

INTERNATIONAL PACIFIC



HALIBUT COMMISSION

Generation of genomic resources for the Pacific halibut (*Hippoglossus stenolepis*) to assist in population biology studies informing fisheries management

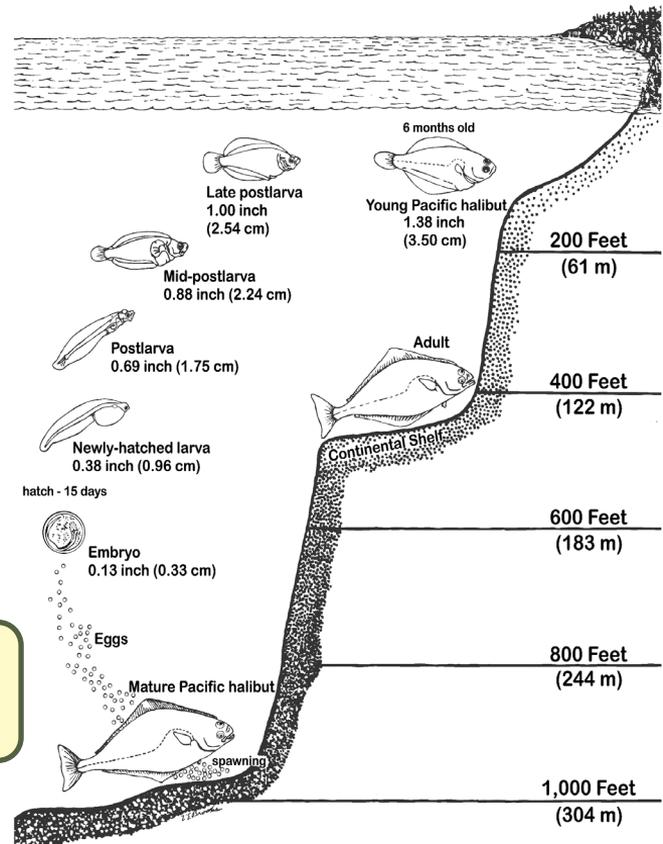
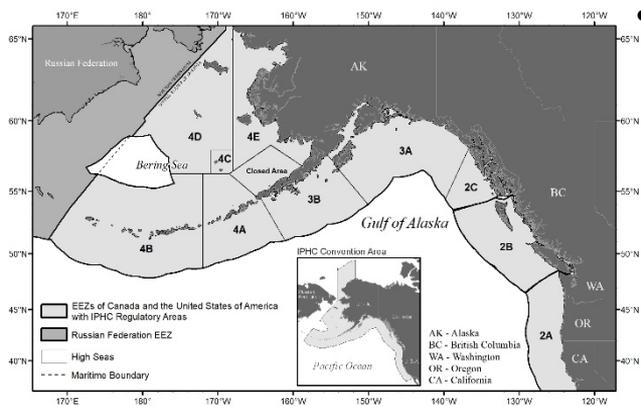
Andy Jasonowicz, Josep V. Planas
International Pacific Halibut Commission
Seattle, WA, USA

Pacific Halibut (*Hippoglossus stenolepis*)

Widely distributed in the North Pacific

- Spawning occurs offshore
- Pelagic eggs and larvae
- Juveniles use shallow areas on continental shelf
- Move offshore at age ~2 – 3
- Seasonal migrations between spawning grounds (winter) and feeding areas (summer).

Managed as a single stock in IPHC Convention Waters



Population Genomics

Define population structure
(Area 4B - Western Aleutian Islands)

