

Genetic identification of *Lampetra* ecotypes in watersheds of the Northeastern Pacific Ocean



Nass River,
BC



Ksi Ts'oolh Ts'ap
Creek

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Problem:

Differentiating taxa (ecotypes) within the genus *Lampetra* can be challenging with current morphological and genetic identification (ID) methods



Proposed Solution:

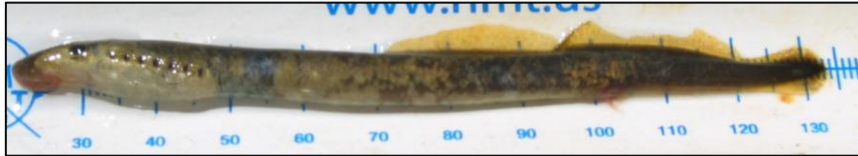
Develop a novel genetic method to differentiate W. North American *Lampetra* taxa at all life stages



Lampetra Life History

- Western Brook (*Lampetra richardsoni*) and Western River (*L. ayresii*) are two most common *Lampetra* taxa in PNW/Columbia basin
- Ranges generally overlap (BC to CA)
- Previously paired species, now ecotypes or life history variants (i.e., rainbow trout/steelhead)

WBL: Western Brook (*Lampetra richardsoni*)

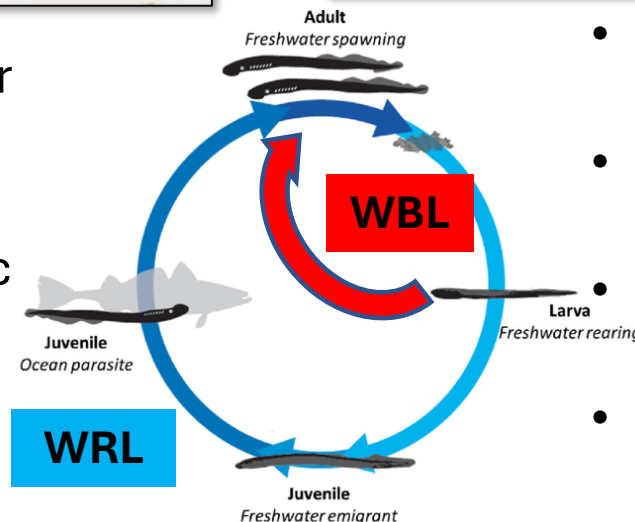


- Non-migratory freshwater resident
- Larvae metamorphose directly into non-parasitic adults
- Adult are small ~90-200 mm TL

WRL: Western River (*Lampetra ayresii*)



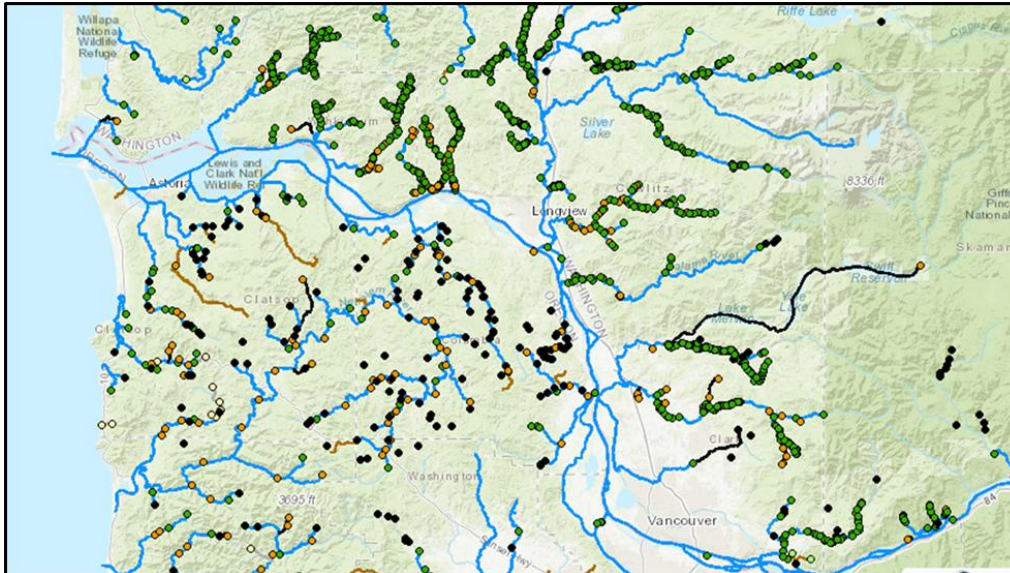
- Anadromous (like Pacific lamprey)
- Larvae metamorphose into migratory juvenile
- Feeds parasitically in saltwater
- Adults grow larger ~200-330 mm TL





Lampetra Critical Needs

- Knowledge gaps: *Lampetra* ecotype distribution, status, trends in abundance, etc.
- Understanding *Lampetra* ecotype biology and ecology important for future conservation and management
- Challenges with *Lampetra* ecotype identification (morphological and genetic methods) may hamper research on these fish



USFWS PLCI Lamprey Mapper

[https://databasin.org/maps/new/
#datasets=a243fb1346ca4258b63
88c5f7a90aee4](https://databasin.org/maps/new/#datasets=a243fb1346ca4258b6388c5f7a90aee4)



Morphological ID

Morphological ID of *Lampetra* ecotypes (WBL vs. WRL) can be difficult! (Even for experienced observers!!)

