



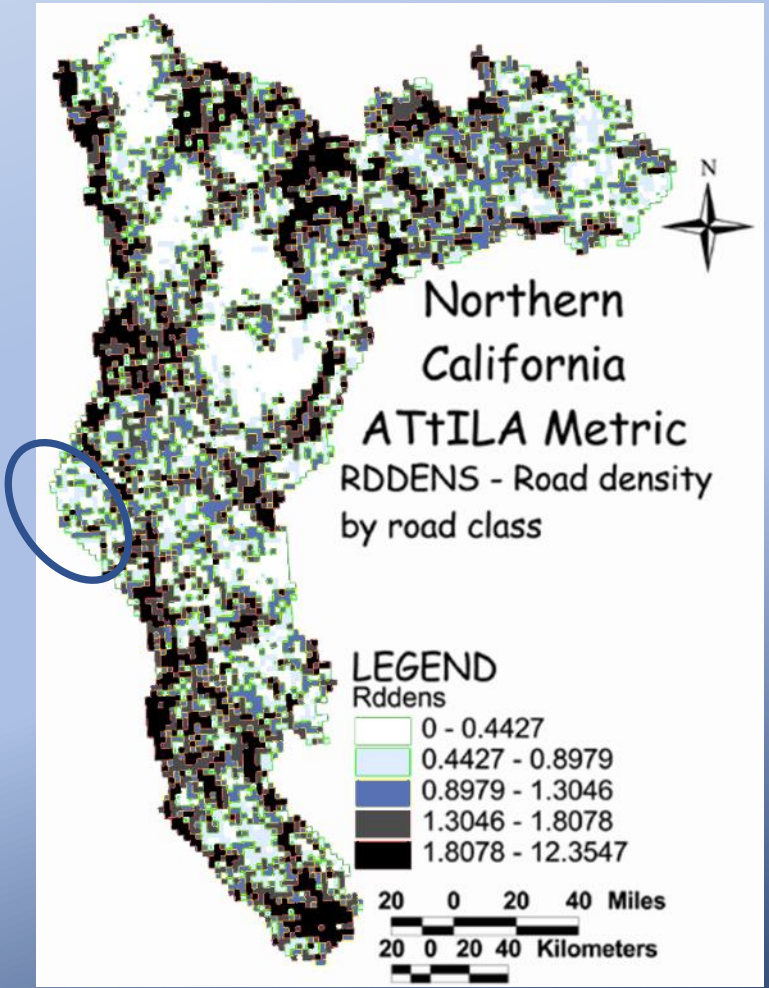
Amphibian Diversity Assessment of Northern California's Lost Coast Region.

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Institute for Wildlife Studies

King Range National Conservation Area: A rare coastal wilderness



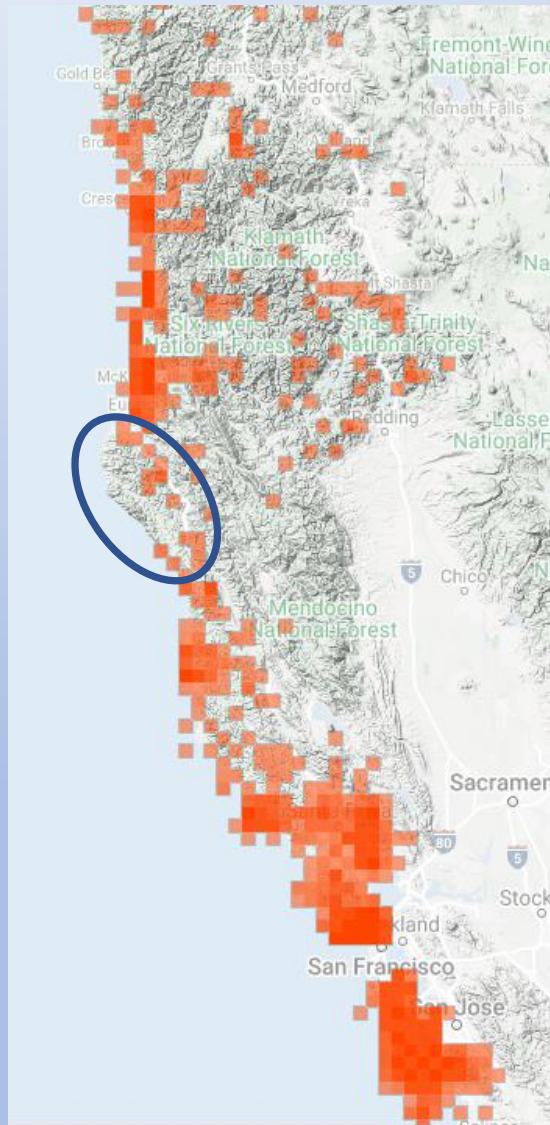
Longest stretch of undeveloped coast in the U.S. outside of Alaska.



