Genome-wide association study of survival in acoustically tagged steelhead smolts in Puget Sound

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ASILOMAR CONFERENCE GROUNDS
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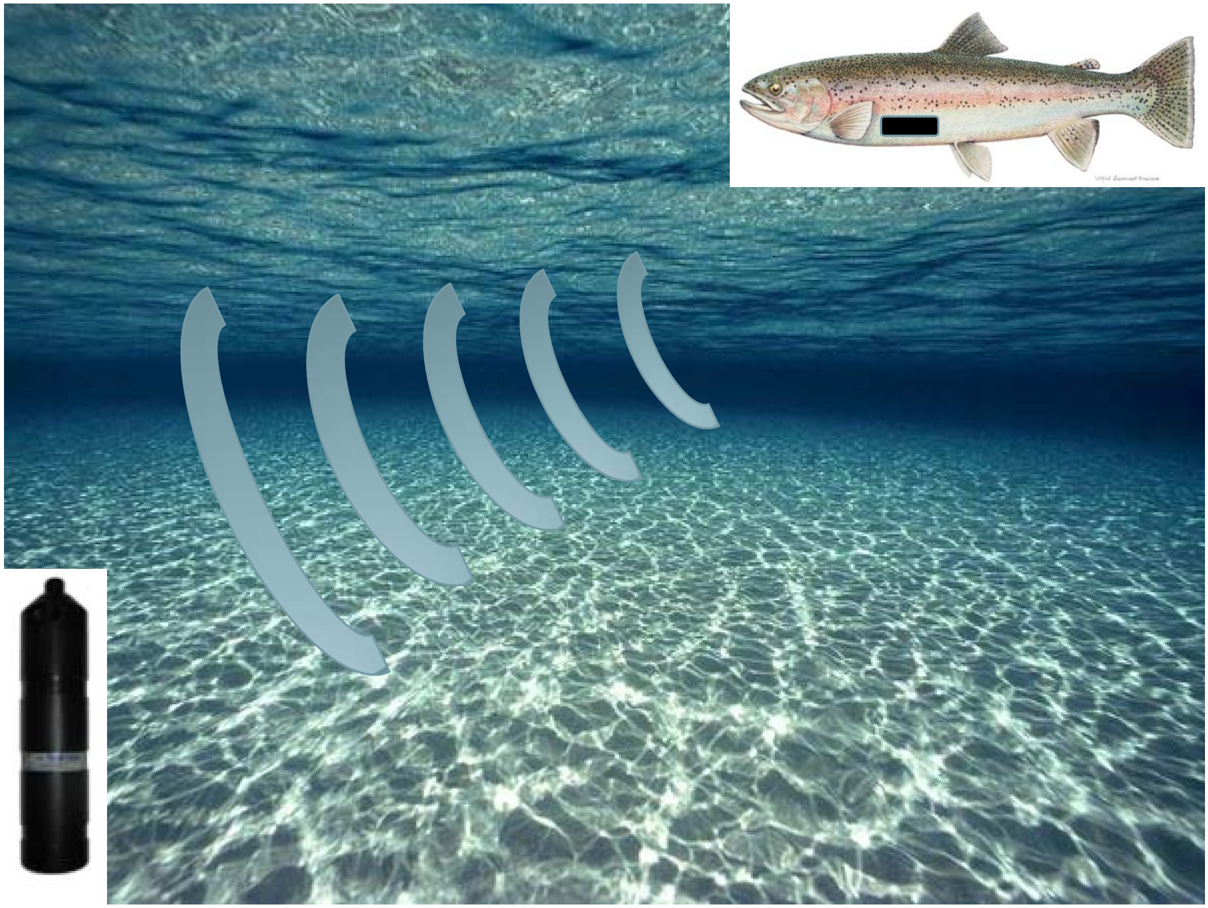
Definitions

- Genome-wide association study (GWAS)
  - Correlation between genotype – genetic “fingerprint” (e.g., SNPs) and phenotype (e.g., QTLs, fate – survival)
- Steelhead
- Smolt

