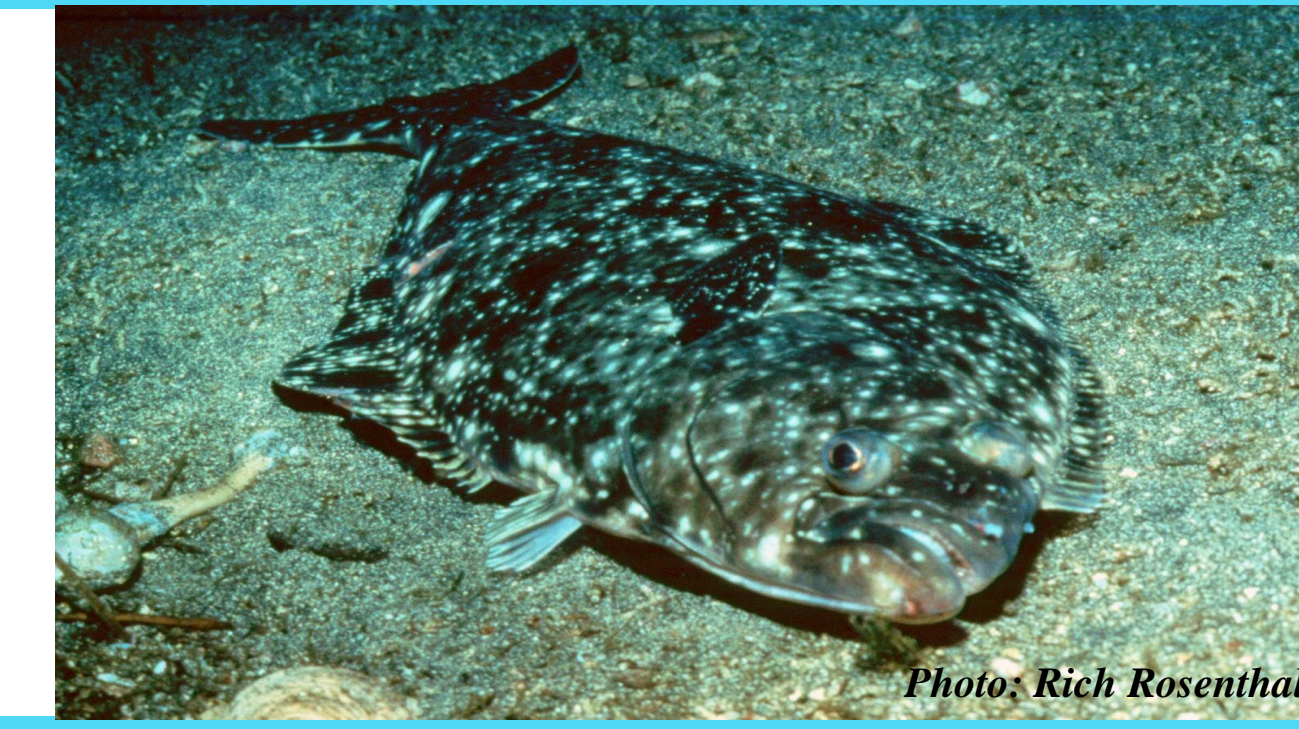


Genetic population structure of Pacific halibut (*Hippoglossus stenolepis*): progress to date



Species distribution and management structure

Pacific halibut (*Hippoglossus stenolepis*) are distributed throughout the North Pacific Ocean (Fig. 1), from northern California in the east, northward throughout the Bering Sea, and westward throughout the Sea of Okhotsk and northern Bering Sea. Stocks in the eastern Pacific are managed by the US and Canada via the International Pacific Halibut Commission, with legal retention restricted largely to hook-and-line fisheries that are composed of commercial, recreational, and subsistence sectors. Mortality rates in all harvest sectors and catch limits in target fisheries (Fig. 2, upper) are calculated and applied, respectively, within a series of regional regulatory areas (Fig. 2, lower), and a variety of policy analyses and harvest considerations are structured similarly. However, the numerical stock assessment is conducted at the coastwide scale on an assumption of population-level panmixis.

