals and the number of loci examined. Polymorphism estimates were determined by dividing the number of loci possessing more than a single allele by the total number of loci.

Results

The best resolution of the various enzyme systems was obtained with the following buffer systems and running times: Tris-citrate pH 6.7 for 4 h (ADH, AK, Aldolase, GPDH, Glucuronidase, CK, Fumerase, IDH, LDH, ME, MDH, MPI, ODH, 6-PGD, PGI, PGM, SDH, XDH); Tris maleic EDTA pH 7.4 for 6 h (Alk. Phos., G-6-P); and Tris HCI pH 8.5 for 2 h (GOT). Resolution of the remaining enzymes appeared to be the same regardless of the buffer system employed.

For Northwest Atlantic harp seals, fifty-five presumptive gene loci were resolved and assayed (Table 1) from 18 of the 30 investigated enzyme systems. No activity was encountered in three enzyme systems (G-3-PDH, GDH, and Hexokinase) in any tissue. The electrophoretic mobilities of four additional enzymes (Adenosine Deaminase, Glucuronidase, Fumerase, and IPO) were very diffuse and loci could not be resolved. Loci were observed in the remaining five enzymes (Acid Phosphatase, Esterase, LAP, Peptidase, and 6-PGD). They were omitted from the analysis because loci could not be consistently scored. One of the four CK loci (CK-2) was also deleted from analysis for the same reason. Of the 55 loci which

were examined, only five (GOT-1, MDH-1, MPI-1, IDH-2, and G-6-P-1) were found to be polymorphic. The remaining 50 were monomorphic.

Most of the enzymes which were assayed from samples obtained in the Gulf and Front could not be resolved in samples from the West Ice. Only three enzymes (Alk. Phos., GOT, and MDH) encoded by five presumptive gene loci were evident in West Ice samples. Like the Northwest Atlantic samples, those from the West Ice were found to be polymorphic for MDH-1, GOT-1, and Alk. Phos. -1.

The number of samples assayed from each region and for each locus is summarized in Table 1. The number of genes sampled (= 2 x the number of individuals) per locus varied considerably and this was due mainly to the large sample sizes which were employed for those isozymes which were found to be variable (Table 1). An extremely low level of genetic variation was observed in Gulf and Front harp seals (Table 2). The frequency of polymorphic loci was found to be only nine per cent in both areas using the criterion that any locus found to vary, irrespective of gene frequency, was designated polymorphic. If rare alleles with a gene frequency of <0.05 are omitted, as is customary, frequency of polymorphic loci was only two per cent. The higher proportion of polymorphism or heterozygosity encountered in the West Ice is not realistic as only five loci could be resolved and these loci included most of the variation found in samples from the Northwest Atlantic.

Table 1. Enzyme loci and number of samples examined in harp seal (Pagophilus groenlandicus) tissues.

Locus	Gulf	Front	West Ice
AK-1	55	56	-
AK-2	55	56	-
AK-3	55	56	-
AK-4	55	56	-
AK-5	55	56	-
ADH-1	63	88	-
ADH-2	63	88	-
ADH-3	63	88	-

Table 1. (continued)

Locus	Gulf	Front	West Ice
ADH-4	63	88	-
ADH-5	63	88	-
ADH-6	63	88	-
Aldolase-1	73	72	-
Aldolase-2	73	72	-
Aldolase-3	73	72	-
Alk.Phos.-1**	95	97	92
Alk.Phos.-2	95	97	92
CK-1	48	24	-
CK-3	48	24	-
CK-4	48	24	-
G-6-P-1*	120	120	-
GOT-1*	379	461	82
GPDH-1	55	56	-
GPDH-2	55	55	-
GPDH-3	55	55	-
IDH-1	120	120	-
IDH-2*	120	120	-
LDH-1	96	96	-
LDH-2	96	96	-
MDH-1*	289	294	87
MDH-2	289	289	87
ME-1	55	56	-
ME-2	55	56	-
MPI-1*	96	95	-
ODH-1	73	72	-
ODH-2	73	72	-
ODH-3	73	72	-
ODH-4	73	72	-
ODH-5	73	72	-
ODH-6	73	72	-
PGI-1	63	64	-
PGI-2	63	64	-

Table 1. (continued)

Locus	Gulf	Front	West Ice
PGI-3	63	64	-
PGI-4	63	64	-
PGM-1	63	64	-
PGM-2	63	64	-
PGM-3	63	64	-
SDH-1	63	64	-
SDH-2	63	64	-
SDH-3	63	64	-
XDH-1	63	64	-
XDH-2	63	64	-
XDH-3	63	64	-
XDH-4	63	64	-
XDH-5	63	64	-
XDH-6	63	64	-

* Loci which demonstrated polymorphic isozymes (see Tables 3-8).
** Polymorphism demonstrated by Alk. Phos.-1 is considered to be artifactual (see Discussion).

Table 2. Genetic variation in harp seals (Pagophilus groenlandicus)

	Gulf	Front	West Ice
Number of loci	55	55	5
Number of genes sampled per locus (±2SE)	164.8±32.8	174.3±37.4	176.0±5.4
Total Number of alleles	113	112	11
Alleles per locus (±2SE)	2.05±0.06	2.04±0.04	2.2±0.4
Polymorphic loci[1]	0.09	0.09	0.4
Polymorphic loci (omitting rare alleles)[2]	0.02	0.02	0.4
Frequencies of heterozygotes:			
average over loci (obs)	0.01	0.01	0.15
average over loci (exp)	0.01	0.01	0.16

[1]using the criterion that all polymorphic loci were included irrespective of gene frequency.

[2]using the criterion that polymorphic loci with the gene frequency of the rare allele <0.05 were omitted.

Isozymic gene frequencies for polymorphic loci are given in Tables 3 to 8. In each table the numbers of heterozygotes observed in the present study are compared with expected frequencies, assuming Hardy-Weinberg equilibrium. Good agreement between observed and expected numbers of heterozygotes was found. The observed number of heterozygotes was not statistically ($p>0.05$) different from the expected for any of the isozymes. The genotypic frequencies in all areas sampled are thus in Hardy-Weinberg equilibrium.

Table 3. Distribution of GOT-1 isozyme gene frequencies in harp seals. O = observed and E = expected Hardy-Weinberg distributions.

		GOT-1 Isozymes														
Area	No.	(45 / 45)		(45 / 73)		(73 / 73)		(45 / 100)		(73 / 100)		(100 / 100)		χ^2	d.f.	χ^2(0.95)
		O	E	O	E	O	E	O	E	O	E	O	E			
Gulf	379	168	163.1	150	160.1	44	39.3	11	10.9	6	5.4	0	0.2	1.607	3	7.81
Front	461	196	191.8	196	202.8	56	53.6	7	8.3	6	4.4	0	0.1	1.309	3	7.81
West Ice	82	42	38.3	28	35.5	12	8.2	0	0	0	0	0	0	3.670	3	7.81

		Gene Frequencies ($\bar{X}$ ± 2 SE)		
Area	No.	45	73	100
Gulf	379	0.656±0.070	0.322±0.034	0.022±0.010
Front	461	0.645±0.063	0.341±0.032	0.014±0.008
West Ice	82	0.683±0.146	0.317±0.072	0

Table 4. Distribution of MDH-1 isozyme gene frequencies in harp seals. O = observed and E = expected Hardy-Weinberg distributions.

		MDH-1 Isozymes														
Area	No.	(-20 / -20)		(-20 / -55)		(-55 / -55)		(-20 / -100)		(-55 / -100)		(-100 / -100)		χ^2	d.f.	χ^2 (0.95)
		O	E	O	E	O	E	O	E	O	E	O	E			
Gulf	289	1	0.4	18	19.5	270	269.1	0	0	0	0	0	0	1.30	3	7.81
Front	294	0	0.4	23	21.9	267	267.6	0	0.2	4	3.9	0	0	0.68	3	7.81
West Ice	87	0	4.4	39	30.2	48	52.4	0	0	0	0	0	0	7.27	3	7.81

		Gene Frequencies ($\bar{X}$ ± 2 SE)		
Area	No.	-20	-55	-100
Gulf	289	0.035±0.015	0.965±0.015	0
Front	294	0.039±0.016	0.954±0.017	0.007±0.007
West Ice	87	0.224±0.063	0.776±0.063	0

Table 5. Distribution of MPI-1 isozyme gene frequencies in harp seals. O = observed and E = expected Hardy-Weinberg distributions.

		MPI-1 Isozymes														
Area	No.	(72 / 72)		(72 / 85)		(85 / 85)		(72 / 100)		(85 / 100)		(100 / 100)		χ^2	d.f.	χ^2 (0.95)
		O	E	O	E	O	E	O	E	O	E	O	E			
Gulf	96	0	0	1	0.9	93.0	92.9	0	0	2.0	2.0	0	0	0.03	3	7.81
Front	95	0	0	1	0.9	90.0	90.1	0	0	4.0	3.9	0	0	0.07	3	7.81

		Gene Frequencies ($\bar{X}$ ± 2 SE)		
Area	No.	(72)	(85)	(100)
Gulf	96	0.005±0.010	0.984±0.018	0.011±0.015
Front	95	0.005±0.010	0.974±0.023	0.021±0.02

Table 6. Distribution of IDH-1 isozyme gene frequencies in harp seals: O = observed and E = expected Hardy-Weinberg distributions.

