RESEARCH/PD ANNUAL REPORT - FINAL REPORT

2015 annual report - final
Lorenz Hauser
Local adaptation in Puget Sound Pacific cod (Gadus macrocephalus): phenotypic and genomic differentiation and the conservation of a depleted population in a warming environment
R/LME-6
Submitted On: 04/29/2016 01:35:39 AM

METRICS & MEASURES

Metric/Measure	Value	Note
Acres of coastal habitat	0	
Fishermen and seafood industry personnel	0	
Communities - economic and environmental development	0	
Stakeholders - sustainable approaches	0	
Informal education programs	0	KL NOT DOUBLE-COUNTING BETWEEN PROJECTS Estimated annual number of visitors to our exhibit at the Discover Science weekend in the Seattle Aquarium and Husky Weekend activities (200)
Stakeholders who receive information	20	Attendees in outreach talks by co-PI Canino
Volunteer hours	0	KL NOT COUNTING Estimated annual numbers of hours spent by project members at the Discover Science weekend in the Seattle Aquarium and Husky Weekend activities
P-12 students reached	0	KL NOT DOUBLE-COUNTING BETWEEN PROJECTS Estimated annual number of kids to our exhibit at the Discover Science weekend in the Seattle Aquarium and Husky Weekend activities
P-12 educators	0	

REQUESTED INFORMATION

Publications

No **Publications** information reported

Students Supported

Susie Dobkins (New Student)
sdobkins@uw.edu
University of Washington, Aquatic and Fishery Sciences

Field of Study: Aquatic and Fishery Sciences

Advisor: Lorenz Hauser / Mike Canino
Degree Type: BS
Degree Year: 2015

Student Project Title: Evidence for selection at the pantophysin locus in Pacific cod

Involvement With Sea Grant This Period (capstone, fellow, intern, etc.): Intern

Post-Graduation Plans (employer, grad school, etc.): teaching High School in Kentucky, now applying for graduate school

Was this thesis/dissertation supported by Sea Grant?: No

Thesis / Dissertation:

New or Continuing?: New

Degree awarded this reporting period?: Yes

Financially supported?: No

Mary Fisher (New Student)
mfisher5@uw.edu
University of Washington, School of Aquatic and Fishery Sciences

Field of Study:
Advisor: Lorenz Hauser
Degree Type: MS
Degree Year:

Student Project Title:

Involvement With Sea Grant This Period (capstone, fellow, intern, etc.): scholarship student

Post-Graduation Plans (employer, grad school, etc.):

Was this thesis/dissertation supported by Sea Grant?: No

Thesis / Dissertation:

New or Continuing?: New

Degree awarded this reporting period?: No

Financially supported?: Yes

Narratives

Cod Final Report
Uploaded File: WSG_Final_Report_Cod_v3.pdf

Partners This Period

Washington Department of Fish and Wildlife
Types: Government
Scale: STATE
Notes:

Alaska Fisheries Science Center (US DOC, NOAA, NMFS)
Types: Government
Scale: FEDERAL or NATIONAL
Notes: Mike Canino, co-PI

University of Washington
Types: Academic Institution
Scale: STATE
Notes: Sam Wasser's lab

STANDARD QUESTIONS

Community Hazard Resilience

No **Community Hazard Resilience** information reported

Economic Impacts

No **Economic Impacts** information reported

Impacts and Accomplishments

(1)

Type	impact
Title	Washington Sea Grant sponsored research develops tools to establish seasonal migration routes and identify population of origin
Relevance	Genetic stock identification is widely applied in salmon management and allows real time management of diverse population as well as forensic identification of the origin of fish products. Such analyses were impossible in marine fish until recently because of low genetic differentiation among populations.
Response	Washington Sea Grant sponsored researchers developed genetic markers that allow accurate identification of population of origin.
Results	Using novel genetic markers, WSG funded researchers can determine the origin of individual fish, thus determining the distribution of Puget Sound cod (species of concern) in relation to the coastal fishery, as well as identify the population of origin in coastal fish.
Recap	Washington Sea Grant funded researchers can identify the population of origin of individual cod and so help management and conservation of the depleted Puget Sound population. Similar approaches are also possible in Alaska, greatly helping sound management of cod fisheries

Comments	
Primary Focus Area	Healthy Coastal Ecosystems
Secondary Focus Areas	Healthy Coastal Ecosystems
Goals	Ocean and coastal resources are managed using ecosystem-based approaches.|The public is ocean literate.
Partners	NOAA Alaska Fisheries Science Center, Washington Department of Fish and Wildlife

(2)

Type	accomplishment
Title	Washington Sea Grant-supported researchworks to unravel the genetic factors that distinguish Puget Sound's Pacific cod and predict their responses to environmental change
Relevance	Pacific cod in Washington's inland waters are a genetically distinct population, once abundant and commercially important but now listed as a species of concern. They are near the southern limit of their range and could be further impacted by oceanic warming. On the East Coast, Atlantic cod are already shifting northward and showing strong temperature-related genetic gradients. Future management and possible aquaculture or supplemental stocking will depend on how the depleted local population responds to a warming environment and whether lines that are more adaptable can be identified.
Response	Washington Sea Grant-sponsored researchers are identifying molecular genetic markers correlated with environmental conditions.
Results	researchers have identified genetic markers that are highly differentiated between Puget Sound and coastal cod. These results will be compared to selective changes in common garden experiments and genetic differentiation between year classes withing Puget Sound.
Recap	Molecular genetic data suggest selective differentiation between Puget Sound and coastal cod, which will be confirmed by results from captive rearing and temporal comparison of wild samples.
Comments	NOAA Mukilteo Lab, NOAA Northwest Fisheries Science Center, Montlake, NOAA Alaska Fisheries Science Center, Washington Department of Fish and Wildlife
Primary Focus Area	Sustainable Fisheries and Aquaculture
Secondary Focus Areas	Healthy Coastal Ecosystems

