

Update Report

Period 2/1/2013 - 1/31/2014

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 cont, field of study Population Genomics, advisor Lorenz Hauser, degree type PhD, no degree date, 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 Student withdrew because of pregnancy

CONFERENCES / PRESENTATIONS

Lorenz Hauser Finding a Nemo in a Haystack _Reconciling dispersal and gene flow in marine species. Invited seminar at University of California, Santa Barbara, public/profession presentation, 40 attendees, 2014-02-05

ADDITIONAL METRICS

K-12 Students Reached	0	Acres of degraded ecosystems restored as a result of Sea Grant activities	0
Curricula Developed	1	Resource Managers who use Ecosystem-Based Approaches to Management	0
Lorenz Hauser FISH 510 Studies on local adaptation why, what, how and where? Graduate seminar class in SAFS, SP2013		HACCP - Number of people with new certifications	0
Volunteer Hours	0		

Cumulative Clean Marina Program - certifications 0

PATENTS AND ECONOMIC BENEFITS

Description	Patents	Economic Benefit (\$)	Businesses Created	Businesses Retained	Jobs Created	Jobs Retained
None	Actual (2/1/2013 - 1/31/2014)	0	0	0	0	0
	Anticipated (2/1/2014 - 1/31/2015)	0	0	0	0	0

TOOLS, TECH, AND INFORMATION SERVICES

No Tools, Tech, or Information Services Reported This Period

HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

ADDITIONAL MEASURES

Safe and sustainable seafood

Number of stakeholders modifying practices
 Actual (2/1/2013 - 1/31/2014)
 Anticipated (2/1/2014 - 1/31/2015)

Number of fishers using new techniques
 Actual (2/1/2013 - 1/31/2014)
 Anticipated (2/1/2014 - 1/31/2015)

Sustainable Coastal Development

Actual (2/1/2013 - 1/31/2014)
 Anticipated (2/1/2014 - 1/31/2015)

Coastal Ecosystems

Actual (2/1/2013 - 1/31/2014)
 Anticipated (2/1/2014 - 1/31/2015)

PARTNERS

Partner Name NOAA Manchester, type Government, scale Federal or National

Partner Name NOAA Mukilteo Lab, type Government, scale Federal or National

Partner Name NOAA Northwest Fisheries Science Center, Montlake, type Government, scale Federal or National

IMPACTS AND ACCOMPLISHMENTS

Title Washington Sea Grant research investigates genetic factors in Pacific cod's adaptation

to warming ocean waters

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 NOAA species of concern. They live near the southern limit of the species' range and could be further impacted by oceanic warming. Along the Eastern Seaboard, 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 lineages that are more adaptable can be identified. Response Washington Sea Grant-sponsored researchers are undertaking a common garden experiment. They will rear cod from Puget Sound and the other regions under identical conditions and subject both to warmer water, then identify population adaptations to these changing conditions and explore the genetic factors associated with adaptation. Results With the help of commercial fishermen and NOAA personnel, researchers succeeded in capturing and rearing Pacific cod; they anticipate having larvae soon for experiments. Using the latest genetic technologies they recorded 135 million sequences and 60,000 unique genetic markers in the cod genome. Sixty percent of these markers may be matched to the Atlantic cod genome, providing a powerful basis for further experiments.

Recap Washington Sea Grant-funded research is identifying genetic markers in Pacific cod associated with adaptation to warming waters, which will inform future conservation of depleted Puget Sound stocks.

Comments Primary Focus Area LME (HCE) Secondary Focus Area LME (SSSS) State 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).

Related Partners

PUBLICATIONS

No Publications Reported This Period

OTHER DOCUMENTS

No Documents Reported This Period

LEVERAGED FUNDS

No Leveraged Funds Reported This Period

UPDATE NARRATIVE

Uploaded File Hauser_8894_update_nar....1.pdf

Progress Report, 2013/14

Progress in 2013/14 was somewhat compromised by the departure of the graduate student, Shannon O'Brien, who left the program for personal reasons in the spring of 2013. Because of difficulties in obtaining broodstock in 2013, we decided to wait before refilling this position. Unfortunately, the remaining funds were insufficient to employ another (beginning) student, so we decided to search for a post-doctoral research assistant. Even that option was difficult, because relocation to Seattle on the promise of approximately 10 months' salary failed to attract external candidates. We therefore concentrated the search locally, and indeed managed to hire Dr Kristen Gruenthal, an experienced molecular geneticist with expertise in next generation sequencing. Kristen previously worked for NOAA as a contractor and will be employed on the project at 75% FTE for 2014. To accommodate these changes, we applied and were granted a Change of Scope and reorganized the project budget. Despite these setbacks, some progress was achieved both in broodstock collection and the optimization of molecular methods.