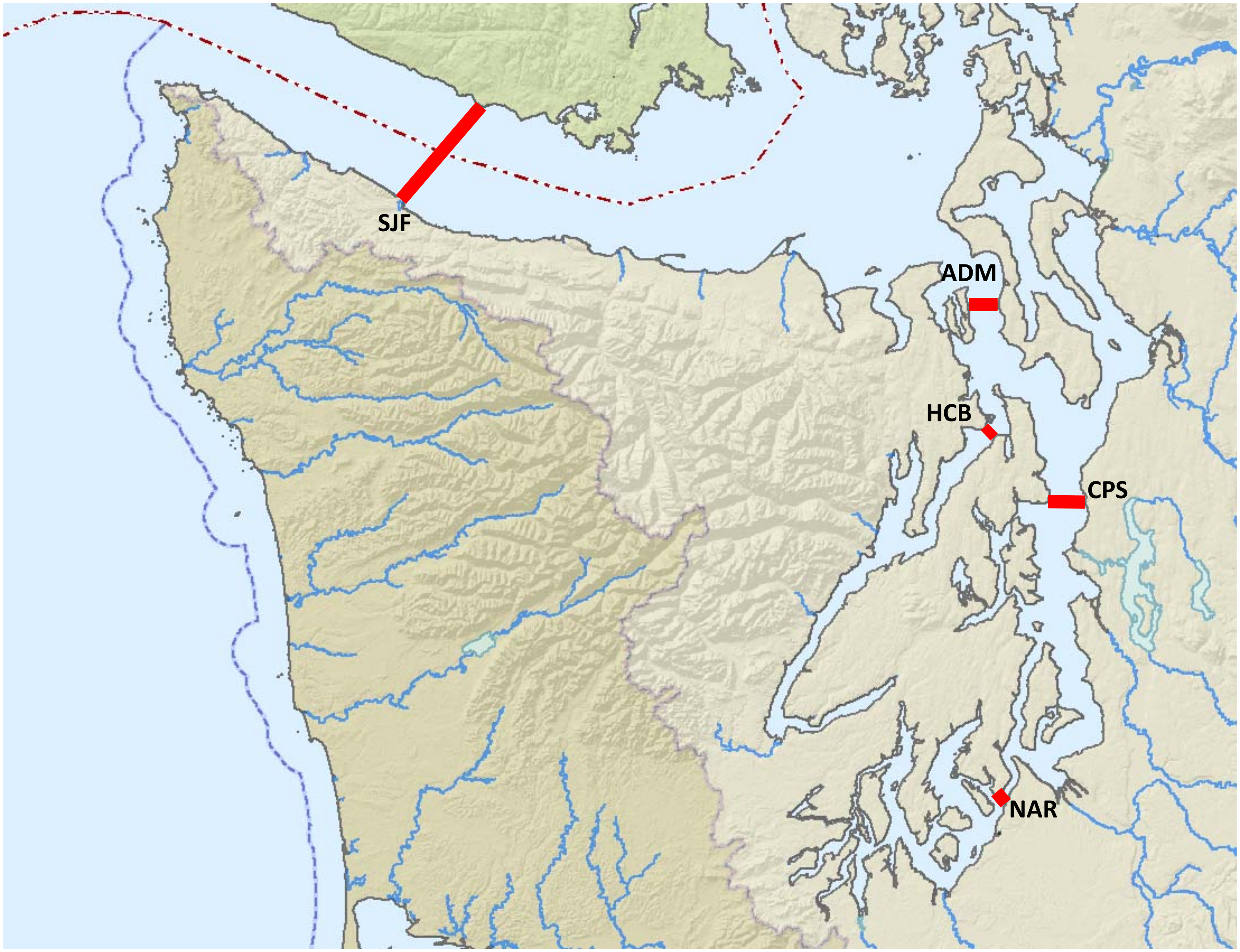
GWAS between smolt survival while out-migrating through Puget Sound, WA and smolt’s genome (reduced-representation of genome)