Samples Collected From
Spawning Groups

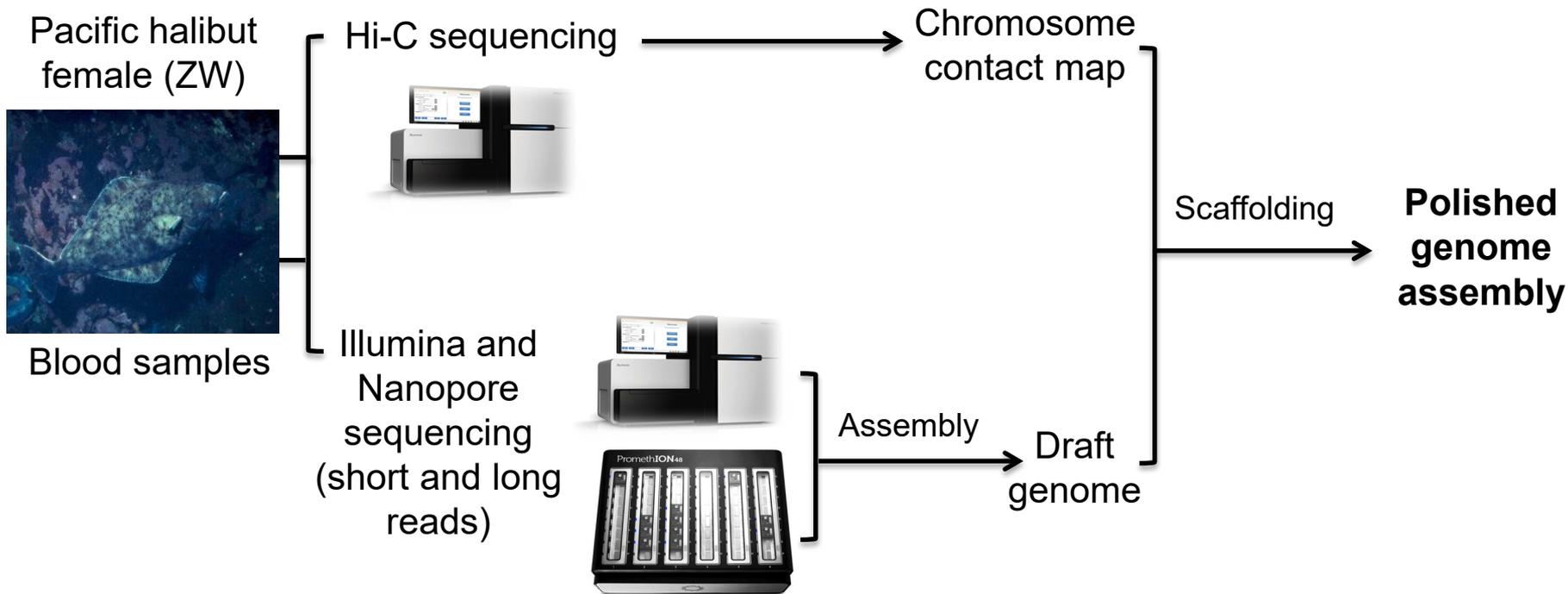


Establish Genetic Baseline

- N=50 per collection (600 total)
- **Low-coverage whole-genome resequencing (lcWGR)**
 - 3x coverage targeted
 - Millions of **single nucleotide polymorphisms (SNPs)**
- **A reference genome is needed**
- **A reference transcriptome is also needed to identify functionally important coding genes in the genome**



