

Update Report

Period: 2/1/2014 - 1/31/2015

Project: R/LME-6 - Local adaptation in Puget Sound Pacific cod (*Gadus macrocephalus*): phenotypic and genomic differentiation and the conservation of a depleted population in a warming environment

STUDENTS SUPPORTED

No Students Reported This Period

CONFERENCES / PRESENTATIONS

Hauser L, Gruenthal K, Drinan D, Lowry D, Canino M (2014) Selective differentiation and population boundaries from RAD sequencing along a microsatellite isolation-by-distance gradient in Pacific cod (*Gadus macrocephalus*). Annual AFS meeting, Quebec, Canada, 17-21 August 2014, public/profession presentation, 40 attendees, 2014-08-18

ADDITIONAL METRICS

P-12 Students Reached:	100	P-12 Educators Trained:	0
A significant number of kids visited our exhibit at the Discover Science weekend in the Seattle Aquarium, 8 Nov 2014			
Participants in Informal Education Programs:	200	Volunteer Hours:	0
Estimated number of visitors to our exhibit at the Discover Science weekend in the Seattle Aquarium, 8 Nov 2014			
Acres of coastal habitat protected, enhanced or restored:	0	Resource Managers who use Ecosystem-Based Approaches to Management:	0
Annual Clean Marina Program - certifications:	0	HACCP - Number of people with new certifications:	0

ECONOMIC IMPACTS

Description	Patents	Market	Non-	Business	Business	Jobs	Jobs
		Impact	Market				
		ts (\$)	Impact	es	es	Created	Retained
None	0	0	ts (\$)	0	0	0	0

SEA GRANT PRODUCTS

Description	Developed?	Used?	ELWD?	Number of Managers	Names of Managers
Data on summer distribution of Puget Sound cod and Washington coast cod in the Salish Sea	Yes	No	No	0	
Data on population differentiation of cod along the northeast Pacific coast and between Puget Sound and the Washington coast	Yes	No	No	0	
Development and successful optimization of molecular protocols to screen cod at 1000s of genetic markers	Yes	No	No	0	
Samples of cod families for genetic	Yes	No	No	0	

analysis

HAZARD RESILIENCE IN COASTAL COMMUNITIES

Name of coastal community	County	Number of resiliency trainings / technical assistance services provided	Was community hazard resiliency improved (e.g., via changes in zoning ordinances) ?
		0	Yes

ADDITIONAL MEASURES

Number of stakeholders modifying practices: 0

Sustainable Coastal Development

of coastal communities: 0

PARTNERS

Partner Name: NOAA Alaska Fisheries Science Center

Partner Name: NOAA Hadfield Marine Science Center, Newport, Oregon, type: Government, scale: Federal or National

Partner Name: NOAA Mukilteo Lab

Partner Name: NOAA Northwest Fisheries Science Center, Montlake

Partner Name: Washington Department of Fish and Wildlife

IMPACTS AND ACCOMPLISHMENTS

Title: **Washington Sea Grant research identifies genetic markers for Puget Sound's dwindling Pacific cod population and other populations throughout the species range**

Type: impact

Relevance, Response, Results:

Relevance: Pacific cod in Washington's inland waters form a genetically distinct population that was once abundant and commercially important. Today it is listed as a NOAA species of concern and a recent state trawl survey caught only a few dozen fish. While information about genetic makeup throughout a fish species' range is useful for both conservation and management, analysis becomes more complex when fish populations mix at sea. Mixed stock analyses are widely applied to salmon, relying on identification of fish origins to allow real-time management of diverse populations. But such analyses for other marine fish species were, until recently, unfeasible.

Response: Washington Sea Grant researchers initially intended to explore Pacific cod adaptability to a warming climate by examining genetic variation in the distinct Puget Sound population, which is at the southern limit of the species' range. Because Puget Sound fish were not available, the project scope was broadened to compare northern Alaska and southern Washington coastal populations. Researchers sought to identify genetic markers that correlate with environmental conditions and allow accurate identification of population of origin.

Results: Highly differentiated genetic markers were identified in Puget Sound and coastal cod, enabling the determination of the origins of individual fish and relative population distributions. Results will be used to investigate seasonal cod movements, safeguard against further depletion, and guide efforts to explore climate change implications.

Recap:

Recap: Washington Sea Grant-supported genetic research confirms selective differentiation in Pacific cod from Puget Sound and finds markers identifying fish population origin to support future conservation.