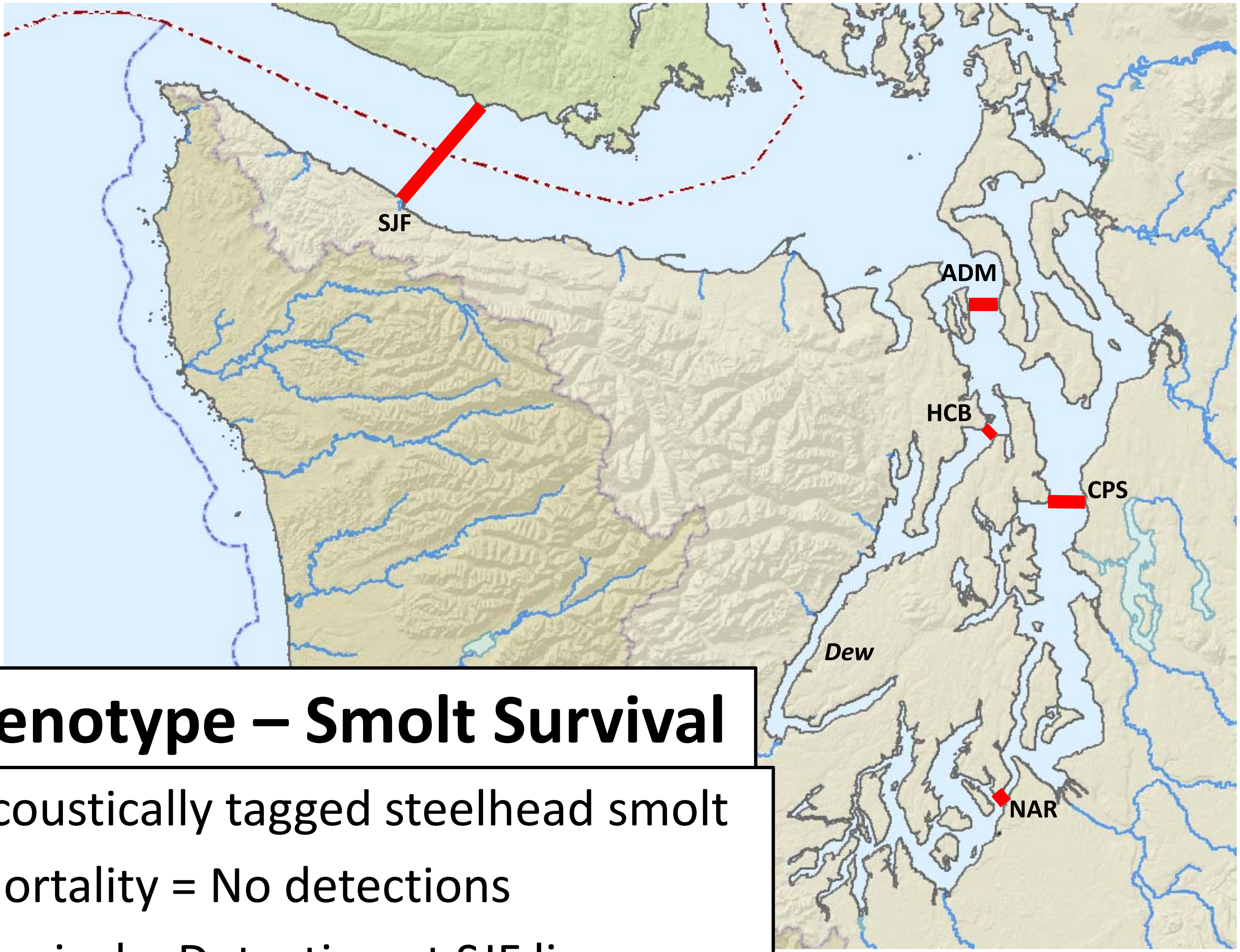
Phenotype – Smolt Survival

- Acoustically tagged steelhead smolt



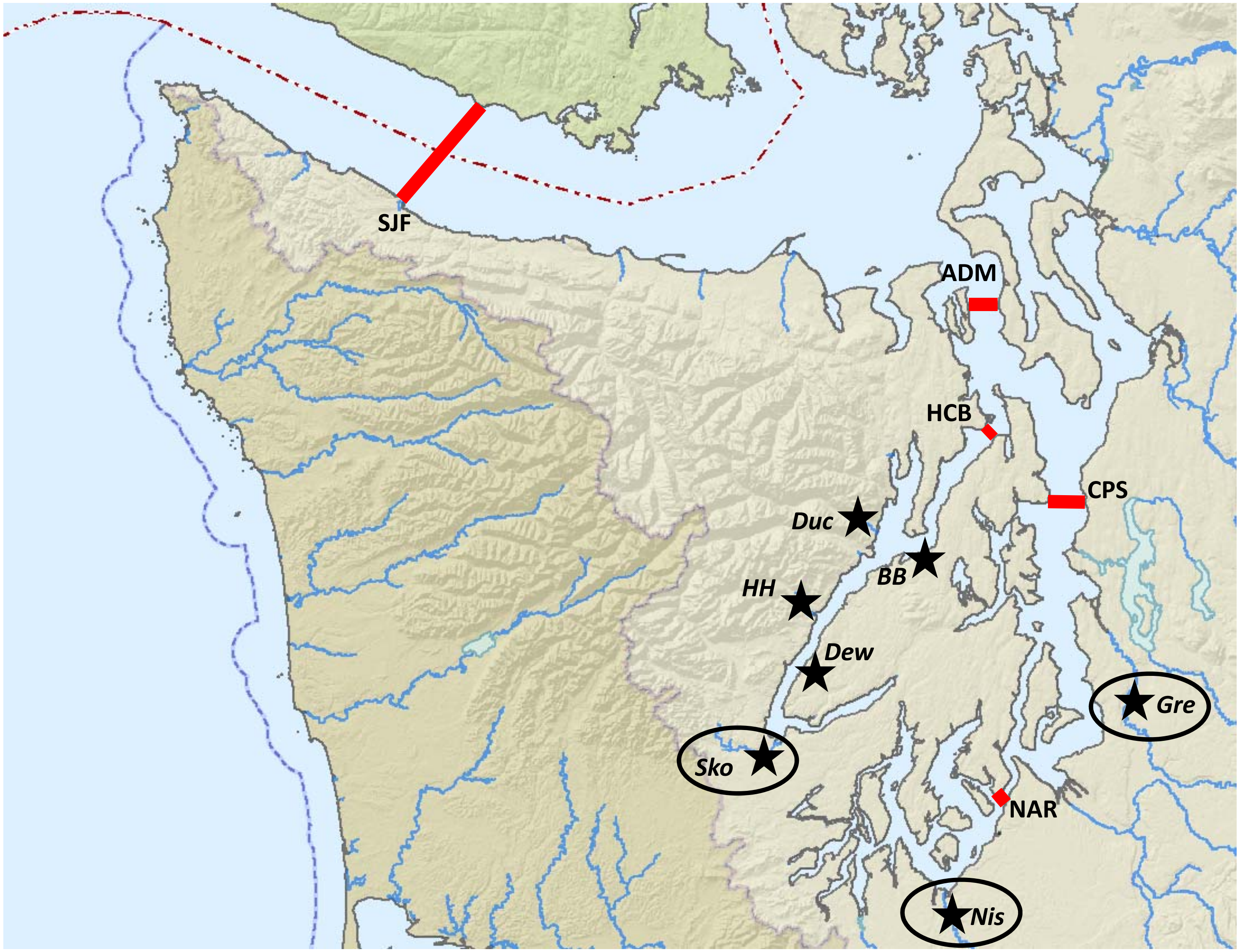
7mm and 9 mm transmitters
@ 69kHz, 136 db