The species can be highly dispersive at nearly all life history stages – from larval through spawning adult (Fig. 3) – at temporal scales ranging from seasonal to generational. But, behaviors such as philopatry, homing (Loher, *Fish. Res.* 92:63-69), and limited home ranges (Nielsen *et al.*, *Mar. Ecol. Prog. Ser.* 517:229-250), in association with bathymetric and environmental heterogeneity, may contribute to isolation and local adaptation that has the potential to result in significant population structure. Here, we endeavor to identify genetic population structure that might warrant attention at scales not addressed within current management structures, as well to identify genetic signatures that may serve as a mechanism for further understanding dispersal and mixing.

Figure 1: Pacific halibut are distributed throughout the North Pacific Ocean to at least 800 m depth, from northern California in the east to Hokkaido in the west, and northward throughout the Bering Sea and Sea of Okhotsk. Larval settlement and early juvenile nursery areas are likely distributed throughout most of this range in relatively shallow (<50 m) water.

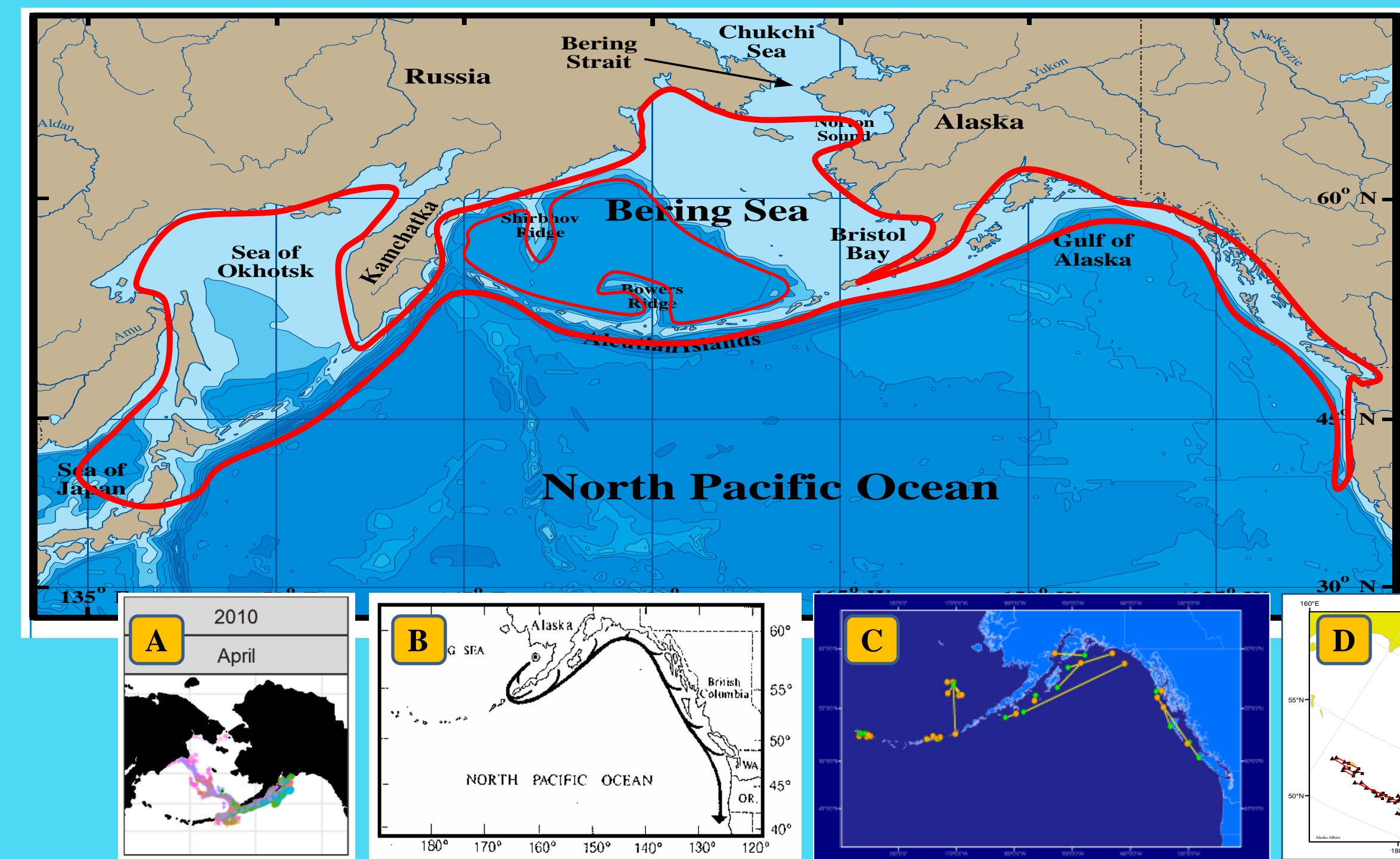


Figure 2: The Pacific halibut resource in the eastern Pacific Ocean is managed within a series of Regulatory Areas, some of which were established to reflect perceived biological stock structure (e.g., Area 4B in the Aleutian Islands region) and others of which were intended to represent socioeconomic or political units within broader biological regions (e.g., Area 2B, defined as all Canadian waters).