		IDH-1 Isozymes							
Area	No.	(92 / 92)		(92 / 100)		(100 / 100)		Gene Frequencies	
		O	E	O	E	O	E	(92)	(100)
Gulf	120	0	0	4	4.0	116	115.9	0.017±0.017	0.983±0.017
Front	120	0	0	0	0.0	120	120	0	1.0

Table 7. Distribtuion of G-6-P-I isozyme gene frequencies in harp seals: O = observed and E = expected Hardy-Weinberg distributions.

		G-6-P-1 Isozymes							
Area	No.	(86 / 86)		(86 / 100)		(100 / 100)		Gene Frequencies	
		O	E	O	E	O	E	(86)	(100)
Gulf	120	118	118.0	2	1.9	0	0	0.992±0.011	0.008±0.011
Front	120	120	120.0	0	0.0	0	0	1.0	0

Table 8. Distribution of Alk. Phos. - 1 isozyme gene frequencies in harp seals: O = observed and E = expected Hardy-Weinberg distributions[1].

		Alk. Phos. - 1 Isozymes							
Area	No.	(37 / 37)		(37 / 100)		(100 / 100)		Gene Frequencies	
		O	E	O	E	O	E	(37)	(100)
Gulf	95	0	0	0	0	95	95	0	1.0
Front	97	0	0	0	0	97	97	0	1.0
West Ice	92	92	92	0	0	0	0	1.0	0

[1]under the assumption of two alleles at one locus. The data are also consistent with the hypothesis of two separate monomorphic loci.

Frequency of heterozygotes, using both the observed and expected values, was the same for harp seals born in the Gulf and on the Front ($p > 0.01$). Both Rogers' Index (S) and Nei's Index of genetic similarity ($\bar{I}$) between harp seals in the Gulf and Front were >0.999.

Specific results for each of the eighteen enzymes resolved and assayed are given below.

Adenylate Kinase (Fig. 6)

The best resolution for AK was encountered in kidney tissue and, since tissue specificity was not evident, only the kidney phenotype was used for this analysis. The five bands which were resolved could be interpreted as a tetrameric configuration involving only two loci. However, multiple alleles of AK may not form subunits (Markert and Whitt 1968) and this would suggest that AK is not polymeric. Thus, the five-banded pattern would be indicative of five separate loci, consisting of four anodal bands, AK-2, AK-3, AK-4, AK-5, and one cathodal band, AK-1.

Alcohol Dehydrogenase (Fig. 4)

ADH is not considered to be a polymeric enzyme (Ward 1977); it is thus assumed that ADH in the harp seal is encoded by six presumptive gene loci which demonstrate tissue specificity. Four anodally migrating bands, ADH-3, ADH-4, ADH-5, and ADH-6, of equal staining intensity were found in heart and kidney samples. Liver had a distinctive cathodal band, ADH-1,

as well as ADH-3 and ADH-4. Muscle demonstrated ADH-3, ADH-4, and an additional weakly-migrating cathodal band, ADH-2.

Aldolase (Fig. 4)

Five anodal bands indicative of a tetramer (Markert and Whitt 1968) were found from extracts of heart and kidney. Resolution for liver and muscle extracts was poor but indicated tissue specificity. A distinct cathodal band in liver seems unrelated to the anodal tetrameric series and was considered to be an independant locus (Aldolase-1). Anodal bands in muscle and liver could not be resolved and may indicate additional loci whose migration is complexed with the migration of the homotetrameric band (Aldolase-2) and one or more of the heterotetrameric bands. In harp seals, Aldolase thus appears to be encoded by at least three loci. Aldolase-1 is represented by a liver specific cathodal band; Aldolase-2 and Aldolase-3 form a tetramer.

Alkaline Phosphatase (Fig. 7, Table 8)

Alk. Phos. did not exhibit tissue specificity and was encoded by two loci. Alk. Phos.-2 was found to be monomorphic in all harp seals examined. Alk. Phos.-1 may be polymorphic in this species as two distinct isozymes were encountered. Front and Gulf seals invariably had Alk. Phos.-$1^{(100)}$, whereas all of West Ice samples had Alk. Phos.-$1^{(37)}$. The possibility exists that each Alk. Phos.-1 isozyme represents a separate locus but the interpretation would be the same since there is apparent fixation for different isozymes (or loci) between Northwest Atlantic seals and those from the West Ice. This interpretation may not, however, be correct (see Discussion).

Creatine Kinase (Fig. 6)

Extracts from kidney provided the best resolution for CK. This enzyme appears to be encoded by four loci, CK-1, CK-3, and CK-4 were monomorphic in all samples which could be resolved. CK-2 appeared to be polymorphic. Owing to poor resolution and close proximity of bands, it was not possible to consistently score the isozymes.

Glucose-6-Phosphate (Fig. 7, Table 7)

Only one locus was demonstrated for G-6-P. Two heterozygous individuals were encountered in Gulf seals and these had a two-banded phenotype indicative of a monomeric isozyme. The gene frequency for the rare allele

(0.008±0.011) indicates that harp seals from Gulf and Front are essentially monomorphic for G-6-P.

Glutamic Oxaloacetic Transaminase (Fig. 6, Table 3)

Only one locus was consistently resolved for GOT and this locus proved to be polymorphic in animals from the Gulf, Front, and West Ice. It is evident that GOT-1 is a dimeric isozyme which shares two of three variant alleles, GOT-$1^{(100)}$, GOT-$1^{(73)}$, and GOT-$1^{(45)}$. Heterozygotes all demonstrated the typical three-banded pattern. Genotype frequencies matched Hardy-Weinberg expectations (Table 3). GOT-$1^{(100)}$, the rarest allele, was not encountered in West Ice samples. Cathodal migration was observed but loci could not be resolved.

Glycerophosphate Dehydrogenase (Fig. 4)

The five anodal bands which were obtained from heart and kidney extracts may be interpreted as a tetrameric series in the harp seal, consistent with the fact that GPDH forms subunits between multiple alleles (Markert and Whitt 1968). Muscle and liver extracts demonstrated only 3 of the five bands. Liver extracts had a presumably unrelated cathodally migrating band (GPDH-1). Thus, this enzyme is encoded by 3 presumptive monomorphic loci consisting of a liver specific locus (GPDH-1) and the tetramer which is made up of two anodally migrating loci (GPDH-2 and GPDH-3).

Isocitrate Dehydrogenase (Fig. 7, Table 6)

Two loci encoded isozymes of IDH. IDH-1 was represented by a cathodally migrating band and was monomorphic in all Gulf and Front samples examined. IDH-2 is weakly polymorphic and heterozygotes demonstrated a monomeric two-banded pattern. IDH-$2^{(92)}$ was a relatively rare allele, encountered in a heterozygous state in four of 120 Gulf seals examined (Table 6).

Lactate Dehydrogenase (Fig. 4)

LDH demonstrated a typical tetrameric pattern (Markert and Whitt 1968, Ward 1977). The homotetramer, LDH-1 was predominant in liver and muscle extracts whereas LDH-2 was most active in heart and kidney extracts.

Malate Dehydrogenase (Fig. 7, Table 4)

Two loci were observed for MDH. MDH-2 is represented by an anodally migrating band and in one of 578 samples was found to be a dimeric heterozygote. Considering the extremely low frequency of this variant allele,

MDH-2 was considered to be monomorphic. Cathodally migrating MDH-1 is a polymorphic, dimeric isozyme with three alleles which separated in Hardy-Weinburg equilibria. The rarest allele, MDH-$1^{(-100)}$ was not encountered in West Ice or Gulf samples, and was only present in a heterozygous condition in 4 of 294 Front seals examined.

Malic Enzyme (Fig. 5)

ME appeared to be encoded by two anodally migrating, monomorphic isozymes which demonstrated tissue specificity. Neither was evident in liver extracts, and kidney extracts demonstrated only ME-1. Both loci, ME-1, ME-2, were found in heart and muscle extracts.

Octanol Dehydrogenase (Fig. 5)

The six ODH loci appeared to be identical to ADH phenotypes with respect to tissue specificity and electrophoretic mobility. ODH-1 is a liver-specific, cathodally migrating isozyme and ODH-2 is muscle specific. Heart and kidney extracts all exhibited four anodal loci and ODH-3 and ODH-4 were present in all tissues.

Phosphoglucose Isomerase (Fig. 6)

The best resolution for PGI isozymes was found using kidney extracts which demonstrated three cathodally migrating loci, PGI-1, PGI-2, PGI-3, and one anodally migrating locus, PGI-4. Gulf and Front harp seals were found to be monomorphic for all four loci.

Phosphoglucomutase (Fig. 5)

Three monomorphic loci were found to encode isozymes for PGM. Heart and muscle extracts only revealed one isozyme, PGM-2, whereas liver and kidney extracts exhibited all three.

