NORTH PACIFIC RESEARCH BOARD PROJECT FINAL REPORT

A landscape genetics approach to Pacific cod (*Gadus macrocephalus*) population structure in the Bering Sea and Aleutian Islands; investigation of ecological barriers to connectivity between potentially distinct population components

NPRB Project 817 Final Report

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Abstract

This study tested the hypothesis that more than one discrete population of Pacific cod (*Gadus macrocephalus*) exists within the Bering Sea/Aleutian Islands (BSAI) management area of Alaska. This is a fine scale study of genetic population structure which took into account oceanographic and landscape features that may act as barriers to migration. Nine locations were surveyed (96 fish per sample), with one temporal sample, using 17 microsatellite DNA markers. Samples were taken from spawning fish collected from the western Aleutian Islands east to Unimak Pass and as far north as the Pribilof Island area. The data provides evidence for limited connectivity among spawning groups. In particular, there appear to be three distinct populations among the samples studied; Unimak and Pribilof samples were distinct from each other and from the Aleutian Island samples. Overall, dispersal appears to be limited by distance rather than oceanographic features.

Keywords

Pacific cod, *Gadus macrocephalus*, Bering Sea, Aleutian Islands, Alaska, spawning groups, genetic population structure, population subdivision, genetics, microsatellites

Citation

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Study Chronology

- Observer collected Pacific cod fin clips in Pribilof Island area (February, 2004).
- Fin clips were collected in the western Aleutians in the Near, Kiska, Amchitka, Tanaga, and the Great Siskin areas by UAF researcher on board F/V Seafisher (February-March, 2005). During this same time period, AFSC researchers on board the R/V Ballyhoo collected fin clips from Unimak Pass.
- National Marine Fisheries Service Observers and Dave Fraser of Adak Fisheries of Adak, AK collected fin clips in the Amlia Is. area (February, 2006).
- National Marine Fisheries Service Observers and Dave Fraser of Adak Fisheries of Adak, AK collected additional samples from Amlia Is. and Atka Is. areas (February-March, 2007).
- DNA was extracted from all samples December, 2008.
- New microsatellite markers were screened January, 2008-September, 2009.
- Genotyping was performed on 21 microsatellite loci and all samples July 2009-September 2010.

Introduction

Lack of migration among groups within a species results in restricted gene flow, reproductive isolation, and complex population structure. When two populations are separated for many generations, their signature at neutrally evolving DNA loci will diverge and may become distinct, primarily due to the evolutionary force called genetic drift. Over long time periods, such isolated groups may diverge into distinct species or remain as distinct populations within a species. On land, barriers to gene flow may consist of fragmented habitat due to human land use, or landscape features such as rivers or mountain ranges. Similar barriers exist in the ocean, in the in the form of currents (Gaylord and Gaines 2000), bathymetry (Schüller 2010), and temperature gradients, (Wyllie-Echeverria 1995). A goal of marine resource management is to match management units with populations, because when a management unit consists of more than one distinct stock, unintended depletion of one or more population can occur (Laikre 2005, Taylor 1997).

Pacific cod (*Gadus macrocephalus*) comprise the second largest fishery in the United States, next to walleye Pollock (*Theragra chalcogramma*), both by weight and value (Hiatt et al. 2009). The largest catch is taken from the Bering Sea/Aleutian Island (BSAI) management area of Alaska (Hiatt et al. 2009). They are a key species in the ecosystem, both as predators, primarily of walleye pollock and other fish, crab, and shrimp (Lang and Livingston 1996), and as prey for halibut, seabirds, and marine mammals, including the endangered Steller sea lion, *Eumetopias jubatus* (Sinclair and Zeppelin 2002). They reside

near but not on the bottom, generally from 80-260m, and are not found deeper than about 500m (Bakkala et al. 1984, Nichol et al. 2007). Tagging studies have shown that they undertake both summer feeding migrations and winter spawning migrations, with individual migrations ranging as far 900 km (Shimada and Kimura 1994, Shi et al. 2007). Indirect evidence associated with tagging studies suggests that cod may exhibit site fidelity associated with spawning areas (Gustafson et al. 2000). Within the BSAI, cod are primarily found along the Bering Sea shelf and the Aleutian chain, and spawning areas have been identified throughout this range (Logerwell and Neidetcher 2008).

There is strong evidence that Atlantic cod (*Gadus morhua*) undertake long-distance migrations to feeding areas and return to specific spawning areas for consecutive years (Robichaud and Rose 2001). Results from an extensive tagging effort between 1982 and 1990 indicate that like Atlantic cod, Pacific cod migrate seasonally for reasons related to water temperature, food supply, and spawning grounds (Shimada and Kimura 1994). Seasonal migrations to feeding areas occurred in the summer and spawning grounds in the winter. Tracking data from ultrasonic transmitters in Atlantic cod show that adults have a strong homing tendency, a mechanism that has been attributed to localized population structure (Green and Wroblewski 2000, Wright 2005). Population units in Atlantic cod have been shown to exist in geographic areas as small as 30 km, and gene flow appears to be influenced more by dispersal of pelagic larvae than by adult movement (Jorde et al. 2007, Knutsen et al. 2003).

Oceanographic flow has the potential to constrain marine species dispersal and gene flow (Gaylord and Gaynes 2000), and complex oceanography and bathymetry along the Aleutian chain could act as barriers to migration (Figure 1). Large changes in species composition and diversity have been observed at Samalga pass (169W), an area that divides waters of the Alaska Coastal Current (ACC) to the east and the Alaskan Stream to the west. There are four major passes, Amukta, Amchitka, Buldir, and Near Strait, the deepest of which is over 1000 m (Hunt and Stabeno 2005). In addition, clockwise circulation patterns in the Aleutians have been identified that could work to entrain larvae within a limited geographic area (Ladd et al. 2005).

The same forces that limit species dispersal can structure populations within a species, and several marine species display limited dispersal within the Aleutian Islands archipelago. Data from AFSC bottom trawl surveys from 1980 – 2003 identified 245 fish species across the Aleutian chain, and 63 of these are not continuously distributed (Loggerwell et al. 2005). The Aleutian passes define all of these species ranges, suggesting that the passes themselves may act as physical barriers to gene flow in this region. Pacific cod appear to be more demersal than either walleye pollock or the congeneric Atlantic cod. Bentzen et al.

(1996) found significant differentiation among three northwest Atlantic cod (*Gadus morhua*) populations (Flemish Cap, Scotian Shelf, and northern cod), each separated by submarine trenches that were up to 1000 m deep. Thus, the passes of the Aleutian Archipelago, several of which are over 500 m, may represent significant barriers to dispersal.