	2A	2B	2C	3A	3B	4A	4B	4CDE	Total
O2E Non-FCEY									
Commercial discard mort.	0.01	0.07	NA	NA	0.13	0.06	0.03	0.02	0.32
Bycatch	0.11	0.23	0.02	1.01	0.45	0.29	0.20	1.96	4.28
Recreational	NA	NA	1.43	1.86	0.91	0.02	0.00	0.00	3.31
Subsistence	NA	0.41	0.44	0.22	0.01	0.01	0.00	0.00	1.14
Total Non-FCEY	0.12	0.71	1.89	3.09	0.61	0.37	0.22	2.04	9.04
O2E FCEY									
Commercial discard mort.	NA	NA	0.06	0.30	NA	NA	NA	NA	0.36
Recreational (discard mort.)	0.21	0.43	0.59	1.70	NA	NA	NA	NA	3.08
Subsistence	0.03	NA	NA	NA	NA	NA	NA	NA	0.03
Commercial landings	0.23	2.65	3.01	6.59	1.85	1.32	0.99	1.36	18.49
Total FCEY	0.47	3.14	3.76	8.98	1.95	1.32	0.99	1.36	21.96
TCEY	0.59	3.84	5.65	12.07	2.56	1.69	1.21	3.34	31.00

Figure 3: Pacific halibut may be highly dispersive across life-history stages. Advection modelling suggests that dispersal during the 6-month pelagic larval phase (panel A) may be on the order of 1000s of kilometers (E. Goldstein, NMFS-AFSC Seattle, unpublished), balanced by contrainant migrations of on average the same magnitude conducted as benthic juveniles (panel B) (Hiborn *et al.*, IPHC Sci. Rep. 31). As adults, basin-scale seasonal (panel C) and interannual (panel D) migrations have been documented (Seitz *et al.*, *Fish. Mgmt. Ecol.* 24:339-346; Loher and Clark, IPHC Rep. Assmt. Res. Act. 2010:537-552).

