

Update Report

Hauser, Lorenz

Period: 2/1/2012 - 1/31/2013

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

O'Brien, Shannon, smobrien@uw.edu, University of Washington, School of Aquatic and Fishery Sciences, status:new, field of study:Population Genomics, advisor:Lorenz Hauser, degree type:PhD, degree date:2015-12-01, degree completed this period:No

Student Project Title:

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

Involvement with Sea Grant This Period:

Graduate student

Post-Graduation Plans: *none*

:: CONFERENCES / PRESENTATIONS

Canino M (2012) Genetic structure in Pacific cod. Coastal Cod Workshop, School of Aquatic and Fishery Sciences, University of Washington, public/profession presentation, 17 attendees, 2012-10-18

:: ADDITIONAL METRICS

K-12 Students Reached:0	Acres of degraded ecosystems restored as a result of Sea Grant activities:0
Curricula Developed:0	Resource Managers who use Ecosystem-Based Approaches to Management:0
Volunteer Hours:9 Fishing for cod broodstock (Dan Cooper)	HACCP - Number of people with new certifications:0
Cumulative Clean Marina Program -0 certifications:	

:: PATENTS AND ECONOMIC BENEFITS

Description	Patents	Economic Benefit (\$)	Businesses Created	Businesses Retained	Jobs Created	Jobs Retained
None	Actual 0	0	0	0	0	0
	(2/1/2012 -					

1/31/2013) :
Anticipated 0 0 0 0 0 0
 (2/1/2013 -
 1/31/2014) :

:: TOOLS, TECH, AND INFORMATION SERVICES

Description	Developed	Used	Names of Managers	Number of Managers
High differentiation markers for Pacific Cod population identification. R/LME-6	Actual 0 (2/1/2012 - 1/31/2013) : Anticipated 1 (2/1/2013 - 1/31/2014) :	0	0	1
Markers for genome regions related to temperature stress in Pacific Cod. R/LME-6	Actual 0 (2/1/2012 - 1/31/2013) : Anticipated 1 (2/1/2013 - 1/31/2014) :	0	0	0
Rapid genotyping by next generation sequencing technology for Pacific cod. R/LME-6	Actual 0 (2/1/2012 - 1/31/2013) : Anticipated 1 (2/1/2013 - 1/31/2014) :	0	0	0

:: HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

:: ADDITIONAL MEASURES

<u>Safe and sustainable seafood</u>	
Number of stakeholders modifying practices Actual (2/1/2012 - 1/31/2013) : Anticipated (2/1/2013 - 1/31/2014) :	Number of fishers using new techniques Actual (2/1/2012 - 1/31/2013) : Anticipated (2/1/2013 - 1/31/2014) :
<u>Sustainable Coastal Development</u>	
Actual (2/1/2012 - 1/31/2013) : Anticipated (2/1/2013 - 1/31/2014) :	<u>Coastal Ecosystems</u> Actual (2/1/2012 - 1/31/2013) : Anticipated (2/1/2013 - 1/31/2014) :

:: PARTNERS

Partner Name: Kitsap Poggie Club, type: NGO, scale: local

Partner Name: NOAA Manchester lab, type: government, scale: federal

:: IMPACTS AND ACCOMPLISHMENTS

Title: Washington Sea Grant-supported research works to unravel the genetic factors that distinguish Puget Sound's Pacific cod and predict their responses to environmental change

Type: accomplishment

Description:

Relevance: The 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 will perform a common garden experiment, rearing cod from Puget Sound and the outer coast in identical conditions and subjecting both to warmer water. They will work to identify population adaptations to changing conditions, and uncover the genetic factors associated with adaptation.

Results: In the initial year, the project established rearing facilities and obtained necessary fish collection and transport permits and university approvals. Researchers have contacted numerous commercial fishermen and charter operators to collect coastal broodstock, and begun direct collection efforts in Puget Sound. They have also optimized high-throughput molecular methods for analyzing cod genetics, which can use minute quantities of DNA from small larvae.

Recap:

Washington Sea Grant-funded researchers are seeking to identify genetic and other characteristics that will contribute to conservation of depleted Pacific cod stocks in Puget Sound and adaptation to warming waters.

Comments:

Associated Goals: 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 (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).

