Using Environmental DNA to Monitor Eulachon (*Thaleichthys pacificus*) Abundance Northern Southeast Alaska (aka hooligan, Saak) Meredith Pochardt^{1,3}, Ted Hart², Taal Levi ³

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Overview:

- Eulachon background
- Population Monitoring the beginning
- eDNA for population monitoring
- Challenges, successes, next steps

Eulachon Background





Endangered Species Act Recovery Plan for the Southern Distinct Population Segment of Eulachon (*Thaleichthys pacificus*)

Prepared by: National Marine Fisheries Service, West Coast Region







Life History

- Columbia River spawning 2yrs.
- Alaska spawning 4-5yrs.
 - Otolith & isotope
- Discrepancy in semelparous (Clark et al. 2007) VS. iteroparous (Patrick R. Biondo (WDFW), Personal communication & Barraclough, 1964)
 - Ovary & isotope
- Regional population structure



Flannery et al. 2013. Microsatellite Analysis of Population Structure in Alaska Eulachon with Application to Mixed-stock Analysis

Indigenous-led Population Monitoring

How does the spawning population vary on an annual basis – one river (Chilkoot) 2010-2018



Method 1: Mark-Recapture













Chilkoot River Mark-Recapture

Challenges

- Active subsistence fishing & foraging does not meet closed population assumptions
- Single river
- Costly: ~ \$20,000 annually





Method 2:

ENVIRONMENTAL DNA (eDNA)

- eDNA is found in a variety of substrates: soil, water, air
- Noninvasive, efficient, and relatively inexpensive
- A relatively new but established tool for detecting the presence and absence of fishes in freshwater systems
- Relies on mitochondrial genome



Origin of eDNA for monitoring aquatic vertebrates



Biol. Lett. loi:10.1098/rsbl.2008.0118 Published online

Species detection using environmental DNA from water samples

Gentile Francesco Ficetola^{1,2,*}, Claude Miaud², François Pompanon¹ and Pierre Taberlet¹



First Paper in 2008

eDNA research started slow

LETTER

2011

"Sight-unseen" detection of rare aquatic species using environmental DNA

Christopher L. Jerde¹, Andrew R. Mahon¹, W. Lindsay Chadderton², & David M. Lodge¹

OPEN CACCESS Freely available online

2011

2012

Molecular Detection of Vertebrates in Stream Water: A Demonstration Using Rocky Mountain Tailed Frogs and Idaho Giant Salamanders

Caren S. Goldberg¹*, David S. Pilliod², Robert S. Arkle², Lisette P. Waits¹

MOLECULAR ECOLOGY

Molecular Ecology (2011) FROM THE COVER doi: 10.1111/j.1365-294X.2011.05418.

PLos one

Monitoring endangered freshwater biodiversity using environmental DNA

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Now many hundreds of papers **Primary focus on technical aspects**

Mitochondrial Genome



- More copies of mtDNA in each cell
- Shorter sequence
 - Genome mapped for more species



Concentrate all cells via filtering



Collect sample

Isolate eulachon specific DNA



Merkes, C. (2017) USGS National Water Quality Monitoring Program

Isolate eulachon specific DNA





Merkes, C. (2017) USGS National Water Quality Monitoring Program

Isolate eulachon specific DNA

How do we detect DNA from one organism and not others?

Fluorescent signal will only be detected if Primer sequence matches AND Probe sequence matches