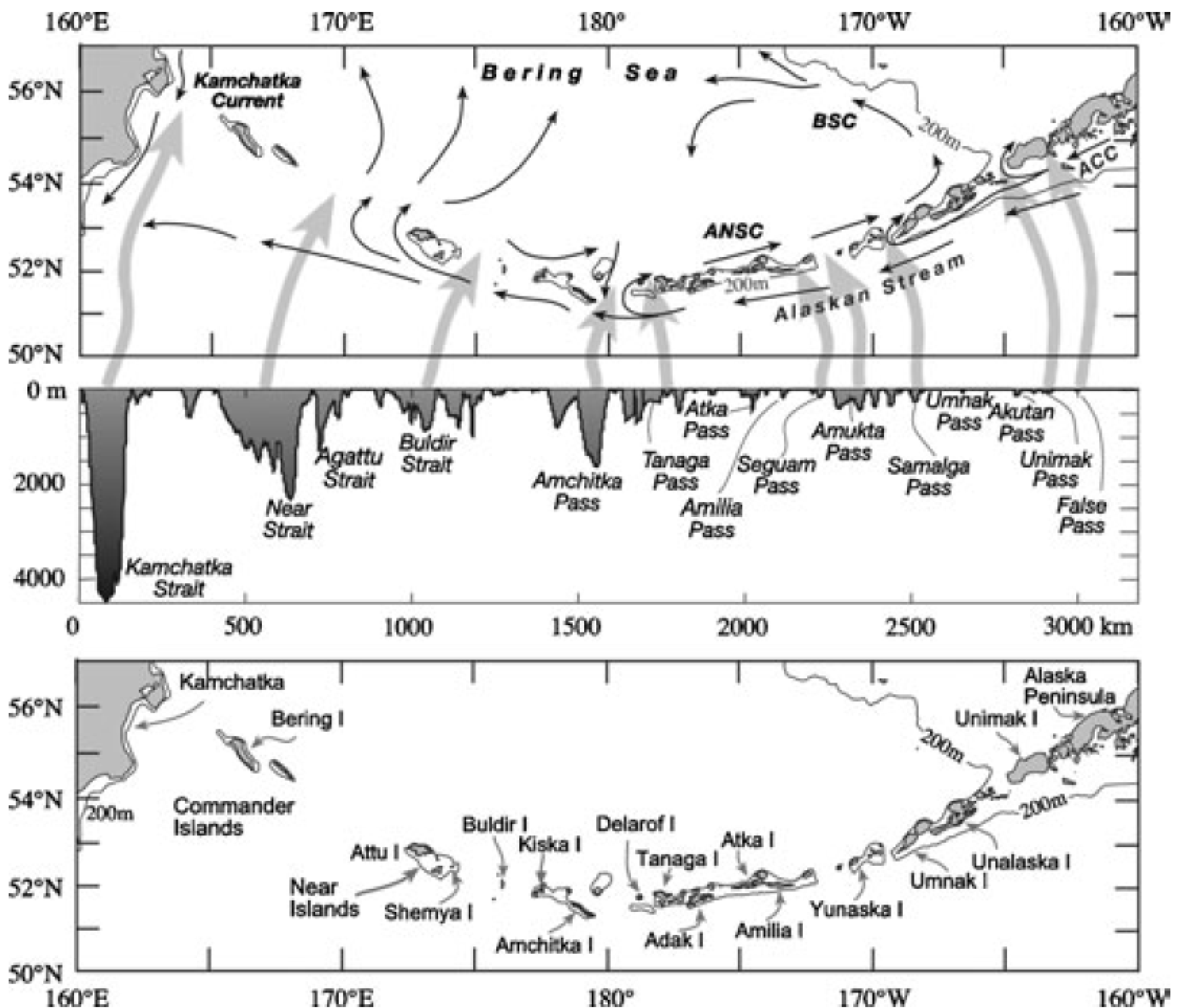

Figure 1. Aleutian Islands with 200m isobath (bottom), depth and passes (middle), and currents (upper), figure taken from Hunt and Stabeno (2005).

Together, biological and oceanographic factors suggest that more than one discrete population may exist within the BSAI. Neutral genetic markers (such as microsatellite, SNP, and mtDNA) are useful for determining whether spawning occurs in a relatively closed system or whether some level of reproductive mixing occurs between spawning groups. These markers are able to show long-term patterns in gene flow that can infer presence or absence of local populations. A study of nuclear microsatellite markers (Cunningham et al. 2009) found significant genetic heterogeneity in pooled samples of spawning adult

cod from Kodiak Island, Unimak Pass, and the central Aleutian Islands east of Tanaga Pass. An analysis of molecular variation (AMOVA) supported partitioning the variance components into either two or three regional groupings; both central Aleutians and Unimak Pass versus Kodiak Island or with all three regions representing separate groups. The AMOVA results did not support grouping all three locations as a single population or grouping the central Aleutians sample with any other region, implying that there may be significant genetic changes among Pacific cod from east to west along the Aleutian archipelago.

Whether distinct population components exist across the range of Pacific cod in Alaska is an important question for management and conservation. This study examined fine scale population structure of Pacific cod using neutral genetic markers, and compared gene frequency differences to physical oceanographic features that may act as barriers to gene flow.

Objectives:

1. To examine population structure of Pacific cod in the BSAI on a fine scale using genetic markers.
2. To compare population structure to physical oceanographic characters.

Methods

All samples were taken from commercial fishing trawlers, with the exception of the Unimak pass sample which was taken on board a research survey using pot gear. All samples were collected during spawning season (February-March) and consisted of fin clips stored in non-denatured ethanol. The Pribilof Island sample was frozen until ethanol became available. Two samples were collected north of Amlia Island, in 2006 and 2007, in order to investigate whether the genetic composition is temporally stable. Overall, the samples span the range of the entire Aleutian chain, and partially up the Bering shelf, from the Near Islands in the west, Amlia Island in the south, Unimak Pass in the east and Pribilof Island in the north (Table 1). DNA was extracted using Qiagen 96 Blood and Tissue kits (QIAGEN Inc., Valencia, CA) according to manufacturer's instructions.

Table 1. Sample locations, date, and number of samples at each location.

Location	*Number*	*Latitude*	*Longitude*	*Date (month/year)*
Near Islands	192	52.56°N	174.29°E	2/2005
S. Kiska Is.	96	51.80°N	177.79°E	3/2005
S. Amchitka Is.	50	51.26°N	179.23°W	2/2005
S. Tanaga Is.	96	51.67°N	178.27°W	3/2005
N. Great Siskin Is.	118	52.10°N	175.86°W	3/2005
S. Atka Is.	96	51.97°N	174.38°W	3/2007
N. Amlia Is.	96	52.42°N	173.80°W	2/2006
N. Amlia Is.	96	52.28°N	173.69°W	2/2007
Unimak Pass	96	54.50°N	165.30°W	3/2005
Pribilof Is.	96	56.83°N	170.00°W	2/2004

In order to determine how many samples and how many microsatellite loci are required to provide sufficient statistical power to reject the null hypothesis when it is false, I analyzed a simulated dataset with similar allele frequencies to the dataset of Cunningham et al. (2009) using POWSIM software. POWSIM estimates the power and alpha error associated with rejecting the null hypothesis of

homogeneity when varying numbers of microsatellite loci and sample sizes are used (Ryman and Palm, 2006). Results from the POWSIM analysis indicated that 20 microsatellite loci would be sufficient to detect significant levels of allele frequency differentiation if F_{ST} was on the order of 0.001. With the goal of 20 loci, I screened 19 walleye Pollock loci (O'Reilly et al. 2000) and 19 loci isolated from Atlantic cod (Miller et al. 2000, Stenvik et al. 2006, Wesmajervi et al. 2007, Skirnisdottir et al. 2008) in addition to the eleven microsatellite loci screened in Cunningham et al (2009) and *Gma*107 (Canino et al. 2005). Although 59 microsatellite loci have been developed for Atlantic cod, I limited my choice of microsatellites to tetranucleotides because they tend to have less stutter than dinucleotides and are easier to analyze. Of these 38, 14 were variable and amplified well: *Gmo*G13, *Gmo*G16,*Gmo*8, *Gmo*G18, *Gmo*19, *Gmo*C82, *Gmo*C83, *Gmo*C88, *Gmo*G5, *Tch*5, *Tch*9, *Tch*13 *Tch*17, and *Tch*18. Further screening of 96 samples revealed that only *Gmo*G13, *Gmo*G16, *Gmo*19, *Gmo*C82, *Gmo*C83, *Gmo*G5, and *Tch*13 were in Hardy-Weinberg equilibrium (HWE) and could be scored reliably (Table 2), for a total of 19 loci screened included in the study.

Table 2. Microsatellite loci used in this study and associated information, in addition those described in Canino et al. 2005.