Related Partners:

Kitsap Poggie Club

Northwest Fisheries Science Center (US DOC, NOAA, NMFS, NWFSC)

University of Washington, School of Aquatic and Fishery Sciences, College of the Environment (UW)

:: PUBLICATIONS

Title: The Thermal Environment of Cascadia Basin

Type: Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year: 2012

Uploaded File: *none*

URL: *none*

Abstract:

Located adjacent to the NE Pacific convergent boundary, Cascadia Basin has a global impact well beyond its small geographic size. Composed of young oceanic crust formed at the Juan de Fuca Ridge, igneous rocks underlying the Basin are partially insulated from cooling of their initial magmatic heat by a thick layer of pelagic and turbidite sediments derived from the adjacent North American margin. The igneous seafloor is eventually consumed at the Cascadia Subduction Zone (CSZ), where interactions between the approaching oceanic crust and the North American continental margin are partially controlled by the thermal environment. Within Cascadia Basin, basement topographic relief varies dramatically, and sediments have a wide range of thickness and physical properties. This variation produces regional differences in heat flow and basement temperatures for seafloor even of similar age. Previous studies proposed a north-south thermal gradient within Cascadia Basin, with high geothermal flux and crustal temperatures measured in the heavily-sedimented northern portion near Vancouver Island and lower than average heat flux and basement temperatures predicted for the central and southern portions of the Basin. If confirmed, this prediction has implications for processes associated with the Cascadia Subduction Zone, including the location of the 'locked zone' of the mega-thrust fault. Although existing archival geophysical data in the central and southern Basin are sparse, non-uniformly distributed and derived from a wide range of historical sources, a substantial N-S geothermal gradient appears to be confirmed by our present compilation of combined water column and heat flow measurements.

Citation:

Johnson, H. P., S. Hautala, and T. Bjorklund. 2012. The Thermal Environment of Cascadia Basin. *Geochem. Geophys. Geosyst.* in press. 10.1029/2011GC003922

Copyright Restrictions + Other Notes:

Journal Title: *Geochem. Geophys. Geosyst.*

:: OTHER DOCUMENTS

No Documents Reported This Period

:: LEVERAGED FUNDS

No Leveraged Funds Reported This Period

Progress Report

Progress was achieved in two areas: preparation for broodstock collection and development of molecular methods. The former also provided some outreach opportunities.

Preparation of brood stock collection:

All necessary fish collection and transport permits from the National Marine Fisheries Service (NMFS), Washington Department of Fish and Wildlife (WDFW) and the Institutional Animal Care and Use Committee (IACUC) have been obtained. The rearing facility at NOAA Sand Point has been approved for use under UW Animal Care IACUC guidelines.

Facilities for brood stock maintenance are provided by the NOAA Northwest Fisheries Science Center in Manchester, WA. We currently have a large (20' diameter x 5' depth) covered tank with flow-through seawater, plus four smaller tanks, for brood maintenance. Several mesh cages have been constructed within the large tank for isolation of individual female cod as they approach spawning. Co-PI Canino has been trained in the use of ultrasound equipment to determine sex and maturity. Sources of food for broodstock (frozen squid and fish) have been identified and can be exploited at short notice.

Co-PI Canino has also made numerous contacts with commercial fishermen and recreational fishing charters for collection of broodstock from the outer Washington coast. Most of these outfits operate from Bellingham, incurring considerable fuel costs to reach the fishing grounds on the coast. Recently, we have made contact with a commercial fisherman operating out of Neah Bay, who may provide the most cost-effective source of live broodstock. Negotiations are still ongoing but we anticipate a collection attempt within the next two weeks.

Within Puget Sound, collections are planned in close collaboration with co-PI Lowry from WDFW. To date, one collection has been attempted at Agate Pass in central Puget Sound, a short run by boat from the NOAA Manchester facility. This attempt was successful in terms of logistics, but did not catch or observe any live cod. The historical time for peak spawning activity in this region ranges from early February through mid-March and is tied with the spawning time of Pacific herring. We anticipate making weekly collection trips to the Agate Pass and Neah Bay regions through mid-March. As cod are likely following their main prey, herring, we are closely tracking independent monitoring by WDFW of herring spawning activity in the Agate Pass area and will flex weekly sampling effort to coincide with observations of high herring abundance.

Outreach:

PI Canino has been in contact with several recreational angling groups in the Puget Sound region that have expressed interest in the project and have served as a reporting source for incidental cod catches during the winter blackmouth (Chinook) salmon fishing season. PI Canino is scheduled to give a talk on the project to the South Kitsap Poggie Club in Bremerton on February 13th, and continued interaction with sport anglers is anticipated during the winter/spring months.

Molecular Genetics

The primary focus during the reporting period was on the development of methods optimal for our specific project. These development efforts included both empirical laboratory work and computer simulations based on the recently published Atlantic cod genome.

Laboratory work

Wild adult Pacific cod and larval walleye pollock samples were used to start optimizing sequencing library creation methods. Additional samples of wild Puget Sound Pacific cod were acquired from WDFW (2003 [n=211]; 2009 [n=205]; 2012 [n=51]). Pacific cod larvae are currently not available, and therefore, wild and lab-reared pollock larvae were used as the best available substitute for preliminary laboratory work. DNA extractions were successful for all wild Pacific cod samples and larval samples tested to date. However, samples of early larvae yielded relatively little DNA (500-600 ng).