Sorbitol Dehydrogenase (Fig. 5)

Typical, five-banded, tetrameric phenotypes were found in heart and kidney extracts. The most anodally migrating homotetrameric band (SDH-3) was not resolved in muscle or liver, and liver extracts demonstrated a unique, presumably unrelated, cathodally migrating band (SDH-1).

Xanthine Dehydrogenase (Fig. 5)

The banding pattern for XDH was identical to the patterns observed for ADH and ODH. The coding of XDH loci follows that used for these other enzymes although there is some evidence that XDH is a polymeric enzyme (Markert and Whitt 1968).

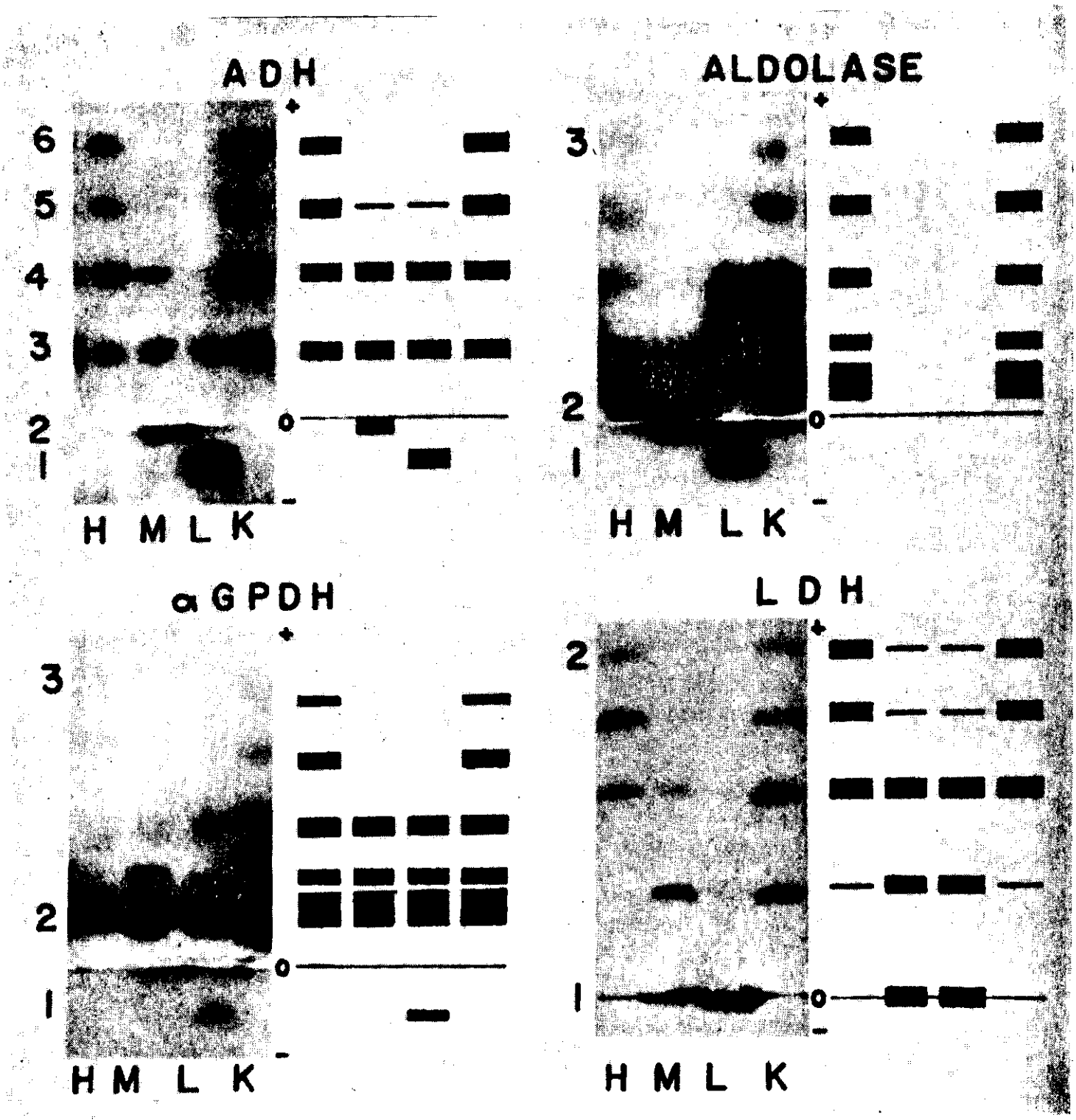

Fig. 4. Phenotypes of ADH, Aldolase, GPDH, and LDH. These enzymes appear monomorphic in harp seals. Tissues are denoted by H-heart, M-muscle, L-liver, and K-kidney. Migration is towards the positive or anodal pole.

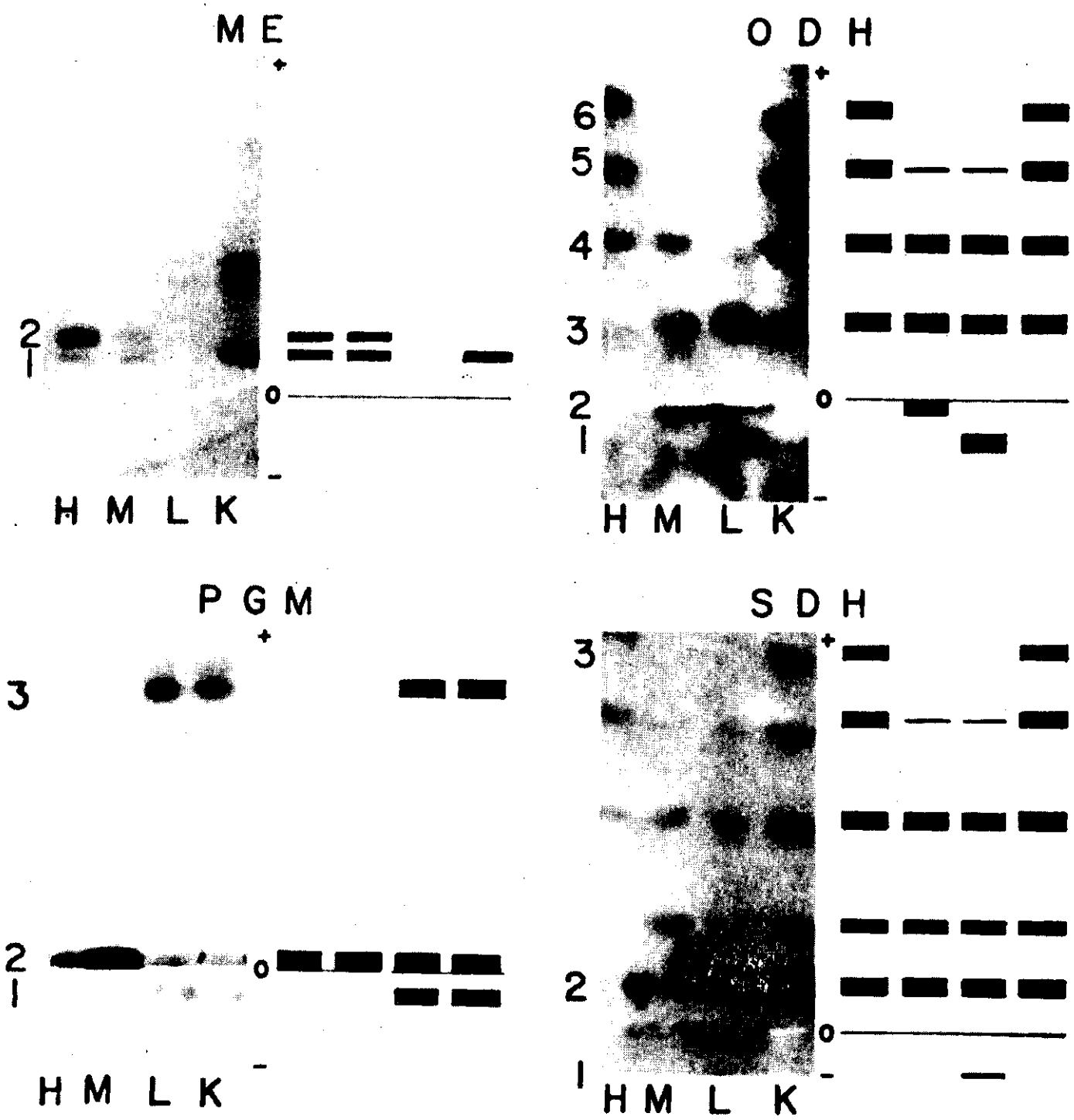

Fig. 5. Phenotypes of ME, ODH, PGM, and SDH. These enzymes appear monormorphic in harp seals. Tissues are denoted by H-heart, M-muscle, L-liver, and K-kidney. Migration is towards the positive or anodal pole.