Sequencing of the Pacific halibut genome



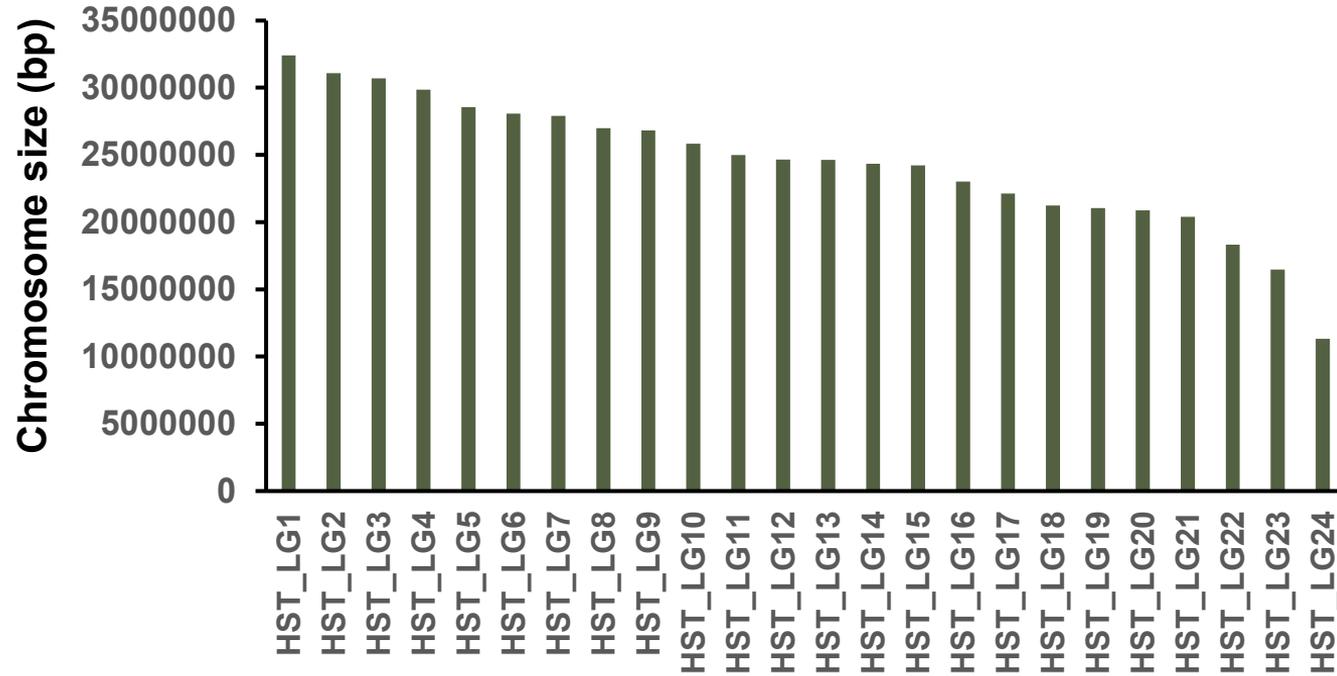
Pacific halibut genome: assembly data

		Complete assembly	Chromosomes only
Assembly metrics	Number of scaffolds	52	24
	Total size of scaffolds	602,147,347	600,901,890
	Longest scaffold	32,845,235	32,845,235
	Shortest scaffold	582	11,267,222
	Mean scaffold size	11,579,757	25,037,579
	Median scaffold size	184,878	25,468,064
	N50 scaffold length	27,288,303	27,288,303
	L50 scaffold count	11	11
	% of assembly in chromosomes	-	99.78 %
	% of assembly in unanchored scaffolds	-	0.22 %
Assembly completeness	Complete BUSCOs (C)	3,580 (98.4%)	
	C and single-copy BUSCOs	3,540 (97.3%)	
	C and duplicated BUSCOs	40 (1.1%)	
	Fragmented BUSCOs	17 (0.5%)	
	Missing BUSCOs	43 (1.1%)	



Pacific halibut genome: assembly data

Pacific halibut chromosome size



Pacific halibut genome: assembly data

Comparative genome metrics with other related species:

Assembly metrics	Pacific halibut	Atlantic halibut	Greenland halibut
Genome size (Mb)	602.1	596.8	598.5
Chromosome-level scaffolds mapped to total assembly (%)	99.8	99.8	96.3
N50 (Mb)	27.3	26.3	25.0
Complete BUSCO (%)	98.5	96.9	96.5



Pacific halibut genome: NCBI annotation



National Library of Medicine
National Center for Biotechnology Information

Accession: [GCF_022539355.2](https://www.ncbi.nlm.nih.gov/assembly/GCF_022539355.2)

Log in

Please send us [Feedback](#) to let us know how we're doing!

Genome Data Viewer

Home Share this page Reset All More Info

Hippoglossus stenolepis

(Pacific halibut)

Assembly: HSTE1.2 (GCF_022539355.2) · Chr 9 (NC_061491.1)

Search assembly

grid2

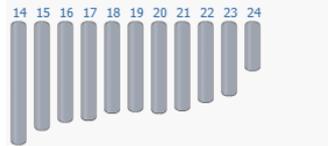
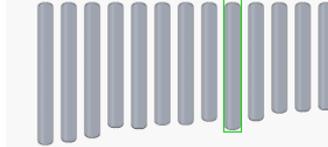
Examples

Assemblies

Ideogram View

Unplaced/unlocalized scaffolds: 28

MT 1 2 3 4 5 6 7 8 9 10 11 12 13



Tracks and User Data

BLAST

Tracks by Accession

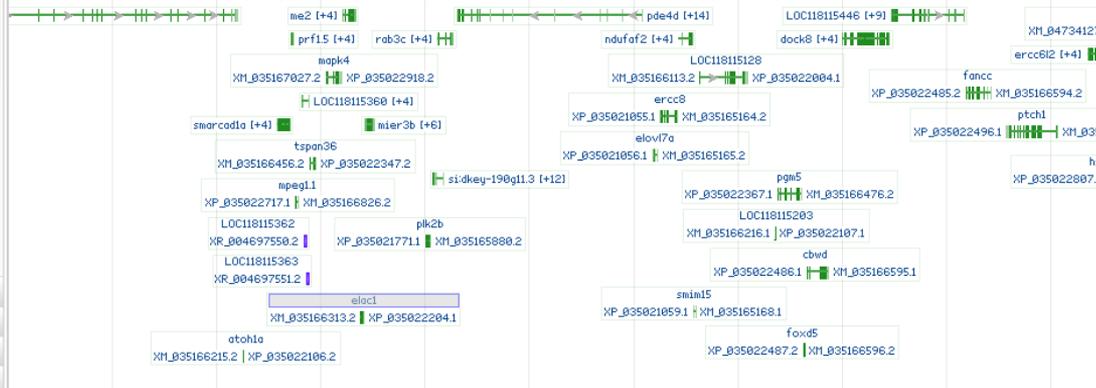
History

NC_061491.1: 18,600,482 - 19,660,277

Region: grid2 | Gene: XM_035167012.2 | Transcript: XM_035167012.2



Genes, NCBI Hippoglossus stenolepis Annotation Release 101, ...



27,944
annotated
genes



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Pacific halibut genome: first applications

Objectives

Core Methods

Research Outcomes

Task 1

Analysis of Population Structure

Low coverage whole genome resequencing

- Population structure
- Genetic baseline

Task 2

Develop Marker Panel

Genotyping-in-thousands by sequencing (GT-seq)

- Genomic marker identification
- Development of GT-seq panel

Task 3

Range Expansion

Assignment testing and cluster analyses

- Stock composition at latitudinal extremes of range
- Genetic differentiation in latitudinal extremes

Task 4

Mixed Stock Analysis

- Proportion of genetic baselines contributing to mixed fishery samples
- Stock composition differences from separate fishery samples