SJF
ADM
HCB
CPS
NAR



Phenotype – Smolt Survival

- Acoustically tagged steelhead smolt
- Mortality = No detections
- Survival = Detection at SJF line


SJF
ADM
HCB
CPS
Duc
BB
HH
Dew
Gre
Sko
NAR
Nis

Source	Year	Origin	Release	Mortality	Survival	Total
Skokomish	2006	N	Skokomish	5	6	11
Skokomish	2007	N	Skokomish	5	5	10
Skokomish	2008	N	Skokomish	8	4	12
Skokomish	2009	N	Skokomish	4	3	7
Skokomish	2010	N	Skokomish	7	0	7
Green	2014	U	Nisqually	4	2	6
Green	2014	U	Green	16	7	23
Green	2008	N	Green	7	2	9
Nisqually	2014	U	Nisqually	2	2	4
Nisqually	2014	U	Green	12	3	15
				70	34	104

RAD-seq

- Restriction-site associated DNA (RAD) (sbfI)
- Used to discover 1000s – 10,000s SNPs
- Distributed throughout the genome
- 80 bp fragment

Category	**# Loci**
Initial set of sequences	~650 million
Catalog 1 (m5M2)	109,571
Catalog 2 (m3M4)	137,521
Overlap, filters	5,702

GWAS Methods

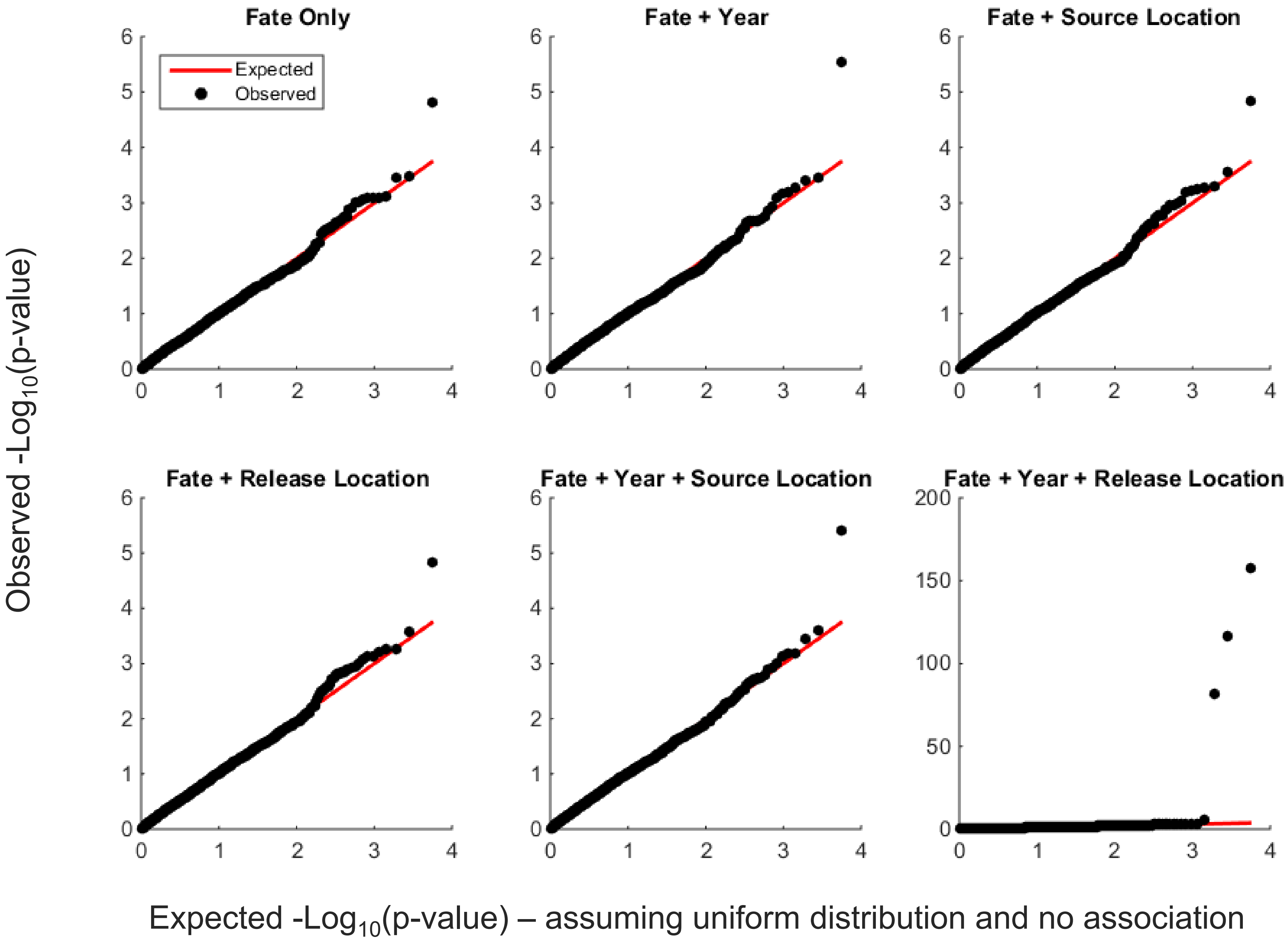
- Mixed linear model (MLM) implemented in program *TASSEL*
- Population structure and kinship included to eliminate false association and reduce Type 1 error
  - Population structure: program *STRUCTURE*
  - Kinship (relatedness): *TASSEL*
- Grouping factors as covariates
  - Year, source location, release location
- Genotype: 5702 SNP loci
- Phenotype: Fate (survival v. mortality)