Originally, we planned to use RAD sequencing, but due to the low amount of DNA recovered from the lab-reared larvae, we are investigating alternative sequencing methods. Basic RAD sequencing (RADseq) protocols create a reduced representation library by using one restriction enzyme to cut the genome, a shearing step to further reduce the fragment sizes, a ligation step to attach primer and barcode sequences to the end of fragments, and finally a fragment size selection step. Due to the number of steps in this protocol and the associated loss of DNA, a large amount of starting DNA is needed (0.5-1 μ g). This would only allow one sequencing attempt on larvae and, if problems occurred during the library preparations steps, could potentially result in a loss of samples. An additional problem with standard RAD sequencing is that the random nature of shearing one end of the fragment results in large variability in coverage between fragments (i.e., the number of times each fragment is sequenced) and thus a large proportion of missing data in the final data set.

Because of these issues, we are testing alternate library preparation methods that use less DNA (200 - 300 ng), including a two-enzyme GBS (Genotype-by-sequencing) approach (Poland et al. 2012) and a double digest RADseq (ddRAD) (Peterson et al. 2012). These two methods are identical to RAD sequencing with the exception that they both use two restriction enzymes to obtain fragments of appropriate length for sequencing rather than using a shearing step. By adjusting the restriction enzymes, it is possible to adjust the number of loci that are sequenced. For example, two enzymes that cut the DNA into smaller fragments will produce more fragments than enzymes that cut only rarely. An estimate of the number of fragments is important, because it will determine the coverage (number of times each fragment is sequenced) and the number of individuals that can be sequenced in each lane of the sequencer. A GBS DNA library was sent to the University of Oregon Sequencing Center for evaluation and sequencing in September 2012, but was not sequenced because a preliminary control revealed low concentrations of fragments in the library. We are in the process of testing additional libraries for sequencing.

Computer simulations

To select restriction enzymes needed to produce the appropriate target number of fragments, custom Python scripts were written to perform an *in silico* restriction digest of the current Atlantic cod draft genome. These results were used to estimate the number of potential loci that would be obtained from the cod genome using a single restriction enzyme (RADseq) as well as a double restriction enzyme digest with different combinations of two restriction enzymes (Figure 1). Although the number of fragments estimated from these scripts is an underestimate due to sequence gaps in the draft genome, they provide good approximations for empirical data. Based on these results, we will further evaluate the PstI-MspI combination.

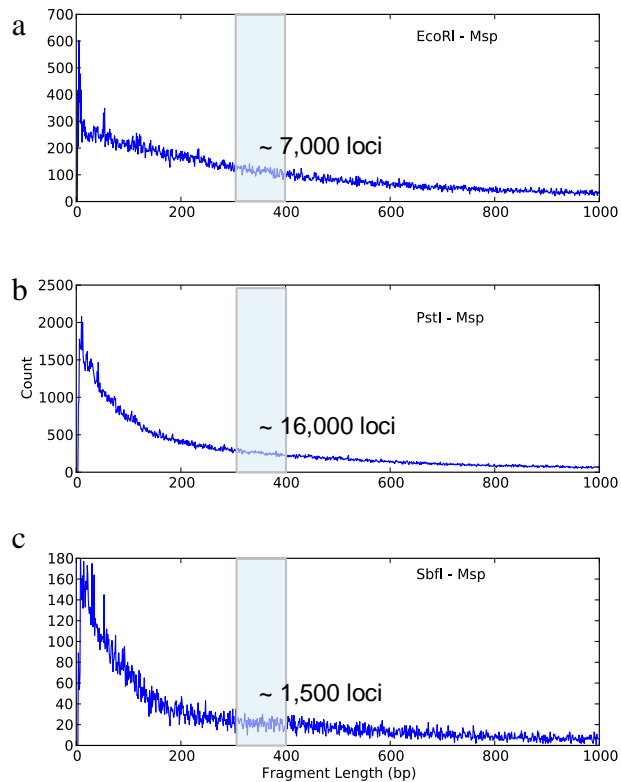


Figure 1: Expected number of different sized fragments resulting from an *in silico* digest of the Atlantic cod draft genome, using three pairs of restriction enzymes (a) EcoRI – MspI (b) PstI – MspI (c) SbfI – MspI. The number of loci within the targeted size range (300–400 bp; blue box) was estimated using Python scripts from Peterson et al. (2012).

References

- Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE (2012) Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. *PLoS ONE*, **7**.
- Poland JA, Brown PJ, Sorrells ME, Jannink JL (2012) Development of High-Density Genetic Maps for Barley and Wheat Using a Novel Two-Enzyme Genotyping-by-Sequencing Approach. *PLoS ONE*, **7**.