

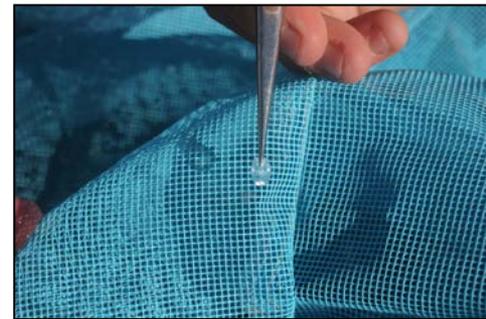
Summary of diet information by season based on prey remains and fecal samples

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DFO: John Ford, Graeme Ellis

Data sources for seasonal variation

- Prey remains (scales, tissue)
- Fecal samples
 - PCR based presence/absence
 - Quantification of species-specific DNA
 - PCR – cloning
 - PCR – high throughput sequencing



Methods: Cloning & NGS – 454Roche

1. Extract DNA from fecal samples (collected over a period of 8 years !)
2. Pool DNA extractions by month (all months *except* Jan, Feb, Mar, and Apr are well represented)
3. PCR amplify 16s region using custom primers that amplify all prey species simultaneously (but do not amplify killer whale DNA).
4. Clone & Sequence individual PCR products
5. NGS: Label and Sequence individual PCR products using 454Roche platform.

Sequence Data Analysis / Bioinformatics

How do you analyze 40,000 DNA sequences?

- Quality filter for read length
- Reference database determines the reliability and accuracy of taxon identification.
 - Used two independent alignment programs (blastn & bowtie)
 - Aligned to both genbank and to custom prey database
- Post processing spot checking of sequences that hit to unexpected targets

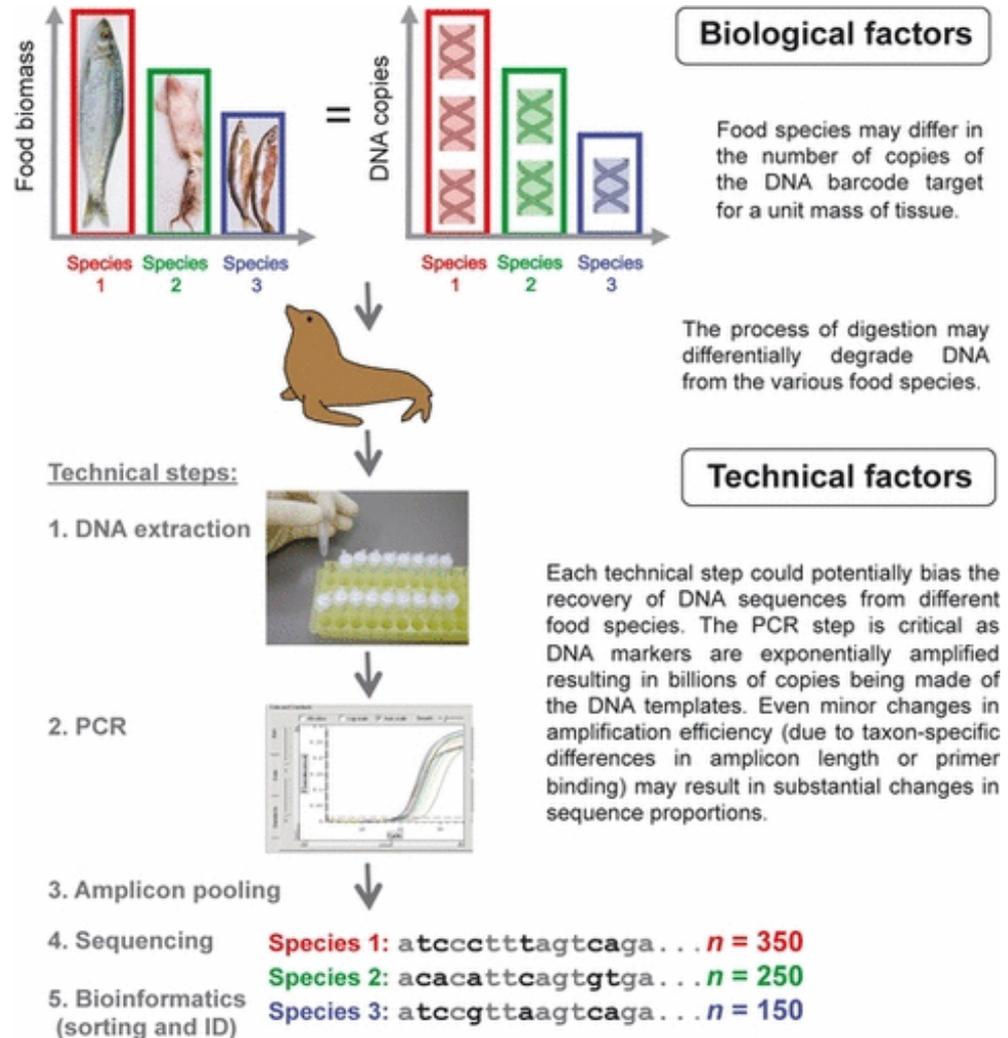
From Pompanon et al. "Who is eating what: diet assessment using next generation sequencing"