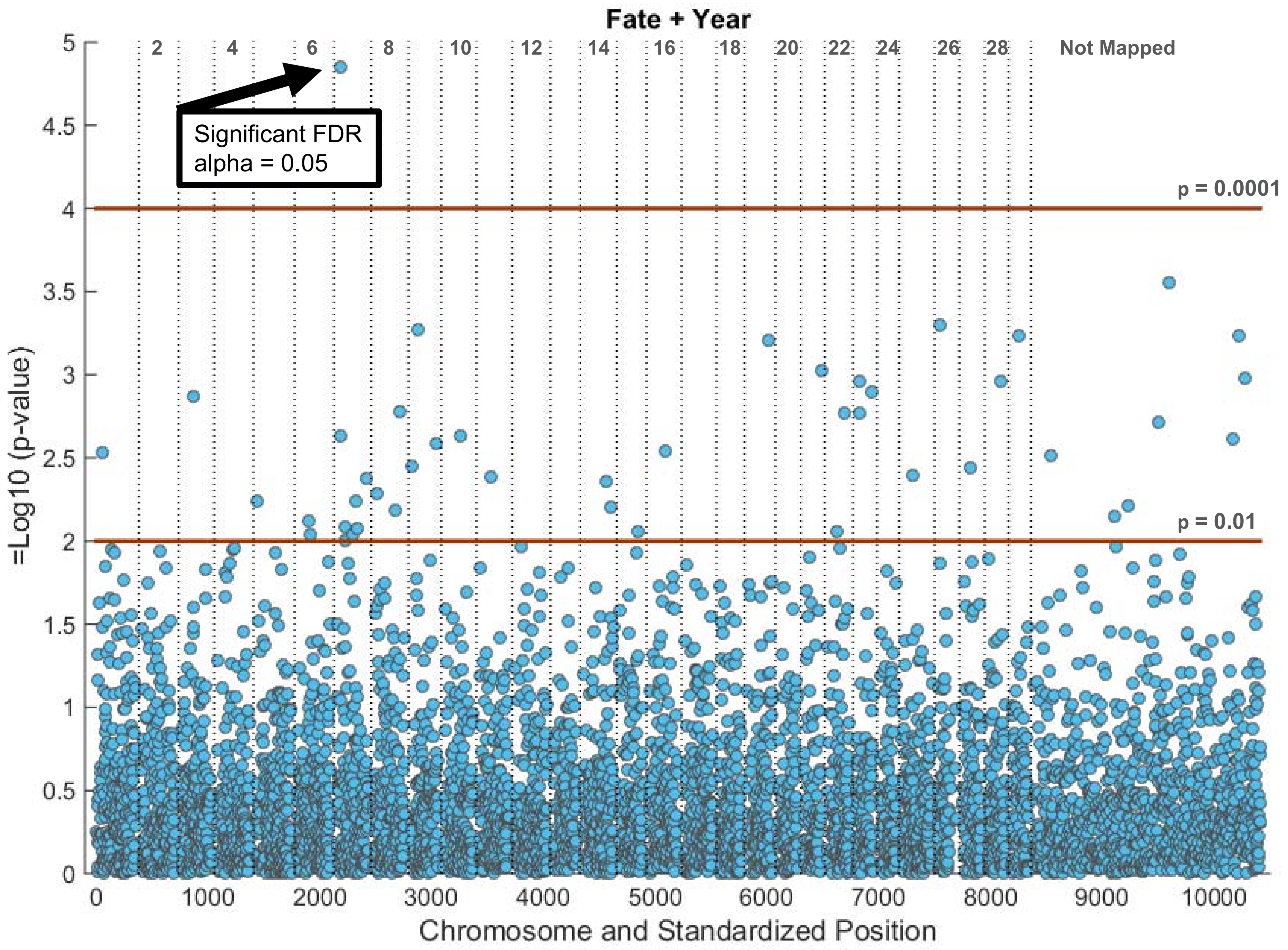
Calculate probability for each SNP H_o: no association