Adult ID (i.e., WRL returning from ocean)

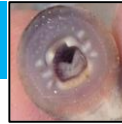
Size and dentition

- WBL: small, dull teeth
- WRL: larger, sharp teeth

WBL



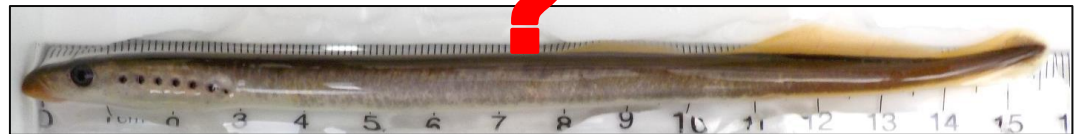
WRL



Lampetra ID Challenges

Recently metamorphosed fish

- Size overlap of adult WBL and juvenile WRL
- Dentition can be ambiguous



Larvae

- WBL and WRL larvae identical





Genetic ID

Genus Level ID: *Entosphenus* (Pacific) vs. *Lampetra* (WBL/WRL)

Resolved through:

- MtDNA markers
- Nuclear DNA markers (Microsatellites, Single Nucleotide Polymorphism (SNP) markers)

Lampetra Ecotype ID: WBL vs. WRL

Unresolved through:

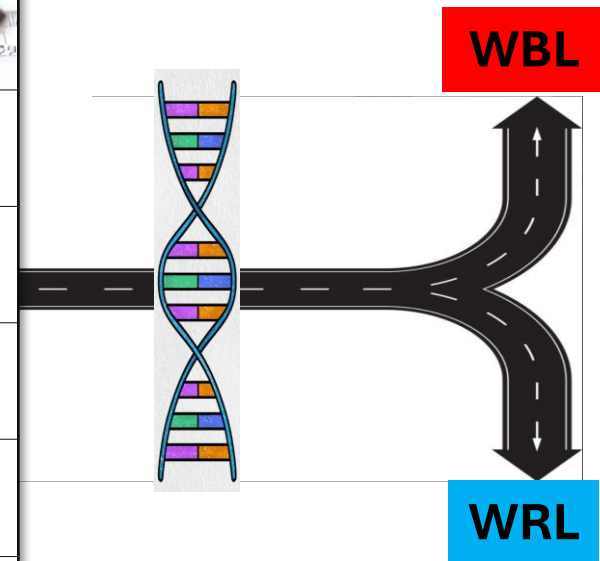
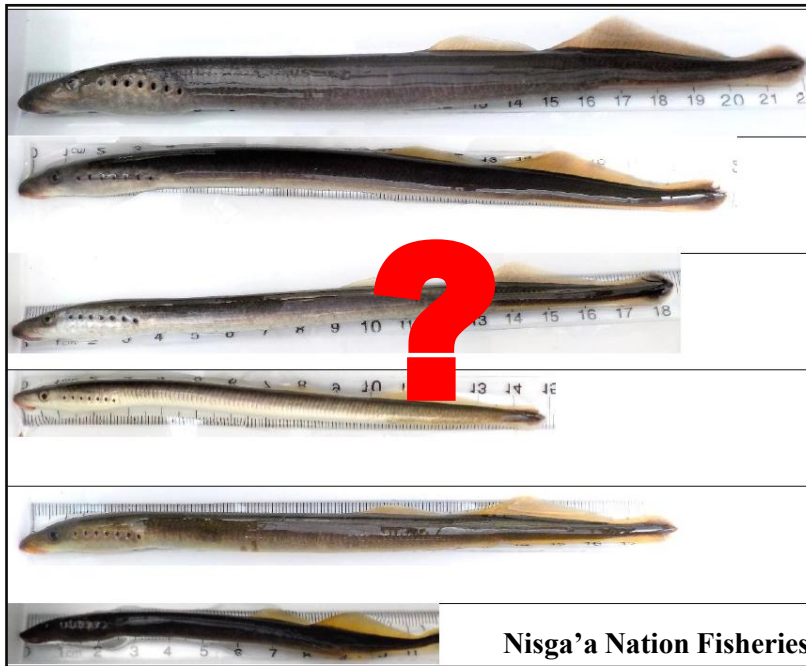
- MtDNA markers
- Nuclear DNA, including low-resolution survey of hundreds of SNP markers (Hess et al. 2015)
- Therefore, the need still exists for a genetic method to ID *Lampetra* ecotypes



Lampetra Ecotype Genetic ID: A Path Forward

High resolution genomic survey

- Millions of SNPs in *Lampetra* genome
- Develop novel diagnostic SNP marker(s) to differentiate *Lampetra* ecotypes





Study Objectives

Development of SNP

1. **Develop and validate novel genetic ID SNP** for *Lampetra* ecotypes (WBL vs. WRL)

Application of SNP

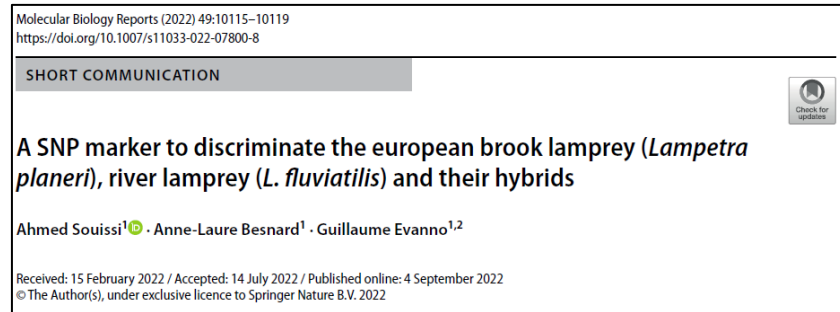
2. **Test concordance** of genetic and morphological ID methods for
 1. Genera (*Entosphenus* vs. *Lampetra*)
 2. *Lampetra* ecotypes (WBL vs. WRL)
3. **Characterize composition** of lamprey genera and *Lampetra* ecotypes at tributary collection sites in Northeastern Pacific region