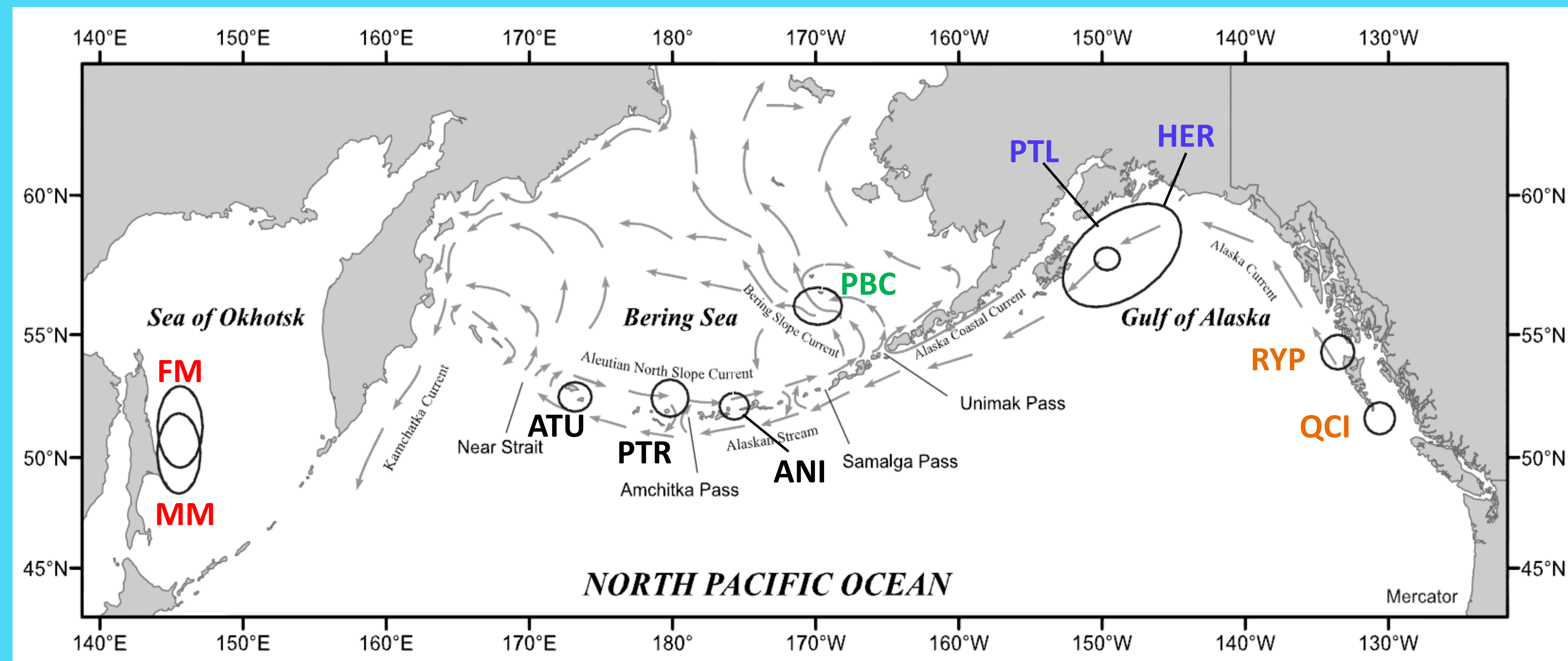


Figure 4: Locations at which adult Pacific halibut were sampled for genetic analyses; note that the 3-letter site designations used here are the same as those used in the table of results and DAPC plot, presented subsequently. Locations spanning British Columbia (QCI and RYP) through the eastern Bering Sea (PBC) and central Aleutian Islands (ANI) were visited during the winter and targeted halibut spawning aggregations. Samples from the western Aleutians (PTR and ATU) were collected during the IPHC's summer stock assessment survey; Russian samples (FM and MM) were also collected during summer.

Prior research

Results from previous analyses of genetic population structure have suggested subtle genetic differentiation between the eastern and western Pacific, despite the fact that the signal of each individual study has been limited. Early allozyme analyses (Grant *et al.*, *Can. J. Fish. Aquat. Sci.* 41:1083-1088) observed differentiation between samples from Japan compared to those from the Bering Sea and Gulf of Alaska; subsequent microsatellite analyses detected differences between Russia and both the Gulf of Alaska and Washington (Bentzen *et al.*, IPHC Rep. Assmt. Res. Act. 1998:229-241). Most recently, Nielsen *et al.* (*Genetics* 11:999-1012) observed significant differentiation between samples collected in the Aleutian Islands relative to individuals from the eastern Bering Sea and Gulf of Alaska.