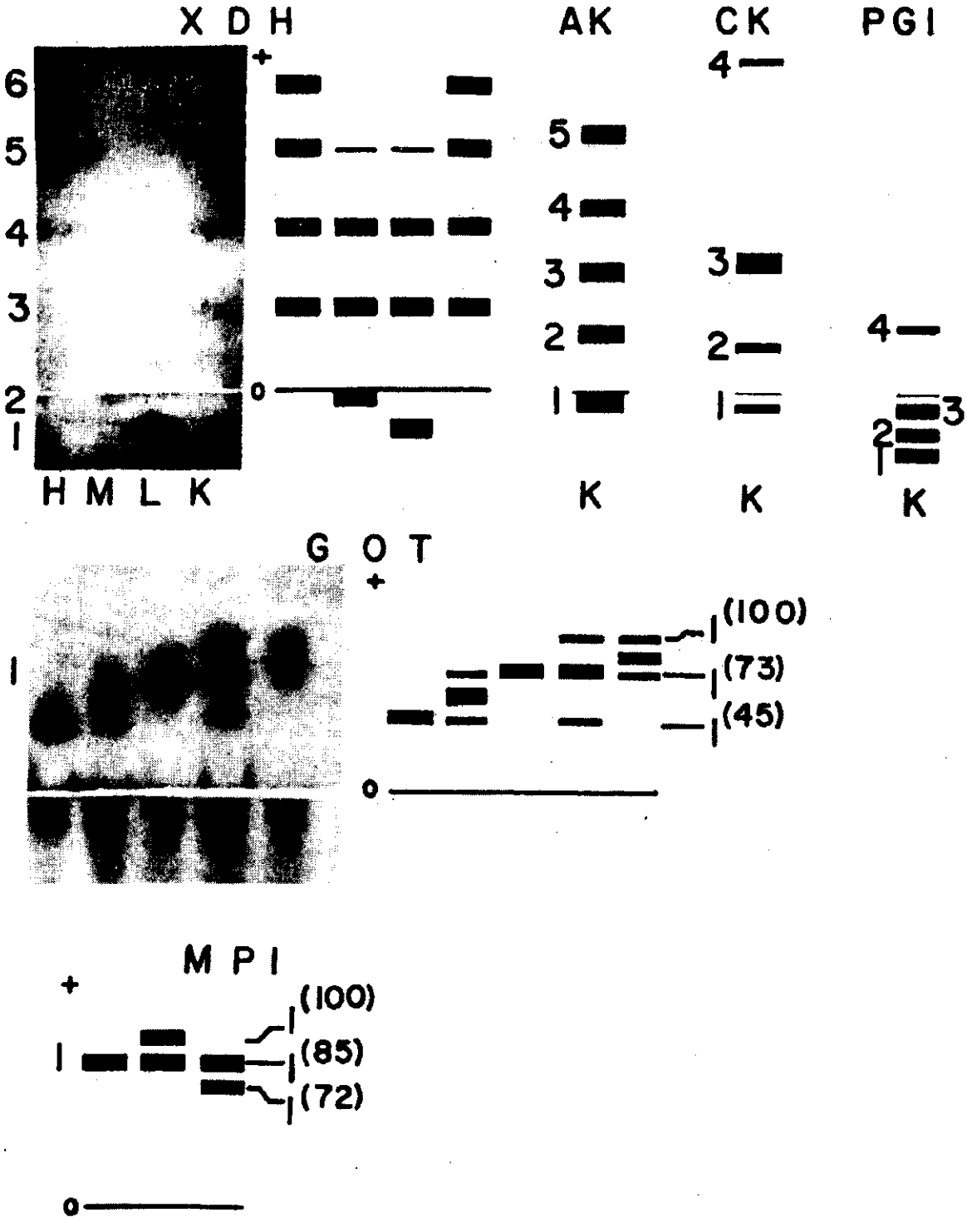

Fig. 6. Phenotypes of XDH, AK, CK, PGI, and GOT. XDH, AK, CK, and PGI all appear monomorphic; GOT appears polymorphic, and exhibited similar mobility in all tissues; MPI also appears polymorphic, but could only be clearly resolved in kidney tissues. Tissues are denoted by H-heart, M-muscle, L-liver, and K-kidney. Migration is towards the positive or anodal pole.

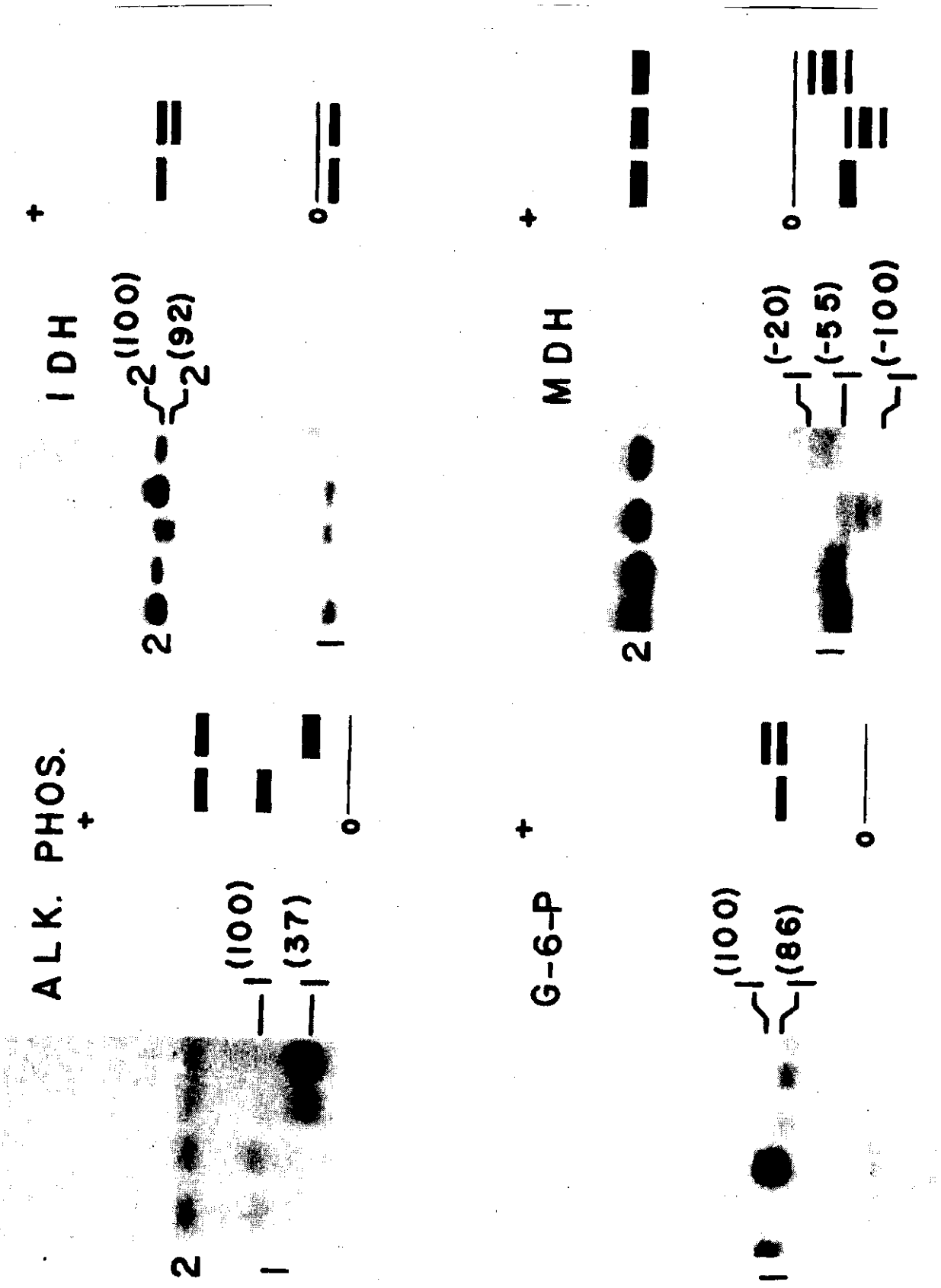

Fig. 7. Phenotypes of Alk. Phos. IDH, G-6-P, and MDH. These enzymes appear polymorphic in harp seals. All phenotypes shown are from kidney tissue.

Discussion

The low genetic variability observed in the harp seal makes it one of the least variable vertebrate species examined to date. Low levels of genetic variability may be attributed to a variety of evolutionary events. If a population is small, then the genetic consequences of drift, inbreeding, or founder effects may be increased homozygosity. Genetic drift in small populations is believed to have mediated reduced heterozygosity in cave fish (Avise and Selander 1972), island populations of mice (Selander et al. 1971), lizards (Webster et al. 1972), and isolated populations of Drosophila (see Prakash et al. 1969). "Bottlenecking" also results in increased homozygosity in a population. This occurs when a population is reduced to small numbers of reproductive individuals which form the nucleus of a subsequent population expansion. In pinnipeds, exploitation of the elephant seal is thought to have reduced effective breeding populations to very low levels. Lack of variability in the southern elephant seal M. leonina, and homozygosity encountered in the northern elephant seal M. angustirostris may be accounted for by the bottleneck phenomenon (Bonnell and Selander 1974).