Isolation and ruggedness means little disturbance



Isolation means seldom surveyed

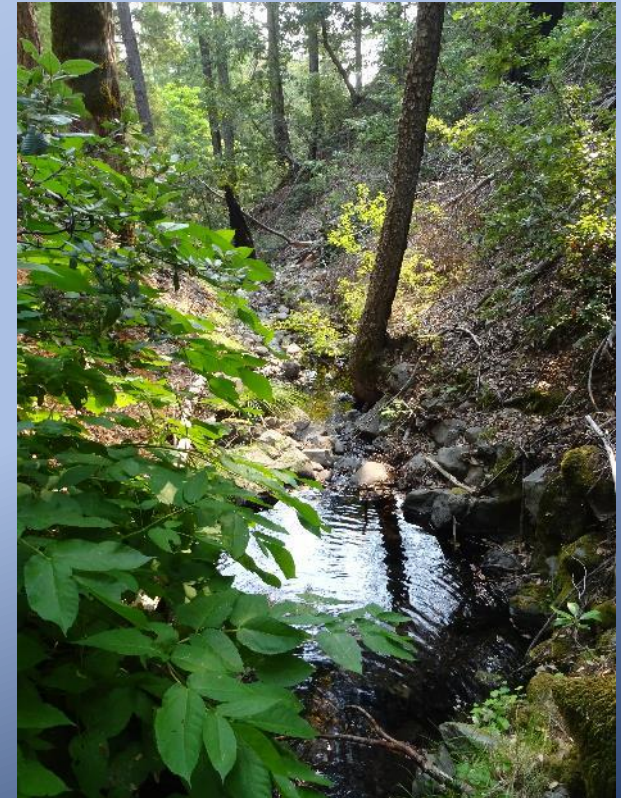
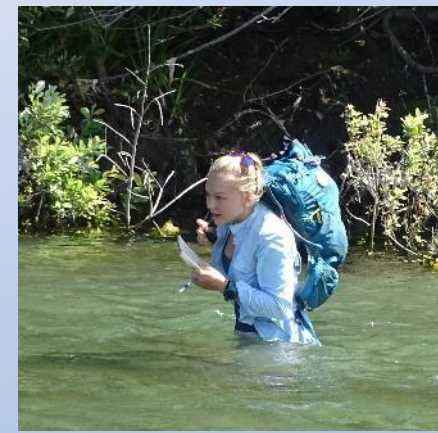