Comments:

Primary Focus Area: LME (HCE)

Secondary Focus Area: LME (SSSS)

Associated Goals: Strengthen ecosystem approaches to management of living marine resources through improved understanding of marine biodiversity, marine and coastal ecosystem function, climate change, and other sources of variability. (HCE Science)

Support conservation and sustainable use of living marine resources through effective and responsible approaches, tools, models, and information for harvesting wild and cultured stocks and preserving protected species. (SSSS Supply)

Partners:

NOAA Alaska Fisheries Science Center

NOAA Newport Research Station

NOAA Mukilteo Lab

NOAA Northwest Fisheries Science Center, Montlake

Washington Department of Fish and Wildlife

Related Partners: NOAA Alaska Fisheries Science Center, Washington Department of Fish and Wildlife

PUBLICATIONS

No Publications Reported This Period

OTHER DOCUMENTS

No Documents Reported This Period

LEVERAGED FUNDS

No Leveraged Funds Reported This Period

UPDATE NARRATIVE

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Local adaptation in Puget Sound Pacific Cod (*Gadus microcephalus*): 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

Progress on the project was greatly aided by the employment of Dr Kristen Gruenthal, who carried out the molecular work, did most of the analyses and helped with the larval rearing. Dr Gruenthal went on maternity leave (baby number 2 for this project!) in December 2014, but given her efficiency, we expect no major delay in progress.

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. These observations by themselves shows the critical status of the species in Puget Sound, which now appears to have reached very low abundances indeed. Nevertheless, the comparison between a northern (Alaska) and southern (Washington) population will still be useful for the project aims, and the samples collected in Puget Sound were very useful for molecular screening.

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 in within a half sib family. Approximately 12,000 fertilized eggs (7,000 from WA coast and 5,000 from Alaska) were 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 in Seattle. 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. 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. The resulting

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
warm	20	6	4	48	1	48	20	13	48	48
	31			24		1	35		46	17
	55			23						

Table 1: Larval samples obtained from the common garden rearing experiment in 2014. Larvae were sampled at approximately 20 and 31 days post spawning.

samples have not been screened for genetic variation yet but will allow, at least, the construction of a map and possibly a comparison of selection within two contrasting cod populations in a common garden experiment.

Molecular work

Fin-clips from spawning cod collected for our previous Sea Grant funded work (year and sample size follow abbreviation: Washington State coast (WC05, n = 48); Hecate Strait (HS04, n = 48); Kodiak Island (KOD03, n = 48), Unimak Pass (UP03, n = 48), and Adak Island (AD06, n = 48)) as well as new collection from the Salish Sea (JDF12, n = 21; SS12/13, n = 42) and Prince William Sound (PWS12, n = 48)) were used. Preparation of pooled RAD-tagged DNA fragments, including SbfI restriction enzyme digestion, adapter ligation, shearing, and PCR, was conducted on 500ng DNA per fish according to previously published methods, with modification to include SPRI bead cleanup and size selection. Final libraries were submitted to the University of Oregon Genomics Core Facility (UOGCF) for next-generation DNA sequencing (single-read, 100 bp target length) on a HiSeq 2000 (Illumina, Inc., San Diego, CA). Raw reads were quality filtered and demultiplexed using Stacks v1.21. Individuals and SNPs were filtered to minimize physical linkage and sequencing errors and to retain markers in Hardy-Weinberg equilibrium. A total of 6,756 loci and 299 individuals (WC05 = 40, JDF12 = 18, SS12/13 = 31, HS04 = 38, PWS12 = 46, KOD03 = 43, UP03 = 43, AD06 = 40) were retained after filtering.

	JDF12	SS12/13	WC05	HS04	PWS12	KOD03	UP03	AI
JDF12	0							
SS12/13	0.0168	0						
WC05	0.0003	0.0196	0					
HS04	0.0035	0.0188	0.0034	0				
PWS12	0.0156	0.0310	0.0150	0.0095	0			
KOD03	0.0206	0.0372	0.0193	0.0132	0.0022	0		
UP03	0.0225	0.0369	0.0199	0.0138	0.0036	0.0011	0	
AD06	0.0297	0.0450	0.0276	0.0189	0.0065	0.0042	0.0035	

Table 2: Estimates of pairwise F_{ST} between populations. Values in red are significant ($P < 0.05$). Cells are shaded according to F_{ST} values (red – high, green – low). Note the high differentiation of the Salish Sea sample.

The global F_{ST} was 0.017 and highly significant. Pairwise F_{ST} values showed low but highly significant differentiation between all pairs of samples, with the exception of the Strait of Juan de Fuca and the Washington coast (Table 2). Similar to our previous study with microsatellites (Cunningham et al. 2009), there was a strong isolation by distance pattern among coastal populations (Fig. 1). In contrast to microsatellites, however, we were able to re-assign fish with high confidence to their population of origin. Excluding the Strait of Juan the Fuca sample (JDF12), which clearly consisted of Washington coast cod, over 90% of fish were assigned to the correct origin. We will further try to optimize the assignment process, and have submitted a

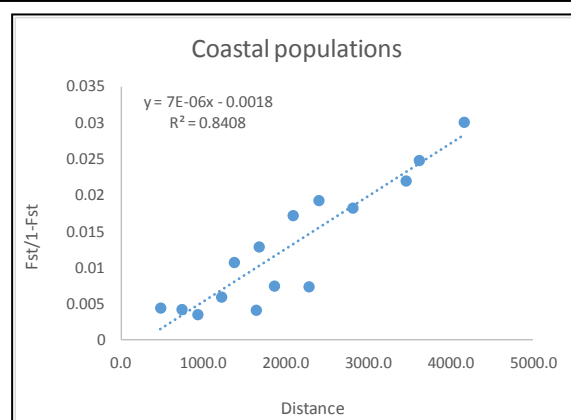


Fig 1: Isolation by distance pattern in coastal populations of Pacific cod.

proposal to the North Pacific Research Board using this approach to investigate seasonal migration of cod in Alaska.

