Contents

Microsatellite analysis of the population structure of the Bering Sea pollock

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The study continues a series of effort aimed at genetic certification of the spawning concentrations of the Bering Sea pollock using microsatellite markers. The numerous microsatellite loci are relatively evenly distributed throughout the genomes and, as a rule, are marked by a high allele polymorphism. Such polymorphism emerges thanks to the DNA-polymerase sliding through along one of the chains in the process of replication. The allele variants are inherited according to Mendel laws, and are customarily considered to be selectively neutral. Despite the very low values of differentiation indices caused by the large effective population size and a high level of gene flow, the sea fishes, including pollock, are weakly but significantly genetically structured by neutral loci within a vast spatial scale (Bailey et al., 1999, DeWoody&Avise, 2000, Bentzen et al., 1996). The Bering Sea pollock was analyzed previously using Karagin, Olutor, Koryak, Navarin and North Kuril concentration samples. It was shown, however, that the genetic distances between the territorially separated spawning concentrations are short, and the dendrograms founded on those distances are not stable. The microsatellite DNA molecular features marked by homoplastics (i.e. differences in the ways of evolution of the same allele variants), and the high probability of reverse mutations slow down the allele frequencies becoming divergent among various spawning concentrations, and entail discrepancies between the results obtained from the samples separated by considerable time intervals (Olsen et al., 2002). The minimum size of sample for the sea fish species of little variance is 70-150 fish depending on the number of allele variants. The number of preparations examined from each sample must not be less than 50. The specimens from territorially distant spawning stocks of an established population status should be introduced into the analysis.

Our study involved the analysis of 306 specimens from six regions of the Bering Sea (Fig. 1): Karagin, Olutor, Shirshov Ridge, Navarin, North Kuril and East Bering Sea. The pollock samples from the East Bering Sea were provided by the courtesy of N. Williamson (Alaska Fisheries Research Center).

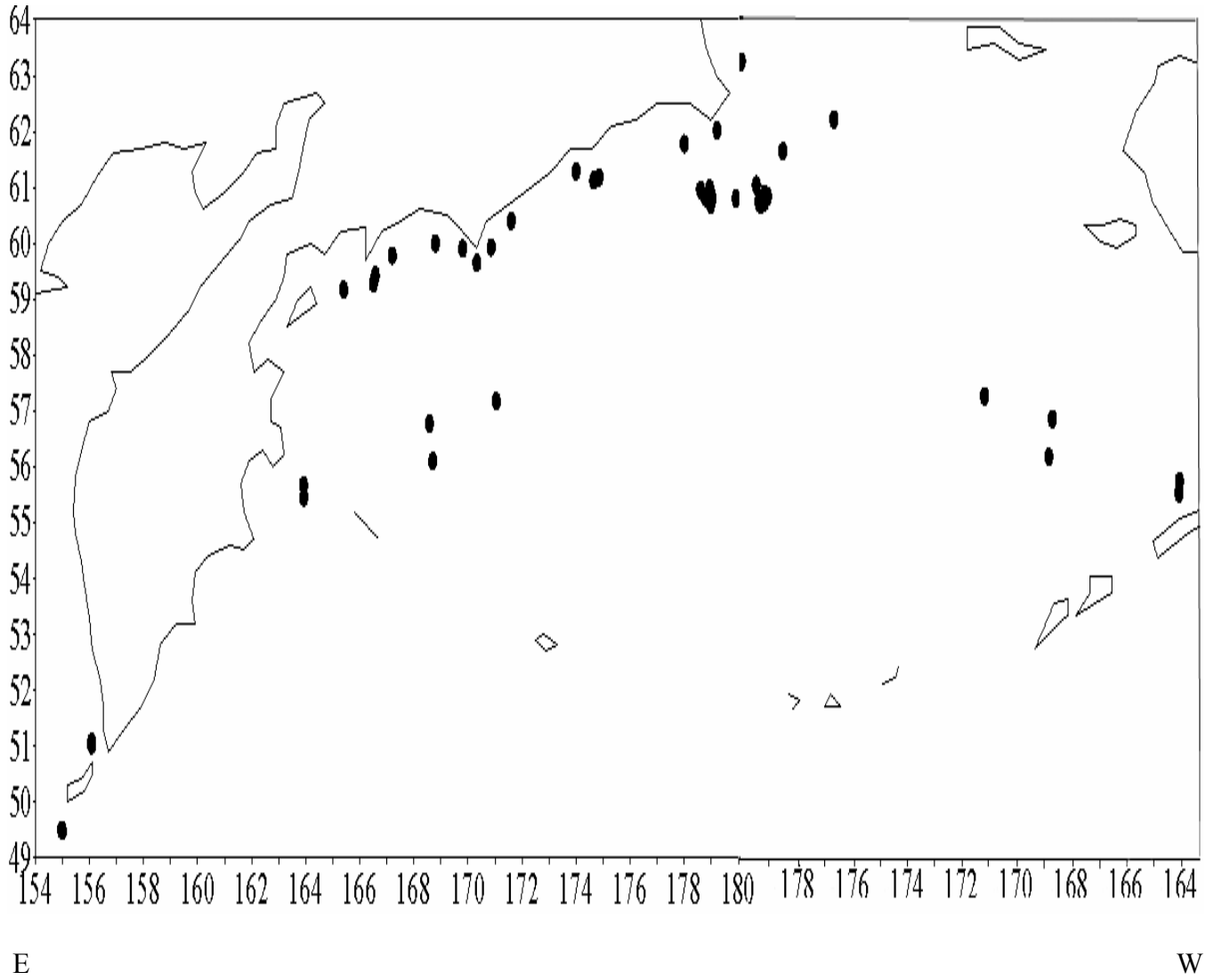


Fig. 1. Sites of genetic sampling.