Locus	*Repeat Sequence*	*Primer Sequence 5'-3'*	*Anneal-ing Temp.*	*Accession No.*	*Reference*
Gmo19	(GACA)	F: CACAGTGAAGTGAACCCACTG R: GTCTTGCCTGTAAGTCAGCTTG	50	AFI159232	Miller, 2000
Gmo37	(GACA)	F: GGCCAATGTTTCATAACTCT R: CGTGGGATACATGGGTACT	46	AFI159237	Miller, 2000
GmoC82	$(TG)_{13}$	F: CCTGGAAGAGAACCCTTTTCA R: GTTTCTTGGGAACTCCTACATTCCT ACTCTC	51	50353897	Stenvik, 2006
GmoC83	$(TG)_{11}$	F: CGGTGCGTTGGATTTCAT R:GTTTCTTAACTGCTCTCCTGATTTT GTTTT	51	50353980	Stenvik, 2006
GmoG5	(AG)	F: GTCTCTTGCCCTACGTTTGTTCG R: GTTTCTTTTCTGGTTGTGGTGTGC CCTGAC	65	DQ836317	Wesmajervi, 2007
GmoG13	(GTCA)	F: ATGCGCTAGACACAGGGCTTGTT R:GTTTCTTGACGGACTGTGTCAGTGT CTGGTG	55	DQ648550	Wesmajervi, 2007
GmoG16	(TGTC)	F: GTCTGCACATCTTGGTGCGTGATT R:GTTTCTTTATGGTTTCAATACCGCC GGTTTC	55	DQ648552	Wesmajervi, 2007
Tch13	(GT)	F: TTTCCGATGAGGTCATGG R:AATCCACTGGTGCAGACC	54	AF178503	O'Reilly et al. 2000
Tch20	$(GA)_6$GGGAA(GGAA$)_3$GGAT(GGAA$)_2$GGAA T$(GAAA)_{10}$GAA G$(GAAA)_5$	F: ACATTGTAAACGGCGATTC R: TGGTTAGTCTGAGACCCAG	54	AF178509	O'Reilly et al. 2000

All polymerase chain reactions were performed according to the cocktail and profile conditions described in Canino et al. (2005), with 55°C used as the annealing temperature for all loci. Reruns for the Pribilof sample were performed slightly differently, because the tissue had degraded slightly prior to being stored in ethanol. For these PCR's, I used the Velocity PCR kit (Bioline, London, UK) in a 5µl volume following manufacturer's instructions, with 3% DMSO (v/v), 2mM $MgCl_2$, 0.4µM each primer, and 0.05U/µl Velocity DNA polymerase.

Due to equipment failures, two different genotyping platforms were used. Seven loci (*Gma*100, *Gma*101, *Gma*102, *Gma*103, *Gma*106, *Gma*109, and *Tch*20) were run on one of two MegaBASE 1000 DNA sequencers (Amersham Biosciences/GE Healthcare, Uppsala, Sweden). All other loci were run on an ABI3730xl (Applied Biosystems/Life Technologies Corporation, Carlsbad, CA) via contract with

Eurofins MWG Operon (Huntsville, AL). Because one of the goals of this project was to standardize data with those of the Cunningham et al. (2009) data, 16 samples (3 samples from 4 locations) from that dataset were run on the MegaBACE and ABI platforms to provide standardization protocols. Additionally, I compared samples between platforms by running the same 96 samples on each

MegaBACE data was scored using Genetic Profiler version 2.2 software (Amersham Pharmacia Biotech, Inc., Piscataway, NJ) and ABI data was scored using GeneMarker v1.85 (SoftGenetics LLC, State College, PA). Data quality was evaluated using several different parameters. A double-blind genotyping protocol, where two individuals score the gel image independently, was employed on all samples run on the ABI platform (12 loci), and multiple runs of the same sample were compared for reliability with the MegaBACE loci. All loci were rerun when scores were questionable to ensure scoring reliability, some as many as four times.

Tests for Hardy Weinberg equilibrium (HWE), F_{IS} calculations and associated *p*-values were performed using Genepop on the web (http://genepop.curtin.edu.au/) with a burn-in period of 10,000 iterations, 1000 batches and 10,000 iterations per batch. Observed and expected heterozygosity were analyzed in Genetix 4.02 (Belkhir et al. 2000). Pairwise tests for linkage disequilibrium were performed in Genetix and significance was determined using 1000 permutations of the data. Because tests of population structure assume that loci are neutral, I used the program fdist2 to examine whether any of the loci appear to be under selection (Beaumont and Nichols 1996). This program provides a comparison between observed F_{ST} values as a function of heterozygosity with those simulated with the same size and number as the actual data set, using the island model with 100 islands and an infinite alleles mutational model. This allows 0.025, 0.5, and 0.975 quantiles for a selectively neutral distribution to be estimated.

Micro-Checker v.2.2.3 was used look for the presence of null alleles, upper allele dropout, and errors due to stutter (Van Oosterhout et al. 2004). Tests for genetic differentiation between samples were performed using several different methods. Pairwise theta (Weir and Cockerham 1984), an estimator of F_{ST} was calculated in Genetix, and significance was assessed with 1000 permutations of the data. Tests for allele frequency differentiation among samples (genic tests) were performed using Genepop with the same burn-in, iterations, and batches as the F_{IS} calculations. Because it is sometimes difficult to visualize the genetic relationship resulting from a table of pairwise comparisons, a multidimensional scaling plot, used to represent the relationships among points spatially, was created in the R statistical package (library "psych") using pairwise F_{ST} values (R development core team 2008).

To compare population structure to physical oceanographic characters, I used two tests that have been specifically designed to determine whether a correlation exists between potential barriers to dispersal and reduced gene flow. The first is the maximum distance Monmonier algorithm, which is designed to visualize data contained in a genetic distance matrix onto a geographical map to identify boundaries (Manel et al. 2003, Monmonier 1973). This was done using the program BARRIER, v2.2. One drawback of this program is that it does not provide evidence for barriers; the user must provide the number or barriers they suspect, and the program provides the location, and how many loci provide support for the barriers. The second test was an analysis of molecular variance, or AMOVA, implemented in Arlequin, ver. 2.000 to examine divergence within and among groups of samples.

Another test that looks for a relationship between genetic differences and geographic distance was implemented in R using linear regression. This test, also known as isolation-by-distance, tests whether distance is a significant factor in determining genetic distinctiveness. Additionally, a factor representing whether any two points span Amchitka pass was utilized, and a variable representing the depth of the deepest pass between two points was considered. I used a step function in R that used Akaike Information Criterion (AIC) to determine the best combination of these three factors. Distances between points were determined using Google earth software, v. 5.2.1.1588 (Microsoft Corp., Seattle, WA).

Results

All loci and populations conformed to Hardy-Weinberg Equilibrium, with two exceptions. Locus *Gma*106 was discarded due a clear excess of heterozygotes (data not shown). Sample and locus statistics are given in Table 3, with significant tests for extreme F_{IS} values shown in bold. The second exception, locus *Gma*107, appeared to be significantly out of HWE in four of 10 populations. The remaining 17 loci all appeared to be in HWE, as did all 10 samples. Fewer than 5% (7 out of 170 locus-sample combinations) were out of HWE overall, which is less than would be expected by random chance alone. All loci conformed to the assumption of neutrality, with the exception of *Gma*107 (Figure 2). The remaining 17 loci were all within the 95% quantiles expected for neutral loci. Allele frequency histograms by population were unremarkable at *Gma*107, discounting the possibility that the locus revealed any selective effect among the different populations. Tests for null alleles, upper allele dropout, and error due to stutter performed in Micro-Checker were not significant for any locus except *Gma*107, which indicated the presence of null alleles. Thus, all 17 loci in Table 3 were retained for further analyses, with the exception of *Gma*107, which was discarded due to the presence of null alleles, contradicting assumptions of neutrality, and Hardy-Weinberg Equilibrium.