Lampetra Ecotype Genetic ID: A Template for SNP Development

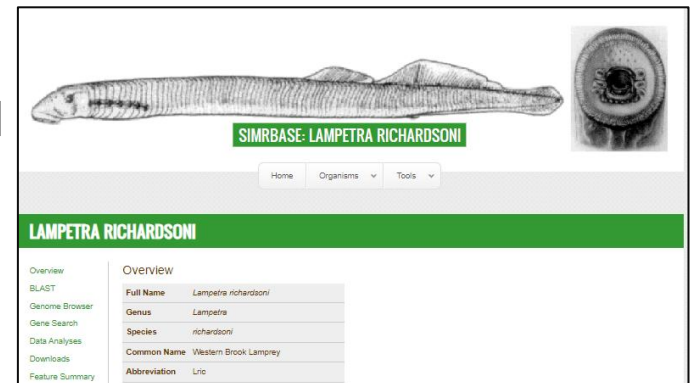
1. Use pioneering work in other paired species to guide SNP development

- Diagnostic SNP marker developed to resolve European Brook (*Lampetra planeri*) and European River lamprey (*L. fluviatilis*)
- Similar approach may work for North American *Lampetra* ecotypes



2. Develop a reference whole genome within North American *Lampetra*

- *Lampetra* reference whole genome assembled from Columbia River basin WBL specimen (GenBank Accession Number: JARYGF000000000, JASBGX000000000).

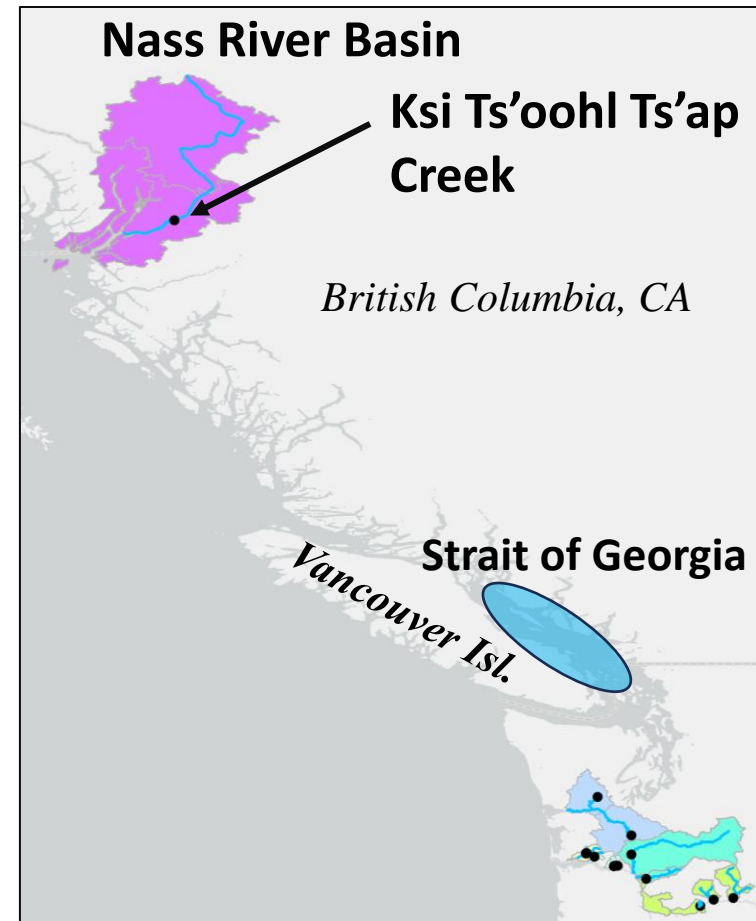




Lampetra Ecotype Genetic ID: A Template for SNP Development

3. Identify suitable study site where WBL and WRL are sympatric

- Collaboration with Nisga'a Nation Fisheries in Nass River basin, Northern BC
- Observed both WBL and WRL ecotypes in Ksi Ts'oohl Ts'ap Creek (formerly Zolzap Creek)
- Appeared to be ideal site for SNP marker development