Potential Sources of Bias:

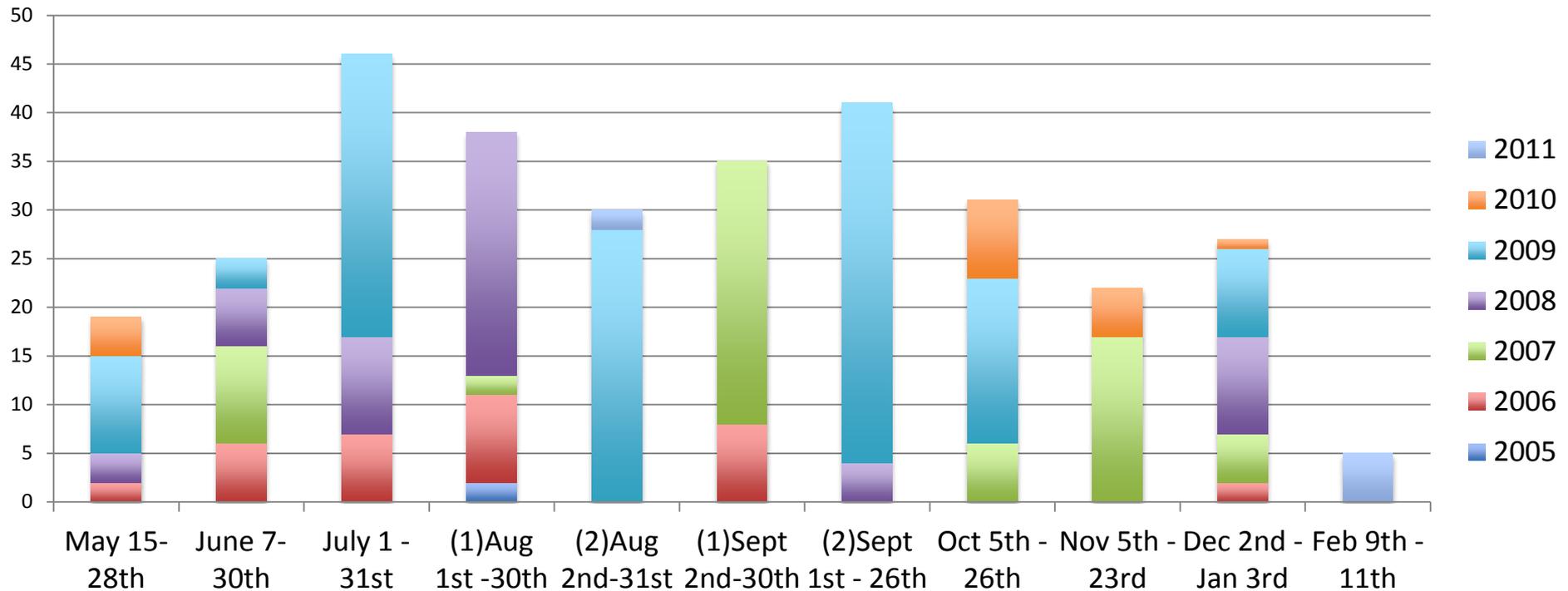
-Inter and intraspecific variation in gene copy number (esp. mtDNA)