Recent methodology

Here, samples (N = 198 female, 168 male) from adult Pacific halibut that were collected in the eastern Pacific Ocean from British Columbia to the southeastern Bering Sea and westward into the Aleutian Islands, plus samples from two locations in the Sea of Okhotsk (N = 56 female, 38 male) (Fig. 4) were genotyped using 23 anonymous microsatellite loci and 38 EST-linked microsatellites to investigate potential selective differences. Expected and observed heterozygosity were calculated using Genepop v4.2 and the allelic richness (A_r) and private alleles (A_p) were estimated for each sampling location using rarefaction with five individuals per location using HP-Rare. After removal of sex-linked loci, loci deviating from Hardy-Weinberg equilibrium, and linked loci, overall and pairwise F_{ST} was estimated and tested using the techniques of Weir and Cockerham (*Evolution* 38:1358-1370) implemented by Genepop v4.2. (For full methodology and results see: Drinan *et al.*, *J. Fish. Biol.* 89:2571-2594.)

Findings

Three out of 16 neutral microsatellites were found to be linked to sex (present in females and generally absent in males;) and suggest that – unlike their Atlantic congener (*Hippoglossus hippoglossus*) – females are the heterogametic sex in Pacific halibut (see Galindo *et al.*, *Mar. Biotechnol.* 13:1027-1037). These loci were used to develop sex-identification markers (Drinan *et al.*, *J. Heredity* 109:326-332) that are now routinely used by the IPHC for stock assessment purposes (see poster by Simeon *et al.*, this session).

Genetic diversity (A_r , A_p , H_o , and H_e) was similar among sampling locations and few private alleles were present. Overall F_{ST} was 0.0032 and highly significant ($p < 0.001$) and median F_{ST} value for all site-based pairwise comparisons (Table 1) was 0.0014 (25 and 75% quantiles = -0.0001 and 0.0074). For individual pairwise comparisons, significant differentiation was found in 17 of 28 tests after correcting for multiple comparisons (Table 1). Pairwise comparisons that included samples from ATU or PTR (western Aleutian Islands) had the greatest median F_{ST} values while other sampling locations had median F_{ST} values an order of magnitude smaller (Table 1). Discriminant analysis of principal components (DAPC) plotting showed evidence of separation into distinct geographic groups with the first dimension separating ATU and PTR (both western Aleutian Islands) from all other samples; a second dimension separated ANI (central Aleutians) from all non-Aleutian Islands samples; and all other samples clustered together (Fig. 5).

	Sea of Okhotsk	Aleutian Islands			Bering Sea	Gulf of Alaska	Haide Gwaii		
	FM	MM	ATU	PTR	ANI	PBC	HER	PTL	
FM									
		0.0006 (0.799)	-0.0012 (0.730)	-0.0015 (0.903)	-0.0007 (0.909)	-0.0003 (0.540)	-0.0007 (0.357)	-0.0008 (0.707)	-0.0015 (0.747)
MM	-0.0004 (0.608)		0.0028 (0.041)	-0.0016 (0.277)	0.0014 (0.050)	0.0015 (0.001)	0.0011 (0.047)	0.0003 (0.437)	0.0023 (0.01)
ATU	0.0060 (0.001)	0.0071 (0.001)		0.0008 (0.262)	-0.0001 (0.586)	0.0014 (0.032)	0.0000 (0.416)	-0.0001 (0.521)	-0.0005 (0.454)
PTR	0.0073 (0.001)	0.0089 (0.001)	0.001 (0.006)		-0.0003 (0.093)	0.0000 (0.105)	0.0018 (0.021)	0.0008 (0.027)	-0.0007 (0.101)
ANI	0.0007 (0.145)	0.0028 (0.001)	0.0096 (0.001)	0.0103 (0.001)		0.0003 (0.339)	0.0000 (0.434)	0.0004 (0.461)	0.0001 (0.701)
PBC	-0.0001 (0.539)	0.0012 (0.046)	0.0088 (0.001)	0.0080 (0.001)	0.0016 (0.006)		0.0001 (0.069)	0.0000 (0.102)	0.0001 (0.567)
HER								0.0003 (0.404)	-0.0003 (0.533)
PTL	-0.0001 (0.574)	0.0002 (0.127)	0.0077 (0.001)	0.0103 (0.001)	0.0013 (0.075)	-0.0012 (0.529)			-0.0003 (0.357)
RYP									-0.0003 (0.916)
QCI	-0.0002 (0.239)	0.0008 (0.046)	0.0063 (0.001)	0.0070 (0.001)	-0.0008 (0.889)	-0.0009 (0.806)		-0.001 (0.667)	