Although all three major stocks of harp seals have been heavily exploited for decades (Sergeant 1976a) and all have undergone a marked decrease in numbers, the harp seal is presently an abundant pinniped species, surpassed in numbers only by the unexploited crab-eater Lobodon carcinophagous in the Antarctic (FAO 1976). Northwest Atlantic harp seals, although perhaps reduced to about one-third their aboriginal level (Lett and Benjaminsen 1977), still number about 1.2 million animals aged one and older. The estimated number of breeding females (or annual pup production) is in the order of 250,000 to 350,000 (Lett et al. 1977), and the gene frequency data reported herein indicate random interbreeding between these animals. The Jan Mayen stock breeding on the West Ice comprises some 100,000 animals producing about 25,000 pups annually: the White Sea stock is now thought to number between 500,000 and 700,000 animals which produce, according to recent estimates, between 110,000 and 175,000 pups annually (FAO 1976, Øritsland 1976). Since there is no evidence that harp seals in these three areas have ever been at significantly lower levels than at present, bottlenecking may be rejected as a viable explan-

ation for low genetic variability (Nei et al. 1975) in this species.

Some enzymes which could not be resolved in the present study might possess variation that was not detected or could not be quantified. Polymorphism was observed in Esterases, LAP, CK-2, Acid Phosphatase, and 6-PGD, but these isozymes could not be successfully resolved for genetic variation analyses. Had these loci been resolved, calculated variability would have been higher. However, considering the large number of monomorphic loci observed, this increase would not have resulted in a substantial increase in heterozygosity. Some of the enzymes which were considered monomeric in the present study might well be polymeric in harp seals and this would result in a reduction of the total number of monomorphic loci reported. If heteropolymers had been observed, polymeric isozymes could have been identified and the loci classified more accurately. Nevertheless, the actual number of loci involved would not be sufficient to bias the reported results substantially. Techniques involving heat denaturation have been used recently to detect some variable alleles in esterases (Bonhomme and Selander 1978). Use of these or other techniques might have increased the absolute genetic variability in the present study. However, many enzymes do not demonstrate heat sensitive alleles and the vast majority of studies dealing with levels of heterozygosity have not used these techniques. For comparative purposes, all studies using a large number of loci and similar techniques should underestimate total heterozygosity to a similar degree (Ayala 1978), perhaps by a factor of three according to Powell (1975). Even if average heterozygosity per individual in Northwest Atlantic harp seals is 3 x 0.01 or 0.03, it still represents one of the lowest values encountered in a bisexually reproducing species (Powell 1975, Selander 1976, Nevo 1978).

Low levels of genetic variability in some species have been attributed to a relatively stable environment. This tenet was reviewed by Bryant (1974) and has been offered as an explanation for low levels of heterozygosity in some fossorial mammals (Spalax, Nevo and Shaw 1972; Thomomys talpoides, Nevo et al. 1974, the American Alligator Alligator mississippiensis, Gartside et al. 1977), as well as an alternative explanation to bottlenecking in the northern elephant seal (Bonnell and Selander 1974). Conflicting results from other fossorial rodents (Geomys bursarius, Selander et al. 1974; Thomomys bottae, Patton and Yang 1977) detract from the suggestion

that the subterranean environment is "selecting for" homozygosity. Thus Patton and Yang (1977) preferred to invoke bottlenecking and reproductive isolation to account for low levels of heterozygosity observed in some populations of fossorial rodents.

The correlation of low genetic variability in homogeneous environments, aside from the limited and somewhat contradictory empirical evidence noted above, has also been postulated on theoretical grounds (Levene 1953, Levens 1968, Van Valen 1965, Rothstein 1973). According to the niche-variation model, specialized organisms occupy a narrow niche and exhibit low genetic variation; generalists occupy a wider niche and consequently exhibit more genetic variation (Rothstein 1973).

Although little is known of the evolutionary history of pinnipeds (McLaren 1975, Mitchell 1975) it seems reasonable to assume that there was strong selection pressure on ancestral seals (and other marine mammals) in order to successfully adapt to a relatively narrow and specialized (for mammals) niche in the marine environment. Perhaps then, founding effects and strong selection pressures (Lewontin 1978) can be invoked for early pinnipeds, such that ancestors which passed through the transition to aquatic life became essentially homozygous for those enzyme systems which were of greatest adaptive value in this "new" environment. Large, interbreeding populations would, under these circumstances, reinforce fixation of any rare or mutant allele which demonstrated a selective advantage. The constraints of the marine environment on the mammalian body plan may well have selected against variant alleles and thus maintained low genetic heterozygosity.

Evidence of specialized modifications in the basic mammalian body plan resulting from reinvasion of the aquatic environment is abundant. Distantly related marine mammals - Pinnipedia (Order Carnivora), Cetacea, and Sirenia, demonstrate convergent evolution in several morphological traits and physiological adaptations (Hart and Fisher 1964). These include specialized body shapes (Lewontin 1978), elaborately modified and/or unique appendages for locomotion in water; thermoregulatory adaptations - principally the elaboration of an insulating layer to copy with the thermal properties of water (Hart and Fisher 1964); cardiovascular adjustments related to tolerance apnea during proionged dives (Andersen 1966); and

adaptations of visual (Schusterman and Balliet 1971, Jamieson and Fisher 1972, Lavigne et al. 1977) and auditory (Ramprashad et al. 1972, Ramprashad 1975, Schusterman 1975) systems for use underwater.

Although the suggestion that low genetic variability in seals is related to specialization of the terrestrial mammalian plan for life in water is consistent with the niche-variation model, other considerations are relevant. Benthic marine invertebrates live in one of the most temporally and spatially stable environments on Earth, both in terms of physical parameters, e.g. temperature, light, and biotic characteristics, e.g. trophic resource supply (Ayala et al. 1975a, Sanders 1968, Hessler and Jammars 1974). Yet benthic organisms examined to date exhibit relatively high to normal levels of genetic variability (Ayala et al. 1975a, 1975b, Nevo 1978). Furthermore a comparison of genetic variation as a function of latitude in marine invertebrates (Ayala et al. 1975a, Gould 1975) reveals a gradient in variability which is negatively correlated with trophic resource variability as one moves from low latitudes towards the poles. Low genetic variability in marine invertebrates thus appears to be related more to resource variability than to the homogeneity of the environment (Ayala et al. 1975a). To account for these observations Ayala and Valentine (Ayala et al. 1975a, Gould 1975) suggested that, in a variable environment, selection would favour "phenotypic plasticity". Individuals best able to cope with a variety of environmental conditions would be able to locate and utilize many alternate food resources, and as a result would have a higher fitness than less adaptable individuals. In such situations, they concluded that selection would favour "generalized alleles" whereas any variant alleles which lowered average fitness would be selected against. As a result, species adapted to a variable resource base would have few alleles at an average locus and individuals would tend to be highly homozygous. On the other hand, in homogeneous and trophically stable environments variant alleles may not be selected against and this may lead to high levels of genetic polymorphism in the population and of high heterozygosity in individuals (Ayala et al. 1975a).

The above hypothesis appears to account for variability in some deep sea invertebrates and may well be relevant to the lack of variability observed in harp seals and other phocids examined to date. This hypothesis also appears to contradict the niche-variation model. On close examinat-

ion however, some of the apparent contradiction is generated by semantics, specifically the continued use of such imprecise and subjective concepts as "habitat specialist" and "habitat generalist" (Nevo 1978), and homogeneous, heterogeneous, stable and variable environments. When used in general ecological and evolutionary contexts without precise specification of what individual trait or environmental component is being considered such concepts become meaningless. For example, using circular reasoning, a species characterized by high genetic heterozygosity and capable of adapting to a variety of random or stochastic changes in the environment is usually called a generalist. Another species may be termed a generalist because it utilizes a wide variety of prey species; yet this species might well be characterized by very low genetic heterozygosity (Ayala et al. 1974a; Pianka 1978). In the terms of Roughgarden (1972, 1974) the first species is emphasizing the between-phenotype component of niche breadth, the latter, the within-phenotype component. The latter species would be termed a "pure generalist" by Pianka (1978), possessing the "generalized" alleles of Ayala and Valentine (Ayala et al. 1975a). However, in reality, it is a specialist, with the ability to adapt to non-random and seasonal (and in long-lived species, annual) events in the ecosystem. Its adaptations define broad, but delineated, and temporally varying, tolerance limits which permit survival in such environments. The environment, although variable on an annual basis is still homogeneous in the sense that all individuals in the population are exposed to the same environmental conditions at the same time throughout the year. True to the niche-variation model, these "generalized specialists" are characterized by low genetic variability and must be considered susceptible to random or stochastic perturbations natural or man-made, consistent with the mass extinction hypothesis (Bretsky and Lorenz 1970).

Harp seal range limits in the North Atlantic (Sergeant 1976) are correlated with the seasonal limits of the Arctic pack ice (Baird 1964, p 103). More important to the present discussion, the distribution of harp seals is essentially contiguous with the Subarctic of the sea as defined by Dunbar (1968, p 44). According to Dunbar the subarctic marine environment is characterized by being more variable than the true Arctic zone. It is an area of mixing which leads to "instability and increased produc-

tion", which is characterized by two peaks in productivity as occurs in temperate waters; it has a greater variety of fauna and flora and thus greater ecosystem complexity than the Arctic, and exhibits greater sensitivity to marine climatic change. The harp seal spends the year migrating throughout the Subarctic marine environment (Sergeant 1965), spending the summer in waters surrounding the eastern Canadian Arctic Archipelago and off West Greenland, moving to the whelping grounds in the Gulf of St. Lawrence and off Newfoundland in late winter and early spring, an annual migration of perhaps 6000 km.