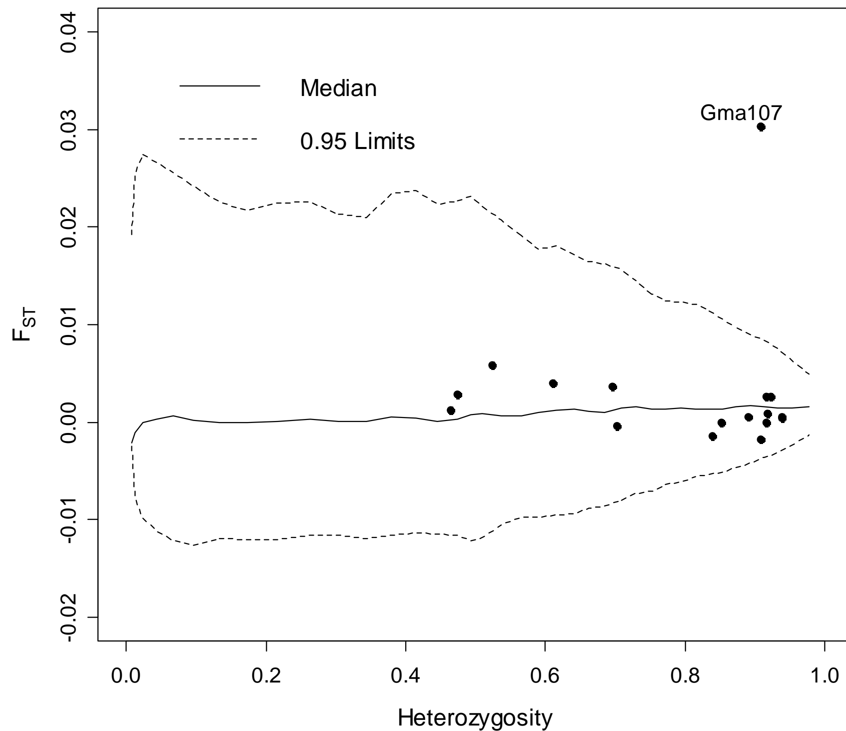

Figure 2. F_{ST} values estimated from 17 microsatellite loci plotted with respect to heterozygosity. The 95% quantiles were estimated using an island model and expected F_{ST}=0.0017.

Nine of 136 locus-by-locus comparisons were significant at the $p \leq 0.05$ significance level for linkage disequilibrium. This is approximately 6.6%, close to what might be expected by chance alone. For perspective on the increased odds of type I error, I applied both a strict and sequential Bonferroni test to the data (Rice 1989). Under the strict Bonferroni, no locus comparisons were significant, while the sequential Bonferroni indicated that locus comparisons *Gma*100 vs. *Gma* 102 and *Gma*102 vs. *Gma*108 were in disequilibrium. Considering that these loci were previously examined and found not to be in linkage disequilibrium (Cunningham et al. 2009), and the fact that *Gma*100 vs. *Gma*108 did not appear to be linked ($p \leq 0.15$), this test result did not appear to be entirely reliable, and we consider all 17 loci to be in linkage equilibrium.

Tests for population differentiation are shown in Table 4; F_{ST} values are above the diagonal and genic differentiation results are below. It is notable that several comparisons at the largest distances are significant, such as Near Islands and Pribilof both with the genic test and F_{ST}. Also, the temporal sample

at Amlia Is. is significantly differentiated between 2006 and 2007. The 2006 sample is significantly different from the Pribilof sample but the 2007 sample is not. A multidimensional scaling plot that represents the relationship among F_{ST} values in two dimensions is shown in Figure 3. Note that the dimensions are meaningless and the spatial relationship among points demonstrates genetic differences. This clearly shows that Aleutian Islands samples cluster, and that the Unimak and Pribilof samples are distinct (to the bottom and left of the plot). In addition, the 2007 Amlia sample is located far from the 2006 Amlia sample. Within the Aleutian samples, Near and Kiska are off to the side, while the remaining samples are more central in the plot.

Results from the Monmonier algorithm implemented in the program BARRIER indicated several barriers. The Amlia 2007 sample was removed because a barrier between temporal samples was not relevant to this analysis. When one barrier was imposed, there was equal weight (5 loci each) between a barrier between Pribilof and Unimak, and a barrier between Unimak and the Aleutian Island samples. When two barriers were imposed, nine loci suggested a barrier between Unimak and the Aleutians and seven supported a barrier between Unimak and Pribilof. For three barriers, these two barriers again received the strongest support (11 loci supported the Pribilof-Unimak barrier, 9 supported the Unimak – Aleutians barrier) and a third barrier was suggested at Amchitka Pass, between the Near and Kiska sample and the rest of the Aleutians.

Using the step function in R, the best model describing the pairwise F_{ST} values was one that included only distance between points. Neither depth of passes or whether two points spanned Amchitka pass were significant. There was a significant isolation-by-distance relationship ($p \leq 9.58e^{-13}$), and the results are shown in Figure 4 and Table 5. The AMOVA results are shown visually in Figure 5 and summarized in Table 6. The pattern including three groups shown in Figure 5 (Unimak Pass, all Aleutian Island samples, and Pribilof sample) indicated significant differentiation among groups (F_{CT} p-value = 0.00391) while no additional significant differentiation was detected among groupings (F_{SC} p-value = 0.09482).

Table 4. Above diagonal, F_{ST} values. Negative values were replaced with zeroes. Below diagonal, *p*-values associated with the genic test of differentitation. In both cases, significance is given as *<=0.05,**<=0.025,***<0.001.

	N.Amlia '06	*N.Amlia '07*	*G.Siskin*	*Near*	*Pribilof*	*S.Amchitka*	*S.Atka*	*S.Kiska*	*Tanaga*	*Unimak*
N.Amlia '06		0.00167	0	0.00094	0.00225**	0	0	0.00167*	0.00078	0.0019**
N.Amlia '07	0.0130**		0	0	0.00158	0	0.00032	0.00047	0.00076	0.0028**
G.Siskin	0.2558	0.2454		0	0.0025**	0	0	0.00211**	0.00099	0.00056
Near	0.0094**	0.0476*	0.1368		0.00336*	0.0008	0.00049	0.00107*	0.00011	0.0021***
Pribilof	<0.0001***	0.0796	0.0004***	0.0004***		0.00061	0.00164*	0.00317***	0.00188**	0.00232**
S.Amchitka	0.0317*	0.1384	0.1070	0.0256*	0.0092**		0	0	0	0
S.Atka	0.2511	0.1399	0.2289	0.1033	0.0060**	0.3887		0.0004	0	0.00134*
S.Kiska	0.0161**	0.2608	0.0711	0.2041	0.0013**	0.1473	0.1954		0.00109	0.00257**
Tanaga	0.0731	0.2874	0.0521	0.6103	0.0009**	0.2178	0.3930	0.0475*		0.0022**
Unimak	0.0017**	0.0113**	0.0542	0.0391*	0.0596	0.2700	0.0142	0.0065**	0.0399*	