Dicamptodon spp



Survey methods

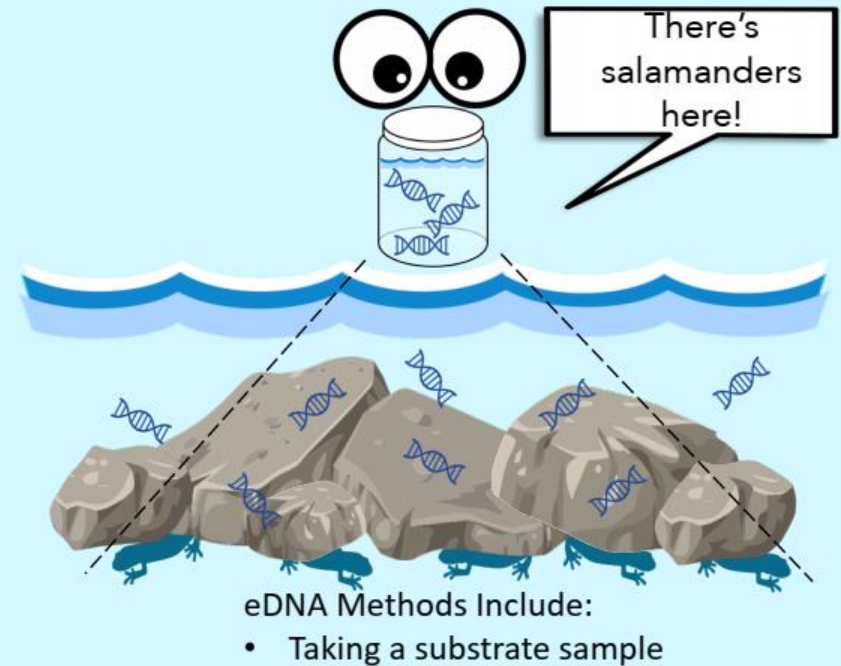
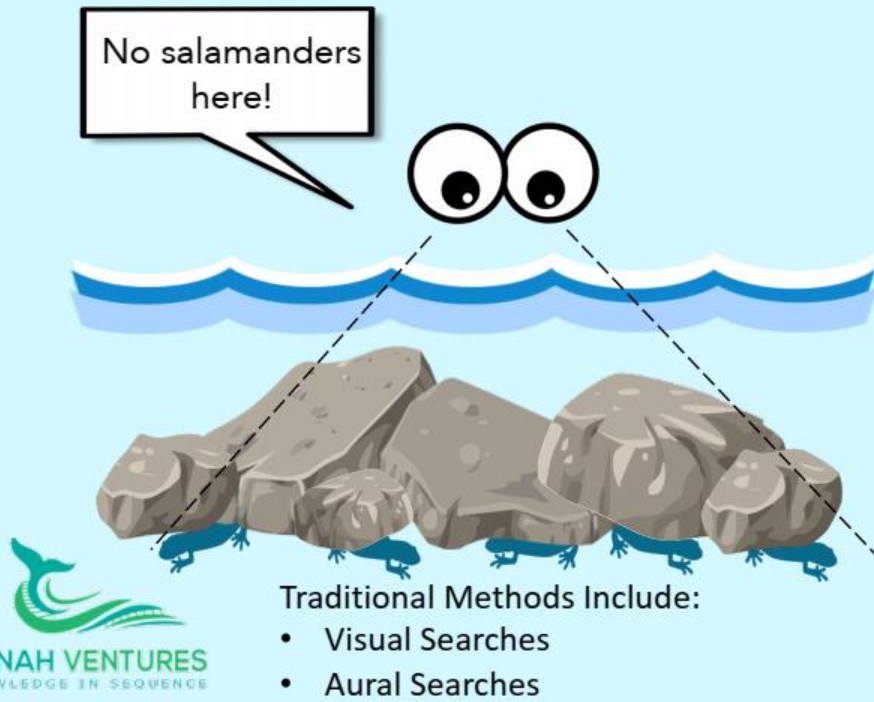
- Identified 29 sites with summer stream flow
- Visited each site 3 times May-Aug 2021
- During each visit we conducted a Visual Encounter Survey (VES)
- On final visit collected eDNA sample



Survey methods: eDNA

Advantages of eDNA

- Using eDNA can be more effective at finding species than traditional methods
 - An organism may be able to hide if you're looking for it, but it can't hide its DNA in the environment!