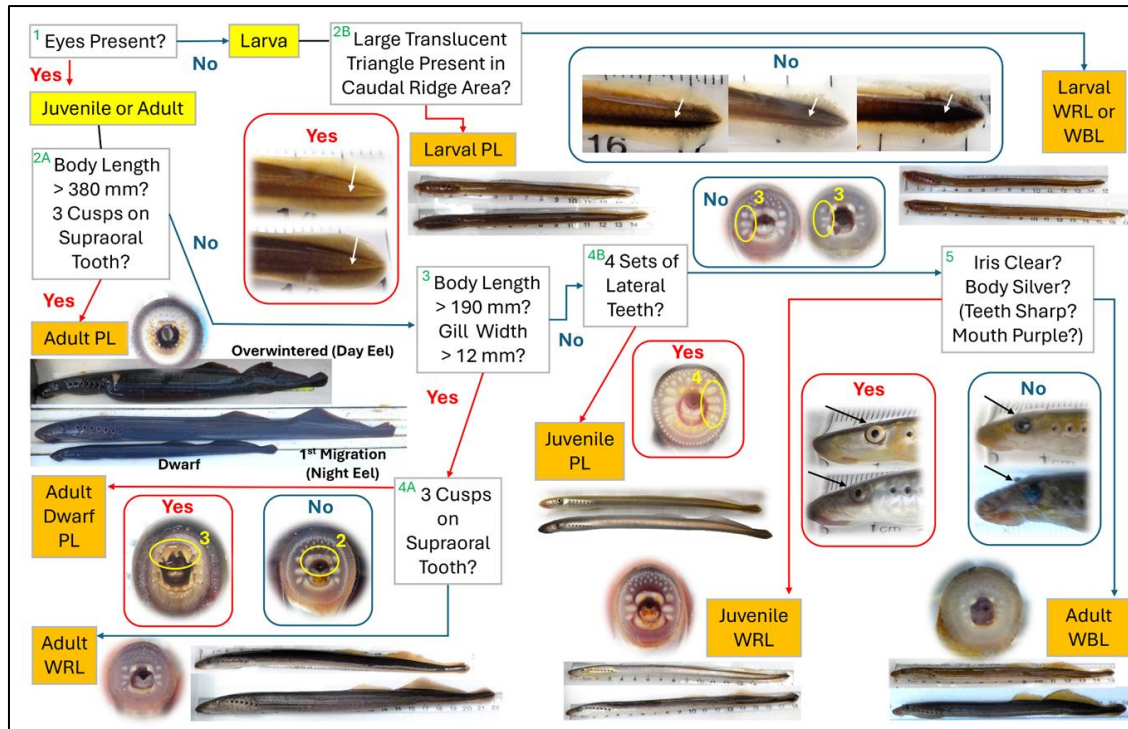




Lampetra Ecotype Genetic ID: A Template for SNP Development

4. Use systematic method for morphological ID of WBL and WRL

- Identified 'voucher' specimens of WBL and WRL from Ksi Ts'oohl Ts'ap Creek for SNP marker development



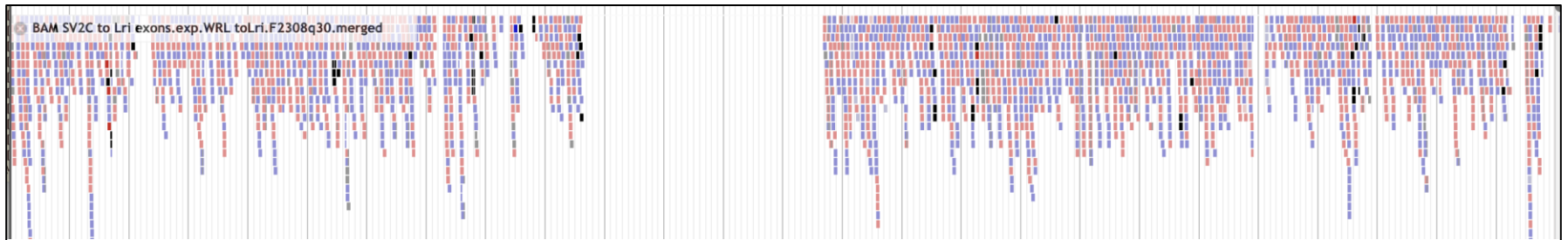
Dichotomous key adapted from Yakama Nation ID guide



Lampetra Ecotype Genetic ID: A Template for SNP Development

5. Utilize shotgun sequencing of WBL and WRL voucher specimens from Ksi Ts'oohl Ts'ap Creek

- Align WBL (N=24) and WRL (N=15) voucher specimen sequences with WBL reference genome

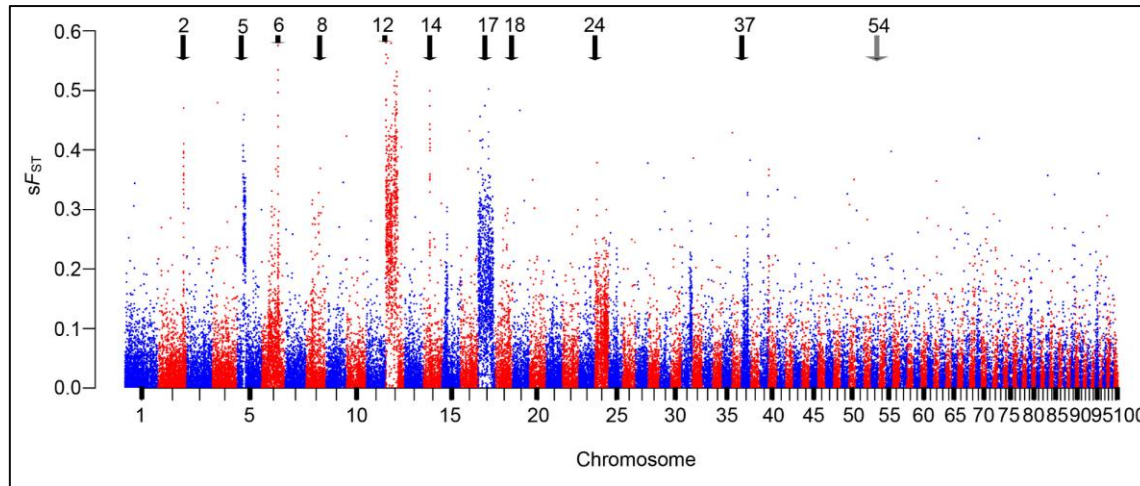




Lampetra Ecotype Genetic ID: A Template for SNP Development

6. Compare WBL and WRL allele frequencies to find highly divergent SNPs

- High F_{ST} SNPs on 10 chromosomes (2, 5, 6, 8, 12, 14, 17, 18, 24, 37)



7. Design primers on subset of SNPs at these peaks

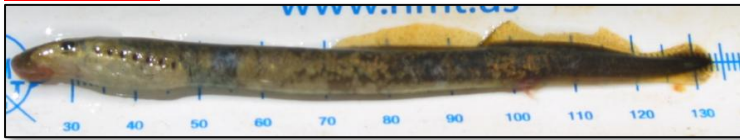
- Developed GT-seq assays for 47 candidate SNPs
- Top candidate **SNP *Lri2P16367064*** selected as putative genetic ID for *Lampetra* ecotypes (WBL/WRL)
(High F_{ST} , most successful genotyping rate)