Table 1: Genetic differentiation among Pacific halibut from the sites depicted in Fig. 4. Below the diagonal are pairwise F_{ST} and P -values (in parentheses) ultimately based on 14 anonymous and 32 expressed sequence-tag (EST) linked microsatellite markers. Above the diagonal are measures that were based solely upon anonymous microsatellites, representing those which had been included in a prior analysis of Pacific halibut population structure (Nielsen *et al.*, *Genetics* 11:999-1012).

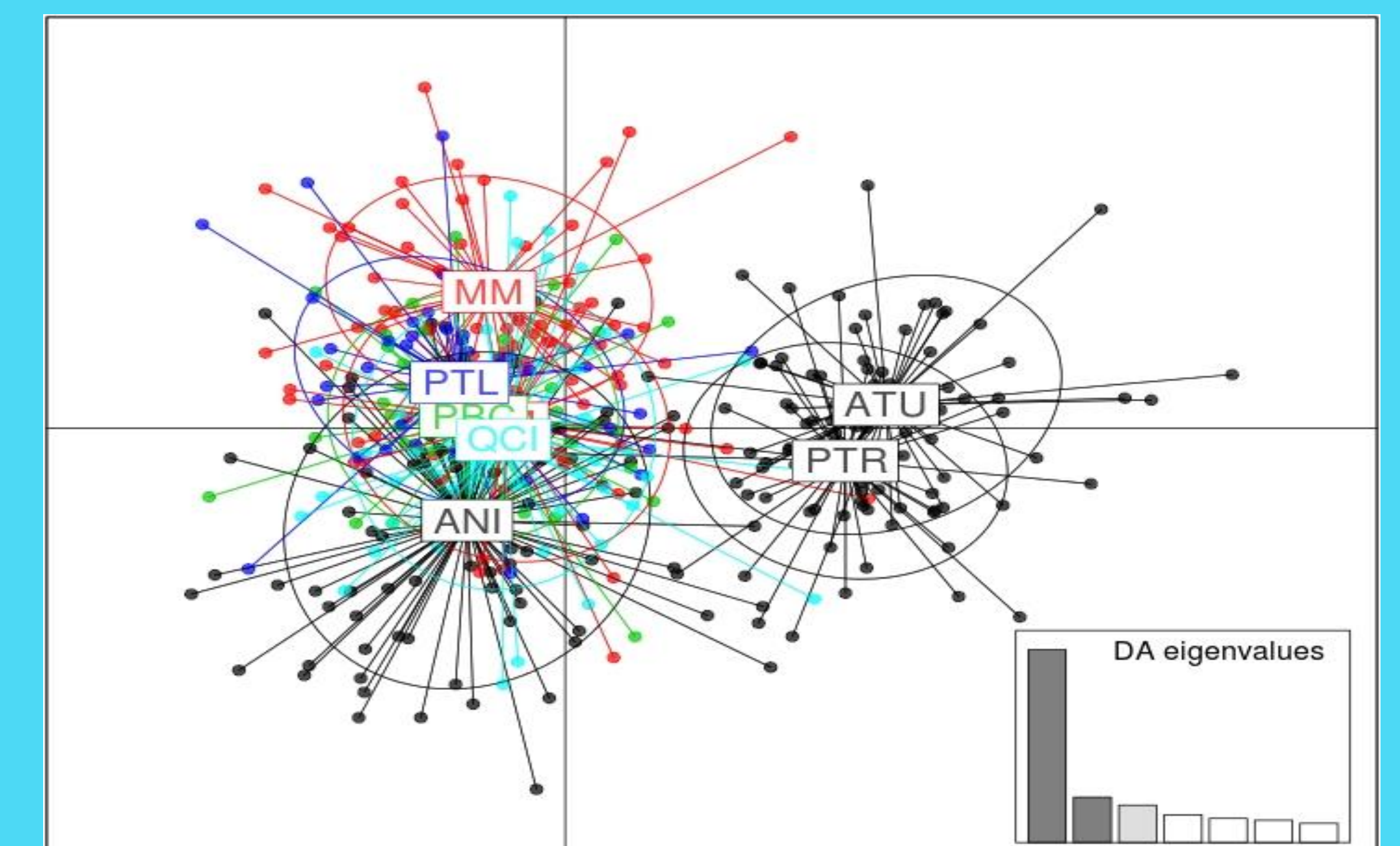


Figure 5: Discriminant analysis of principal components (DAPC) and DA eigenvalues from a study of Pacific halibut population structure using 14 anonymous and 32 EST-linked microsatellite markers. Samples from the western Aleutian Islands (ATU and PTR) show distinct separation from the remaining regions along the first dimension; the central Aleutians (ANI) show weaker separation along the second dimension.

Implications

The results of the population genetic study suggest that IPHC Regulatory Area 4B may be composed of two distinct substocks of Pacific halibut units: its eastern half representing a westerly extension of a genetically well-mixed population inhabiting the continental shelf of North America; and its western half representing a somewhat-isolated substock. If true, regional productivity and stock dynamics may in western Area 4B relative to eastern 4B, warranting the development of spatially-explicit assessment or management procedures that can accommodate any significant differences between these substocks.

We hypothesize that isolation of the Western Aleutian Pacific halibut may arise is due to limited migration of benthic-phase individuals across Amchitka Pass (minimum depth ~1150 m) in conjunction with localized spawning (Seitz *et al.*, *Fish. Mgmt. Ecol.* 24:339-346), retention of those larvae within cyclonic currents that encircle the major Aleutian Island Groups (i.e., the Fox, Andreanof, and Near-Rat Islands), and localized settlement (IPHC unpublished data) that results in regional self-recruitment.

Ongoing and future research

At this juncture, the study's main result is considered provisional due to the nature of the sample collections: i.e., the western Aleutians were sampled during summer, which represents the species' dispersive feeding period and therefore represents the highest probability of encountering reproductive stock mixtures. Efforts are under way to obtain winter samples from both the central and western Aleutian Islands for further analysis. Additionally, modern sequencing technologies including whole-genome resequencing may provide further insights into population structure, dispersal, and mixing; and may allow for the identification of genes under selection. Such genes may reveal temporal adaptation to rapidly changing environments and to spatial and temporal variance in fishing pressure.