Goals	Ocean and coastal resources are managed using ecosystem-based approaches.|Fisheries are safe, responsibly managed and economically and culturally vibrant.
Partners	NOAA Mukilteo Lab, NOAA Northwest Fisheries Science Center, Montlake, NOAA Alaska Fisheries Science Center, Washington Department of Fish and Wildlife

Leveraged Funds

(1)

Purpose	Local adaptation in Puget Sound Pacific cod (Gadus macrocephalus): phenotypic and genomic differentiation and the conservation of a depleted population in a warming environment - graduate student support
Source	School of Aquatic and Fishery Sciences and College of the Environment, University of Washington
Amount	84924
Start Date	09-16-2015
End Date	09-16-2017

Meetings, Workshops, Presentations

(1)

Type of Event	Public or professional presentation
Description	Gruenthal K (2015) Genetics, life history, and the management of highly fecund marine species. Departmental Seminar, School of Aquatic and Fishery Sciences
Event Date	05-27-2015
Number of Attendees	50

(2)

Type of Event	Public or professional presentation
Description	Gruenthal K, Canino M, Lowry D, Hauser L (2015) Assigning Individual Pacific Cod Gadus macrocephalus to Population of Origin Along an Isolation-By-Distance Gradient Using RAD Sequencing. Annual AFS meeting, Portland, Oregon,
Event Date	08-18-2015
Number of Attendees	40

(3)

Type of Event	Public or professional presentation
Description	Canino M (2015) “Pacific cod in the Salish Sea”. Coastal Conservation Association Sno-King Chapter, August, 2015.
Event Date	08-19-2015
Number of Attendees	20

Tools, Technologies, Information Services / Sea Grant Products

(1)

Description	Genetic tool to assign Pacific cod to population and geographic region.
Developed (in the reporting period)?	Yes
Used (in the reporting period)?	No
Used for EBM?	Yes
ELWD product?	No
Number of managers	0
Description/Names of managers	
Reported in previous year?	

Local adaptation in Puget Sound Pacific Cod (*Gadus macrocephalus*): phenotypic and genomic differentiation and the conservation of a depleted population in a warming environment

Lorenz Hauser, Kristen Gruenthal, Mike Canino and Dayv Lowry

Introduction

One of the four primary goals of Sea Grant is "understanding the marine environment and conserving marine resources while providing for sustainable use and ensuring healthy populations in the future" (Living Marine Ecosystems). This task is particularly important in species, which support a large fishery in part of their range while being depressed in others. Understanding the reasons for such differences in population status would not only "support the recovery of depleted species" but would also help understanding "natural variability in marine biodiversity". Pacific cod is one such species: it supports the second largest US Pacific fishery in Alaska, far exceeding US catches of the better-known Atlantic cod, while being critically depleted near the southern end of its distribution, the Salish Sea. Indeed, the declining population of Pacific cod in the Salish Sea has recently been listed as a NMFS Species of Concern, based largely on genetic evidence from our previous Washington Sea Grant (WSG) research demonstrating the long-term isolation of Salish Sea cod from coastal populations (Cunningham et al. 2009; Canino et al. 2010). What is still unknown is the adaptive significance of this genetic differentiation, that is, whether Pacific cod in the Salish Sea are adapted to local conditions. Such local adaptation may increase the biocomplexity of the species as a whole, thus preventing extreme abundance fluctuations in a 'portfolio effect' (Schindler et al. 2010). Furthermore, populations at the southern edge of a species' distribution may be old and harbor genetic variation that may be particularly important for the survival and evolution of a species, a theory known as the 'rear edge' effect (Hampe & Petit 2005). On a practical level, these populations may be particularly valuable as broodstock adapted to warming conditions, while on the more fundamental level such differences in local adaptation will become increasingly important as climate change speeds up.

The abundance of Pacific cod in Puget Sound has been declining for several decades, but the causes of this decline, especially in relation to the abundant northern stocks, are uncertain. In particular, it was unknown whether such declines reflect a geographic shift in abundance or a reduction in abundance of a local population. In 1999, Pacific cod in Puget Sound were petitioned for consideration under the Endangered Species Act (Wright 1999). The resulting investigation by the Biological Review Team commented on the limited data available on stock structure but found the greatest support for a large Distinct Population Segment (DPS) extending to Dixon Entrance (Alaska). Consequently, the review team concluded that there was low risk of extinction given the many spawning locations and the abundance of cod in that area (Gustafson et al. 2000). Within Puget Sound, cod abundance has continued to decline from a population that once supported thriving commercial fisheries to low abundances despite protection from directed fisheries for more than 25 years. The Washington Department of Fish and Wildlife (WDFW) has undertaken strong conservation measures by eliminating target bottom trawl and set net fisheries for cod, limiting the bycatch of cod in commercial fisheries, eliminating or reducing the allowed recreational catch, and protecting known spawning habitats. However, the stock has not recovered, and recreational and commercial fisheries are still closed or limited given the overall depressed level of the cod population. The reasons for this lack of recovery are uncertain, but a warming climate as well as habitat loss and by-catch are likely factors (Beamish 2008).