NPRB Project # 2110



**NOAA
FISHERIES**

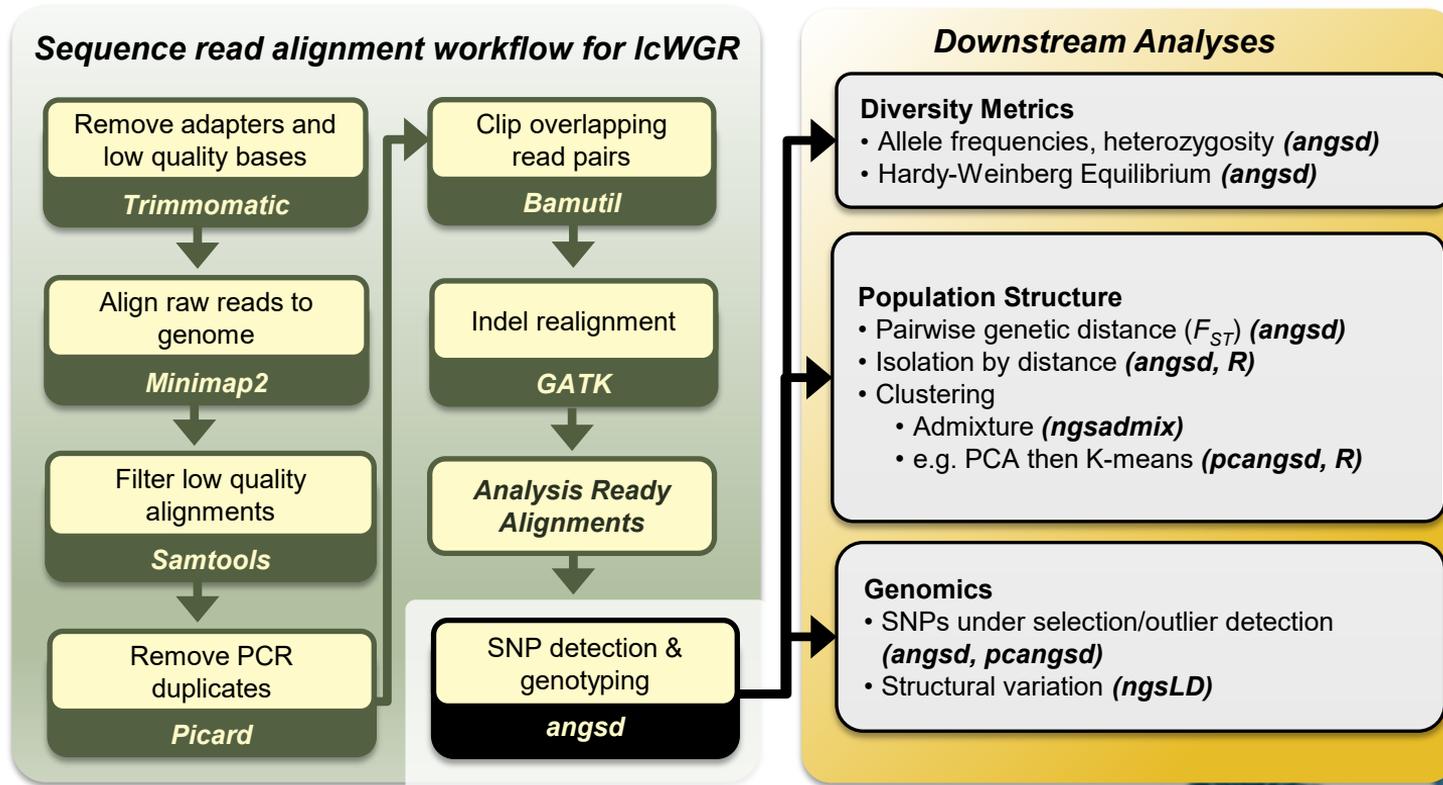


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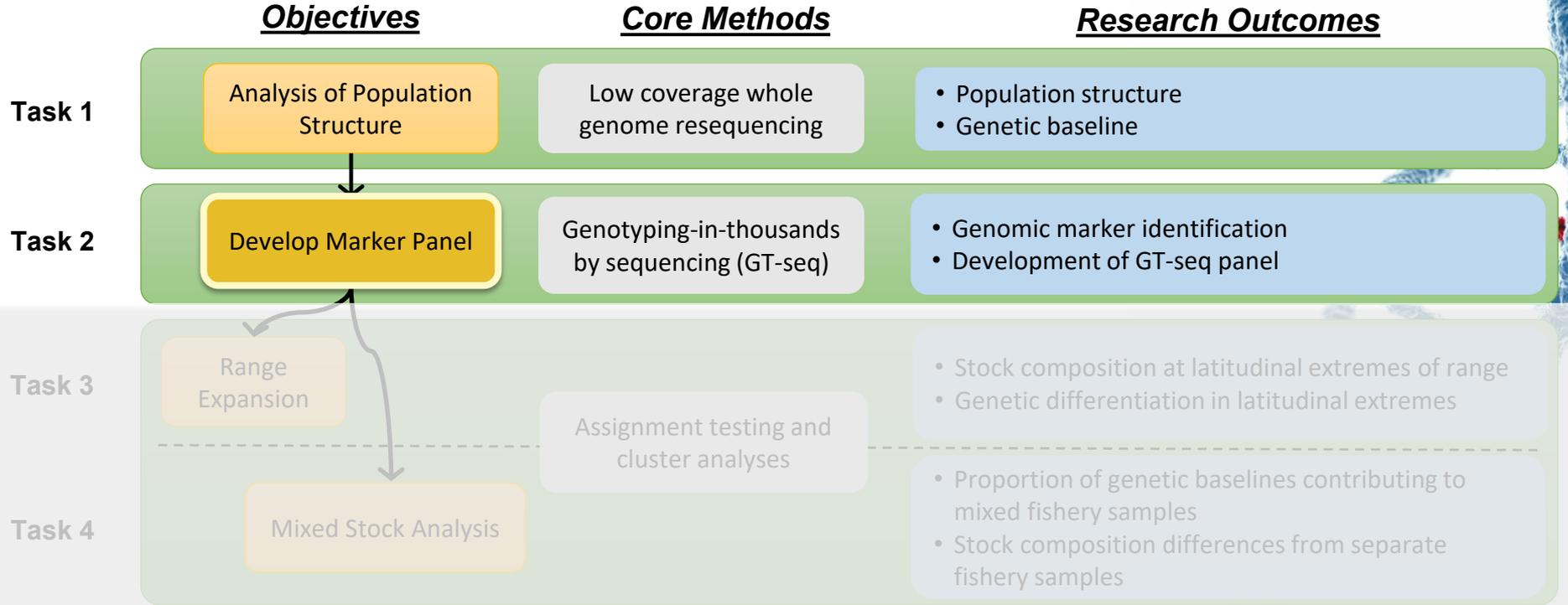
IPHC