Materials and methods

Nine microsatellite loci identified by O'Reilly (O'Reilly et al.., 2000) were selected for analysis: Tch5, Tch10, Tch12, Tch13, Tch14, Tch15, Tch18, Tch19 and Tch22. The sequences of nine microsatellite sites and their primers are given in Figure 2.

Tch5 $(GATA)_{14}$
F: gcc tta ata tca cgc aca
R: tcg cat tga gcc tag ttt

Tch10 $(GGCT)_6CTCT\ (GTCT)_2$
F: gtc tct atg tct gtc ttt cta ttt g
R: acg aaa ccc aac cct gat t

Tch12 $(GGTT)_{22}$
F: caa ttt gtc agc ctc tgt tac c
R: agt aca gct tga ttg ttt ctg gg

Tch13 $(GT)_9$
F: ttt ccg atg agg tca tgg
R: agt aca gct tga ttg ttt ctg gg

Tch14 $(GAAA)_{31}$
F: cat aca ttg gtc act ctt tct tac
R: aaa ctg ata tac gcc caa ct

Tch15 $(GA)_3(CA)_2GACA\ (GA)_5CAGATA(GA)_8$
F: aaa ctt cac ctg acc aac
R: gca aca caa ctt aat cat ct

Tch18 $(GT)_{15}$
F: gga gat ggt gct aac tgg
R: aac gca cat gca cat acg.

Tch19 $(GTCT)_{15}$
F: tat gct gat tgg tta ggc
R: gat cat ttg ttt cag aga gc

Tch22 $(GACA)_6$
F: atc ata tct ggc caa gtt c
R: ctc tct ctg aat ccc tct g

Fig. 2. The sequences of nine microsatellite sites and their primers (F-forward; R-reverse).

Electrophoretic subdivision of allele variants and genotyping

The PCR products containing microsatellite fragments were divided in a 6% or 8% polyacrylamid gel in TB acetate. Upon the completion of electrophoresis the gels were colored with ethidium bromide and photographed in UV light. Allele variants were typed with KODAK 1D Image analysis software. When data on marker fragment size is introduced this program allowed us to determine the absolute size of microsatellite alleles. An example of stage of typing process is given in the Figure 3.

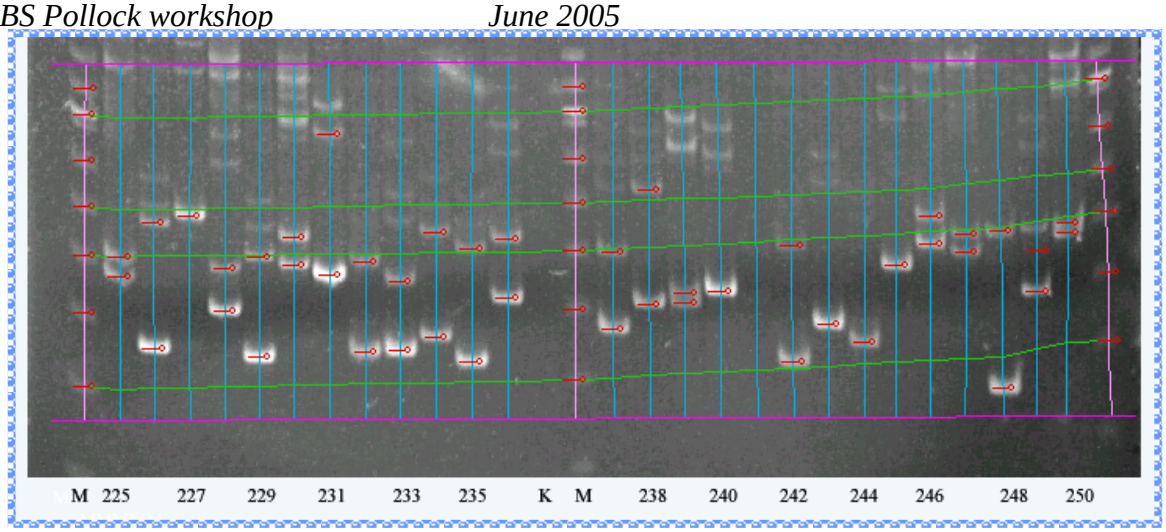


Fig. 3. Locus Tch19 type setting stage.

The products of typing of Tch13, Tch15, Tch18 and Tch22 loci were chemically not stable; that is why those loci were excluded from the analysis.

Tables of allele variants were compiled on the basis of typing.