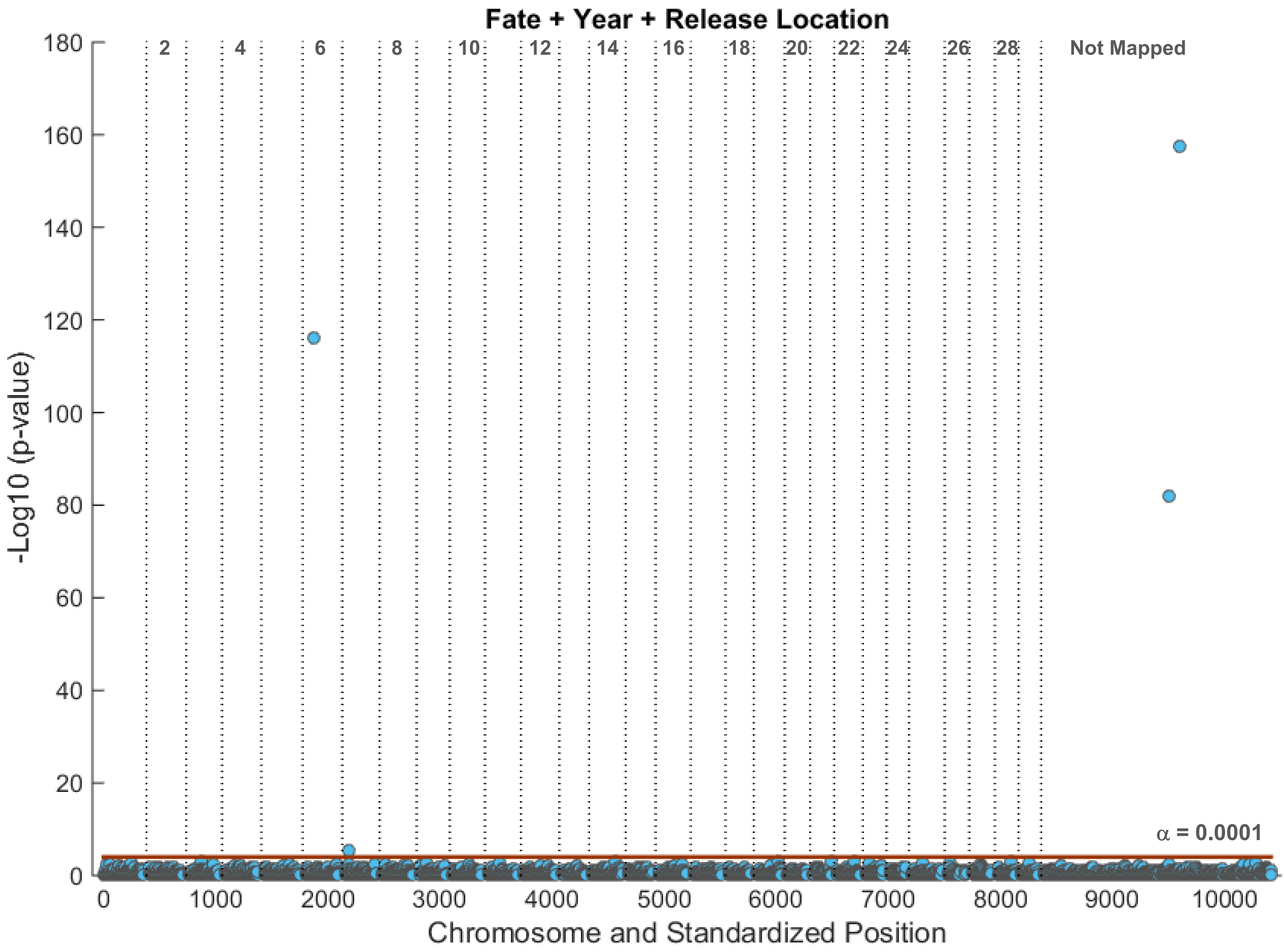
Grouping Factors

- Fate only (no grouping factor)
- Fate + Year
- Fate + Source
- Fate + Release
- Fate + Year + Source
- Fate + Year + Release
- Fate + Year + Source + Release[1]

[1] No results: design matrix not full column rank


Fate Only
Fate + Year
Fate + Source Location
Fate + Release Location
Fate + Year + Source Location
Fate + Year + Release Location
Expected
Observed
0
1
2
3
4
5
6
50
100
150
200
Observed -Log$_{10}$(p-value)
Expected -Log$_{10}$(p-value) – assuming uniform distribution and no association