Environmental DNA analysis

Samples sent to Jonah Ventures for processing

Next Generation Sequencing used to search for all amphibians

- eDNA in sample amplified via PCR using amphibian specific primers
- Amplified strands sequenced using Illumina MiSeq machine
- Sequences compared to eDNA library
- Matches and near matches reported



Results: Species observed

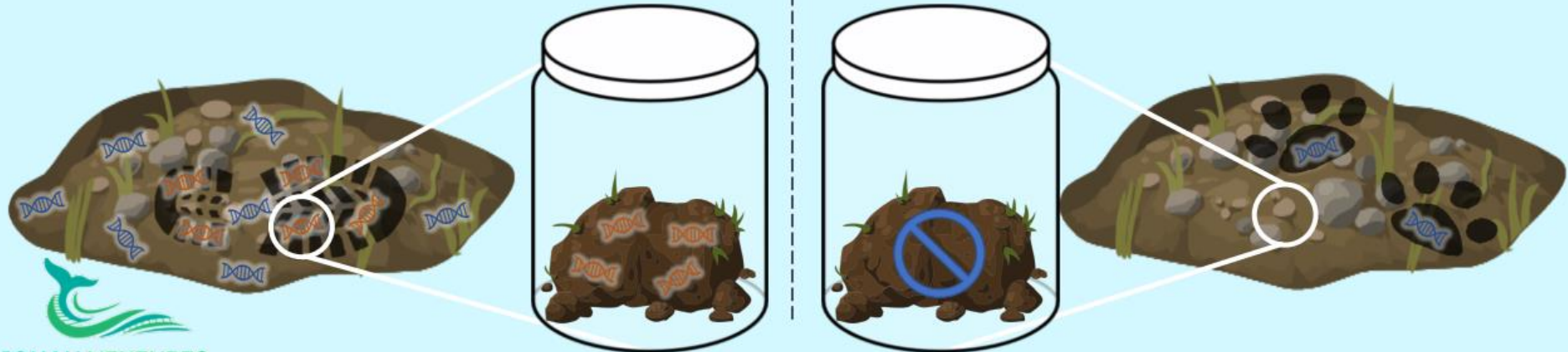
Amphibians with published species ranges encompassing study area

Common name	Genus species	Primarily Aquatic	Observed
California slender salamander	<i>Batrachoseps attenuatus</i>	N	-
Ensatina	<i>Ensatina eschscholtzii</i>	N	-
Arboreal salamander	<i>Aneides lugubris</i>	N	-
Black salamander	<i>Aneides flavipunctatus</i>	N	V
Wandering salamander	<i>Aneides vagrans</i>	N	V
Southern Torrent Salamander	<i>Rhyacotriton variegatus</i>	Y	V
Northwestern salamander	<i>Ambystoma gracile</i>	Y	V
Coastal giant salamander	<i>Dicamptodon tenebrosus</i>	Y	V,e**
Rough-skinned newt	<i>Taricha granulosa</i>	Y	V
Red-bellied newt	<i>Taricha rivularis</i>	Y	e
Pacific chorus frog	<i>Pseudacris regilla</i>	tadpole only	V
Foothill yellow-legged frog	<i>Rana boylei</i>	Y	V,e
Northern red-legged frog	<i>Rana aurora</i>	Y	V,e
Western toad	<i>Anaxyrus boreas</i>	Y	V,e*
Tailed frog	<i>Ascaphus truei</i>	Y	V,e
Bullfrog	<i>Lithobates catesbeianus</i>	Y	-

- Results: A deeper look at eDNA results
- False positives and false negatives

Limits of eDNA

- False positives: when a species is detected in a sample even though it does not occur there
 - Can be caused by DNA being brought to a site on equipment or other contamination
- False negatives: when a species is NOT detected in a sample even though it occurs there
 - Can be caused by collecting samples that are too small, not collecting in the correct area, DNA degradation, etc.