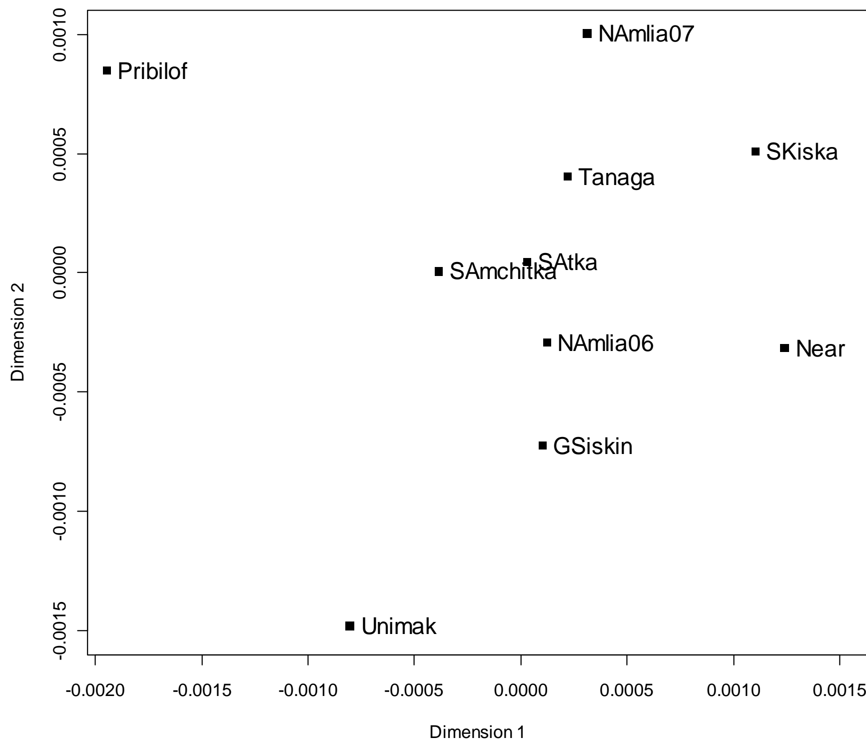

Figure 3. Multidimensional scaling plot of all samples using pairwise F_{ST} values.

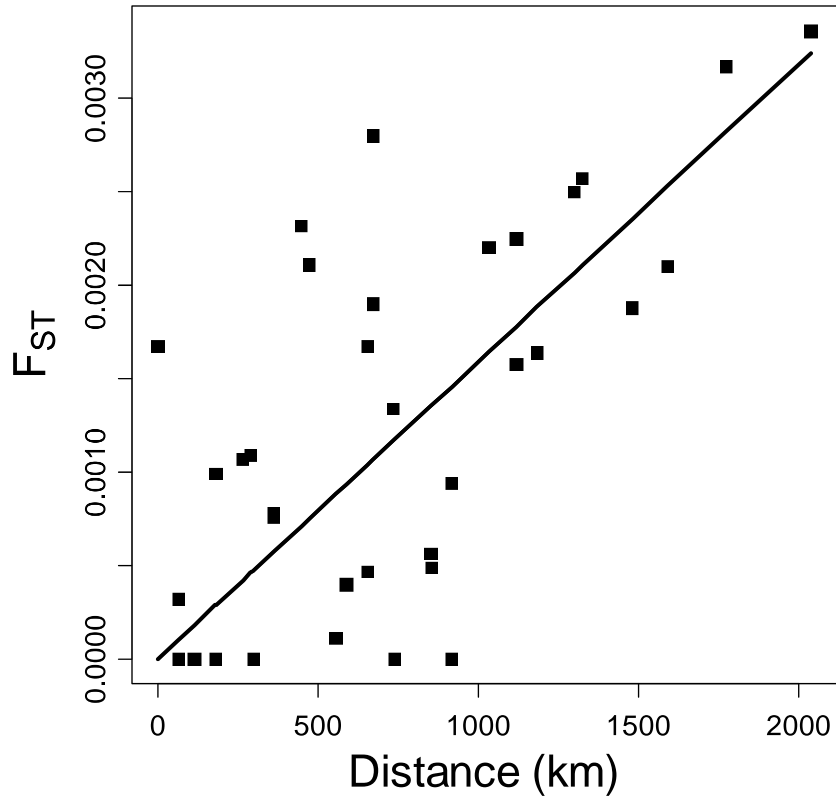

Figure 4. Genetic vs. Geographic distance. Genetic "distance" is represented by pairwise F_{ST} values (black squares).