Slide 9

Population Genomics – Analysis Workflow



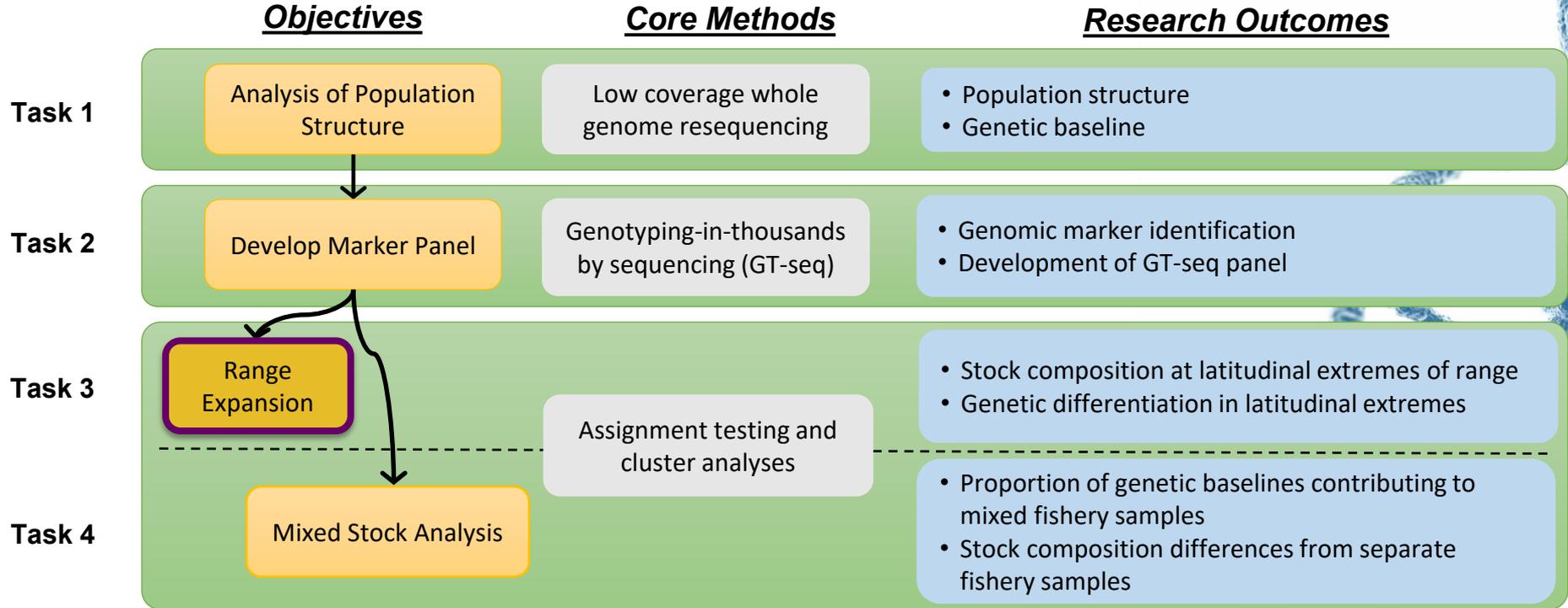
Pacific halibut genome: first applications