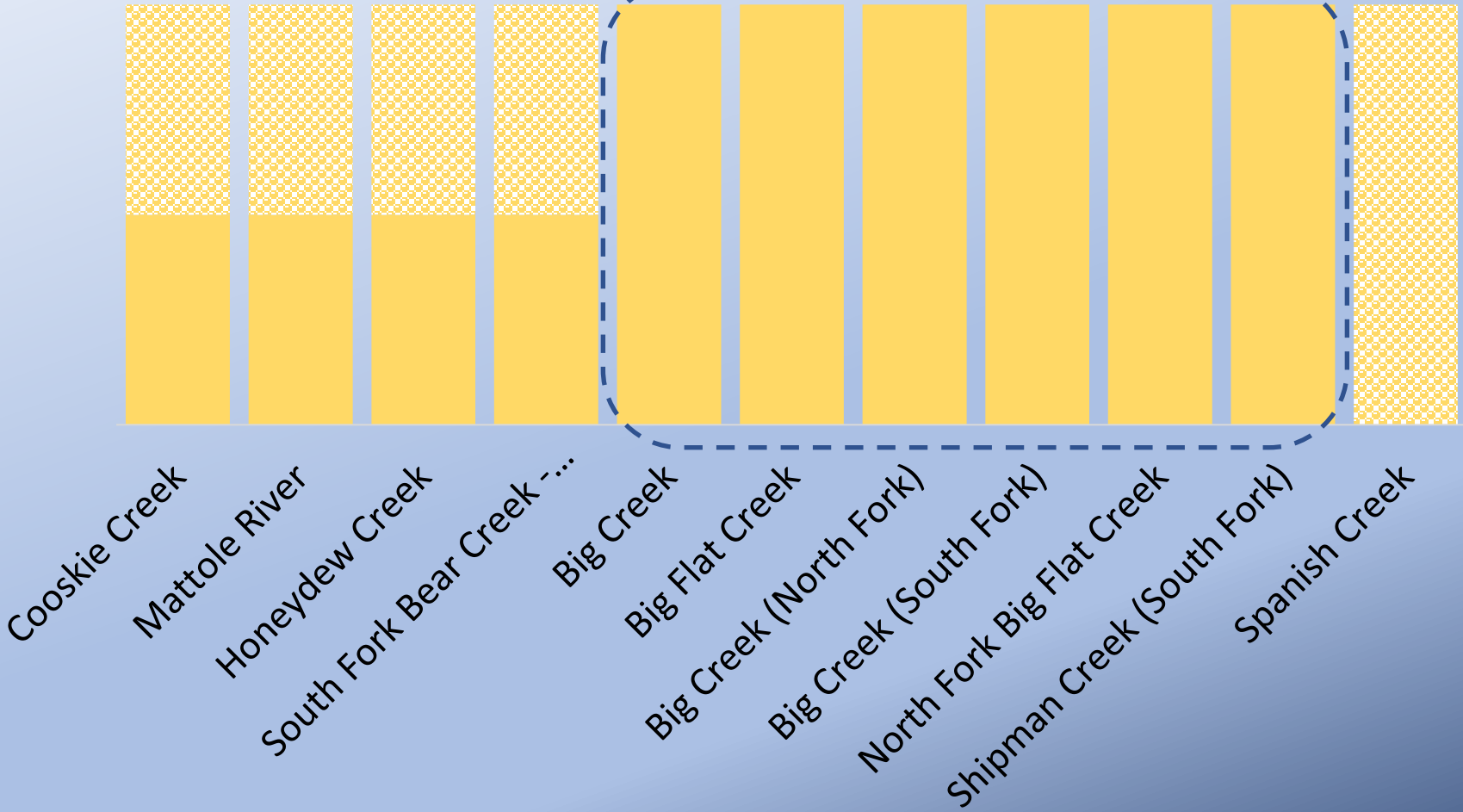
Results: A deeper look at eDNA results
 - False positives