Table 5. Summary of isolation by distance, linear regression between pairwise F_{ST} values and geographic distance between points.

```
Call:
lm(formula = fst ~ dist - 1)

Residuals:
       Min         1Q     Median         3Q        Max
-0.0014612 -0.0004751  0.0001438  0.0005727  0.0017319

Coefficients:
      Estimate Std. Error t value Pr(>|t|)
dist 1.592e-06  1.467e-07   10.85 9.58e-13 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.0007774 on 35 degrees of freedom
Multiple R-squared: 0.7709,     Adjusted R-squared: 0.7643
F-statistic: 117.7 on 1 and 35 DF,  p-value: 9.585e-13
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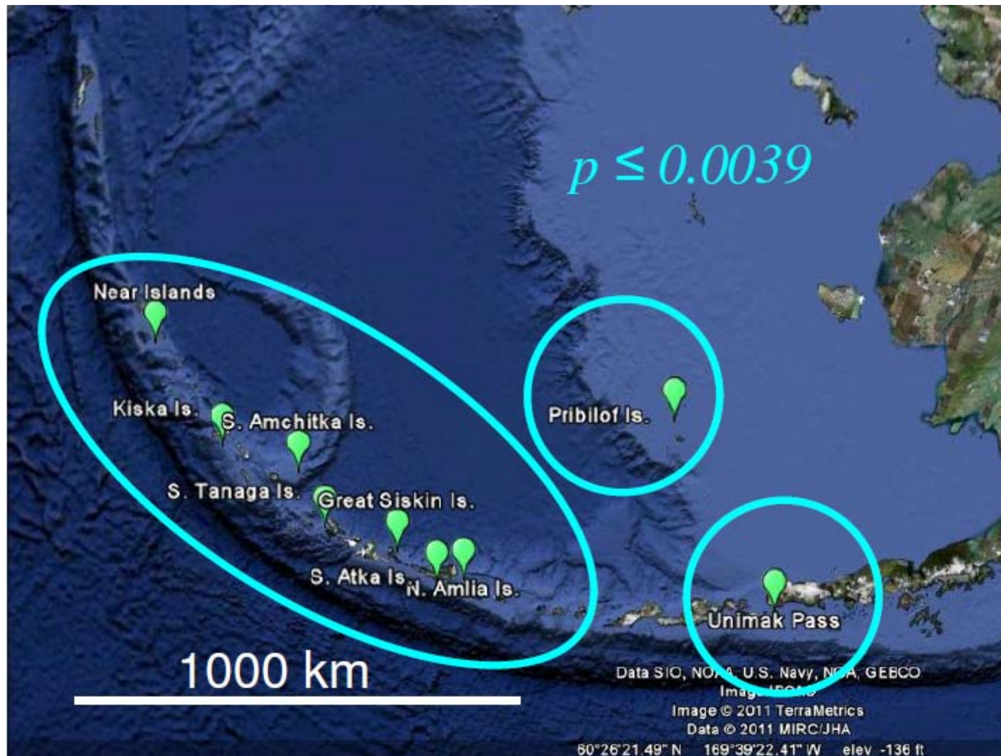

Figure 5. Visual representation of the AMOVA results.

Table 6. Summary of AMOVA results, which contains three groupings, Unimak Pass, all Aleutian Island samples, and Pribilof sample.

Source of variation	Sum of squares	Variance components	Percentage variation
Among groups	21.136	0.01092	0.16174
Among populations Within groups	51.216	0.00306	0.04531
Within populations	12563.069	6.73556	99.79295

Vc and FST : P(rand. value < obs. value) = 0.00000
Vb and FSC : P(rand. value > obs. value) = 0.09482
Va and FCT : P(rand. value > obs. value) = 0.00391

Discussion

Overall, results strongly reject the null hypothesis of panmixia, or genetic homogeneity of Pacific cod within the Bering Sea and Aleutian Islands. This study has the most statistical power of any performed to date on Pacific cod, due to the large number of microsatellite loci, and is the first to examine population structure at such a fine scale. Many tests were performed on the data, and the analyses were fairly concordant in their results, with two main themes. First, as distance increases between two samples, the genetic differences observed between these points increases. Second, the strongest evidence for barriers to migration appear between the Unimak, Pribilof, and the Aleutian Island samples.

Isolation by distance has been shown by other studies of Pacific cod (Cunningham et al. 2009, Canino et al. 2010) and was confirmed here as well. A close examination of Figure 4 shows that the variance around the slope decreases with longer distances, particularly around 1000 km. It is relevant to note that the length of the Aleutian chain, from the Near Islands to Amlia Is. is approximately 1000 km. The slope of the IBD line was almost identical to that observed with coastal North American samples; BSAI, the Gulf of Alaska, and Hecate Strait in Canada (1.57×10^{-6} vs. 1.59×10^{-6}, Cunningham et al. 2009). This prior analysis concluded that the mean dispersal distance by a single cod throughout its lifetime was less than 100 km per generation, and our results support this result.

Biological and environmental factors should be considered when evaluating the limited dispersal, and whether further patterns exist. The data suggests that the Bering Sea and Aleutian Islands area contains three distinct groups of Pacific cod; Aleutian Islands, Unimak, and Pribilof. Surprisingly, of all the Aleutian passes hypothesized to act as barriers, only Samalga Pass appears to have a strong effect. In addition, there appears to be a barrier between Unimak Pass and the Pribilof Island area despite the lack of oceanic passes and fairly consistent bathymetry. In order for passes to act as barriers, they must restrict movement at both adult and larval phases. While circular current patterns have been observed, strong currents could also result in mixing, particularly of pelagic larvae. Samalga Pass, on the other hand, is the site of a major split between two current systems, the Alaska Coastal Current and the Alaska Stream. Similarly, the current northward along the Bering Sea slope may act differently on larval cod spawned in Unimak Pass and those from the Pribilof area.

Demersal Pacific cod eggs hatch between 15 and 45 days depending on temperature (Laurel et al., 2008), and larvae are pelagic until 3-4 months after hatching (Narimatsu et al. 2007); thus currents may affect their distribution. Flow north of Unimak Pass diverges into an eastward component (the Bering Coastal Current, BCC) and a northward component toward the middle and outer Bering Sea shelf while larvae spawned on the slope are transported northward along the eastern Bering Sea shelf via basin and shelf

currents (Stabeno et al. 1999). In addition, due to the presence of ice along the Bering Sea shelf through spring months (Overland and Stabeno, 2004), spawning at the Pribilof Islands may be later than at Unimak Pass. Thus, both current patterns and spawn timing may prevent mixing of larvae spawned at Unimak and those from the Pribilof Islands area.

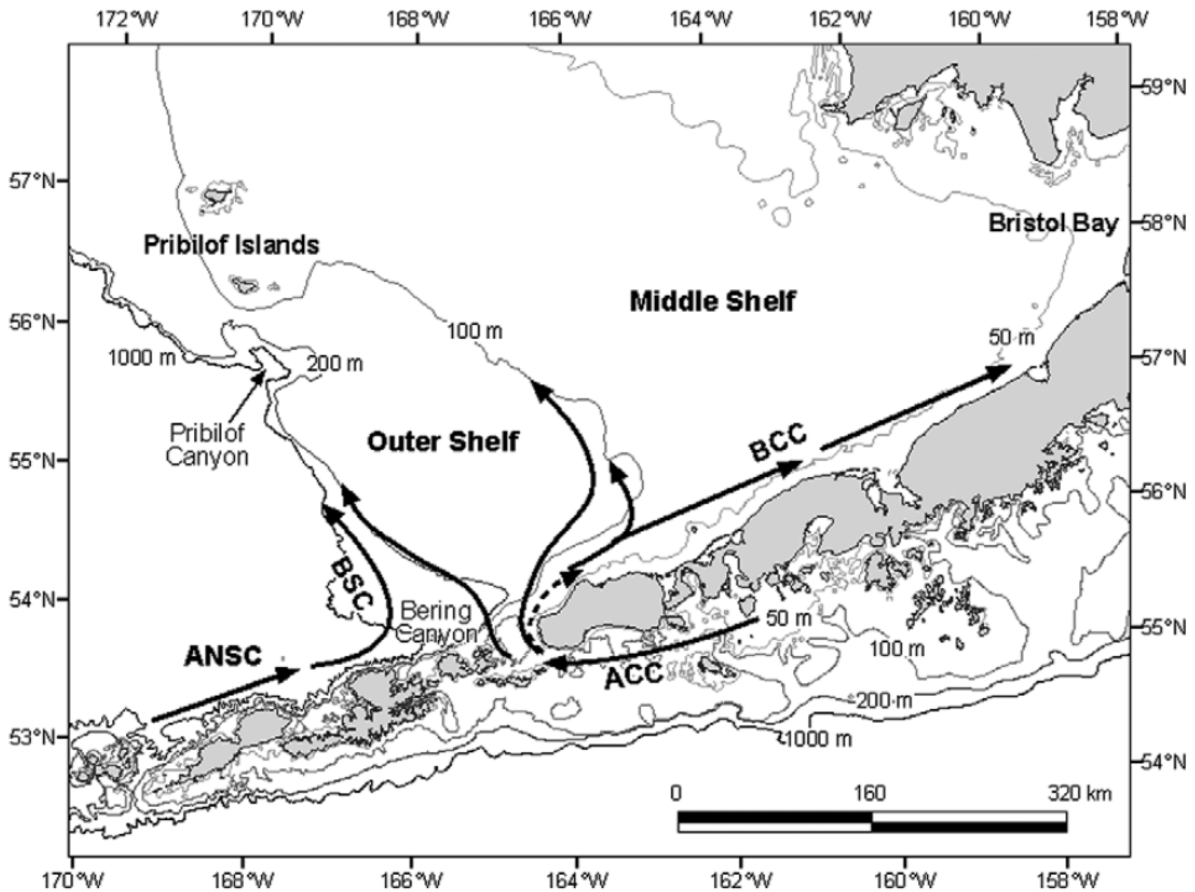

Figure 6. Predominant currents of the eastern Bering Sea (from Lanksbury et al. 2007).

While differentiating between connectivity at the larval and the adult phase would require further study, genetic differentiation is caused by a lack of migration at any life history phase. This study provides no information on whether connectivity occurs at the larval or the adult phase where populations appear continuous, such as along the Aleutian chain. Adult spawning site fidelity and some degree of larval mixing does provide a scenario consistent with the observed data. This scenario does not provide evidence that deep water passes act as barriers to adult movement, although larval drift across passes could mask barriers specific to adults.

Currently, the total allowable catch (TAC) of Pacific cod in the Bering Sea and Aleutian Islands management area are assessed as one unit (Thompson et al. 2010). Relative fishery exploitation rates in