Fate + Year
2
4
6
8
10
12
14
16
18
20
22
24
26
28
Not Mapped
Significant FDR
alpha = 0.05
p = 0.0001
p = 0.01
=Log10 (p-value)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
Chromosome and Standardized Position



Fate + Year + Release Location
2
4
6
8
10
12
14
16
18
20
22
24
26
28
Not Mapped
α = 0.0001
-Log10 (p-value)
0
20
40
60
80
100
120
140
160
180
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
Chromosome and Standardized Position

Phenotype (MLM)	Locus			
	39529_18	55970_7	12301_21	51226_71
Fate	1.54E-05	3.49E-04	5.07E-01	9.14E-02
Fate + Year	**2.92E-06**	2.16E-03	3.96E-01	1.40E-01
Fate + Source	1.42E-05	2.80E-04	4.74E-01	1.51E-01
Fate + Release	1.46E-05	2.63E-04	5.00E-01	1.40E-01
Fate + Year + Source	**3.97E-06**	2.53E-03	3.74E-01	1.21E-01
Fate + Year + Release	**4.18E-06**	**3.62E-158**	**6.63E-117**	**9.44E-83**
Blast to N Sequences	2	0	5	14
Morphogenesis	1	0	0	1
Immunological	0	0	0	6
Other	1	0	5	7

Bold = significant using FDR (Benjamini – Hochberg) at alpha = 0.05

Bonferroni @ alpha 0.05 = 8.8E-6

Bonferroni @ alpha 0.10 = 1.8E-5

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	39529_18			51226_71
Fate	1.54E-05			9.14E-02
Fate + Year	**2.92E-06**			1.40E-01
Fate + Source	1.42E-05			1.51E-01
Fate + Release	1.46E-05			1.40E-01
Fate + Year + Source	**3.97E-06**			1.21E-01
Fate + Year + Release	**4.18E-06**			**9.44E-83**
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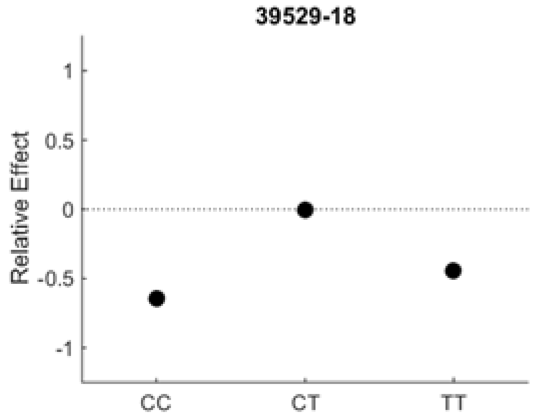
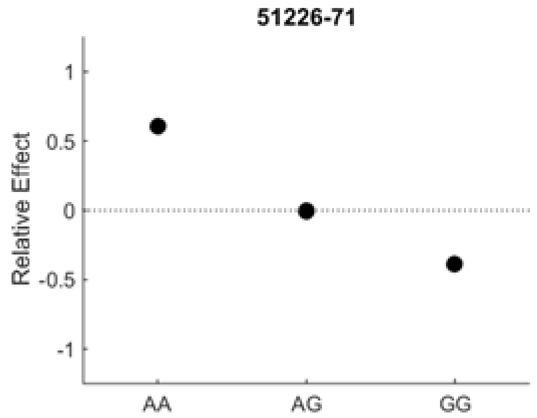
Bonferroni @ alpha 0.10 = 1.8E-5



Blast to N Sequences	2
Morphogenesis	1
Immunological	0
Other	1

Basin	Fate	CC	CT	TT	N	*G*-test *P*-value
Green	Mortality	0.19	0.16	0.65	31	0.019 ✓
	Survival	0.00	0.80	0.20	10	
Nisqually	Mortality	0.50	0.17	0.33	6	0.017 ✓
	Survival	0.00	1.00	0.00	4	
Skokomish	Mortality	0.04	0.30	0.67	27	0.050
	Survival	0.00	0.56	0.44	18	



Blast to N Sequences	14
Morphogenesis	1
Immunological	6
Other	7

Basin	Fate	AA	AG	GG	N	*G*-test *P*-value
Green	Mortality	0.52	0.28	0.21	30	0.028 ✓
	Survival	0.78	0.22	0.00	9	
Nisqually	Mortality	0.57	0.00	0.43	6	0.028 ✓
	Survival	1.00	0.00	0.00	2	
Skokomish	Mortality	0.78	0.13	0.09	23	0.048
	Survival	0.83	0.17	0.00	12	