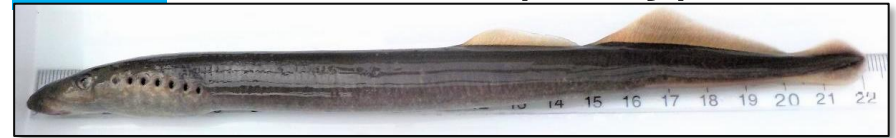
Lampetra Ecotype Genetic ID: A Template for SNP Development

8. Test candidate SNP on WBL and WRL voucher fish from Ksi Ts'oohl
Ts'ap Creek (Objective 1)

WBL N=21 Morphotypes

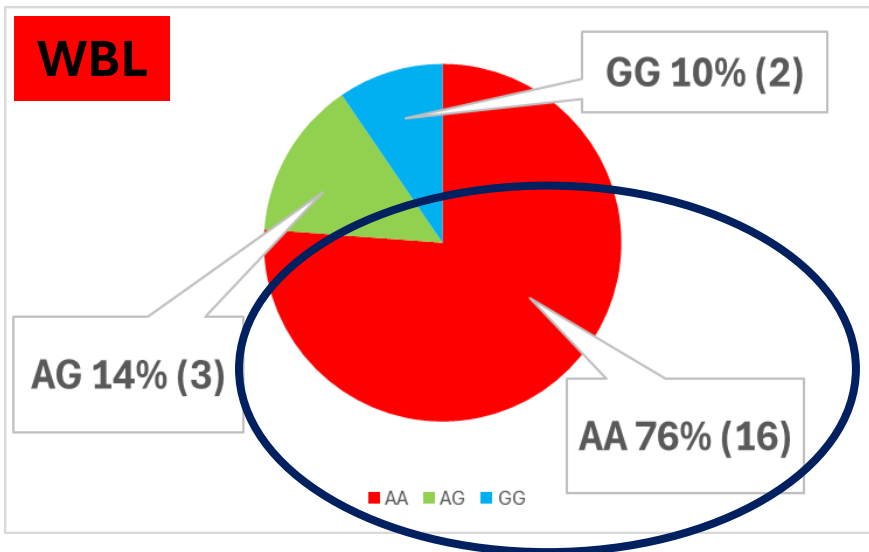


WRL N=11 Morphotypes



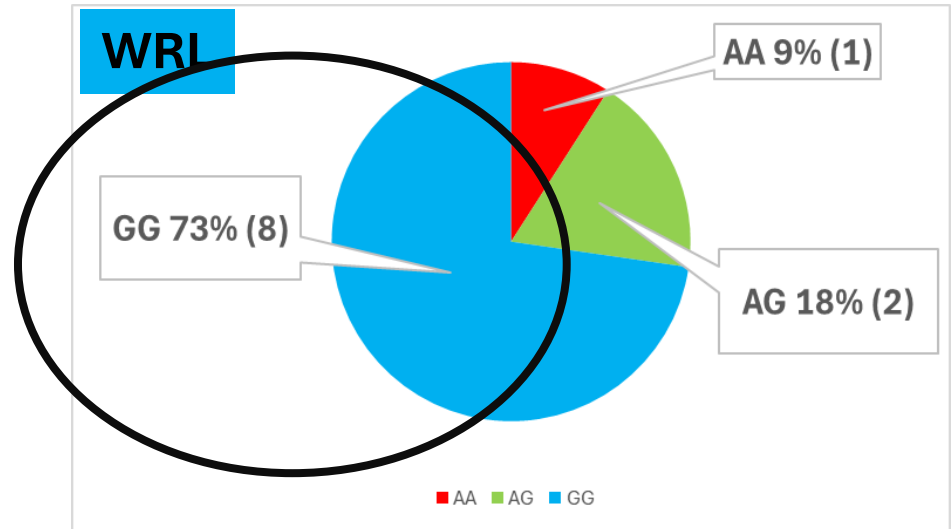
Genotype Results for SNP LRI2P16367064

WBL



'A' allele diagnostic for WBL

WRL



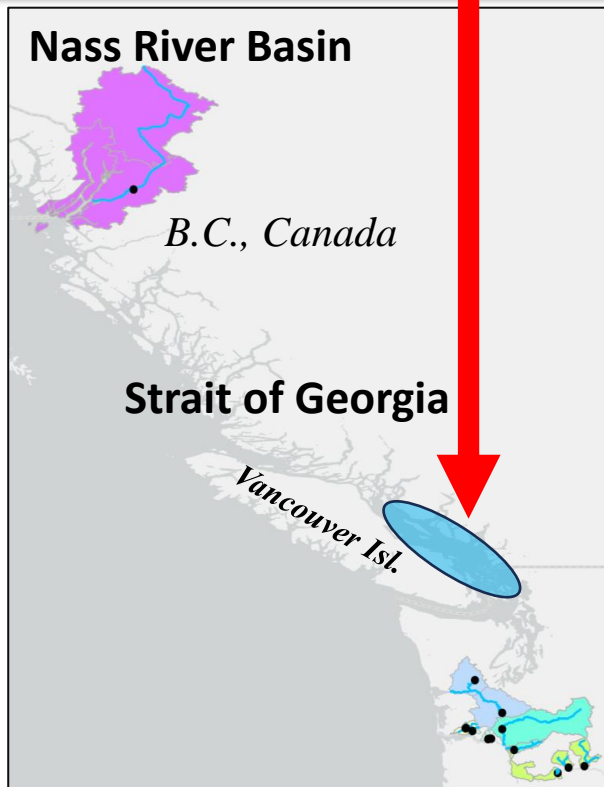
'G' allele diagnostic for WRL



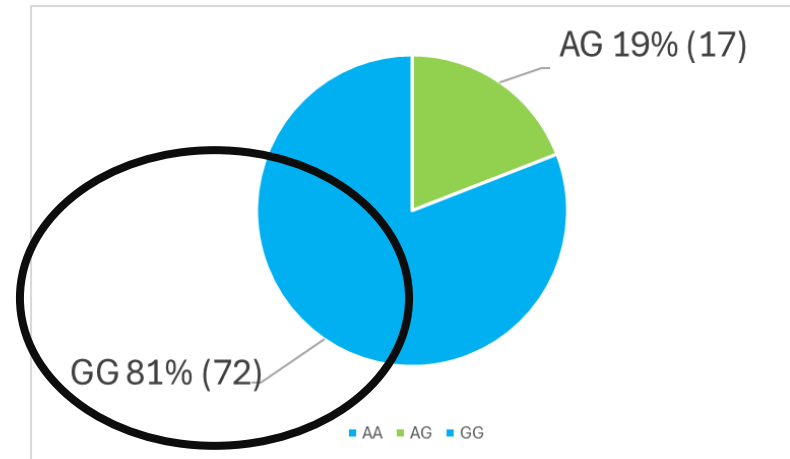
Lampetra Ecotype Genetic ID: A Template for SNP Development

9. Validate candidate SNP on marine-phase WRL as positive control (Objective 1)

WRL N=89 ocean collected WRL



Genotype Results for SNP LRI2P16367064



- 81% homozygous 'G' (WRL) allele
- 0% homozygous 'A' (WBL) allele

Objective 1: Results!

Develop and validate novel SNP marker for *Lampetra* ecotypes

- **Voucher Specimens + Positive Controls = Candidate SNP marker**
- Next: apply *Lampetra* ecotype SNP marker