the Aleutian Islands are higher than in the eastern Bering Sea (22% vs 17%) despite the fact that exploitable biomass in the Aleutians is more than five times smaller (Ormseth et al. 2008). Single and multi-species estimates of biomass in the Aleutian Islands indicate that since the 1970's, populations may have been in decline (Kinzey and Punt 2009). Because population genetics can only document differences in allele frequencies in populations that have been separated for long enough for the effects of genetic drift to act, when differences are noted, they not only indicate population subdivision, but subdivision that has been in existence for hundreds of generations. Therefore, consequences of this research provide the most comprehensive evidence to date that multiple populations of Pacific cod exist in the BSAI and that a precautionary approach to management would be to split this management area into more than one group. While the results of this study are limited to the location of cod during the winter months, this is also the time during which the majority of cod are commercially caught (Thompson et al. 2010). It has been shown that when such stocks are depleted, they are generally not replaced by immigration (Hauser and Carvalho 2008) and preservation of distinct stocks is important to ensure resilience (Schindler 2010).

While a previous study of Pacific cod indicated genetic temporal stability at Unimak Pass and Kodiak Island, our temporal sample at Amlia Island was genetically different in 2006 and 2007 (Cunningham et al. 2009). Interestingly, the 2007 sample was more similar to the Pribilof group, but more data is needed to examine this effect. Although every effort was made to collect all fish from the same year, this was not possible and samples were taken from 2004-2007. Due to evidence that cod populations are typically temporally stable (Cunningham et al. 2009), and the fact that both years of the Amlia sample grouped into the Aleutian Island group with the AMOVA analysis, the lack of stability at Amlia Island does not contradict the conclusions of this paper. However, further work would benefit from more samples, particularly from the Pribilof area, because this is the first genetic analysis of cod from that spawning group.

Conclusions

This work indicates that Pacific cod in the Bering Sea and Aleutian Islands management area of Alaska are not genetically homogeneous. There is a strong relationship between geographic distance and genetic distinctiveness, and further evidence that cod disperse less than 100 km over their lifetime. In addition, the data indicate not only that cod in the Aleutian Islands are distinct from those that spawn in the eastern Bering Sea, but that further stock differentiation may exist along the Bering Sea shelf between the Unimak Pass and Pribilof Islands spawning areas.

Outreach

Presentations in schools (K-12, undergraduate):

1. I presented this research at a career fair at Brier Terrace Middle School in Brier, WA on January 26, 2010 to three consecutive groups of 8th graders. In a mixed media presentation, I first provided an overview of Mendelian genetics and how it could be useful for discerning population structure. Then I discussed the utility of knowing genetic population structure; when unrecognized distinct stocks exist, fishing can result in unintended depletion of some stocks. I used the example of Pacific cod in Alaska and demonstrated the concept using a small model of the Aleutian Islands, with distinct stocks represented by differently colored Swedish fish. I asked 3 volunteers from each group of students to close their eyes and "fish" by taking a handful of Swedish fish. Each student started at the main port, which in the model was identified as Dutch Harbor. I reminded them that they would be penalized for gas and time expenses if they traveled too far from the port. Most students chose the orange fish, which were closest to the port in the model, reinforcing the point that these fish might be the first group to be depleted.

2. Thomas Hollowed, who interned with me on this project during the summer of 2009, presented his work to a panel of Willamette University faculty on April 21, 2010. Thomas, then a sophomore at Willamette University, extracted DNA, performed PCR's, and screened microsatellite loci from Atlantic cod and walleye Pollock for cross-species amplification in Pacific cod. He helped me identify a total of 9 loci that amplify in addition to the 11 that were screened previously (Cunningham et al. 2009).

3. I presented this work at the University of Washington School of Fishery and Aquatic Sciences to a group of fisheries students on 4/5/2010.

4. I presented this work at the University of Washington Center for Environmental Genomics to a group of oceanography students on 4/5/2010.

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Appendix

Table 3. Sample size (N), number of alleles (N_A), expected and observed heterozygosities (H_e and H_o) calciulated in Genetix, and estimates of F_{IS} (Weir and Cockerham 1984), with p-values<0.05 indicated by bold font calculated in Genepop.

		Samples									
Locus		N. Amlia 2006 (83)	N. Amlia 2007 (81)	G. Siskin (117)	Near Is. (192)	Pribilof Is. (95)	S. Amchitka (50)	S. Atka (95)	S. Kiska (95)	Tanaga (95)	Unimak (95)
Gma100	N	74	59	110	181	86	41	91	83	78	90
	N_A	58	45	61	70	55	44	58	59	58	57
	H_e	0.973	0.9695	0.9721	0.9749	0.9712	0.9664	0.9734	0.9727	0.9721	0.9733
	H_o	0.9595	0.9661	0.9455	0.9724	0.9186	0.9512	0.956	0.9518	0.9744	0.9667
	F_{IS}	0.0207	0.0121	0.0320	0.0054	0.0600	0.0280	0.0233	0.0275	0.0042	0.0124
	p	0.3518	0.1718	**0.0391**	0.3930	**0.0059**	0.5017	**0.0415**	0.0928	0.1219	0.0430
Gma101	N	74	74	105	178	64	38	85	94	87	90
	N_A	21	22	22	24	17	15	21	22	23	20
	H_e	0.9145	0.9137	0.9143	0.9106	0.9088	0.8979	0.9066	0.9182	0.9113	0.9022
	H_o	0.8919	0.8784	0.8952	0.9101	0.9063	0.9211	0.8706	0.8936	0.8851	0.8444
	F_{IS}	0.0316	0.0455	0.0256	0.0034	0.0107	-0.0125	0.0457	0.0322	0.0346	0.0695
	p	**0.0172**	0.1484	0.4646	0.3783	0.1284	0.7524	0.4881	0.1416	0.1958	0.1179
Gma102	N	75	81	115	181	95	48	90	94	89	93
	N_A	13	17	12	15	14	14	12	13	14	16