NPRB Project # 2110



Pacific halibut genome: first applications

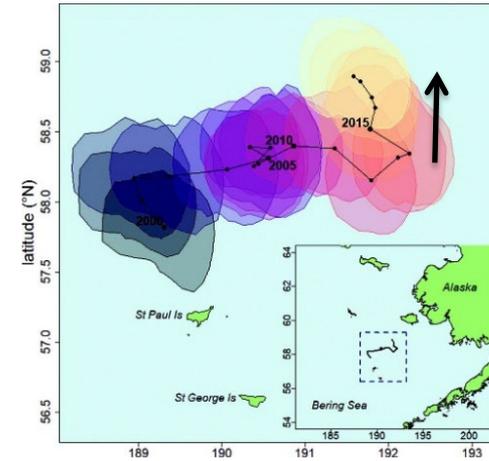


NPRB Project # 2110

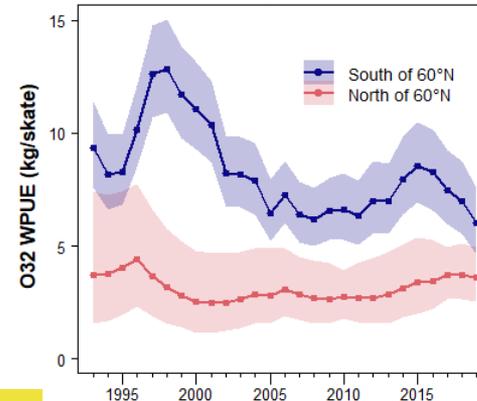


Climatic Change – Eastern Bering Sea

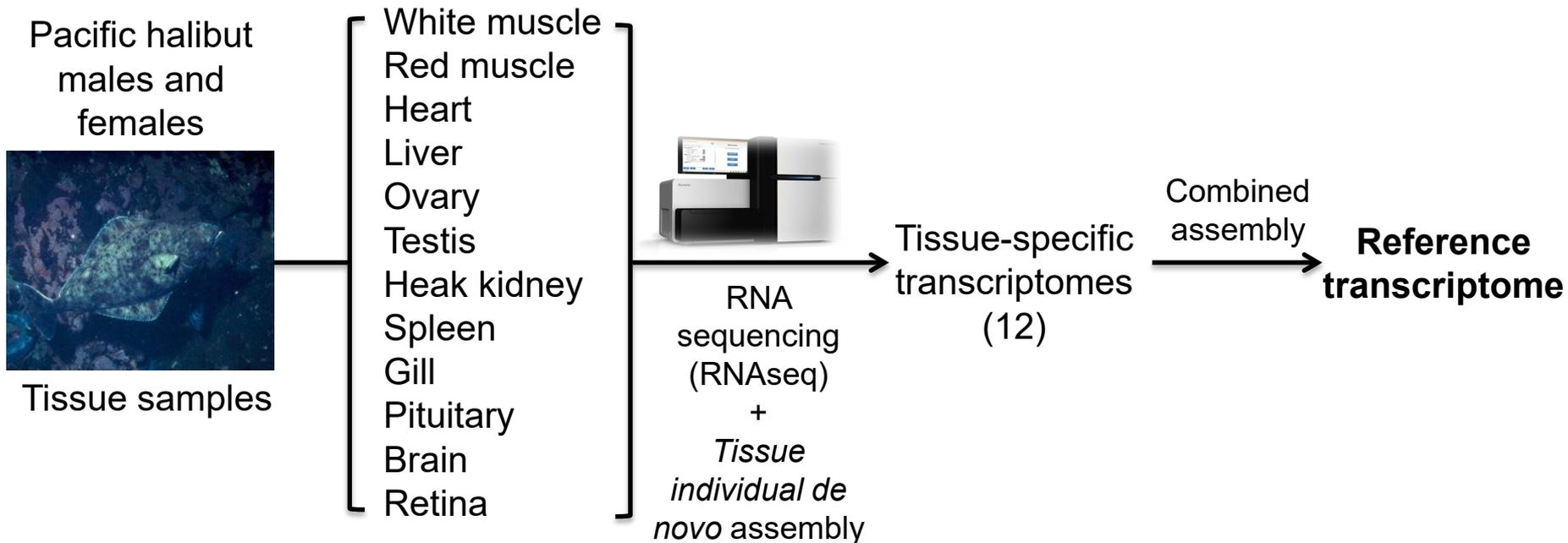
- Patterns of sea ice extent and bottom temperatures (cold pool) have shifted from historic patterns.
- Observed changes in groundfish distribution
 - Walleye pollock
 - Pacific cod
- Northward shift of the Pacific halibut stock distribution in the Eastern Bering Sea (since 2012)



(c) Bering Sea < 137 m



Generation of transcriptomic resources



Transcriptomic resources: Ovary (example)

Transcriptome statistics

- RNA Sequencing

	R1 Reads Before	R2 Reads Before	R1 Reads After	R2 Reads After	R1 Reads Dropped	R2 Reads Dropped	
Ovary	21,650,949	21,650,949	21,633,579	21,633,579	17,370	17,370	43,267,158 reads 39,567,890 reads
Testis	19,792,232	19,792,232	19,783,945	19,783,945	8,287	8,287	

- De novo transcriptome assembly

	Total trinity 'genes'	Total trinity transcripts	Percent GC	Contig N50	Median contig length	Average contig	Total assembled bases
Ovary	48,573	60,084	48.89	2,494	582	1,240.16	74,513,854
Testis	74,363	87,644	47.10	2,004	489	1,014.53	88,917,698