The genetic parameters of the expected populations examined were determined using TFPGA (Miller, 1997) and GENEPOP 3.1. (Raimond and Rousset, 1995) programs. Hardy-Weinberg equilibrium performance was verified by individual loci of specific populations using criterion X^2 and Haldane (1954) test, with a 99% significance limit (TFPGA), and Guo-Thompson method for polymial distribution (GENEPOP). The equilibrium of populations in Markov chain algorithms was verified by Haldane (1954), Weir (1990) and Robertson-Hill (1984) summary test and Fisher probability test based on tables of conjugated characteristics for each locus, for the given population, and for all loci and populations in general. The genotype imbalance by conjugation was found by pairs between loci within each population, and for all populations simultaneously. The values of F_{is}, F_{it} and F_{st}. (f, F and Θ in Weir-Cockerham algorithms) was determined for all populations by individual loci and alleles, and for all population pairs. The jacknife procedure applied by the loci totally made it possible to estimate F-statistic variance, while the bootstrap procedure set up confidence limits. In the given case one thousand bootstrap steps were made for the 95% level. The interpopulation genetic distances (Nei, 1972, 1978) were calculated as based on the value of F_{st} ($F_{st} = 2D^2$) and are expressed both as a diagonal matrix, and graphically as an unrooted UPGMA dendrogram (TFPGA, PHYLIP).

The genotype imbalance by linkage was determined by pairs between loci within each population, and by all populations together.

Results and discussion

The results of genotyping of DNA preparations by loci are represented in histograms. The height of tiers corresponds to the absolute values of allele frequencies in the given sample; X-axis shows the allele variants of loci while the Y-axis shows the frequency of their occurrence.

Small differences in histograms from various samples were recorded in locus Tch5 (Fig. 4).

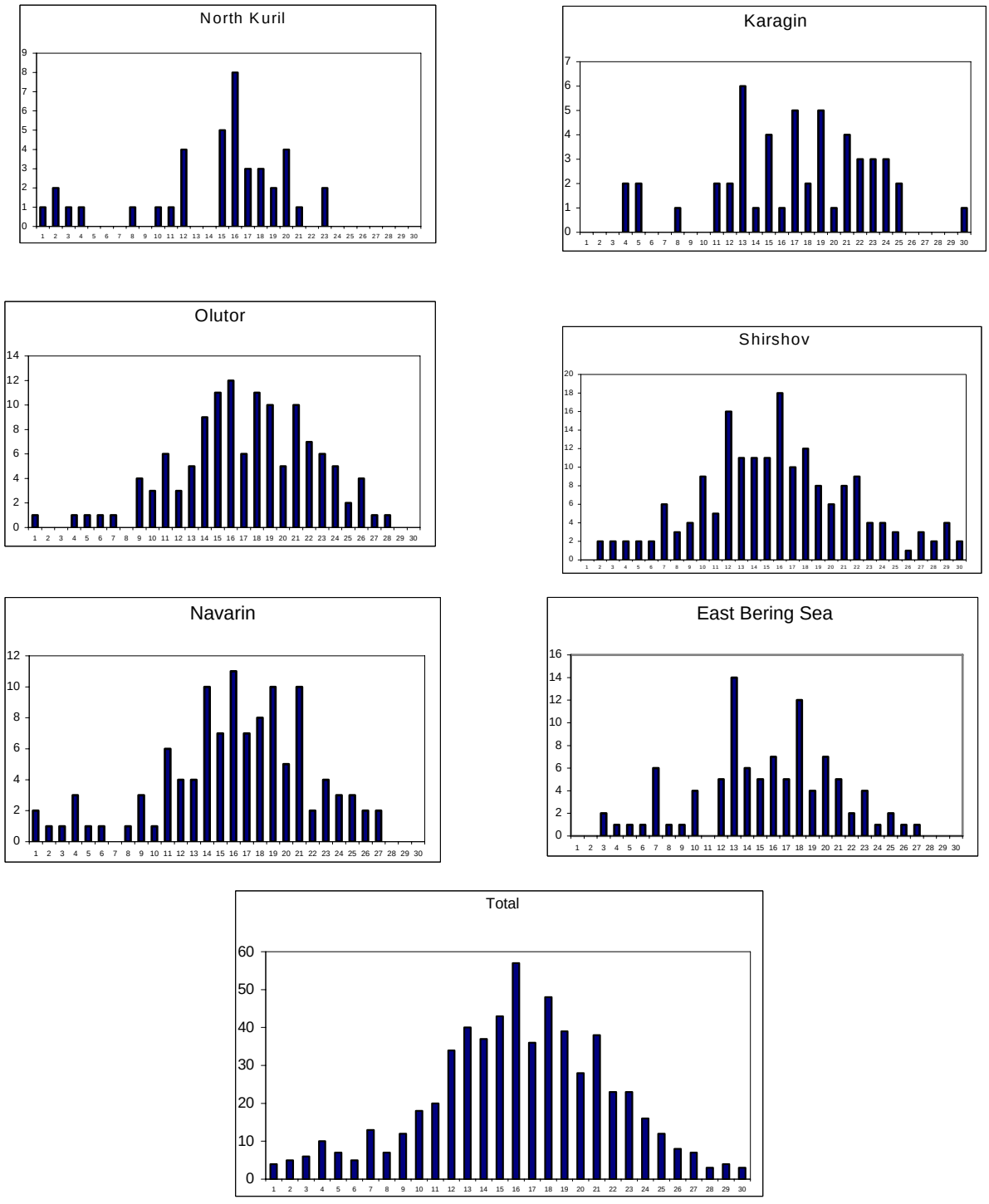


Fig. 4. Distribution of allele frequencies in locus Tch5.

The distribution of allele frequencies in Tch10 varies slightly between the Olutor – Shirshov and East Bering Sea samples. Differences from the small North Kuril sample were observed too. On the whole, however, the profiles were similar (Fig. 5).