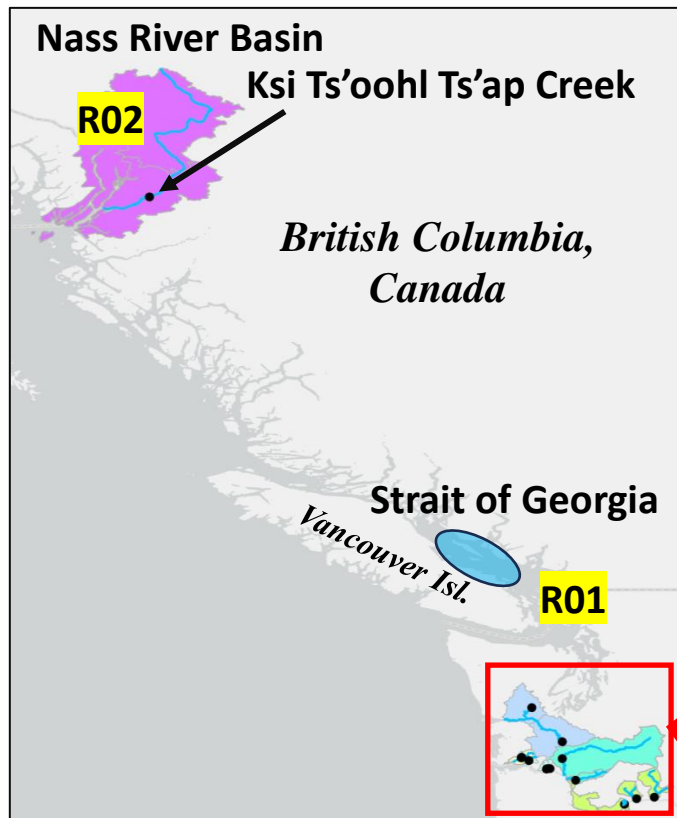


Objectives 2 and 3

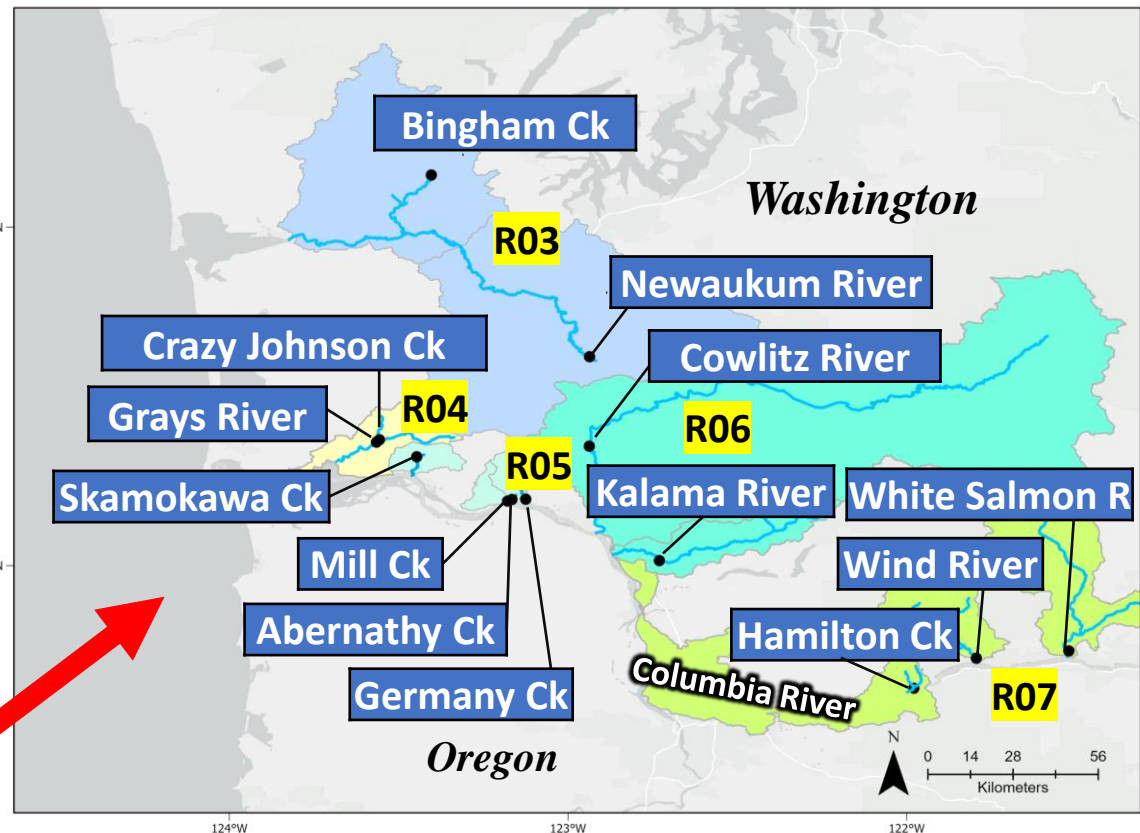
Apply *Lampetra* Ecotype ID SNP Marker

- Collections of mixed lampreys of all life stages (N = 1079)
- NE Pacific region tributary collections

Nass River Basin



Columbia River Basin and W. Washington





Objective 2 Results

Test concordance of genetic and morphological ID

Genus level: *Entosphenus* vs. *Lampetra* (Hess et al. 2020 SNP markers)

Genetic ID		Morphological ID		Concordance
		<i>Entosphenus</i>	<i>Lampetra</i>	
<i>Entosphenus</i>	645	634	11	98%
<i>Lampetra</i>	434	0	434	100%
Total Correct	1079	634	434	99%