- Annotation

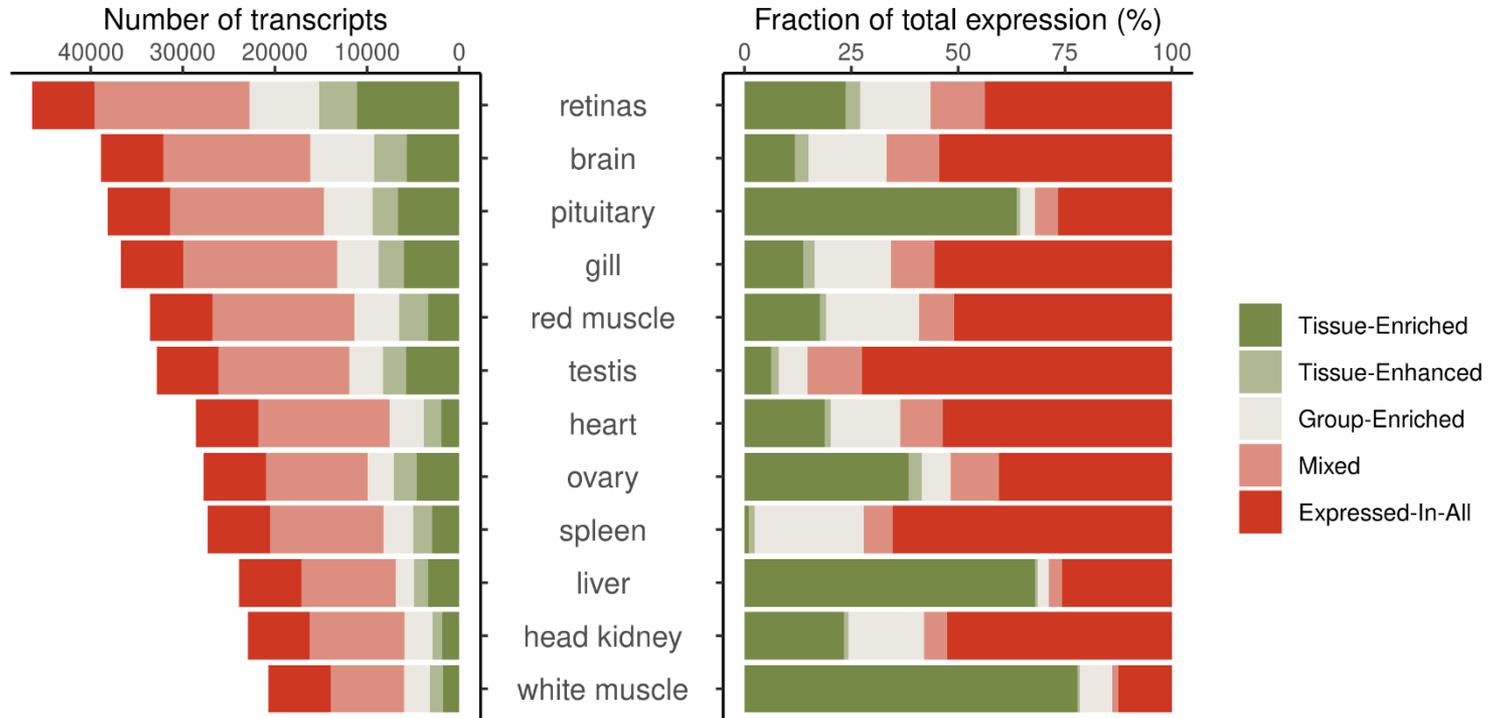
	Danio rerio	uniprot	est others	total	unmapped	Danio%	uniprot%	est others%	unmapped%
Ovary	18,426	4,259	37,267	60,084	132	30.67%	7.09%	62.02%	0.22%
Testis	23,644	5,539	58,303	87,644	158	26.98%	6.32%	66.52%	0.18%