Within this subarctic marine ecosystem the harp seal is usually considered to exist near the top of the trophic web but in reality it is an opportunistic feeder consuming primary consumers e.g. mysids, euphausids, and higher consumers e.g. capelin Mallotus villosus and cod Gadus morhua, sometimes simultaneously (Lavigne et al. 1976). Harp seals along west Greenland during the summer have been observed eating exclusively capelin in one area, while others farther north, were apparently eating invertebrates (Kapel 1975). At other times of the year such as during whelping and nursing, harp seals undergo a period of negative energy balance. Thus the harp seal does exist in a variable environment, eats a wide spectrum of prey species and with its low genetic variability satisfies the criteria of the almost idealized "pure generalist".... with each member of the population exploiting the entire range of resources used by the total population (Pianka 1978). The harp seal has maximized the within-phenotype component of niche breadth, while minimizing the between-phenotype component. The between-phenotype component of niche breadth is presumably realized to some extent by behavioural segregation which results in somewhat different feeding habits associated with age, body size, and sex (Sergeant 1973b, 1976, Lavigne et al. 1976). These differences only involve, as far as is known, tendencies of smaller and younger seals to consume smaller components of the adult prey spectrum (Sergeant 1973b) and do not involve exploitation of very different types of organisms. The low genetic variability observed in harp seals and other phocids is thus consistent with the hypothesis that low genetic heterozygosity is associated with adaptations for a specialized mammalian life style; it is also consistent with the hypothesis that low variability is correlated with variable, but predictable, resource availability (Ayala et al. 1975a).

The apparent benefits of low heterozygosity in organisms such as seals, some fossorial mammals, and marine invertebrates exploiting a variable trophic resource superficially appears contrary to basic evolutionary theory which dictates that genetic variability is the foundation upon which evolution is structured. Variability and isolation lead to speciation whereas in an evolutionary context a lack of variability (reduction in heterozygosity) is considered to be extremely hazardous (Kimura and Ohta 1971, Dobzhansky 1970, Dillon 1973), often leading to extinction (Bretsky and Larenz 1970). However, "Selection has nothing to do with what is necessary or unnecessary, or what is adequate or inadequate for continued survival" (Williams 1966). Any trait of an organism relates to how quickly it accumulates resources for reproduction, how likely it is to stay alive to use those resources for reproduction, and how it distributes those resources in reproduction (Smith 1976). Selection can only operate in the present, "it is not a mechanism that can anticipate possible extinction and take steps to avoid it". Since history suggests that extinction is the ultimate fate of all species (Lewontin 1978), it matters little if adaptations to current conditions are mediated by low genetic variation within a population or species. Similarly, according to Williams (1966) there is no support for the general conclusion that heterozygosity will guarantee rapid evolutionary responses to changing conditions. Heterozygosity within a population coincidentally increases the probability of certain individuals surviving random environmental events by providing variability on which natural selection can act. Species, such as the harp seal, which exhibit low genetic variability, may thus be extremely vulnerable to any significant changes in their ecosystem.

The results of the present study also suggest that random interbreeding occurs between Northwest Atlantic harp seals. Harp seals born in the Gulf of St. Lawrence and on the Front were essentially identical for all fifty-five presumptive gene loci examined. Two rare isozymes, IDH-$1^{(92)}$ and G-6-P-$1^{(100)}$ were found only in Gulf seals, and one rare allele, MDH-$1^{(-100)}$ was observed only in Front animals. Comparing gene frequencies for the few variable isozymes revealed no significant differences ($p>0.01$) between seals sampled in the Gulf and on the Front. These results are consistent with those obtained using less sensitive measures of reproductive isolation (Naevdal 1969b, ICNAF 1977). Tag

returns (Sergeant 1977, ICNAF 1977, Winters 1978), although few in number, suggest some exchange of female harp seals between the Gulf and Front. However, whether the interchange involves both males and females is not presently known and as long as one sex intermixes consistently the present results would be expected. Therefore on the basis of current evidence Gulland's (1971) suggestion that, for most purposes, Northwest Atlantic harp seals can be treated as one population (or stock) remains valid. It may well be, as recently suggested by Winters (1978), that homing of harp seals to the Gulf and Front for whelping and breeding is a facultative rather than an obligatory behavioural response. Since harp seals are highly gregarious and apparently intermix freely in the Arctic during the summer (Sergeant 1976), animals may also segregate in the Gulf and on the Front on the basis of where particular groups of seals locate food during January and February. Segregation of whelping concentrations in the Gulf and on the Front may thus be determined by gregarious behavioural interactions about which we know nothing, and such environmental events as the freeze-up of the Straits of Belle Isle, which might well baracade animals in the Gulf in a particular year. Even if homing is a characteristic of harp seals, genetic interchange between Gulf and Front animals may still be possible. If harp seals are induced ovulators, females from the Gulf might well be mated by Front males if the former moved to the Front after nursing to find suitable ice on which to moult. Under such conditions, Gulf males would also have an opportunity to mate with Front females. However, little is known about the duration of the mating season in harp seals.

On the basis of all available information, it now seems unnecessary to formulate management strategies based on the two stock hypothesis (Lett et al. 1977). Since Gulf and Front harp seals apparently comprise one randomly interbreeding unit, differential exploitation should not alter the distribution of gene frequencies or bring about the extinction of genetically discrete subpopulations or demes. Considering the low genetic heterozygosity observed in the Northwest Atlantic, one harp seal is very much like any other, and large numbers could be removed without affecting normal gene frequency distributions. Even if the number of seals was dramatically reduced, inbreeding should not substantially

alter gene frequency distributions in isolated groups or be a factor in concentrating unique, perhaps deleterious, combinations of alleles. The progeny of such isolated groups should be genetically similar and be capable of interbreeding with each other. In this context, low genetic variability is of benefit for species which are subjected to heavy exploitation and rapid recovery from exploitation should be expected as long as current environmental conditions persist. Several species of seals which have been exploited in the past have been reduced to very small numbers. When subsequently protected, a number have demonstrated a remarkable resilience and their numbers have increased markedly (FAO 1976). Elephant seals which have undergone such a recovery, attest to the validity of this hypothesis but additional species of pinnipeds must be examined electrophoretically in order to validate the suggestion that low genetic variability is a general characteristic of this group. Northwest Atlantic hooded seals, Cystophora cristata, appear to exhibit as low a degree of polymorphism and heterozygosity as harp seals from the same region (unpublished data). The ideal experimental animal would be the abundant and essentially unexploited crab-eater seal in the Antarctic.

The present study also suggests that management allocation of hunting effort, since the introduction of quota management in 1971 (Sergeant 1976), should not pose any particular problems for the harp seal. Nevertheless, a conservative management strategy for the Northwest Atlantic would probably attempt to maintain effort in approximate proportion to the relative abundance of whelping harp seals in the Gulf and Front, as suggested by Lett et al. (1977), recognizing that deviation from this strategy in any particular year should not have any profound effect on the stock as suggested by Sergeant (in Litt. 1978). It must be emphasized that these comments relate only to the allocation of quotas between Gulf and Front, and make no reference to the absolute numbers of seals taken. The decision as to the actual quota is based on other kinds of data.

Owing to the disparity in the number of loci which were resolved from West Ice samples, it was not possible to calculate genetic similarities between Northwest Atlantic and West Ice harp seals. From gene frequency probabilities, GOT-1 isozymes revealed no significant difference between the two areas ($p>0.05$) but gene frequency probabilities for MDH-1 isozymes were significantly different ($p<0.001$). This would indicate

that there is not random interbreeding between the West Ice and the Northwest Atlantic harp seals which is consistent with the observed morphological differences between seals in the two areas (Khuzin 1967, Yablokov and Sergeant 1963) and tag return data (Sergeant 1976). The amount of isolation would depend on the amount of inbreeding as compared to the amount of outbreeding of individuals from these two areas and this information is not avialable at this time.

In an attempt to assess the failure to obtain information for the majority of enzyme systems from West Ice seals, it is apparent that the enzyme activity in the tissues was reduced prior to arrival in Guelph. After that, all tissues were processed for electrophoresis using identical procedures. In the field, West Ice tissue samples were taken from the same organs in similar ways from freshly killed harp seal pups. The most obvious differences, were the use of 2-phenoxyethanol (Nakanishi et al. 1969) in all the West Ice samples and the freezing of these samples in the sealing vessel's freezer rather than on dry ice. In addition, some of the West Ice samples thawed during shipment to Guelph.