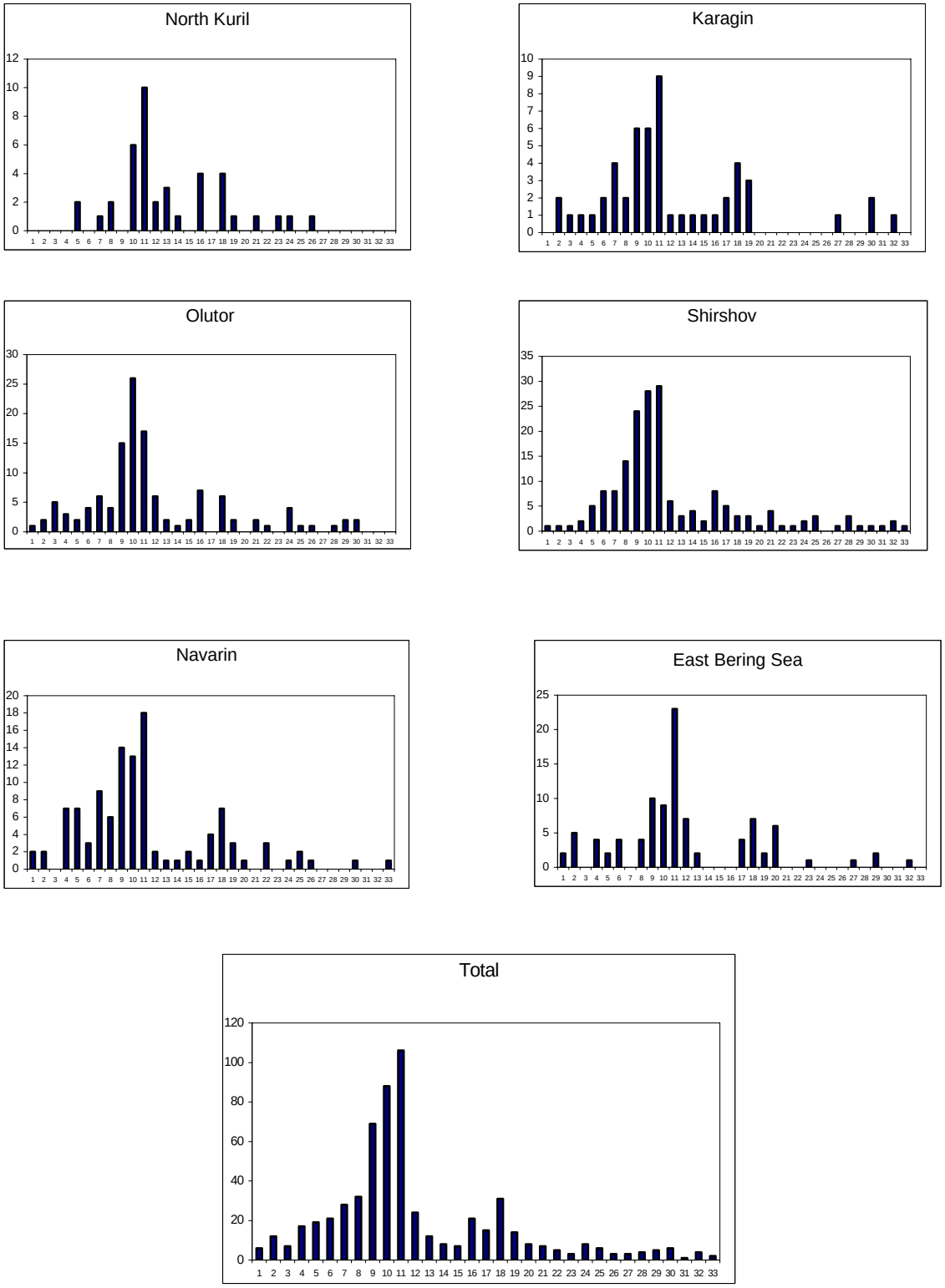


Fig. 5 Distribution of allele frequencies in locus Tch10.

For locus 12 differences were obtained between the North Kuril and Karagin samples only which may result from the insufficient volume of these samples (Fig. 6).