Recently, Salish Sea cod have been listed as a species of concern by the National Marine Fisheries Service http://www.nmfs.noaa.gov/pr/species/fish/pacificcod.htm. This decision to list was strongly influenced by genetic evidence from our previous Sea Grant project demonstrating the isolation of Salish Sea cod from coastal populations (Cunningham et al. 2009), the relatively old age of that

population (Canino et al. 2010), and limited migratory exchange suggested by otolith microchemistry (Gao et al. 2005). All of these scientific results suggest that Salish Sea cod represents a separate DPS from coastal populations of Pacific cod. Two main criteria are needed for a DPS under the Endangered Species Act (ESA): first, "discreteness" in terms of genetic and phenotypic differences from other populations, and second, "significance" in terms of an unusual ecological setting, representing a significant part of the range or the only natural occurrence of a species and significant differences in genetic characteristics (USFWS & NMFS 1996). Salish Sea cod clearly meet the 'discreteness' criterion, in that quantitative measures of genetic discontinuity provide evidence of separation from coastal cod. The population also potentially meets the 'significance' criterion because it persists "in an ecological setting unusual or unique for the taxon" and because it "differs markedly from other populations in the species in its genetic characteristics" (USFWS & NMFS 1996). Finally, the population also may meet the criterion of a threatened or endangered status. Nevertheless, some uncertainties remain: first, although genetic data clearly demonstrate isolation (discreteness) of Salish Sea cod, the adaptive significance of that genetic differentiation is unclear. Second, occasional strong year classes, such as that of 2008, warrant a re-evaluation of the status of the population. Both these issues were mentioned as data deficiencies in the species fact sheet attached to the SOC listing (http://www.nmfs.noaa.gov/pr/pdfs/species/ pacificcod_detailed.pdf). Understanding the nature of stock discreteness and current abundance trends are important for WDFW and its tribal co-managers in order to understand whether fishery and conservation measures are sufficient for protecting and recovering cod stocks in Puget Sound.

Another issue affecting the management of cod on a larger geographic scale is the identification of appropriate management units. Our previous research established a clear isolation-by-distance pattern but could not identify clear population boundaries (Cunningham et al. 2009). A follow-up study in Alaska (Spies 2012) revealed genetic differentiation between the eastern Bering Sea and the Aleutian Islands, leading to the separation of the management of the two areas. Nevertheless, tagging results suggest some seasonal migration within and between these areas (Shimada & Kimura 1994; Shi et al. 2007), and the spatial structure of Pacific cod remains a research priority cited in both the AI and EBS stock assessments (Thompson 2013; Thompson & Palsson 2013). Similarly, the spatial structure of cod in the Gulf of Alaska is uncertain. New next-generation sequencing approaches have been shown to be more discriminatory than traditional microsatellite surveys, and additionally, may allow the assignment of individual fish to their population of origin. Such information would be invaluable for the management of the species coastwide.

Project Objectives:

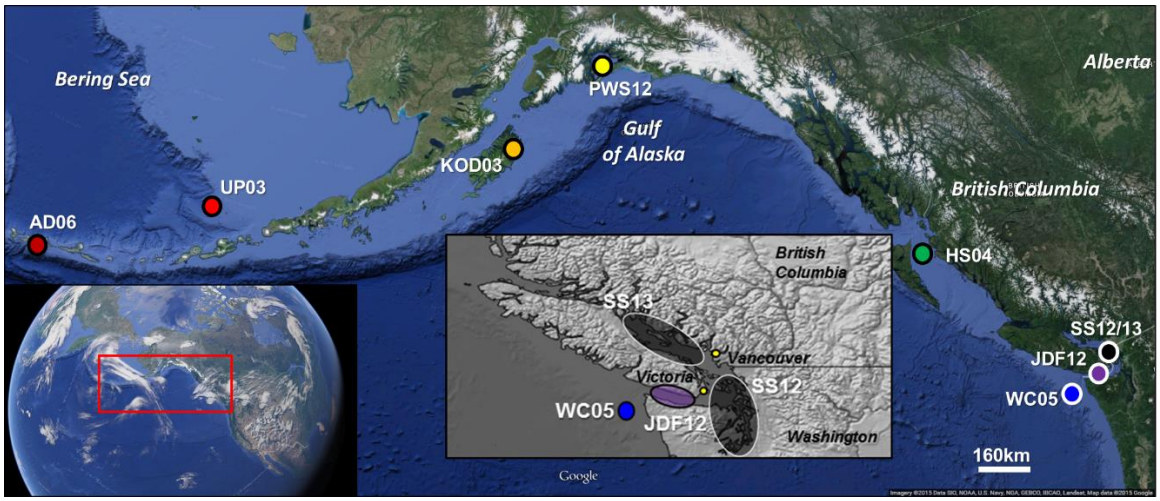
- To identify and measure adaptive genetic differentiation between populations of Pacific cod in Puget Sound (southern Salish Sea), the Strait of Georgia, the Washington coast and Alaska by conducting genome scans with next generation sequencing technologies.
- To detect evidence for selection by comparing year classes of Puget Sound cod born in warm and in cold years.
- To detect stock boundaries in Pacific cod along the west coast of North America and to test the potential for assignment of individual cod to their population of origin.
- To quantify phenotypic differences in larval growth and survival between Pacific cod from Puget Sound and coastal Washington State in common garden rearing experiments and to relate these phenotypic differences to genetic variation from a genome scan in wild populations and selective mortality in captive families reared at different temperatures.