In other studies, certain isozymes were recovered in an apparently unaltered state from phenoxyethanol treated tissues kept at room temperature for a week or sent by air mail without refrigeration from Chile to California (Nakanishi et al. 1969). Merkle et al. (1977) homogenized liver, kidney and spleen tissue samples in phenoxyethanol and stored the supernatants at -20C prior to assaying for 24 presumptive gene loci in hellbenders (Cryptobranchus). Considering the difficulties in obtaining and shipping tissue samples of seals, the usefulness of preservatives such as 2-phenoxyethanol would be a definite advantage in electrophoretic studies. The failure to resolve most of the enzyme systems in the present study was unexpected. Only five presumptive loci could be scored successfully and these loci were evident in all the samples which were assayed. Isozymes of MDH were noted to survive well in phenozyethanol-treated tissues by Nakanishi et al. (1969), and both MDH-1 and MDH-2 were resolved in the harp seal samples from the West Ice. Nakanishi et al. also reported that LDH isozymes were resolved in their tissue samples from a number of vertebrates but LDH isozymes could not be demonstrated in West Ice harp seals. It was, perhaps, fortuitous that the enzyme systems which were demonstrated in the West Ice samples proved to be variable in samples from

the Gulf and Front, and allowed for gene frequency interpretations. The apparent fixation of Alk. Phos-$1^{(37)}$ in West Ice harp seals opposed to A.k. Phos.-$1^{(100)}$ which was found in all the Gulf and Front seals would indicate absolute isolation of the West Ice seals from those of the Northwest Atlantic. No heterozygotes were found in 284 samples (see Table 8). Subsequently, through experimentation, Alk. Phos.-$1^{(37)}$ has been demonstrated from a few phenoxyethanol-treated Gulf harp seals when the tissues were left at room temperature (22°C) overnite. The possibility exists, therefore, that Alk. Phos.-$1^{(37)}$ is an artifact arising from the use of 2-phenoxyethanol and cannot be considered, at this time, an indicator of stock isolation. Alk. Phos. was not examined by Nakanishi et al. (1969) or by Merkle et al. (1977). Since identical phenotypes (genotypes) were encountered for MDH-1 and GOT-1 in Gulf, Front and West Ice seals, there is no reason to suspect that differences in gene frequencies for these loci in West Ice samples are artifacts. It is evident that further study is needed concerning the use of phenoxyethanol before it is used extensively in biochemical population genetics. Additional samples of West Ice, and ideally, White Sea, harp seals should be examined to complete the current discussion of genetic variability and stock delineation in this species.

ACKNOWLEDGEMENTS

This research was funded by a contract from the Fisheries and Marine Service, Department of the Environment (Contract No. KF802-7-2065). Additional support was obtained from the Committee On Seals and Sealing to the Minister of Fisheries and Environment, an N.R.C. operating grant to J.P.B. and a grant from the Donner (Canadian) Foundation to the University of Guelph.

Tissue collection was facilitated by arrangements made by Fisheries and Marine Service. We thank C. Button and M.C. Mercer for various efforts on our behalf. Tissues were collected in the Gulf of St. Lawrence from the M.V. Martin Karlsen. We particularly thank H. Hendriksen, Karlsen Shipping, and Captain Lyngvaer and crew for their assistance and hospitality. Fisheries Officers P. Fleweling and S. Dudka, and W. King and D. Boness provided additional assistance in the field.

On the Front, tissues were collected from the MV Carino. We grate-

fully acknowledge the cooperation of B. Nygaard, Carino Co., St. Johns, Nfld., Capt. Dykes and crew whose hospitality and assistance made the field collections in this region possible. G. Nancekivell got our equipment to St. Johns and safely stowed on board the Carino. We also thank Fisheries Officer Chesley Lethbridge and G. Traverse for their assistance in the field.

Collection of samples from the West Ice was arranged by M.C. Mercer with T. Øritsland, Institute of Marine Research, Directorate of Fisheries, Bergen, Norway. We thank M. Bryden and T. Øritsland for collecting the samples and making arrangements for their shipment to Guelph.

We also thank M. Ferguson for her generous assistance during the laboratory analysis, K. Ronald for the use of laboratory facilities, and Dr. A.W. May, Director-General, Resources Services Directorate, Fisheries and Marine Service, Department of the Environment, Ottawa, who initially suggested that we submit a research proposal for this work, and who was the Scientific Authority for this contract.

REFERENCES

Anderson, H.T. 1966. Physiological adaptations in diving vertebrates. Physiological Reviews 46: 212-243.

Avise, J.C., D.O. Straney, and M.H. Smith. 1977. Biochemical genetics of sunfish. IV. Relationships of Centrarchid genera. Copeia 1977: 250-258.

Avise, J.C. and R.K. Selander. 1972. Evolutionary genetics of cave-dwelling fishes of the genus Astyanax. Evolution 26: 1-19.

Ayala, F.C. 1978. The mechanisms of evolution. Sci. Amer. 239(3): 56-69.

Ayala, F.J., J.W. Valentine, D. Hedgecock, and L.G. Barr. 1975a. Deep-sea asteroids: high genetic variability in a stable environment. Evolution 29: 203-212.

Ayala, F.J., J.W. Valentine, T.E. DeLaca, and G.S. Zumwalt. 1975b. Genetic variability of the Antarctic brachiopod Liothyrella notocadensis and its bearing on mass extinction hypothesis. J. Paleon. 49: 1-9.

Baird, P.D. 1964. The polar world. Longman Group Ltd., London.

Bonhomme, F. and R.K. Selander. 1978. Estimating total genetic diversity in the house mouse. Biochem. Genet. 16: 287-297.

Bonnell, M.L. and R.K. Selander. 1974. Elephant seals: genetic variation and near extinction. Science 184: 908-909.

Borisov, V.I. 1966. Some data of the serological analysis of Pagophilus groenlandicus Erxleben colonies. Zool. Zh. 45: 1890-1892. (In Russian with English summary).

Bretsky, P.W. and D.M. Lorenz. 1970. An essay on genetic adoptive strategies and mass extinctions. Geol. Soc. Amer. Bull. 81: 2449-2456.

Bryant, E.H. 1974. On the adaptive significance of enzyme polymorphisms in relation to environmental variability. Amer. Nat. 108: 1-19.

Comings, D.E. and R.O. Berger. 1969. Gene products in Amphiuma, an amphibian with an excessive amount of DNA. Biochem. Genet. 2: 319-333.

Dillon, L.S. 1973. Evolution, concepts and consequences. C.V. Mosby Co., St. Louis.

Dobzhansky, T. 1970. Genetics of the evolutionary process. Columbia University Press, New York.

Dobzhansky, T., F.J. Ayala, G.L. Stebbins, and J.W. Valentine. 1977. Evolution. W.H. Freeman and Co., San Francisco.

Dunbar, M.J. 1968. Ecological development in polar regions, a study in evolution. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Food and Agriculture Organization of the United Nations. 1976. Mammals in the Seas, Ad hoc Group III on seals and marine otters. UN/FAO/ACMRR/MM/SC/4. Scientific Consultation on marine mammals. 31 August - 9 September 1976, Bergen, Norway.

Gartside, D.F., H.C. Dessauer, and T. Joanen. 1977. Genic homozygosity in an ancient reptile (Alligator mississippiensis). Biochem. Genet. 15: 655-663.

Gould, S.J. 1975. A threat to Darwinism. Natural History, Vol. LXXXIV, No. 10. pp. 4, 9.

Gulland, J.A. 1972. Population dynamics of world fisheries. University of Washington, Seattle.

Hart, J.S. and H.D. Fisher. 1964. The question of adaptations to polar environments in marine mammals. Federation Proceedings 23: 1207-1214.

International Commission for the Northwest Atlantic Fisheries. 1977. Report of the working group on seals. Standing Committee on research and statistics (STACRES) of ICNAF, 15-18 November 1977, Dartmouth, N.S.

International Commission for Northwest Atlantic Fisheries. 1976. Report of Scientific Advisors to Panel A (Seals). Int. Comm. Northwest. Atl. Fish. Summ. Doc. 76/XII/47.

Jamieson, G.S. and H.D. Fisher. 1972. The pinniped eye: a review. In Functional Anatomy of Marine Mammals. Edited by R.J. Harrison. Vol. 1. Academic Press, London and New York.

Kapel, F.O. 1973. Some second-hand reports on the food of harp seals in West Greenland waters. ICES C.M. 1973/N:8.

Kimura, M. and T. Ohta. 1971. Theoretical aspects of population genetics. Princeton University Press, Princeton, N.J.

Khuzin, R.S. 1967. The variability of craniological characters of the harp seal. In Research on Marine Mammals. Edited by K.K. Chapskii and M.I. Iakovenko. (In Russian with English summary).

Lavigne, D.M., C.D. Bernholz, and K. Ronald. 1977. Functional aspects of pinniped vision. In Functional anatomy of marine mammals. Edited by R.J. Harrison. Vol. 3. Academic Press, London and New York.

Lavigne, D.M., W.W. Barchard, S. Innes, and N.A. Øritsland. 1976. Pinniped bioenergetics. UN/FAO/ACMRR/MM/SC/112. Scientific consultation on marine mammals, 31 August - 9 September 1976, Bergen, Norway. (In press).

Lett, P.F. and T. Benjaminsen. 1977. A stochastic model for the management of the northwestern Atlantic harp seal (Pagophilus groenlandicus) population. J. Fish. Res. Bd. Can. 34: 1155-1187.