	H_e	0.8875	0.8891	0.8783	0.8897	0.883	0.8904	0.8797	0.883	0.8847	0.8889
	H_o	0.9333	0.9136	0.887	0.8564	0.8316	0.8542	0.9222	0.9149	0.8764	0.8817
	F_{IS}	-0.045	-0.0213	-0.0055	0.0402	0.0635	0.0512	-0.0428	-0.0307	0.015	0.0135
	p	0.6199	0.5705	0.3583	0.9818	0.0652	0.1996	0.3376	0.7373	0.5628	0.8563
Gma103	N	77	77	117	176	82	48	91	94	92	95
	N_A	35	29	39	47	39	29	32	38	37	39
	H_e	0.9132	0.8857	0.9065	0.8945	0.9225	0.9269	0.9121	0.9239	0.9231	0.9058
	H_o	0.9351	0.9481	0.9316	0.9091	0.8902	0.875	0.9121	0.8936	0.913	0.8842
	F_{IS}	-0.0174	-0.0639	-0.0234	-0.0135	0.0411	0.0664	0.0056	0.0381	0.0164	0.0291
	p	0.7880	0.7645	0.9119	0.1566	0.4920	0.0674	0.2394	0.4811	0.1651	0.3144
Gma104	N	81	77	110	183	48	49	88	79	91	94

	N_A	23	22	25	28	16	19	23	21	25	27
	H_e	0.9096	0.9133	0.9102	0.9027	0.8819	0.8955	0.9117	0.8964	0.8988	0.9151
	H_o	0.963	0.9091	0.9091	0.8852	0.8333	0.898	0.8977	0.8861	0.8571	0.9149
	F_{IS}	-0.0525	0.0112	0.0557	0.0221	0.0656	0.0075	0.0211	0.0179	0.0519	0.0056
	p	0.4197	0.8865	**0.0210**	0.0921	0.1791	0.8703	0.4316	0.2441	0.4219	0.9756
Gma105	N	78	80	110	187	81	49	94	91	92	94
	N_A	9	9	8	14	10	8	10	9	8	10
	H_e	0.8328	0.8445	0.8437	0.8414	0.8349	0.8286	0.8292	0.8277	0.8233	0.8476
	H_o	0.7821	0.8375	0.8	0.861	0.8025	0.8367	0.8191	0.8132	0.8043	0.7979
	F_{IS}	0.0673	0.0146	0.0564	-0.0205	0.0450	0.0005	0.0174	0.0231	0.0285	0.0639
	p	0.1061	0.8032	0.4734	0.8477	0.2852	0.4548	0.3674	0.6020	0.5754	0.4797
Gma107	N	75	76	106	165	59	49	85	87	94	93
	N_A	17	16	20	21	15	15	18	17	21	16
	H_e	0.901	0.9029	0.9073	0.9133	0.9018	0.8884	0.8932	0.8753	0.9003	0.9014
	H_o	0.8267	0.7237	0.8491	0.7636	0.9492	0.8163	0.7176	0.9195	0.9043	0.957
	F_{IS}	0.0891	0.2048	0.0689	0.1668	-0.044	0.0913	0.2022	-0.0447	0.0009	-0.0563
	p	**0.0005**	**0.0001**	0.2977	**0.0004**	0.7176	0.1533	**0.0003**	0.1736	0.5816	0.8749
Gma108	N	82	81	116	187	95	46	94	93	95	95
	N_A	10	10	14	11	11	10	12	9	11	10
	H_e	0.5066	0.3829	0.4242	0.4349	0.4622	0.5279	0.4726	0.4775	0.4619	0.4892
	H_o	0.5244	0.3704	0.4052	0.4759	0.4947	0.5652	0.4787	0.4946	0.4737	0.5158
	F_{IS}	-0.0290	0.0388	0.0491	-0.0916	-0.0651	-0.0598	-0.0076	-0.0304	-0.0201	-0.0491
	p	**0.0370**	0.3268	0.3644	0.3413	0.7484	0.9843	0.9591	0.6924	0.9111	0.5498
Gma109	N	74	78	111	174	80	43	91	84	86	90
	N_A	23	25	28	29	28	20	31	28	28	27
	H_e	0.9088	0.9063	0.9224	0.914	0.9125	0.8997	0.9374	0.9285	0.9076	0.9132
	H_o	0.8784	0.8846	0.8829	0.8736	0.9125	0.9103	0.9451	0.9167	0.8721	0.9444
	F_{IS}	0.0403	0.0304	0.0473	0.0471	0.0063	0.0264	-0.0026	0.0187	0.0449	-0.0286
	p	0.2707	0.2747	0.1801	0.4649	0.2627	0.0643	0.7863	0.1107	0.2734	0.8712
Tch20	N	79	74	112	177	75	48	83	91	91	93
	N_A	21	23	23	25	22	22	23	21	24	23
	H_e	0.9194	0.939	0.9305	0.9362	0.9335	0.9249	0.9358	0.931	0.9399	0.9392
	H_o	0.9494	0.9459	0.9107	0.9774	0.96	0.9167	0.8795	0.9451	0.9451	0.9247
	F_{IS}	-0.0262	-0.0006	0.0258	-0.0412	-0.0217	0.0194	0.0662	-0.0096	0	0.0208
	p	0.6793	0.1244	0.1497	0.4484	0.5580	0.7235	0.0883	0.6171	0.3751	0.3460

Gmo37	N	78	78	115	188	73	49	89	89	94	94
	N_A	35	34	36	44	38	31	39	37	35	37
	H_e	0.9384	0.9303	0.9305	0.9309	0.9251	0.9413	0.9322	0.9354	0.9217	0.9388
	H_o	0.9744	0.9615	0.9304	0.9468	0.9452	0.9184	0.9551	0.8989	0.9574	0.9149
	F_{IS}	-0.0318	-0.0271	0.0044	-0.0145	-0.0148	0.0346	-0.0189	0.0446	-0.0335	0.0308
	p	0.1914	0.1641	**0.0425**	0.7773	0.1018	0.0617	0.8106	0.1694	0.1875	0.6855
GmoG13	N	79	80	112	186	71	49	94	93	94	88
	N_A	12	12	14	12	10	11	12	13	13	13
	H_e	0.8519	0.8453	0.8374	0.8471	0.8414	0.8519	0.8289	0.8526	0.8585	0.8539
	H_o	0.8101	0.8125	0.8393	0.871	0.8732	0.898	0.8617	0.8925	0.8936	0.8068
	F_{IS}	0.0554	0.0451	0.0022	-0.0255	-0.0308	-0.0437	-0.0343	-0.0414	-0.0356	0.0608
	p	0.5045	0.1615	0.8107	0.4380	0.4739	0.4823	0.3839	0.8661	0.5428	0.6605
GmoG16	N	79	70	111	190	89	49	94	94	90	90
	N_A	4	5	5	6	3	5	5	4	5	6
	H_e	0.4976	0.483	0.5064	0.4743	0.4992	0.5185	0.5171	0.5332	0.5635	0.5826
	H_o	0.443	0.4143	0.4595	0.4632	0.6067	0.5306	0.4681	0.5426	0.5667	0.5667
	F_{IS}	0.1159	0.1492	0.0972	0.0262	-0.2099	-0.013	0.1002	-0.0122	-0.0001	0.0329
	p	0.6347	0.5260	0.4029	0.3979	0.0952	1.0000	0.6174	0.8669	0.6290	0.9501
Gmo19	N	80	79	113	189	70	49	90	90	93	90
	N_A	28	24	26	30	21	23	33	23	27	29
	H_e	0.9073	0.9222	0.9195	0.9219	0.8747	0.893	0.917	0.9119	0.8898	0.9262
	H_o	0.9	0.9367	0.8761	0.9206	0.9286	0.8776	0.9333	0.9	0.828	0.9444
	F_{IS}	0.0143	-0.0094	0.0516	0.0041	-0.0544	0.0276	-0.0123	0.0186	0.0749	-0.0141
	p	0.0997	0.7447	0.2365	0.3210	0.9662	0.4526	0.3266	0.2384	0.2749	0.7410
GmoC82	N	80	79	115	190	80	49	93	93	94	92
	N_A	6	6	5	8	4	5	5	5	5	7
	H_e	0.5912	0.5922	0.5894	0.6204	0.5588	0.6187	0.6089	0.6087	0.6107	0.6434
	H_o	0.675	0.5823	0.5913	0.5632	0.5875	0.4898	0.6022	0.5914	0.5426	0.6087
	F_{IS}	-0.1355	0.0231	0.0011	0.0949	-0.0450	0.2182	0.0165	0.0339	0.1169	0.0594
	p	0.3381	0.0785	0.2819	**0.0361**	0.6658	**0.0314**	0.4866	0.7739	0.4265	0.1647
GmoC83	N	81	75	111	190	91	49	94	94	94	92
	N_A	3	3	3	3	3	3	3	3	3	3
	H_e	0.48	0.4575	0.4972	0.4829	0.4731	0.479	0.4663	0.4009	0.4595	0.5077
	H_o	0.4568	0.4667	0.5405	0.4895	0.5714	0.449	0.4894	0.3723	0.5426	0.5326

	F_{IS}	0.0546	-0.0133	-0.0828	-0.0109	-0.2026	0.0729	-0.0441	0.0766	-0.1755	-0.0435
	p	0.7801	0.5387	0.7225	1.0000	0.0812	0.5947	0.6626	0.6087	0.0252	0.0818
Tch13	N	80	80	116	184	77	49	91	92	94	94
	N_A	7	8	8	9	7	7	7	6	7	7
	H_e	0.6737	0.6697	0.7323	0.7268	0.666	0.6976	0.6995	0.6779	0.7052	0.7355
	H_o	0.675	0.5625	0.75	0.7065	0.6709	0.7755	0.6703	0.7826	0.7128	0.7766
	F_{IS}	0.0043	0.1662	-0.0199	0.0306	-0.0021	-0.1014	0.0472	-0.1491	-0.0054	-0.0505
	p	0.4098	**0.0263**	0.1753	0.4011	**0.0279**	0.9753	0.7912	0.4961	0.1078	0.9270
GmoG5	N	77	79	116	190	77	49	91	93	93	94
	N_A	5	4	4	6	5	4	4	4	4	5
	H_e	0.6767	0.6787	0.6646	0.6869	0.6861	0.6847	0.7069	0.7101	0.6909	0.6993
	H_o	0.6623	0.6456	0.6724	0.6737	0.7143	0.6327	0.6593	0.7527	0.7204	0.6915
	F_{IS}	0.0277	0.0551	-0.0075	0.0219	-0.0345	0.0863	0.0727	-0.0545	-0.0374	0.0165
	p	0.9474	0.8718	0.2965	0.4692	0.9792	0.1607	0.2724	0.8244	0.5811	0.6847