Taxa	Common name	# sequences	ave seq length (bp)	ave % match
<i>Batrachuperus tibetanus</i>	Tibetan mountain salamander	1	44	77.3
<i>Dicamptodon spp</i>	Unresolved giant salamander	24	49.8	89
<i>Ascaphus truei</i>	Tailed frog	1	55	94.5
<i>Rana boylei</i>	Foothill yellow-legged frog	14	52	97.6
<i>Anaxyrus spp</i>	Unresolved toad	7	52	98.6
<i>Taricha rivularis</i>	Red-bellied newt	2	53	99.05
<i>Rana aurora</i>	Northern red-legged frog	1	51	100

Results: A deeper look at eDNA results

- False negatives

■ Yellow- Legged Frog (VES) ■ Yellow- Legged Frog (eDNA)



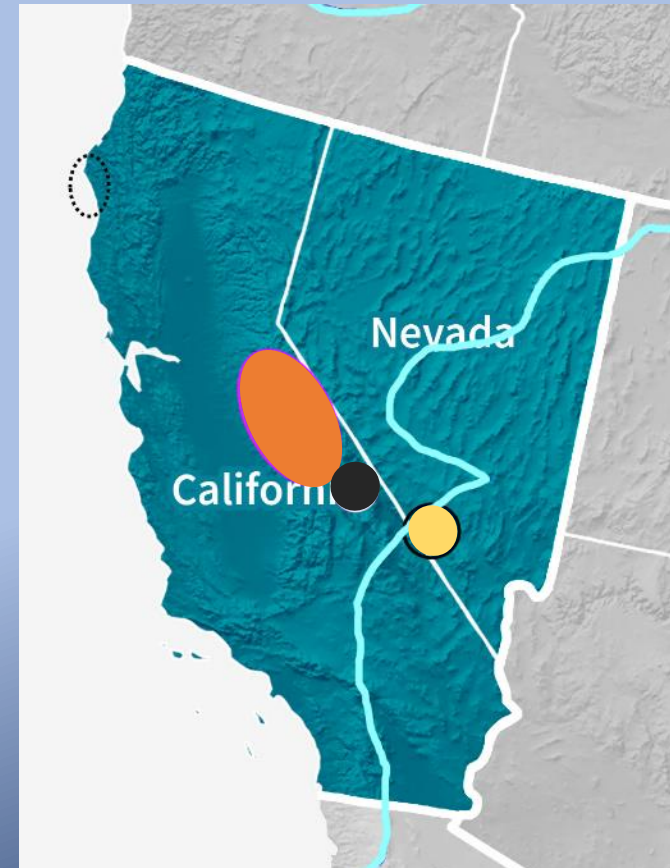
Results: A deeper look at eDNA results

- Unresolved taxa: Unresolved toad

Species	# sequences	Mean % Match	Max % Match
<i>Anaxyrus canorus</i>	7	98.6	100
<i>Anaxyrus exsul</i>	7	99.1	100
<i>Anaxyrus nelsoni</i>	7	98.5	100
<i>Anaxyrus boreas</i>	7	98.8	100



Western toad

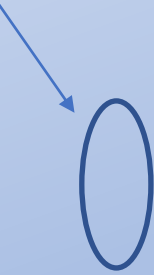


Results: A deeper look at eDNA results

- Unresolved taxa

Species	# sequences	Mean % Match	Max % Match
<i>Dicamptodon aterrimus</i>	24	89.0	94.1
<i>Dicamptodon ensatus</i>	24	89.0	94.1