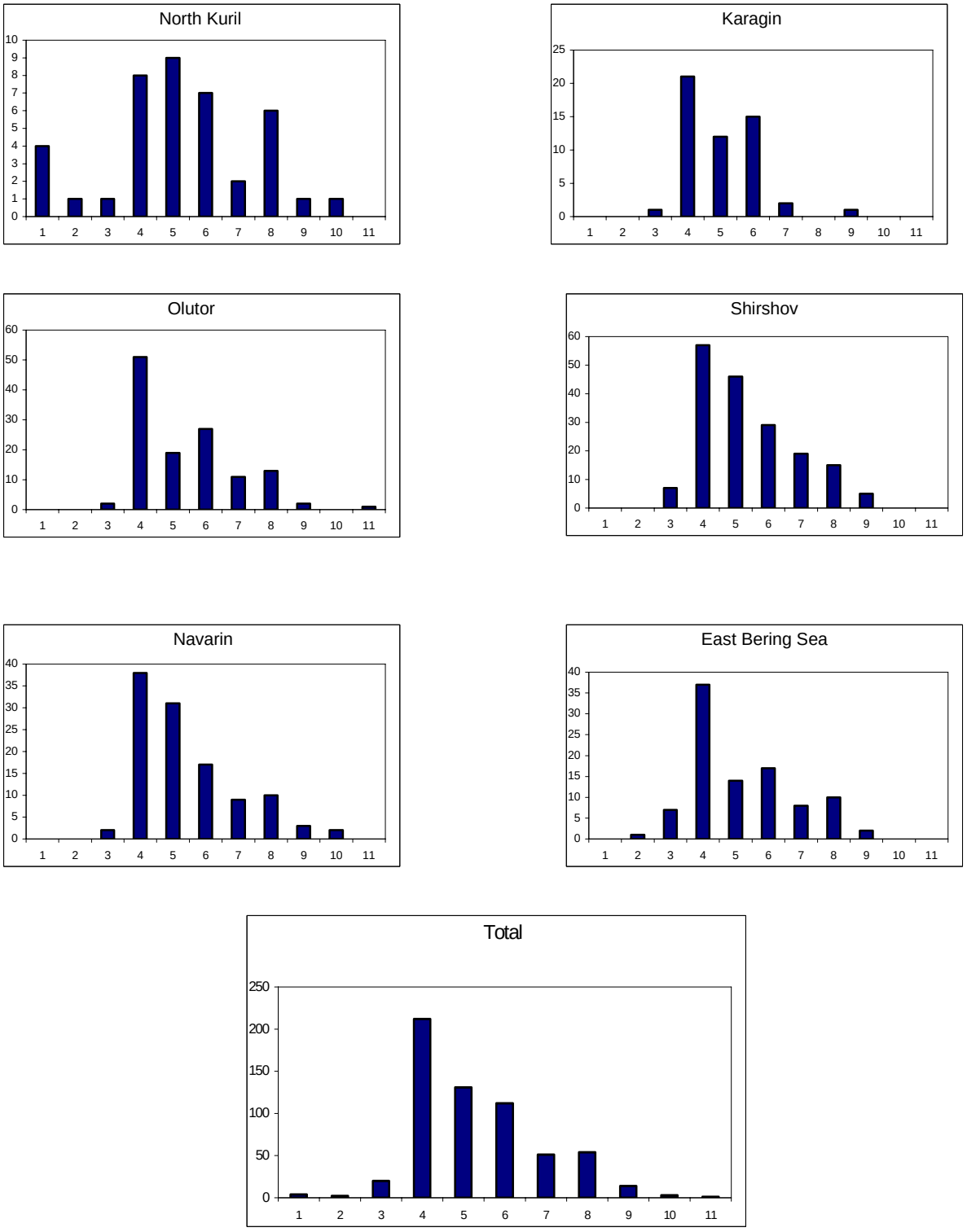


Fig. 6. Distribution of allele frequencies in locus Tch12.

There is some bias in distribution of allele frequencies in the East Bering Sea sample but it occurs smoothly in the series of the Karagin – Olutor – Shirshov– Navarin – East Bering Sea samples (Fig. 7). Totally, the sample's distribution is close to normal.