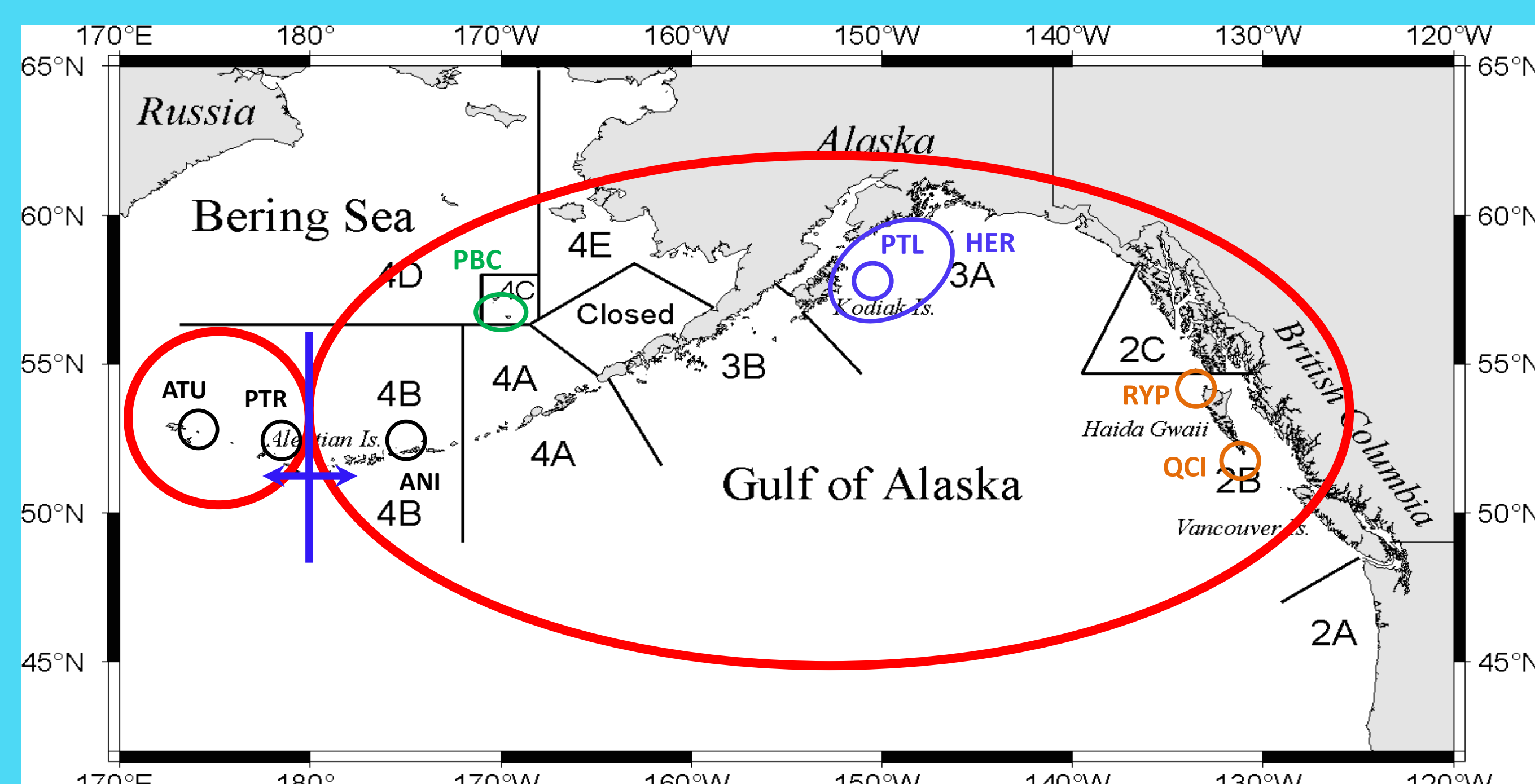


Figure 6: Population structure of Pacific halibut in the eastern Pacific ocean, as implied by genetic analysis. While the population appears to be well-mixed along the coast of North America – and perhaps westward into the central Aleutian Islands – an isolated substock may exist in the Western Aleutians that is separated from the remainder of the stock at Amchitka Pass. Of note is that this separation would not correspond to the boundaries of an existing IPHC regulatory area; rather, Area 4B may be composed of two substocks characterized by different dynamics and productivity characteristics, for which combined stock metrics are calculated and to which equivalent management actions are applied.