Idaho



Results: A deeper look at eDNA results

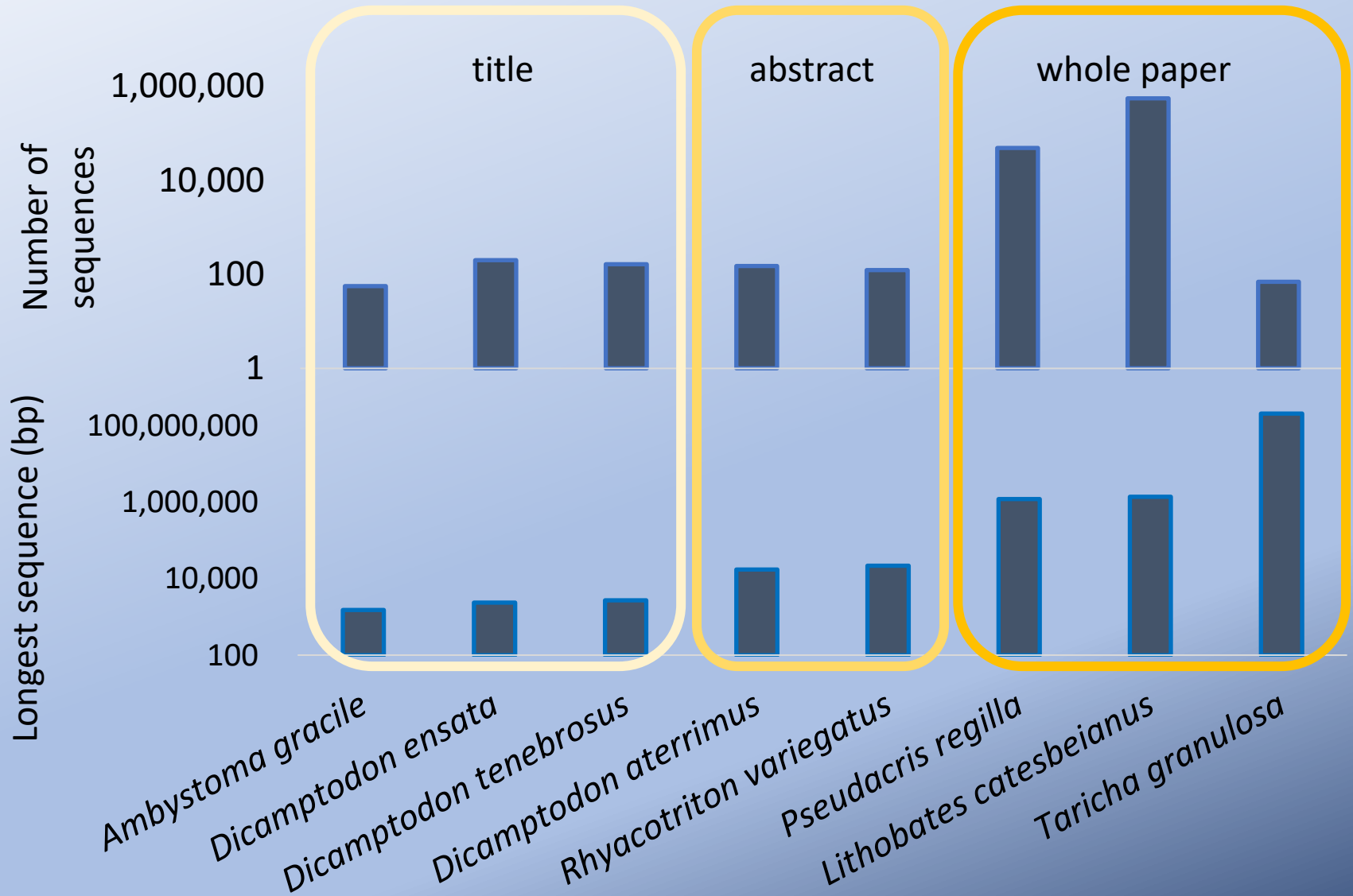
- Unresolved taxa

Species	# sequences	Mean % Match	Max % Match
<i>Dicamptodon aterrimus</i>	24	89.0	94.1
<i>Dicamptodon ensatus</i>	24	89.0	94.1
<i>Dicamptodon tenebrosus</i>	0		

- Range expansion?
- False positive + false negative?



Results: A deeper look at the eDNA library



Conclusions

- Low DNA library coverage affects eDNA survey results and interpretation
- No survey method perfect
- Multiple survey methods are better than one
- Lost coast supports an intact amphibian community



Acknowledgments

BLM:

Zane Ruddy

Michael Holt

CDFW:

Michael Van Hattem