0.05 / 3 = 0.0167
0.10 / 3 = 0.0333

Conclusions

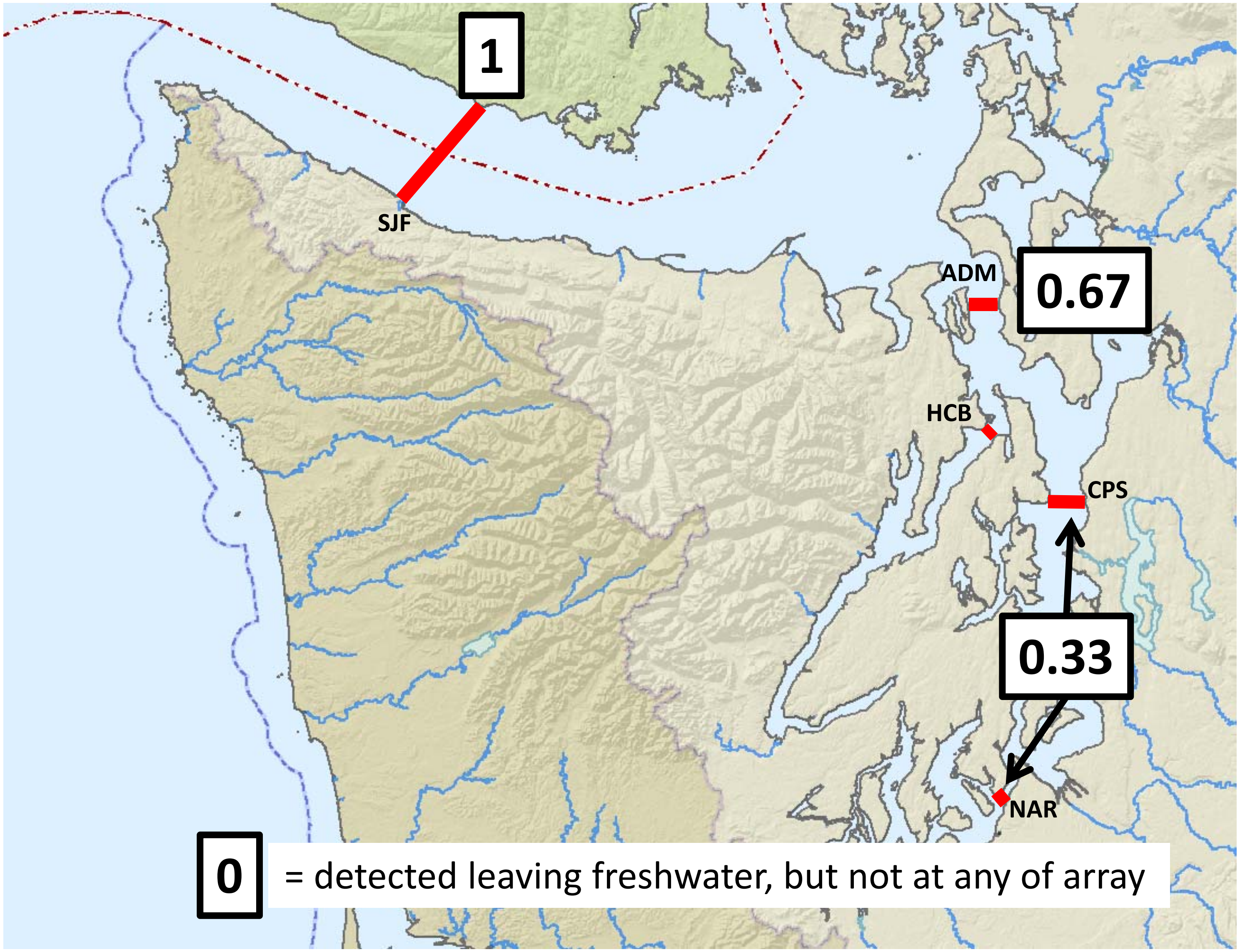
- The fate of out-migrating steelhead smolts is not independent of their genomes
- Two groups of genes appear to have significant association with survival
  - Immunological
  - Developmental
- May depends on time (year) and space (release location)
- Working hypothesis (w/ some arm-waving): Smolts with certain alleles may be compromised by their immunological response or fin development
- Predation, for example, maybe the proximate cause of mortality; but ultimate cause may be fish health and presence of pathogen(s)

Limitations

- Sample size (total and per stratum)
  - Power
  - Prevents post hoc tests
- Lack of independence:
  - between year and source location
  - Between source and release locations
- Design: model is for QTLs, but fate is categorical
- Short fragments (limits annotation)

Next Steps (2016 funding)

- Analysis based on 2014 & 2015 samples from Green and Nisqually rivers
- RAD-seq samples not originally sequenced
- Change fate from binary character to quasi-continuous



0 = detected leaving freshwater, but not at any of array

Next Steps (2016 funding)

- Analysis based on 2014 & 2015 samples from Green and Nisqually rivers
- RAD-seq samples not originally sequenced
- Change fate from binary character to quasi-continuous
- **Sample size increases from 59 to 282**
- Explore other analysis methods (e.g., Random Forest)
- GWAS with phenotype = # of *Nanophyetus* cysts

Acknowledgements

- This study is part of the Salish Sea Marine Survival Project. Funding was provided by Washington State
- Puget Sound Steelhead Marine Survival Workgroup for their support in development and implementation.
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