Methods

We proposed to employ two main approaches to assess the extent of adaptive genetic variation in Pacific cod: (i) spatial and temporal comparison of genome-wide molecular variation in wild populations (genome scans) and (ii) common garden experiments in captivity to estimate phenotypic differences

Table 1: Sample information for collection sites for *G. macrocephalus* in the northeastern Pacific. Data include population location name, month and year collected, abbreviation, approximate latitude and longitude, number of individuals (n), number of SNP loci out of 6442 possible, heterozygosity (H_E), and genetically effective population size (N_e).

Location name	Month/Year	Abbreviation	Latitude	Longitude	n	Loci	H_E	N_e (95% CI)
Salish Sea	05/2012	SS12	48°14'	122°40'W	19	5978	0.193	1041 (919 to 1202)
	08/2013	SS13	49°34'	124°31'W	10			
Strait of Juan de Fuca	05/2012	JDF12	48°8'	122°40'W	18	5904	0.196	2502 (1645 to 5219)
Washington Coast	02/2005	WC05	47°55'	125°33'W	40	6161	0.192	721 (675 to 774)
Hecate Strait	03/2004	HS04	53°13'	130°57'W	38	6173	0.195	3046 (2409 to 4138)
Prince William Sound	03/2012	PWS12	60°32'	147°4'W	46	6262	0.197	4969 (3668 to 7692)
Kodiak Island	03/2003	KOD03	57°48'	152°31'W	43	6158	0.194	1687 (1476 to 1967)
Unimak Pass	01/2003	UP03	54°38'	168°10'W	43	6220	0.198	1666 (1469 to 1925)
Adak Island	03/2006	AD06	51°40'	176°36'W	40	6164	0.196	1362 (1208 to 1560)



between populations and to detect selection at specific regions of the genome.

Genome scans

We used samples from Cunningham et al. (2009) from spawning and pre-spawning aggregations of Pacific cod at six locations between Adak Island in the Aleutians and the Washington coast (Table 1, Fig. 1). In addition, samples of non-spawning fish were collected in Prince William Sound and the Salish Sea in 2012 and 2013. For temporal comparisons within the Salish Sea, we added samples from 2005 (N=22), 2009 (N=23), 2010 (N=38), 2014 (N=14) and 2015 (N=10) collected from WDFW groundfish surveys. Soft ray fin clips were preserved in 95 to 100% non-denatured ethanol and stored at 4°C.

DNA was extracted from fin clip tissue punches in 96-well format using a DNeasy 96 Blood & Tissue Kit (Qiagen, Inc., Valencia, CA). RAD libraries were prepared, including SbfI restriction enzyme digestion, adapter ligation, shearing, and PCR, on 500ng DNA per fish according to Baird et al. (2008) and Hohenlohe et al. (2011), with modification to include Agencourt AMPure XP SPRI beads (Beckman Coulter, Inc., Pasadena, CA) for size selection/exclusion and purification (P.D. Etter, University of Oregon, pers. comm.). Library sizes [300-1000 basepair (bp) target length] were estimated with 1% E-Gel EX agarose gels (Invitrogen, Carlsbad, CA), and concentration and quality were assessed using Quant-iT PicoGreen dsDNA Reagent (Invitrogen, Carlsbad, CA) and a FLx800 Fluorescence Microplate Reader (BioTek Instruments, Inc., Winooski, VT). Libraries were pooled within samples in 10nM concentrations and sequenced in 100bp single end reads on a HiSeq2000 (Illumina, Inc., San Diego, CA).

Raw data were quality filtered and demultiplexed and sequence alignment, SNP discovery, catalog construction, and genotyping were performed in Stacks v1.21 (Catchen et al. 2011; Catchen et al.

2013) according to the methods of Gruenthal et al. (2014), with minor modification. Briefly, catalogs created in the `cstacks` subprogram were generated from the five most data-rich individuals from each sample. Flags (m = 3, M = 2, N = 4, n = 3, max_locus_stacks = 3) associated with increasing the number of loci, while reducing the SNP and allele calling error rates, were set according to Mastretta-Yanes et al. (2015). A genotype file containing putative polymorphic SNPs present in ≥ 80% of fish per sample was filtered to include one SNP per RAD tag (flag: write_random_SNP) to minimize physical linkage. Final filtering removed loci in the last position on the tag (basepair 94) and/or with minor allele frequencies (MAFs) < 0.05 to minimize sequencing errors, as well as loci with uncorrected Hardy-Weinberg equilibrium (HWE) p-values ≤ 0.05.

Locus-specific allele frequencies, expected heterozygosity (H_E), deviations from Hardy–Weinberg equilibrium (HWE) and locus-specific F-statistics (F_{IS}, F_{ST}, and F_{IT}) were estimated with GENEPOP v4.2 (Rousset 2008) using the default parameters. Population pairwise F_{ST} and associated p-values (110 permutations) were estimated using the default parameters in Arlequin v3.5.1.2 (Excoffier et al. 2005), and the results were sequential Bonferroni-corrected at the table-wide $\alpha = 0.05$ level (Rice 1989). The genetically effective size (N_e) of each population was estimated using NeEstimator v2.01 (Do et al. 2014) under the random mating model using the linkage disequilibrium method (Waples and Do 2008), with an MAF cutoff of 0.05 (R. Waples, NOAA, pers. comm.).