Transcriptome dataset: a selection of reproductive markers

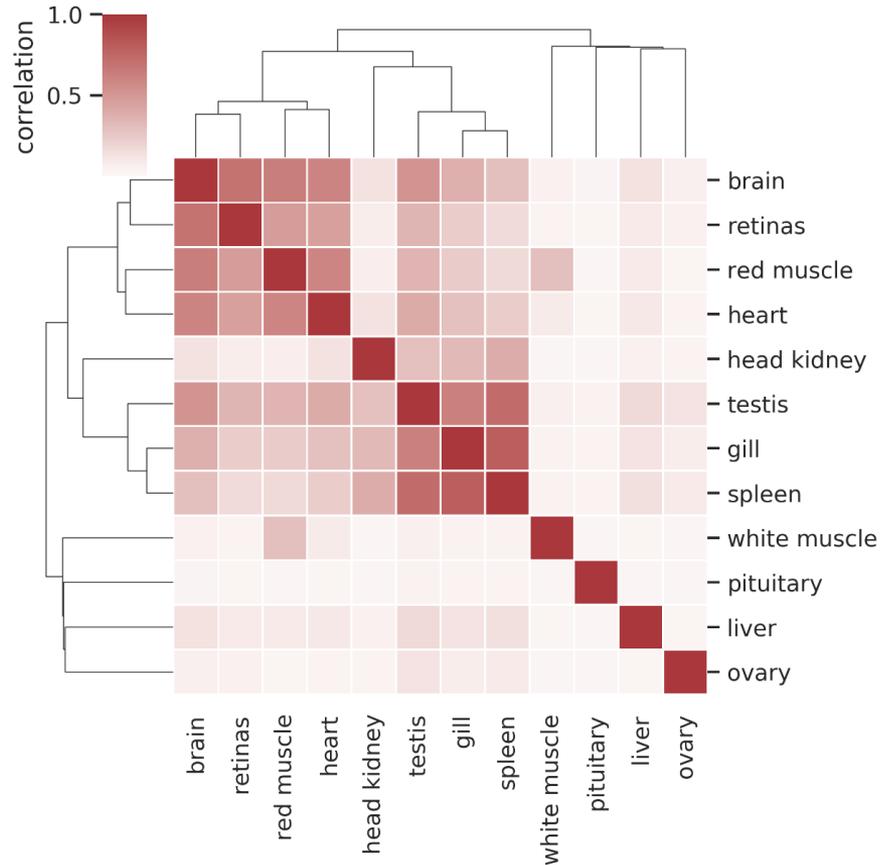
	Sample transcript ID	Length (nt)	Database	Database ID	Identity (%)	Gene_symbol	Annotation	Function
Ovary	TRINITY_DN13531_c0_g1_i1	3754	Danio rerio	ENSDARP00000004431	89,31	acr11ba	activin A receptor, type 1Ba	Oogenesis
	TRINITY_DN31883_c0_g1_i1	585	Danio rerio	ENSDARP00000121689	74,74	adams2	ADAM metallopeptidase with thrombospondin type 1 motif, 2	Ovulation
	TRINITY_DN14738_c0_g1_i1	3062	Danio rerio	ENSDARP00000088795	82,07	ar	androgen receptor	Hormone signaling
	TRINITY_DN18096_c0_g1_i1	1654	Danio rerio	ENSDARP00000076033	76,81	aqp10b	aquaporin 10b	Oocyte hydration
	TRINITY_DN18849_c1_g1_i1	1680	Danio rerio	ENSDARP00000112455	94,97	bmp1a	bone morphogenetic protein 1a	Oogenesis
	TRINITY_DN16877_c1_g1_i1	976	Danio rerio	ENSDARP00000111604	70,78	cyp19a1a	cytochrome P450, family 19, subfamily A, polypeptide 1a	Aromatase (estrogen production)
	TRINITY_DN16252_c0_g1_i1	2580	Danio rerio	ENSDARP00000124026	76,09	ddx4	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4	Oogonia marker
	TRINITY_DN23892_c0_g1_i1	232	Danio rerio	ENSDARP00000073932	71,62	EGFR	epidermal growth factor receptor	Maturation signaling
	TRINITY_DN4356_c0_g1_i1	209	Uniprot	Q4JK73	66,67	HSD17B11	Estradiol 17-beta-dehydrogenase 11	Steroidogenesis
	TRINITY_DN21356_c3_g1_i2	4620	Uniprot	Q9W6M2	80,88	esr2	Estrogen receptor beta	Hormone signaling
	TRINITY_DN6202_c1_g1_i1	287	Danio rerio	ENSDARP00000096529	79,79	fshr	follicle stimulating hormone receptor	Hormone signaling
	TRINITY_DN9106_c0_g1_i1	2006	Danio rerio	ENSDARP00000061827	80,39	foxl2	forkhead box L2	Female sex differentiation
	TRINITY_DN21868_c0_g1_i1	306	Danio rerio	ENSDARP00000055566	70,83	gnrhr4	gonadotropin releasing hormone receptor 4	Hormone signaling
	TRINITY_DN16738_c0_g1_i1	1391	Danio rerio	ENSDARP00000059752	71,32	inhhb	inhibin, beta B	Oogenesis
	TRINITY_DN21305_c0_g1_i1	1466	Uniprot	Q90674	62,65	LHCGR	Lutropin-choriogonadotropic hormone receptor	Hormone signaling
	TRINITY_DN12886_c1_g1_i1	771	Danio rerio	ENSDARP00000109370	82,1	pgr	progesterone receptor	Maturation signal



Tissue-specific transcriptomes: metrics



Tissue-specific transcriptomes



Conclusions

- We have generated the first chromosome-level high-quality assembly of the Pacific halibut genome.
- A full annotation of the Pacific halibut genome is available at NCBI and contains 27,944 genes.
- The generated genomic resource will be instrumental for future research on population genomics informing management and conservation strategies for Pacific halibut.
- We have also generated the first transcriptomic resources for the species, with tissue-specific transcriptomes as well as a combined assembly that constitutes the reference transcriptome.
- The combination of the reference genome and transcriptome will be instrumental for assigning functional significance to the identified genetic variation in the Pacific halibut population.