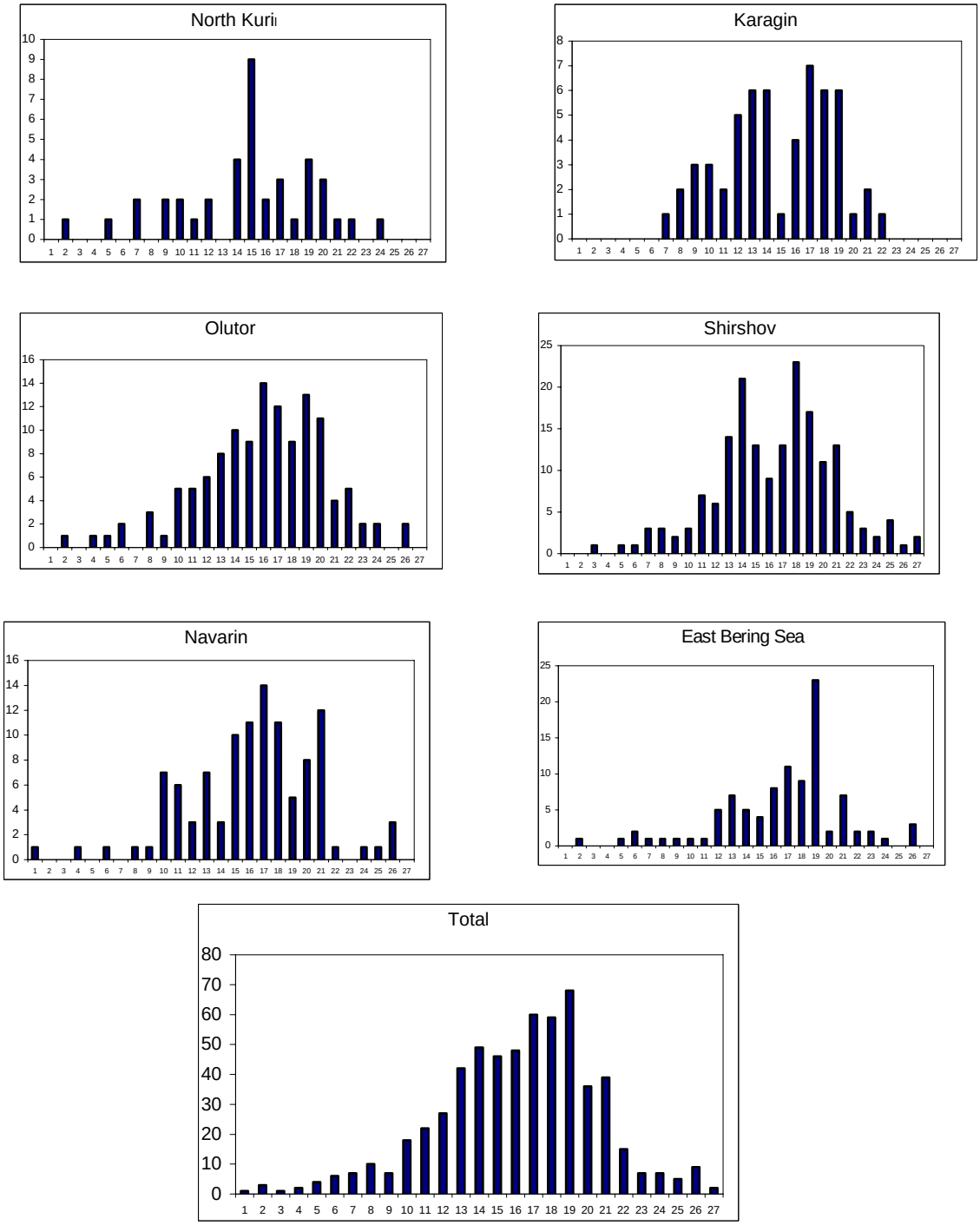


Fig. 7. Distribution of allele frequencies in locus Tch14.

No notable differences were observed in allele frequency distribution in locus Tch19 (Fig. 8).

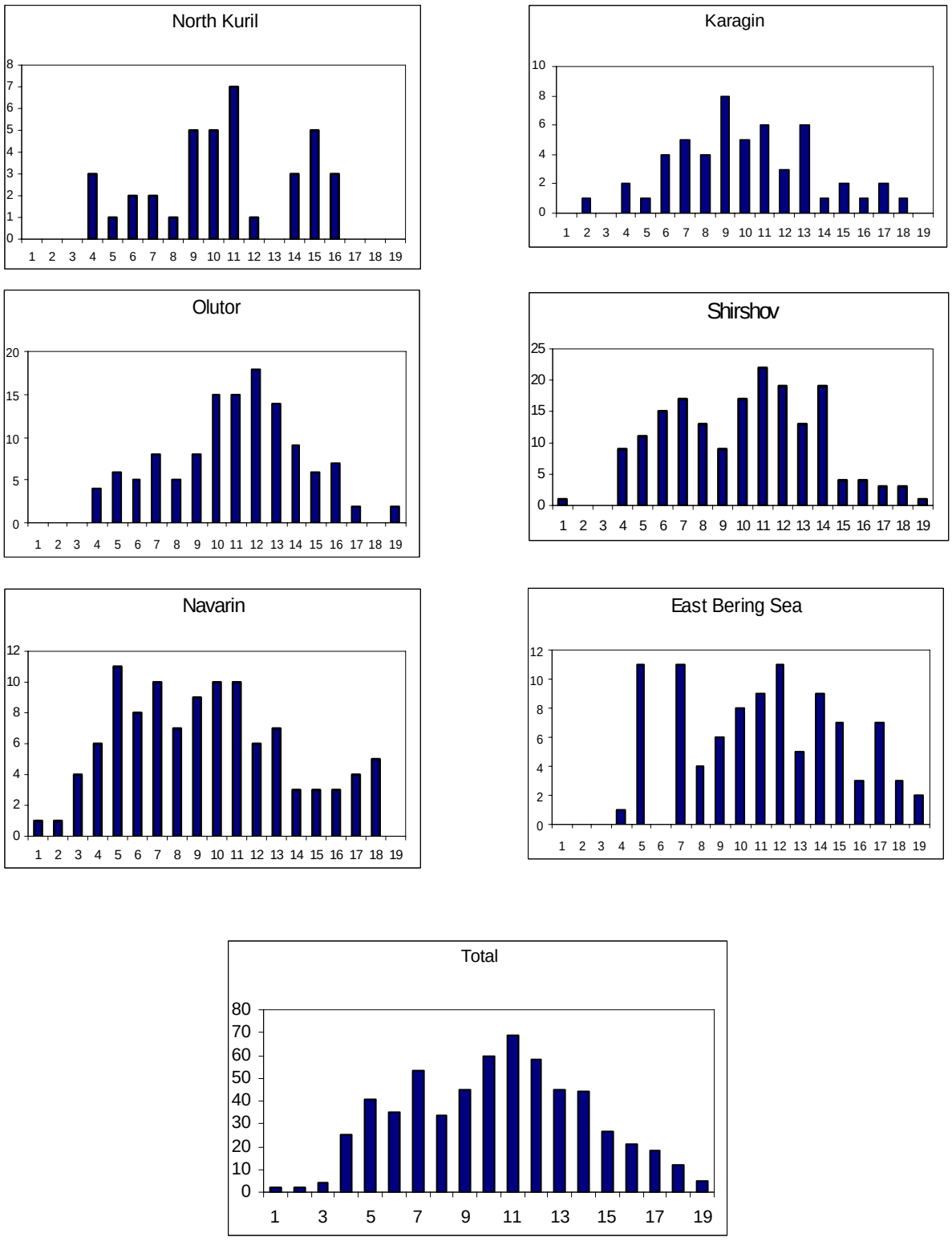


Fig. 8. Distribution of allele frequencies in locus Tch19.