The presence and magnitude of global IBD was assessed with Mantel tests (999 permutations) run in GenAlEx v6.5b4 (Peakall and Smouse 2006, 2012). Pairwise genetic distance (F_{ST} / 1-F_{ST}) was plotted against smallest overwater geographic distance estimated with Google Earth. Discriminant analysis of principal components (DAPC) as implemented in adegenet v1.4-2 (Jombart 2008; Jombart and Ahmed 2011) was used to visualize the relationships among individuals within samples. The number of principal components (PCs) retained during the principal component analysis (PCA) steps of the DAPC was determined using the package function optim.a.score.

To assess the power of the dataset to correctly assign individuals to their population of origin, assignment tests were performed in GeneClass2 (default settings; Piry et al. 2004) using the leave-one-out procedure, with the Bayesian method. All 6442 loci were included in analyses to avoid high-grading bias (Anderson 2010; Waples 2010). In addition, we used SCAT (Wasser et al. 2007) to assign individual cod to a location of origin, using only samples along the coast (i.e. excluding Salish Sea and the Strait of Juan de Fuca). Ten individuals were removed from each sample, allele frequencies were recalculated and removed cod were assigned to location of origin.

Outlier tests to identify putative candidate loci under selection were performed under a Bayesian framework using the differentiation-based method employed by BayeScan v2.1 (Foll and Gaggiotti 2008; Foll et al. 2010; Fischer et al. 2011). Testing was conducted using the default settings, with 20,000 iterations and a false discovery rate of 0.05, on all samples and then again on a subset of samples, including SS12/13, JDF12, WC05, and HS04, to address the significant differentiation associated with SS12/13.

To assess whether the outlier loci co-localized with regions under selection identified in the congeneric Atlantic cod (*G. morhua*) by Hemmer-Hansen et al. (2014), Pacific cod sequences in the Stacks catalog, were aligned to the Atlantic cod genome (ATLCOD1A Newbler genome assembly, www.codgenome.no/data/) using BLASTN according to the methods of Star et al. (2011). Sequences corresponding to the best alignment for each locus, with an e-value less than 10^{-10}, were retained (Hemmer-Hansen et al. 2014). Genome scaffolds and aligned RAD loci were then assigned to Atlantic cod linkage groups using R, based on the linkage map described in Borza et al. (2010) and supplementary annotation information (Online Resource 3) in Hemmer-Hansen et al. (2014).

Pacific cod rearing

Pacific cod broodstock from the Washington coast and from Juneau, Alaska, was successfully

spawned. We were not able to obtain Puget Sound cod because of the low abundance of cod in Puget Sound. A month long survey by WDFW in 2013 only produced 51 fish, all of which were too moribund to be held alive. This observation by itself shows the critical status of the species in Puget Sound, which now appears to have reached very low abundances indeed. Although the planned comparison between Puget Sound and coastal cod was therefore not possible, the comparison between a northern (Alaska) and southern (Washington) population will still be useful for achieving the general sense of the project aims, and the samples collected in Puget Sound were very useful for molecular screening.

Origin		WA coast						AK cod (Newport)		
Family		A	B	C	D	E		F	G	H
sire#		638	638	634	638	638		7416	5891	6611
dam#		602	614	602	608	640		9239	9239	9239
spawn date		3/8	3/8	3/9	3/12	3/12		3/26	3/26	3/26
cold	days						days			
	20	11	24	51	45	30	20		36	36
	31			48			35	54	38	6
control	21	3	5	48	15	41	20		48	28
	31			1		8	35	1	66	71
	20	6	4	48	1	48	20	13	48	48
warm	31			24		1	35		46	17
	55			23						

Table 2: Larval samples obtained from the common garden rearing experiment in 2014. Five families (A-E) from the Washington coast and Alaska (F-H) were reared under cold (4.5°C), control (6°C) and warm conditions (8.5°C). Larvae were sampled at approximately 20 and 31 days post spawning.

Adult Pacific cod from the Washington coast population were obtained from commercial trawlers in Neah Bay, Washington, during February 2014, and transported to the Northwest Fisheries Science Center laboratory facility in Mukilteo, Washington. Eggs from four females were fertilized with sperm from two males to produce five family groups, four of which were half-sibs (*i.e.* shared one parent). Similarly, eggs from a single female Alaskan cod were fertilized with sperm from three males at Hatfield Marine Science Center in Newport, Oregon, to produce three full-sib families nested within a half sib family. Approximately 12,000 fertilized eggs (7,000 from WA coast and 5,000 from Alaska) were incubated at the NOAA Alaska Fisheries Science Center in Seattle, Washington, for about two weeks at 5.1°C and then reared at three experimental temperatures (4.5, 6.0 and 8.5 °C) in a recirculating seawater system at the NOAA Northwest Fisheries Science Center, also in Seattle, Washington. Each family group was reared separately in mesh enclosures housed within 2,000 l tanks and provided with a 14h/10h light/dark diel cycle. At first-feeding readiness, larvae were provided with nominal *ad libitum* densities (5 individuals per ml) of enriched rotifers. Larvae were sampled at hatch, first-feeding and, in some cases, up to 26 days post-spawning (Table 2). Sampled individuals were anaesthetized using MS-222 and measured to the nearest 0.06 mm under a dissecting scope prior to being preserved in 100% non-denatured ethanol.

Major Findings

Genome Scans

So far, data from the spatial comparison of samples along the coast and in the Salish Sea have been analyzed. Samples from the temporal comparison within the Salish Sea, and from the larval rearing experiment, have been

Table 3: Pairwise population estimates of F_{ST} for *G. macrocephalus* in the northeastern Pacific. All pairwise comparisons significant ($P < 0.001$), except between JDF12 and WC05, after sequential Bonferroni correction.