Brood stock collection:

In the original application, we proposed to carry out common garden experiments with cod larvae from broodstock obtained from the Washington coast and Puget Sound. This approach required that we obtain adult cod as broodstock from both the coast and in Puget Sound. Much of the effort this year was spent in obtaining and rearing that broodstock.

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. Furthermore, we have initiated collaboration with the NOAA Mukilteo Laboratory for broodstock maintenance, because NOAA facilities at Manchester are filled to capacity with sablefish. Furthermore, we now collaborate with scientists at the Northwest Fisheries Science Center (NOAA), whose facilities are more advanced than those at Sand Point and therefore provide better chances of success.

As in the previous year, co-PI Canino established and extended numerous contacts to commercial fishermen and recreational fishing charters for collection of broodstock from the Washington coast. Thanks to these efforts, we were successful in obtaining broodstock of Washington coast cod from a commercial fisherman (Roger Bain, F/V Miss Mandy) operating out of Neah Bay. Unfortunately, when 48 of these fish were collected in early March 2013, it turned out that they were all spent (i.e., had spawned already). As keeping these fish over the summer was not an option because of high water temperature in the NOAA Manchester facility, they were donated to another project within NOAA.

During January and February, considerable effort was spent by co-PIs Canino and Lowry with WDFW boat crews to obtain broodstock from Puget Sound. However, despite several trips to known historic fishing ground attempting to catch cod with hook and line, no cod were seen or caught. Furthermore, enquiries at local angling clubs provided no evidence for the presence of cod in Puget Sound. During the annual trawl survey conducted by WDFW in June, several cod were caught in the central and western Strait

of Juan de Fuca, but all were moribund or dead by the time the net was hauled. Therefore, no broodstock could be obtained in Puget Sound – however, we obtained fin clippings from these trawl-caught fish which increased our sample sizes in Puget Sound for genetic analysis.

Because of these difficulties in obtaining broodstock, we had to rethink the project to some degree. Common garden experiments are clearly only possible with two or more populations. If we fail to obtain Puget Sound fish, rearing experiments with larval fish from coastal cod at different temperatures would still provide valuable information in terms of genotype-specific mortality, but the lack of another population for comparison would negate the idea of observing phenotypes of different genotypes in a common environment. We are therefore now planning to compare coastal Washington cod with Alaskan cod reared at the NOAA Hatfield Marine Science Center (HMSC) in Newport, Oregon. Co-PI Canino has established collaboration with Tom Hurst and Ben Laurel of the NOAA Newport lab and managed last year to spawn a cod female and raise some offspring. Based on this experience, we are confident that breeding will be successful with those fish already available in captivity.

In November 2013, we initiated another attempt to obtain coastal cod broodstock in Neah Bay, this time from another operator. On January 23, Co-PI Canino collected 18 cod (about 9 males and 9 females) and brought them to the Mukilteo lab. All females were large and rotund, and obviously ready to spawn.

Molecular Genetics

The primary focus during the reporting period was on the development of methods optimal for our specific project. Molecular work was carried out by Shannon O'Brien before her departure and Isadora Jimenez Hidalgo, and analysis were carried out by Daniel Drinan, a graduate student supported by matching funds starting January 2014.

Wild adult Pacific cod and walleye pollock larval samples were used to start optimizing sequencing library creation methods. Pacific cod larvae are currently not available, and therefore wild and lab-reared pollock larvae were used as the best available substitute for preliminary lab work. DNA extractions were successful for all wild Pacific cod samples but early pollock larvae yielded relatively little DNA (500-600 ng).

As normal RADsequencing requires up to 1 μ g of DNA, and 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.

Four ddRAD libraries consisting of 48 individuals (12 Pacific cod each from the Washington coast, Strait of Georgia and Puget Sound, as well as 12 pollock larvae) were sent to the University of Oregon Sequencing Center to be sequenced on a single lane of the Illumina HiSeq sequencer. This analysis provided 203,789,863 sequence reads of 101 basepairs each, and after removing poor quality reads, we were left with 135,372,745

reads (i.e., 135 million sequences). As typical for these data, sequence quality dropped towards the end of the sequences (Fig 1), but encouragingly no difference in sequence quality was detected between larval and adult samples. After stringent quality controls, we identified 59,351 different loci (markers) in Pacific cod. 8,534 loci were polymorphic. About 60% of all Pacific cod loci (36,693 loci) and 30% of polymorphic loci (2679 loci) aligned to the Atlantic cod genome.

These initial results are encouraging because they show that we can identify many loci in the Pacific cod genome, nearly a third of which can be aligned to the Atlantic cod genome. Furthermore, they show that we can apply these methods to larval samples from our common garden experiment. However, recent literature shows a controversy between different RAD approaches and the optimal method has yet to be identified. We will therefore test additional methods, each with the aim to obtain many reproducible reads from small initial DNA quantities.

Outreach

PIs Hauser and Canino participated at the Discover Science weekend in the Seattle aquarium on November 9th and 10th. 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.

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**.