The main genetic indicators of the pollock samples examined and the characteristics of the microsatellite markers used for that are given in Table 1. It includes the volume of the summary samples examined, for each locus, the number of alleles in locus, the allele size pitch in nucleotide pairs, the expected and observed heterozygosity. The most deviant loci are marked with asterisks. The Hardy-Weinberg equilibrium is followed in loci Tch5 and Tch10 only.

Table 1. Characteristics of the main genetic indicators of samples and microsatellite loci. Notation: N – number of specimens examined; Na – number of alleles in locus; R – allele size pitch in nucleotide pairs; He – expected heterozygosity; Ho – heterozygosity observed.

Sample	Loci					
		Tch 5	Tch 10	Tch 12	Tch 14	Tch 19
North Kuril	N	20	20	20	20	19
	N_a	16	15	10	13	12
	R	186-274	145-187	118-154	116-212	106-162
	H_e	0,90	0,88	0,84	0,90	0,88
	H_o	0,90	0,85	0,60*	0,60***	0,74
Karagin	N	25	26	26	25	26
	N_a	19	21	7	16	16
	R	198-302	139-209	126-150	144-204	94-162
	H_e	0,93	0,92	0,70	0,90	0,91
	H_o	0,96	1,00	0,65	0,56***	0,81
Olutor	N	63	63	63	63	62
	N_a	25	26	8	22	15
	R	186-294	137-199	126-158	116-220	106-166
	H_e	0,94	0,91	0,75	0,93	0,91
	H_o	0,83	0,87	0,48***	0,75***	0,66***
Shirshov	N	90	90	89	89	90
	N_a	29	33	7	24	17
	R	190-302	137-213	126-150	124-224	90-166
	H_e	0,95	0,91	0,79	0,93	0,92
	H_o	0,89	0,81	0,61*	0,65***	0,63***
Navarin	N	56	56	56	54	54
	N_a	26	25	8	21	18
	R	186-290	139-209	126-154	112-220	90-162
	H_e	0,94	0,92	0,77	0,92	0,93
	H_o	0,85	0,84*	0,57**	0,70***	0,57***
East Bering Sea	N	49	49	48	49	49
	N_a	25	19	8	22	15
	R	194-290	137-209	122-150	119-224	106-166
	H_e	0,93	0,90	0,78	0,90	0,92
	H_o	0,80**	0,76	0,69	0,71*	0,45***

The analysis of this table shows that heterozygote deficiency of some degree is a feature of all the samples examined. There is no correlation with the size of samples or the number of alleles in polymorphous loci. O'Reilly and others who have developed the set of microsatellite markers used in this paper recognize that in a number of loci (including Tch14 and Tch19) the level of heterozygosity is low (O'Reilly et al., 2004). However, they ascribe that to technical causes, namely to masking in electrophoresis of long alleles by the short ones, and to a greater number of O-alleles (mutations in flanking sequences – Blankenship et al., 2002), and they believe that the "other methods of electrophoresis" and involvement of programs accounting for the probability of deviation from equilibrium make it possible to track down additional alleles which raises the heterozygosity.

The population differences were analyzed by the genotype variants, allele diversity, and gene frequency variance. The North Kuril sample is a significantly differentiated one both in terms of genotypic and allele variants. The second ranking sample showing loci differentiation comes from the East Bering Sea. The concentrations from the Northwest Bering Sea do not show any significant differentiation. The error in summary testing of loci for genotype and allele differentiation is 0.0226 and 0.000 respectively. It is noteworthy that genotype differentiation was found only in loci 5, 10 and 12, for which Hardy-Weinberg law correspondence was shown (Tch12 has a marginal deviation value).

The quantitative measure of the degree of differentiation in a population is a standardized variance of gene frequencies. It is calculated as difference between the variance in an undivided total population and the intrapopulation variance. Table 2 represents the values of variances calculated using two software which apply different algorithms. F_{it} is variance of allele frequencies in the total undivided sample; F_{is} is the intrapopulation variance; F_{st} is the standardized interpopulation variance. The degree of differentiation which we revealed was very low, though the two estimates of this value obtained by using two software agreed almost fully. Locus Tch14 is the only exception where small differences were recorded.

Table 2. F – statistics estimates obtained with GENEPOP and TFPGA software reflecting allele frequency distribution in five microsatellite loci of pollock. * - Locus where differences in variance estimates were recorded.

Software	**GENEPOP**			**TFPGA**		
Estimates / Loci	**F_{IT}**	**F_{ST}**	**F_{IS}**	**F_{IT}**	**F_{ST}**	**F_{IS}**
Tch5	0,092193	0,001542	0,090791	0,0922	0,0015	0,00908
Tch10*	0,064332	0,002922	0,061589	0,0858	0,0026	0,0834
Tch12	0,244932	0,002893	0,242741	0,2460	0,0031	0,2436
Tch14	0,270590	0,003663	0,267908	0,2706	0,0037	0,2679
Tch19	0,316928	-0,000785	0,317464	0,3313	-0,007	0,3318
average	0,1961	0,0020	0,1945	0,2036	0,0020	0,2020

A precise test for subdivision of samples in general by applying TFPGA program using Markov chains concurrently for each of the five loci showed lack of difference for Tch5 locus only (Raimond and Rousset, 1995).