	SS12/13	JDF12	WC05	HS04	PWS12	KOD03	UP03
JDF12	0.016						
WC05	0.019	0.000					
HS04	0.018	0.004	0.003				
PWS12	0.031	0.016	0.015	0.010			
KOD03	0.038	0.021	0.020	0.014	0.002		
UP03	0.037	0.023	0.020	0.014	0.004	0.001	
AD06	0.046	0.031	0.028	0.019	0.006	0.004	0.003

sequenced, but the data have not been analyzed yet.

A total of 6442 putative biallelic SNP loci and 297 individuals were retained after filtering. Global H_E was 0.196 and averaged 0.195 ± 0.0020 across populations (Table 1). Global F_{IS}, F_{ST}, and F_{IT} were 0.013, 0.015, and 0.028, respectively. Locus-specific F_{ST} ranged from -0.017 to 0.720. Pairwise F_{ST} ranged from zero to 0.046 (Table 3); all comparisons were significant ($P < 0.001$), except between JDF12 and WC05 (F_{ST} = 0.000, P = 0.234), after sequential Bonferroni correction. The SS12/13 sample was the most divergent, and pairwise F_{ST}s including SS12/13 ranged from 0.016 with JDF12 to 0.044 with AD06. There were highly significant positive correlations between pairwise genetic and geographic distance across all samples (R^2 = 0.50, p = 0.005) and the coastal samples (excluding SS12/13; R^2 = 0.82, p = 0.001) (Fig. 2). Finally, N_e averaged 2124 and ranged from 721 (95% CI of 675 to 774) for WC05 to 4969 (95% CI of 3668 to 7692) for PWS12 (Table 1).

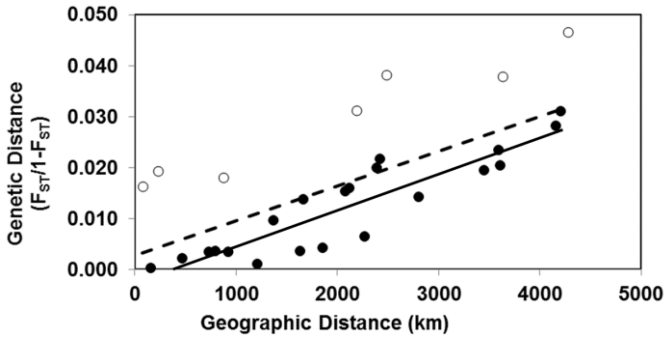


Figure 2: Global IBD in *G. macrocephalus* from the northeastern Pacific. Genetic distance (F_{ST} / 1 - F_{ST}) plotted against geographic distance (km) for each population pair. Black circles represent pairwise coastal population comparisons. Open circles represent pairwise estimates involving SS12/13. Regression line for coastal populations in solid black (R^2 = 0.82) and for all populations, including SS12/13, in broken black (R^2 = 0.50).

Fourteen PCs were retained after a-score optimization for DAPC on the full dataset (Fig. 3 top inset). Three primary clusters are apparent in the DAPC: Alaskan samples (Adak – Prince William Sound), BC and WA samples (Hecate Strait to Washington coast) and the Salish Sea (Fig. 3top). The strong separation between SS12/13 and the remaining samples despite the small geographic separation was supportive of the pairwise F_{ST} results, as well as earlier research by Cunningham et al. (2009) and Canino et al. (2010). In addition, there was a spatiotemporal separation within the Salish Sea between samples from WA collected in 2012 and samples from BC collected in 2013 (Fig. 3 bottom). The reason for the bimodal distribution within each subset is unknown (e.g. was not associated with the specific haul site of individual fish).

Overall, 85% of individuals were correctly (re)assigned to population of origin

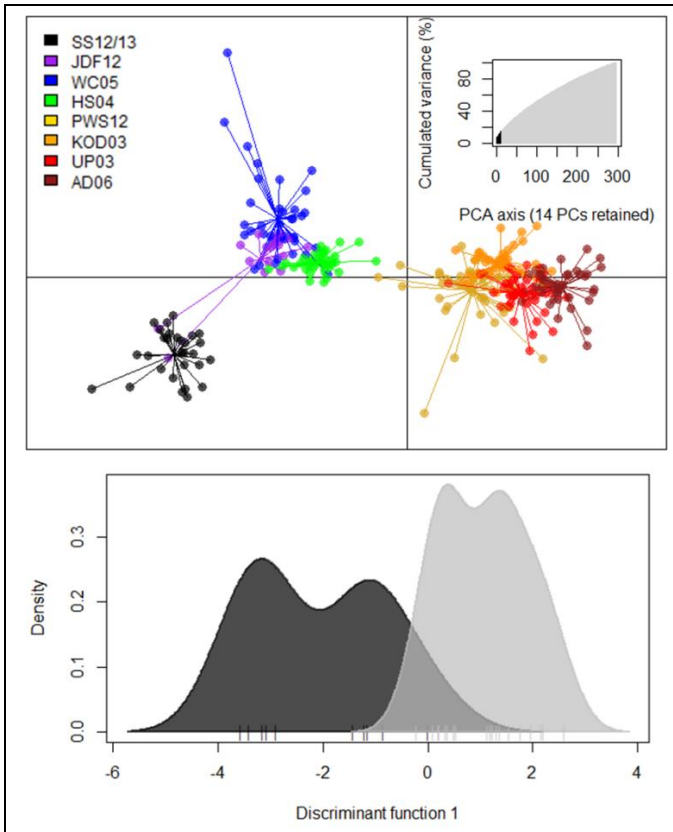


Figure 3: DAPC in *G. macrocephalus* from the northeastern Pacific. Top: DAPC on all samples. Inset shows number of PCs retained (n = 14), based on a-score optimization. Bottom: SS12/13 sample alone in a density chart, with northern Georgia Basin fish in dark grey and fish from U.S. waters (southern Georgia Basin, San Juan Island, and Puget Sound) in light grey. Hash marks along x-axis (DF1) represent individual fish.