- *Entosphenus*: 98% concordance
- *Lampetra*: 100% concordance
- Incorrect IDs = poor image quality of larvae





Objective 2 Results

Test concordance of genetic and morphological ID

***Lampetra* ecotypes** WBL vs. WRL (using new SNP marker)

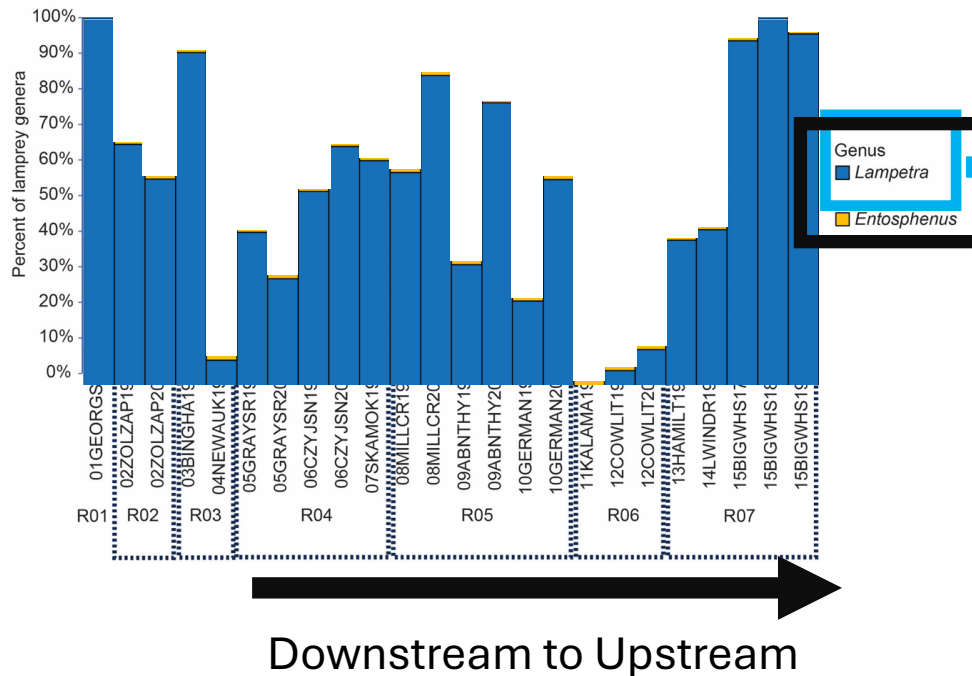
Genetic ID		Morphological ID			Concordance
		WBL	WRL	Larva (UNK)	
WBL	163	160	3	(155)	98%
WRL	11	2	9	(42)	82%
Total Correct	174	160	9	--	97%
Intermediate	17	13	4	(36)	--

- WBL: 98% concordance (excluding larvae)
- WRL: 82% concordance (excluding larvae)
 - 2 ‘incorrect’ morphological IDs
 - 1 intermediate (AG) genotype
 - 2 ‘disagreements’