The multilocus test produced the value of X^2 as 60.5213, the number of degrees of freedom being equal to the 10% and 100% probability of differentiation.

Table 3. Pairs of genetic Nei distances (original ones above the diagonal; unbias ones below the diagonal) between various groups of pollock by five microsatellite loci

Concantration	Shirshov	Olutor	North Kuril	Karagin	Navarin	East Bering Sea
Shirshov	***	0,0701	0,2206	0,1128	0,0708	0,1137
Olutor	**0,0121**	***	0,2375	0,1061	0,0906	0,1148
North Kuril	**0,0890**	**0,0987**	***	0,3331	0,2492	0,3042
Karagin	**0,0150**	**0,0011**	**0,1545**	***	0,11185	0,1441
Navarin	**0,0045**	**0,0172**	**0,1022**	**0,0053**	***	0,1445
East Bering Sea	**0,0473**	**0,0412**	**0,1570**	**0,0307**	**0,0627**	***

As is known, Nei distances are functions of the distances expressed in F_{st} units. That is why it is only the unbias values adjusted by the size of sample that are of interest in Table 3.

The major source of being subdivided in the samples analyzed by genetic distances is the set of the North Kuril samples. The difference between the size of Nei distances for the Bering Sea samples is very small; still, there is some correlation with the sites of samples. At any rate, the East Bering Sea and Navarin samples are genetically somewhat more distant than the Navarin and West Bering Sea ones. The UPGMA cluster based on Nei genetic distances is shown in Figure 9. The bootstrap analysis of the cluster showed that the linkpoint uniting the Shirshov and Olutor samples has a 51% bootstrap coefficient, and is supported by 3 loci; linkpoints 2 and 3 (Navarin and Karagin samples respectively) have bootstrap coefficient of 78% and 66%. Finally, the East Bering Sea and North Kuril samples were 100% supported by all the markers used.

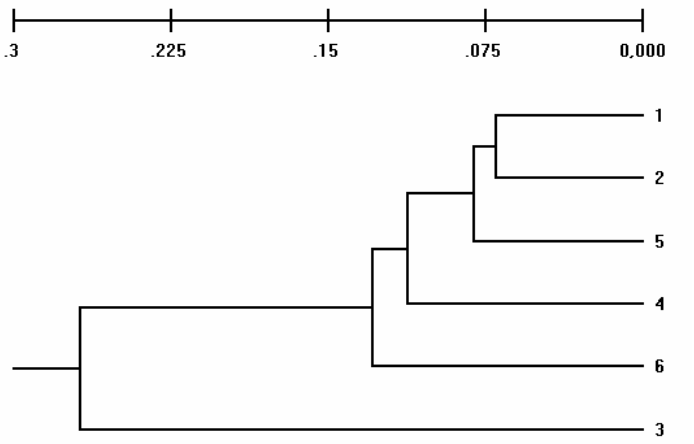
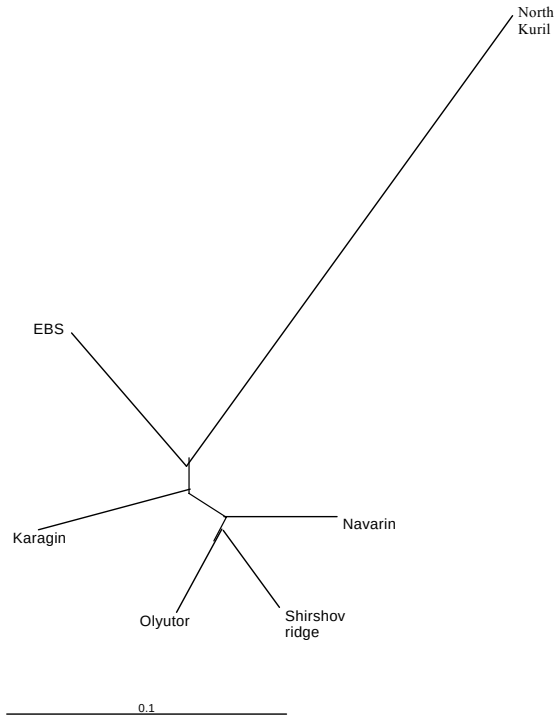


Fig 9. UPGMA dendrogram of pollock concentrations based on Nei genetic distances. Samples; 1. Shirshov; 2. Olutor, 3. North Kuril; 4. Karagin; 5. Navarin; 6 East Bering Sea.

The unrooted dendrogram constructed on the basis of Nei distances shows the degree of genetic distance of pollock groupings from one another which agrees well with the geographic distances between them (Fig. 10).

Fig 10. UPGMA dendrogram of pollock concentrations based on Nei genetic distances (unrooted)



Conclusions

1. Hardy-Weinberg law test confirmed the genetic equilibrium of all the groupings examined.
2. The allele frequency analysis was used as basis for the conclusion regarding the existence of a genetic structure in the sample considered.
3. A quantitative evaluation of the difference showed similarity among the West Bering Sea concentrations, Navarin inclusive. The East Bering Sea samples are very disparate in terms of genetic distances. The North Kuril Grouping stands expressly aside.
4. The genetic distances between the samples on the whole reflect the geographic remoteness of concentrations. The Karagin grouping is an exception. This might result from the insufficiency of the sample.

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