(Table 4). None of the 18 individuals from JDF12 assigned to JDF12; fourteen assigned to WC05, with minimal assignment to SS12/13 and HS04. Thirty-five of 46 individuals from PWS12 assigned to PWS12. Individuals from the remaining samples assigned at high rates (85% to 100%) to their population of origin, with limited assignment to adjacent populations.

Table 4: Assignment results for *G. macrocephalus* populations from the northeastern Pacific. Numbers represent total individuals from samples listed in column at left assigned to samples in row at top. Percent correct assignment within samples listed in column at right.

	SS12/13	JDF12	WC05	HS04	PWS12	KOD03	UP03	AD06	% Correc
SS12/13	29								100
JDF12	2		14	2					0
WC05			38	2					95
HS04			2	36					95
PWS12				1	35	5	5		76
KOD03					3	38	2		88
UP03					2		41		95
AD06							6	34	85

Assignment to location was highly successful (Fig. 4). Individual cod were located on average between 54 and 144 km from the sample location, with the exception of Prince William Sound (434 km) and Adak (339 km). Prince William Sound appeared to be a population mixture of non-spawning fish from outside the sound, and the large assignment distance at Adak was probably an edge effect that could be avoided by increasing sampling density.

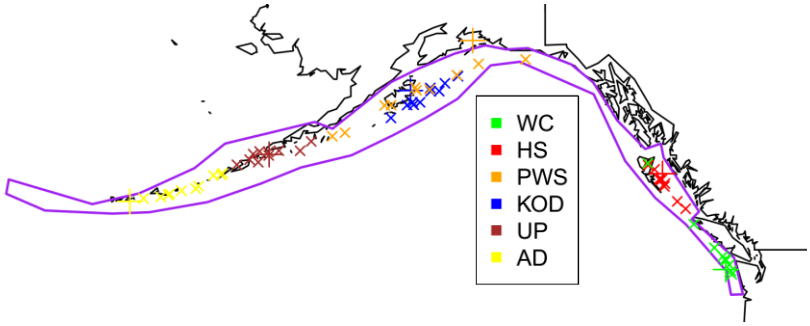


Figure 4: Map of the northeast Pacific showing the assignment of individual cod to geographic locations. X denote assignment location, + the origin of the sample of spawning fish they came from

The log10 probability of the odds (PO) for each locus was estimated for the coastal samples and the southeastern samples, which included SS12/13, JDF12, WC05, and HS04. Across the coastal samples, 106 of 6,442 loci were categorized as exhibiting decisive evidence for selection [log10(PO) > 2, $P(\alpha \neq 0) > 0.99$] (Fig. 5). In the southeast of the sampled range, outlier testing identified 13 loci at log10(PO) > 2 [$P(\alpha \neq 0) > 0.99$] (Fig. 5). Only three loci were deemed outliers in both analyses.

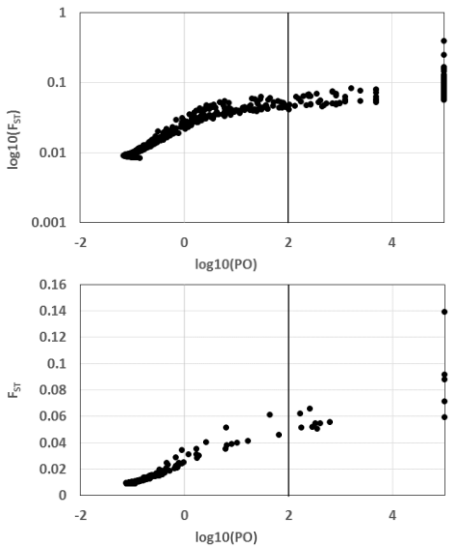


Figure 5: Outlier test results from BayeScan for *G. macrocephalus* from the northeastern Pacific. To provide better resolution, log10 of per locus F_{ST} plotted against log10 probability of the odds (PO) for coastal samples (top). Per locus F_{ST} plotted against log10(PO) for the southeastern portion of the sampled range (bottom). Loci to the right of vertical bar ($\log_{10}(PO) = 2$) are deemed as decisively under selection.

Out of 6442 loci, 3850 aligned to the ATLCOD1A genome assembly, with $e < 10^{-10}$ (Table S2). Of those that aligned, 54 were outlier loci in the coastal samples and five were outliers in the southeast region. For the coastal outliers, tags 51759 and 61894 both aligned to scaffold (s) 5923; tags 917 and 52257 both aligned to s10101; and tags 23193, 26468, and 31611 aligned to s11980. In all other cases, coastal and in the southeast, single tags aligned to single scaffolds. No outlier loci co-localized to candidate gene regions under selection reported by Hemmer-Hansen et al. (2014) for Atlantic cod.

Larval Rearing

Larvae showed very consistent growth among families before introduction to the recirculation system (Fig. 6). Subsequently, daily growth rate differed between families and temperature treatments in the Washington population but not in the Alaska population. Although sample sizes are very small, this could indicate genotype x environment interactions that warrant further investigation.