Objective 3 Results

Characterize composition of **lamprey genera** at tributary collection sites



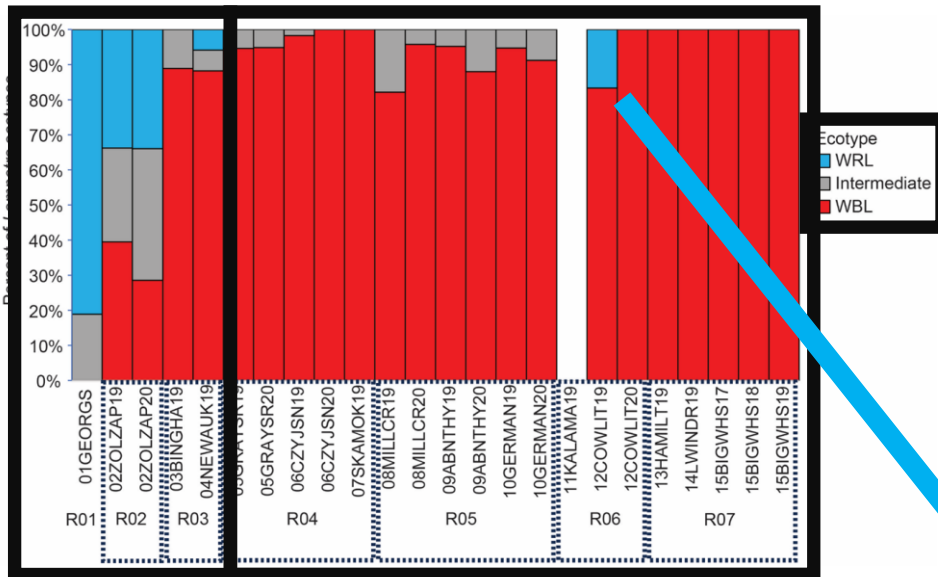
- Breakdown *Lampetra* into ecotypes using new SNP marker
 - Both genera present in 13 of the 15 collection sites
 - Kalama River *Entosphenus* only
 - Strait of Georgia *Lampetra* only (positive control)
- Genera highly variable between years and within regions

Dashed boxes indicate regional groupings



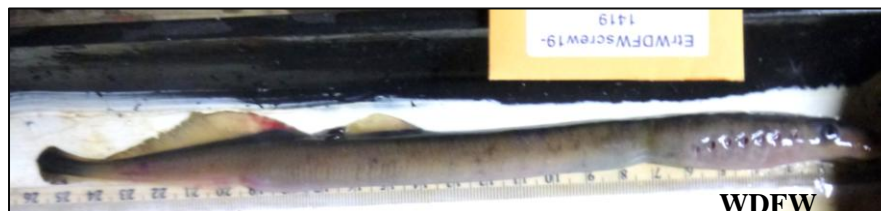
Objective 3 Results

Characterize composition *Lampetra* ecotypes at tributary collection sites



Downstream to Upstream

- WRL more common in north (Nass River, Chehalis River basins)
- *Lampetra* ecotypic proportions stable within regions and across years
- WRL rare in lower Columbia River tribs
 - 1 WRL adult captured in Cowlitz River screw trap



WDFW



WDFW



Summary and Conclusions

- We've developed a putative SNP marker for genetic ID of *Lampetra* ecotypes that has high concordance with morphological ID methods
 - However, analysis limited to northern portion of *Lampetra* range, additional testing in southern portion of range needed
 - Future directions include developing a multi-SNP assay with additional markers for more robust and accurate *Lampetra* ecotype ID versus a single SNP marker
- Genetic ID methods are powerful monitoring tools useful for:
 - Ascertaining genera and ecotypes regardless of life stage (i.e., larval *Lampetra* ecotypes) and size of larvae (i.e., small *Entosphenus* vs. *Lampetra*)
 - Providing larger more representative sample sizes
 - Increasing survey efficiency vs. time-consuming morphological data collection
- Identifying *Lampetra* ecotypes may help:
 - Broaden understanding of WRL distribution, dispersal, and sources/sinks of *Lampetra* anadromous life history variants
 - Inform future conservation actions, ESA petitions, habitat restoration, barrier removal, etc.
- Study supports reclassifying WBL and WRL as ecotypes vs. separate species



Acknowledgements

Washington Dept. of Fish and Wildlife

Jake Hearn (MAG)	Claire Landry (Grays/CJ)
Brad Allen (MAG)	Jenn Eberly (Grays/CJ)
Summer Henricksen (MAG)	Cody Dieterle (Grays/CJ)
Jamie Lamperth (MAG)	Scott Benson (Grays/CJ)
Charlie Cochran (Wind)	Brad Garner (Grays, Duncan, Hamilton Ck/Sp)
Joe Mullen (Wind)	Sean Toomey (Duncan, Hamilton Ck/Sp)
Chase Franklin (Wind)	Jerry Pruiett (Duncan, Hamilton Ck/Sp)
Tim Blubaugh (Wind)	Brianna Murphy (Duncan, Hamilton Ck/Sp)
Tony Bielinski (Bingham)	Laura Lloyd (Duncan, Hamilton Ck/Sp)
Caitlin McNamara (Bingham)	Kevin Fox (Duncan, Hamilton Ck/Sp)
Shayne Noble (Chehalis)	Justin Miller- Nelson (Newaukum)
Kristen McManus (Bingham)	John Winkowski (Bingham, Chehalis, Newaukum)
Sam Williams (Chehalis)	Devin West (Bingham, Chehalis, Newaukum)
Bryan Blazer (Chehalis)	Jeremy Wilson (Kalama)
Ashish Katru (Chehalis)	Joel Quenette (Kalama)
Lindsay Shulock (Chehalis)	Tyson Dammerson (Kalama)
Andrew Kirwan (Newaukum)	Paul Lodholz (Kalama)
Daniel Olson (Newaukum)	Jesse Jones (Grays/CJ)
Fred Bodine (Newaukum)	Rachel Rillera (Cowlitz)
Matt Sturza (Cowlitz)	Carson Swart (Cowlitz)
Erick Rockwood (Cowlitz)	
Vince De Los Santos (Cowlitz)	
Mike Blankenship (Cowlitz)	
Justin Roberts (Cowlitz)	
Josh Harwager (Cowlitz)	
Tony Bielinski (Cowlitz)	

US Geological Survey

Ian Jezorek (White Salmon)
Jill Hardiman (White Salmon)
Brad Liedtke (White Salmon)
Hal Hansel (White Salmon)

Nisga'a Nation Fisheries

Bertram Percival
Zachory Munroe
Frankie McDonald
Branden Azak
James Griffin
Stephen Kingshott

CRITFC Hagerman Genetics Lab

Bonneville Power Administration