Merkes, C. (2017) USGS National Water Quality Monitoring Program

Osmeridae Alignment

Eulachon forward primer has at least 2bp mismatch to other Osmeridae

	240	25	0	260	270	280	290	300	310	320	330	340	350
new mass	CCTTCCCC	CGCATAAA	YAACATAA	GTTTCTGI	ACTTTTACC	TCCCTCCTTYC	TTCTCCTCT	FAGCTTCYT	CUGGTGTTG	AAGCRIGGRIGCO	GGGACCGGCT	GAACAGTTTA	CCGCCAC
Identity													
 Cosmerus mordax den Spirinchus thaleichthy Thaleichthys pacificu 		CG T ATAAA CGCATAAA CGCATAAA	СААСАТАА СААСАТАА ТААТАТАА	GTTTCTGI GTTTCTGI GTTTCTGI	ACTTTTACC ACTTTTACC ACTTTTAC	TCCCTCCTT TCCCTCCTT TCCCTCCTTC EucCOLF	TACTCCTCT TCCTTCTCTCT TTCTCCTT	TAGCTTC T T TAGCTTC C T TAGCTTC T T	CTGGAGTTG. CCGGTGTTG. CTGGTGTTG.	AAGCAGGGGGCC AAGCGGGGGGCC AGCGGGAGCC EucC	GGGACCGGCT GGGAACCGGTT GGGACTGGCT DLI	GAACAGTCTA GAACAGTTTA GAACAGTTTA	CCCCCAC CCACCAC CCGCC <mark>G</mark> C
🖙 4. Mallotus villosus vouc	CGTTCCCA	CGCATAAA	TAACATGA	GTTTCTG	ACTTTTACC	TCCCTCTTTCC	TTCTCCTCT	TA	•		C 1		CAC
	3	60	370	380	390	400	410	>3bj	o mism	atch on '	floresce	nt probe	
	TTGCTGGC	AATCTAGO	CCATGCGG	GAGCTTC	GTAGATYT	AACAATTTTTT	CYCTYCACC	TT -				•	AMA
Identity										-			
 P. ■ 1. Osmerus mordax den P. ■ 2. Spirinchus thaleichthy P. ■ 3. Thaleichthys pacificu 	TTGCTGGT TCGCTGGC TTGCTGGC	AATCTAGO AAT T TAGO AATCTAGO	CCATGC <mark>T</mark> G CCATGCGG CCATGC C G	GAGCTTCC GAGCCTCC GAGCTTCC	GTAGATCI GTAGATTI GTTGATCI	AACAATTTTTT AACAATTTTTT AACAATTTT <mark>C</mark> T	CTCTTCACC CTCTCCACC CCCTTCACC	TTGCGGG A A TTGC A GGGA TTGCGGGGA	ТСТССТСТА ТПТССТСТА ТСТССТСТА	TTCTAGGGGC <mark>A</mark> TTCTAGGGGCA TTCTAGGGGCC	АТСААТТТТА АТСААТТТТА АТСААТТТТА	TTACAACTATT TTACAACTATT TTACAACCAT	'ATTAA <mark>T</mark> A 'ATTAACA ATTAA T A
C 4. Mallotus villosus vouc	TTGCTGGC.	AATCTCGC	TCACGCGG	GGCTTC	GTAGATT	AACCATCTTT	COCTOCACC	TTGCGGGTA	TCTCCTCTA	TTCTAGGGGC	ATTAATTTTA	TTACAACTAT	ATTANCA
	470	480	490	50	0	510	520	530	540	550	560	570	580
	TGAARCCT	CCTGCCAT	TCCCAGT	ACCAGACO	CCCCTTATT	CGTCTGAGCCC	TCCTGATTA	CGGCCGTYC	TTCTTCTCC	TTTCCCTCCCA	GTCHTAGCTG	CTGGRATTACO	ATGCTTC
Identity													
 Def 1. Osmerus mordax den Def 2. Spirinchus thaleichthy Def 3. Thaleichthys pacificu 	TGAA <mark>G</mark> CCT TGAA <mark>A</mark> CCT TGAA <mark>G</mark> CC <mark>C</mark>	CCAGCCAT CCTGCCAT CCTGCCAT	TCCCAGT TCCCAAT TCCCAGT EucCO	ACCAGACO ACCAGACO ACCAGACO LR	CCCTTATI CCCTTATI CCCTTATI	CGTCTGAGCCG CGTCTGAGCAG CGTCTGAGCCG	TCCTGATCA TCCTGATTA TCCTGATTA TCCTGATTA	CGGCCGTCC CGGCCGTCC CGGCCGTTC	TTCTTCTCC TTCTCCTCC TTCTCCTCC	TTTCCCTCCCA TTTCTCTCCCC TTTCCCTCCCA	GT <mark>AC</mark> TAGCTG GTCCTAGCTG GT <mark>TT</mark> TAGCTG	CTGG <mark>G</mark> ATTACC CTGG <mark>G</mark> ATCACC CTGG <mark>A</mark> ATTAC	ATGCTTC ATGCTTC ATGCTTC
🖙 4. Mallotus villosus vouc	TGAAACCT	CCTGCTAT	TTCTCAGT	ACCAGAC	CCTTTATT	TGTTTGAGCTC	TGCTAATTA	CAGCCGTTC'	TTCTTCTAC	TATCCCTTCCT	GTCTTAGCCG	CTGGAATTAC	ATGCTTC

Eulachon reverse primer has 2bp mismatch to longfin smelt and at least 1bp mismatch to other Osmeridae

ddPCR



ww.labcritics.com

Eulachon Tissue



Eulachon Rare



Eulachon Abundant



DNA concentration and species detection is affected by:

- Variance in production among individuals through time (Pilliod et al. 2014, Klymus et al. 2015, Spear et al. 2015)
- Degradations rate UV, temp. (Barnes et al. 2014, Strickler et al. 2015)
- Transport downstream, dilution, diffusion (Wilcox et al. 2016, Deiner and Altematt 2014, Eichmiller et al. 2014)
 - Flow-corrected concentration: DNA Concentration (copies/µl) * Discharge (cfs)



Comparing eDNA & M-R



Does Flow-Correcting Matter?



eDNA for Regional Spatial and Temporal Monitoring

- eDNA peak and AUC
 - Flow-correction
 limitations
- Peak vs. river latitude
- Run timing environmental covariates (Chilkoot)



Results



- Chilkoot had highest NFC eDNA in 2017 & 2018
 - Not always typical
- Taiya was 2nd in 2017
- Chilkat 2nd in 2018
- All rivers showed a decline from 2017 to 2018



Latitudinal Migration – "Grease Wave"



Schindler et al., (2013), Armstrong et al. (2016)

Conclusion

- eDNA cost effective monitoring tool for data-poor species
 - Approx. \$50/sample; \$3,500 per river
- Regional approach for regional populations
- Flow-correction improves abundance estimates



Future Research

- Auto-sampler, OSU OPENS Lab
- Southeast regional tribal eulachon monitoring network
 - Expanding to new areas that are unmonitored
- Regional flow-correction
 - Inter-river comparison
 - Discharge model and/or surrogate flow (Hill et al. 2015)
- Life history investigation
 - Age at spawning, size, reproduction



Thanks to the Partners!



And our Funding Organizations!











Chilkoot River packed with eulachon in 2011!