Samples from family C (Washington coast) have been extracted and sequenced, but data have not yet been analyzed. We are planning to sequence samples from family G (Alaska) in the near future.

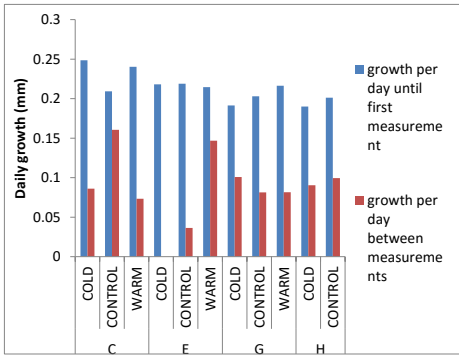


Figure 6: Daily growth rate per day of cod larvae before introduction to the recirculation system (blue) and during the experiment (red). Families C and E are from the Washington coast population, and G and H from the Alaska population. Each family was split into three temperature treatments.

Significance of Results

The results from the coastwide survey of genetic diversity in this project largely confirmed the results of the previous Sea Grant project (Cunningham et al. 2009; Canino et al. 2010) in also detecting a strong isolation by distance pattern and high differentiation of the Salish Sea cod population from coastal stocks. However, the increased power of RAD sequencing now allows accurate assignment of cod to sampled population as well as geographic location and thus greatly increases the utility of molecular markers for applied fisheries management. Using these approaches, it would be possible to identify the provenance of fish products, and maybe more usefully, investigate patterns of seasonal migration in Pacific cod. Indeed, based on these results, we have recently submitted an application to the NOAA Saltonstall-Kennedy fund proposing to investigate seasonal migrations of Alaskan cod by applying these new assignment powers.

In addition, we could clarify the conservation status of Puget Sound cod. Despite reports of a strong year class in 2008, no broodstock could be caught and a month-long sound wide WDFW trawl survey in 2013 caught only 51 cod. It is therefore clear that the abundance of Pacific cod in Puget Sound is very low indeed. Pacific cod appear to be relatively stable in the Strait of Georgia (Beamish, pers. comm), even though earlier reports voiced concerns over increasing bottom temperatures (Beamish & Riddell 2009). Notably, our study provided strong indications that cod in the Strait of Georgia are isolated from those in US waters of the Salish Sea. Therefore, demographic rescue of the Puget Sound populations from Canadian populations is unlikely, and any management efforts would have to concentrate on Puget Sound itself. Such management efforts could include captive rearing and stock enhancement.

In terms of local adaptation, our results identified 13 candidate loci that showed higher than average differentiation between Salish Sea cod and coastal cod. Only three of these loci were also identified as candidate loci in the coastwide comparison. The adaptive significance of these candidate loci will be tested by (i) comparing year classes born in warm and cold years within Puget Sound and (ii) comparing these loci with outliers detected in the comparison among the three temperature treatments of the captive rearing experiment. Any overlap in results between these approaches would be a very clear indication of adaptive genetic differentiation in Salish Sea cod, which would inform both management and ESA listing decisions.

Future Work

This project had to overcome significant challenges that delayed progress and necessitated some adjustment of project objectives. First, we were unable to catch sufficient broodstock of Puget Sound cod for captive rearing experiments, despite extensive targeted efforts by the project team as well as WDFW collaborators; we therefore had to concentrate on Alaskan and Washington coast cod. Second, several

attempts to breed cod failed, and the ultimate experiment resulted in a limited number of families. Finally, and probably most importantly, the graduate student of the project decided to leave her PhD program to start a family – fortunately, we were able to hire Dr Kristen Gruenthal who was an invaluable asset to the project. Because of these challenges and delays, some work remains to be done. First, RAD sequence data from the temporal comparison of Puget Sound age classes remain to be analyzed. Second, RAD data from the captive experiment also need to be analyzed – one family has already been sequenced and another one will be shortly if the results of the first family look promising.

These plans will be facilitated by a new graduate student in PI Hauser's lab, Mary Fisher, who will continue work on Pacific cod. Ms. Fisher will analyze these existing data and collect of new RAD sequences from Alaskan and Puget Sound cod. Furthermore, Hauser and Fisher will attend the World Fisheries Congress in Korea to present their cod results and will take the opportunity to visit a collaborator from Gyeongsang National University in South Korea, who has worked on cod extensively and who collaborates with Korean cod supplementation hatcheries. We are planning to develop collaborative projects based on the results presented here, in particular, larger scale rearing experiments to test the extend of adaptive variation in Korean and North American Pacific cod.

Summary of outreach activities

Members of the project team participated in outreach activities, in particular the annual Discover Science Weekend at the Seattle Aquarium, and the Husky Weekend in 2012 and 2013. The displays included a hands-on demonstration of DNA barcoding developed by Canino, an explanation of herring population structure in Puget Sound as well as displays of salmon hatcheries, species identification and a 'can you jump as high as salmon' activity. The displays were manned by MerLab PIs (Hauser and Naish), MerLab manager Jimenez Hidalgo, graduate students, undergraduate students from Hauser's class, and co-PI Canino. The displays attracted considerable attention, and much fun was had by all.

Co-PI Canino also gave presentations to the South Kitsap Poggy Club in May 2013 and to the Coastal Conservation Association Sno-King Chapter in August 2015. Scientific talks were presented at the Annual Meeting of the American Fisheries Society in Quebec, Canada, August 2014, and in Portland, September 2015. PDRA Gruenthal also presented a departmental seminar in the School of Aquatic and Fishery Sciences in spring 2015.

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