Lett, P.F., D.F. Gray, and R.K. Mohn. 1977. New estimates of harp seal production on the Front and in the Gulf of St. Lawrence and their impact on herd management. Int. Comm. Northwest Atl. Fish. Res. Doc. 77/XI/68 (Revised).

Levens, R. 1968. Evolution in changing environments. Princeton Univ. Press, Princeton, N.J.

Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. Amer. Natur. 87: 331-333.

Lewonton, R.C. 1978. Adaptation. Sci. Amer. 239(3): 212-230.

Markert, C.L. and G.S. Whitt. 1968. Molecular varieties of isozymes. Experientia 24: 977-1088.

Merkle, D.A., S.I. Guttman, and M.A. Nickerson. 1977. Genetic uniformity throughout the range of the hellbender Cryptobranchus alleganiensis. Copeia 1977: 549-553.

McDermid, E.M., R. Ananthakrishnan, and N.S. Agar. 1972. Electrophoretic investigation of plasma and red cell proteins and enzymes of Macquarie Island elephant seals. Anim. Blood Grps Biochem. Genet. 3: 85-94.

McLaren, I. 1975. A speculative overview of Phocid evolution. Rapp. P.-v. Cons. Int. Explor. Mer. 169: 43-48.

Mitchell, E.D. 1975. Parallelism and convergence in the evolution of Otariidae and Phocidae. Rapp. P.-v. Cons. Int. Explor. Mer. 169: 12-26.

Mitton, J.B. 1978. Relationship between heterozygosity for enzyme loci and variation of morphological characters in natural populations. Nature 273: 661-662.

Moller, D., G. Naevdal, and A. Valen. 1966. Rapport om arbeidet med blod-analyser for populasjonsundersøkelser. Fiskin og Havet 1966(2): 1-17.

Naevdal, G. 1971. Serological studies on marine mammals. Rapp. P.-v. Reun. Cons. Int. Explor. Mer. 161: 136-138.

Naevdal, G. 1969. Blood protein polymorphism in harp seals off eastern Canada. Fish. Res. Bd. Can. 26: 1397-1399.

Naevdal, G. 1966a. Protein polymorphism used for identification of harp seal populations. Arbok. Univ. Bergen. Mat.-Naturv. Ser. 1965(8): 1-20.

Naevdal, G. 1966b. Hemoglobins and serum proteins in four north Atlantic seals, studied by electrophoresis. FiskDir. Skr. Ser. HavUnders. 14: 37-50.

Nakanishi, M., A.C. Wilson, R.A. Nolan, G.C. Gorman, and G.S. Bailey. 1969. Phenoxyethanol: protein preservative for taxonomists. Science 163: 681-683.

Nei, M. 1972. Genetic distance between populations. Amer. Natur. 106: 283-292.

Nei, M., T. Marugama, and R. Chakruborty. 1975. The bottleneck effect and genetic variability in populations. Evolution 29: 1-10.

Nevo, E. 1978. Genetic variation in natural populations: patterns and theory. Theor. Pop. Biol. 13: 121-177.

Nevo, E. and C.R. Shaw. 1972. Genetic variation in a subterranean mammal, Spalax ehrenbergi. Biochem. Genet. 7: 235-241.

Nevo, E., Y.J. Kim, C.R. Shaw, and C.S. Thaeler Jr. 1974. Genetic variation, selection, and speciation in Thomomys talpoides. Evolution 28: 1-23.

Ohta, T. and M. Kimura. 1974. Simulation studies on electrophoretically detectable genetic variability in a finite population. Genetics 76: 615-624.

Øritsland, T. 1976. The management of harp and hooded seals in the west ice. ICES C.M. 1976/N:17.

Patton, J.P. and S.Y. Yang. 1977. Genetic variation in Thomomys bottae pocket gophers: macrogeographic patterns. Evolution 31: 697-720.

Pianka, E.R. 1978. Evolutionary ecology, 2nd Ed., Harper and Row, Publ. New York.

Powell, J.R. 1975. Protein variation in natural populations of animals. In Evolutionary Biology. Edited by T. Dobzhansky, M.K. Hecht, and W.C. Steere. Vol. 8: 79-119. Plenum, New York.

Prakash, S. 1977. Genetic divergence in closely related sibling species Drosophila pseudoobscura, Drosophila persimilis, and Drosophila miranda. Evolution 31: 14-23.

Prakash, S., R.C. Lewontin, and J.L. Hubby. 1969. A molecular approach to the study of genetic heterozygosity in natural populations. IV. Patterns of genetic variation in central, marginal and isolated populations of Drosophila pseudobscura. Genetics 61: 841-858.

Ramprashad, F. 1975. Aquatic adaptations in the ear of the harp seal Pagophilus groenlandicus (Erxleben, 1777). Rapp. P.-v. Reun Cons. Int. Explor. Mer. 169: 102-111.

Ramprashad, F., S. Corey, and K. Ronald. 1972. Anatomy of the seal's ear. In Functional anatomy of marine mammals. Edited by R.J. Harrison. Vol. 1. Academic Press, London and New York.

Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. Stud. Genet. VII. Univ. Texas Publ. 7213: 145-153.

Rothstein, S.I. 1973. The niche-variation model - is it valid? Amer. Natur. 107: 598-620.

Roughgarden, J. 1972. Evolution of niche width. Amer. Nat. 106: 683-718.

Roughgarden, J. 1974. Niche width: biogeographic patterns among Anolis lizard populations. Amer. Nat. 108: 429-442.

Sanders, H.L. 1968. Marine benthic diversity: a comparative study. Amer. Natur. 102: 243-282.

Schusterman, R.J. 1975. Pinniped sensory perception. Rapp. P.-v. Reun Cons. Int. Explor. Mer. 169: 165-168.

Schusterman, R.J. and R.F. Balliet. 1971. Aerial and underwater visual acuity in the California sea lion, Zalophus californianus as a function of luminance. Ann. N.Y. Acad. Sci. 188: 37-47.

Selander, R.K. 1976. Genic variation in natural populations. In Molecular evolution. Edited by F.J. Ayala. Sinauer Associates, Sunderland, Mass.

Selander, R.K. and W.E. Johnson. 1973. Genetic variation among vertebrate species. Ann. Rev. Ecol. Syst. 4: 75-91.

Selander, R.K., D.W. Kaufman, R.J. Baker, and S.L. Willials. 1974. Genetic and chromosomal differentiation in pocket gophers of the Geomys bursarius group. Evolution 28: 557-564.

Selander, R.K., M.H. Smith, S.Y. Yan, W.E. Johnson, and J.B. Gentry. 1971. Biochemical polymorphism and systematics in the genus Peromyscus. I. Variation in the old-field mouse (Peromyscus polionotus). Studies in Genetics VI. Univ. Texas Publ. 7103: 49-90.

Sergeant, D.E. 1977. Studies on harp seals of the western north Atlantic population in 1977. Int. Comm. Northwest Atl. Fish. Res. Doc. 77/XI/58.

Sergeant, D.E. 1976. History and present status of harp and hooded seals. Biol. Conserv. 10: 95-117.

Sergeant, D.E. 1973a. Environment and reproduction in seals. J. Reprod. Fert., Suppl. 19: 555-561.

Sergeant, D.E. 1973b. Feeding, growth and productivity of Northwest Atlantic harp seals (Pagophilus groenlandicus). J. Fish. Res. Bd. Can. 30: 17-29.

Sergeant, D.E. 1966. Reproductive rates of harp seals, Pagophilus groenlandicus (Erxleben). J. Fish. Res. Bd. Can. 23: 757-766.

Sergeant, D.E. 1965. Migrations of harp seals Pagophilus groenlandicus (Erxleben) in the Northwest Atlantic. J. Fish. Res. Bd. Can. 22: 433-464.

Smith, C.C. 1976. When and how much to reproduce: the trade-off between power and efficiency. Amer. Zool. 16: 763-774.

Turner, B.J. 1974. Genetic divergence of Death Valley pupfish species: Biochemical versus morphological evidence. Evolution 28: 281-294.

Van Valen, L. 1965. Morphological variation and width of the ecological nich. Amer. Nat. 94: 377-390.

Wallace, B. 1975. Gene control mechanisms and their possible bearing on the neutralist-selectionist controversy. Evolution 29: 193-202.

Ward, R.D. 1977. Relationship between enzyme heterozygosity and quaternary structure. Biochem. Genet. 15: 123-135.

Webster, T.P., R.K. Selander, and S.Y. Yang. 1973. Genetic variability and similarity in the Anolis lizards of Bimini. Evolution 26: 523-535.

Williams, G.C. 1966. Adaptation and natural selection, a critique of some current evolutionary thought. Princeton Univ. Press, Princeton, N.J.

Winters, G.H. 1978. Production, mortality, and sustainable yield of Northwest Atlantic harp seals (Pagophilus groenlandicus). J. Fish. Res. Bd. Can. 35: 1249-1261.

Yablokov, A.V. and D.E. Sergeant. 1963. Cranial variation in the harp seal (Pagophilus groenlandicus Erxleben, 1777). Zool. Zh. 42: 1857-1965. (Fish. Res. Bd. Can. Translation Series 485).