The ability to assign individual cod to the source of origin was particularly useful in Salish Sea samples, which, in contrast to all other collections, were sampled outside the spawning season. Nevertheless, Bayesian clustering techniques clearly identified two populations, and the geographic locations of samples clearly indicated a stock boundary across the shallow sill between Port Townsend, WA, and Victoria, BC (Fig 2). Interestingly, there was no evidence of hybrids between the two populations, suggesting very strong reproductive isolation between populations of Pacific cod.

The precarious situation of the Puget Sound cod population was also indicated by the estimates of effective population size (N_e) from our data (Table 3). N_e estimates ranged from about 1,000 to 10,000 individuals in most populations, but in the Salish Sea, N_e was only 67 individuals, corroborating our observations of extremely low abundance.

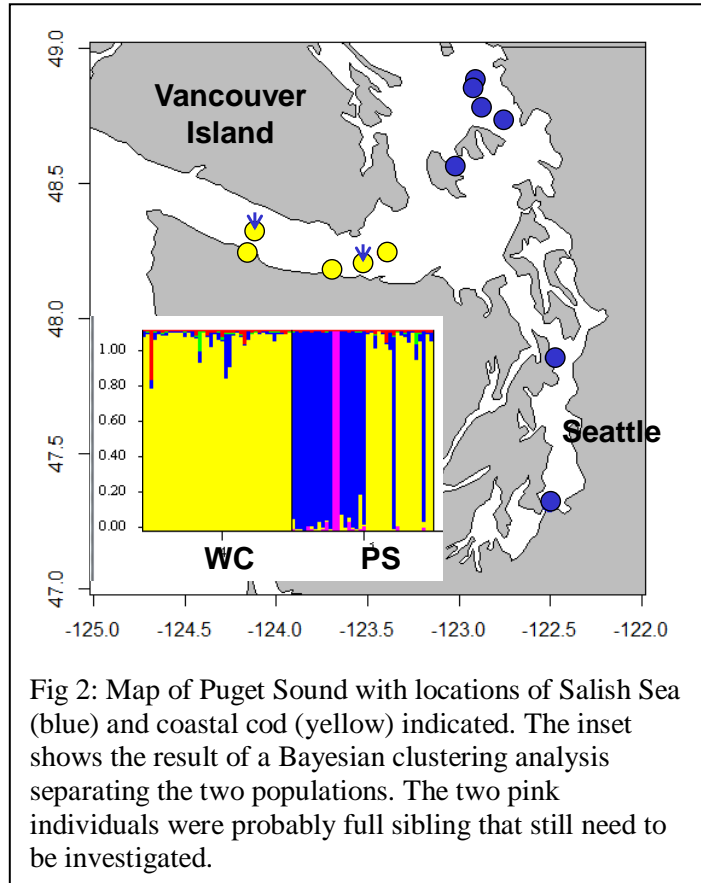


Fig 2: Map of Puget Sound with locations of Salish Sea (blue) and coastal cod (yellow) indicated. The inset shows the result of a Bayesian clustering analysis separating the two populations. The two pink individuals were probably full sibling that still need to be investigated.

We also detected strong evidence for selection: a total of 365 loci exhibit at least “strong” evidence for selection ($q < 0.05$), with 218 of these loci listed as “decisively” (99% probability) under selection. The remaining 6,391 loci were deemed neutral. Loci putatively under selection are currently under further investigation.

	WC05	JDF12	SS12/13	HS04	PWS12	KOD03	UP03	AD06
Estimated N_e	922.8	∞	67.1	9854.8	3352.7	2123.1	2068.3	2485.4
Lower 95% CI	849.8	∞	66.5	5355.4	2723.7	1804.8	1783.8	2033.4
Upper 95% CI	1009.3	∞	67.7	61088.9	4357.4	2576.7	2460.1	3194.0

Table 3: Estimates of effective population size after correcting for linkage disequilibrium. Estimates of infinity for the JDF12 sample are a byproduct of small sample size.

Future Work

In 2015, we will concentrate on (i) finalizing the population screening, (ii) compare different year classes in the Salish Sea population to test for evidence of selective mortality in years with different environmental conditions, (iii) screen families of captive larvae to develop a genetic map and to test for genotype specific mortality in the common garden experiment, and (iv) prepare manuscripts for publications. We will also attend the Annual AFS meeting in Portland to disseminate our results.

References

Cunningham KM, Canino MF, Spies IB, Hauser L (2009) Genetic isolation by distance and localized fjord population structure in Pacific cod (*Gadus macrocephalus*): limited effective dispersal in the northeastern Pacific Ocean. *Can J Fish Aquat Sci*, **66**, 153-166.