-Differential survival of DNA during digestion and differences in the state of digestion.

- 2% difference in amplification efficiency between two initially equal targets can lead to a 30% divergence in DNA copy number over 35 cycles.

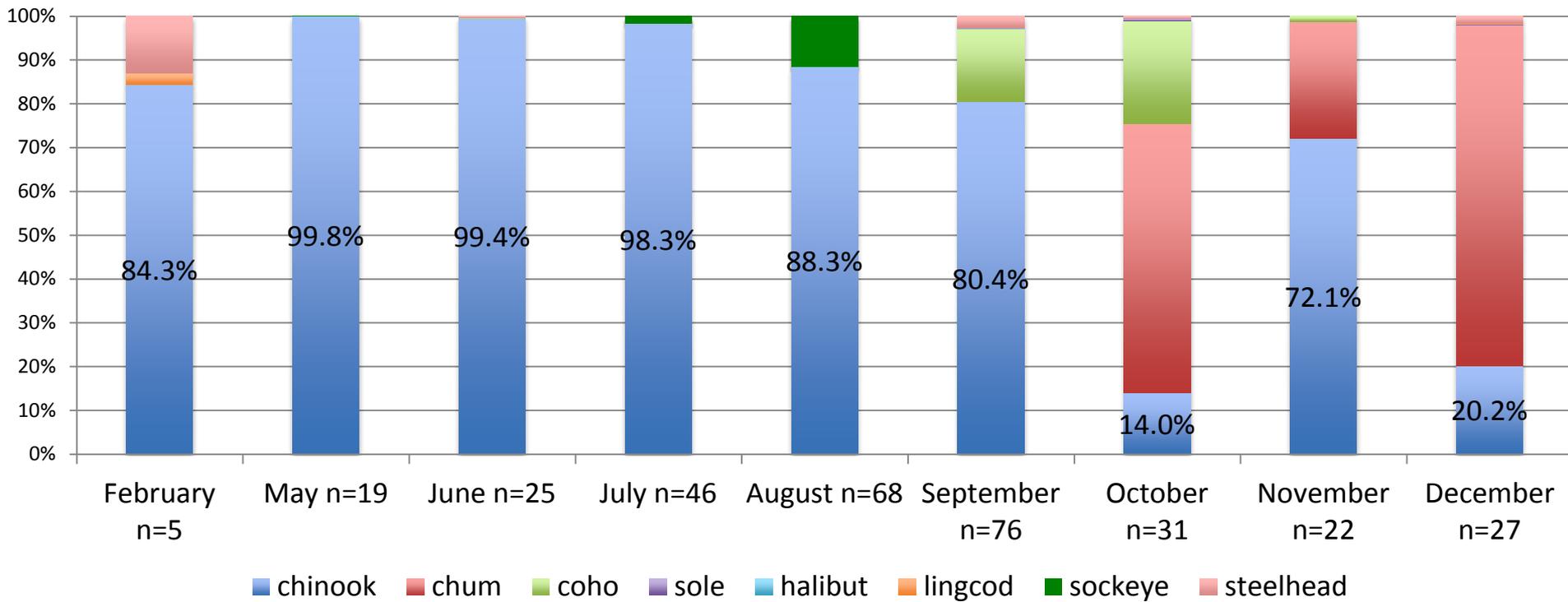


Sequencing – samples sizes



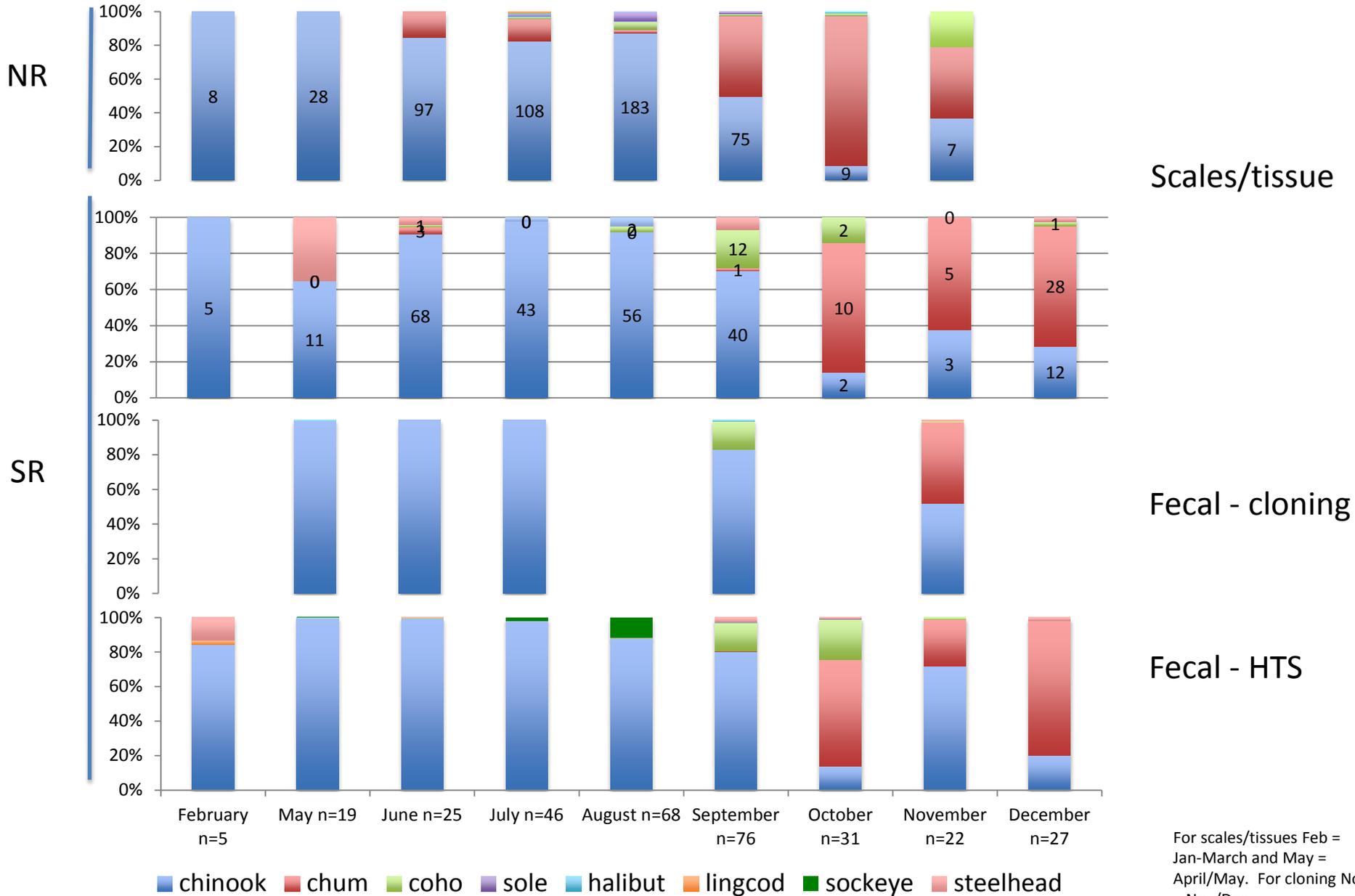
Total sample size = 319 feces

Sequencing -- results



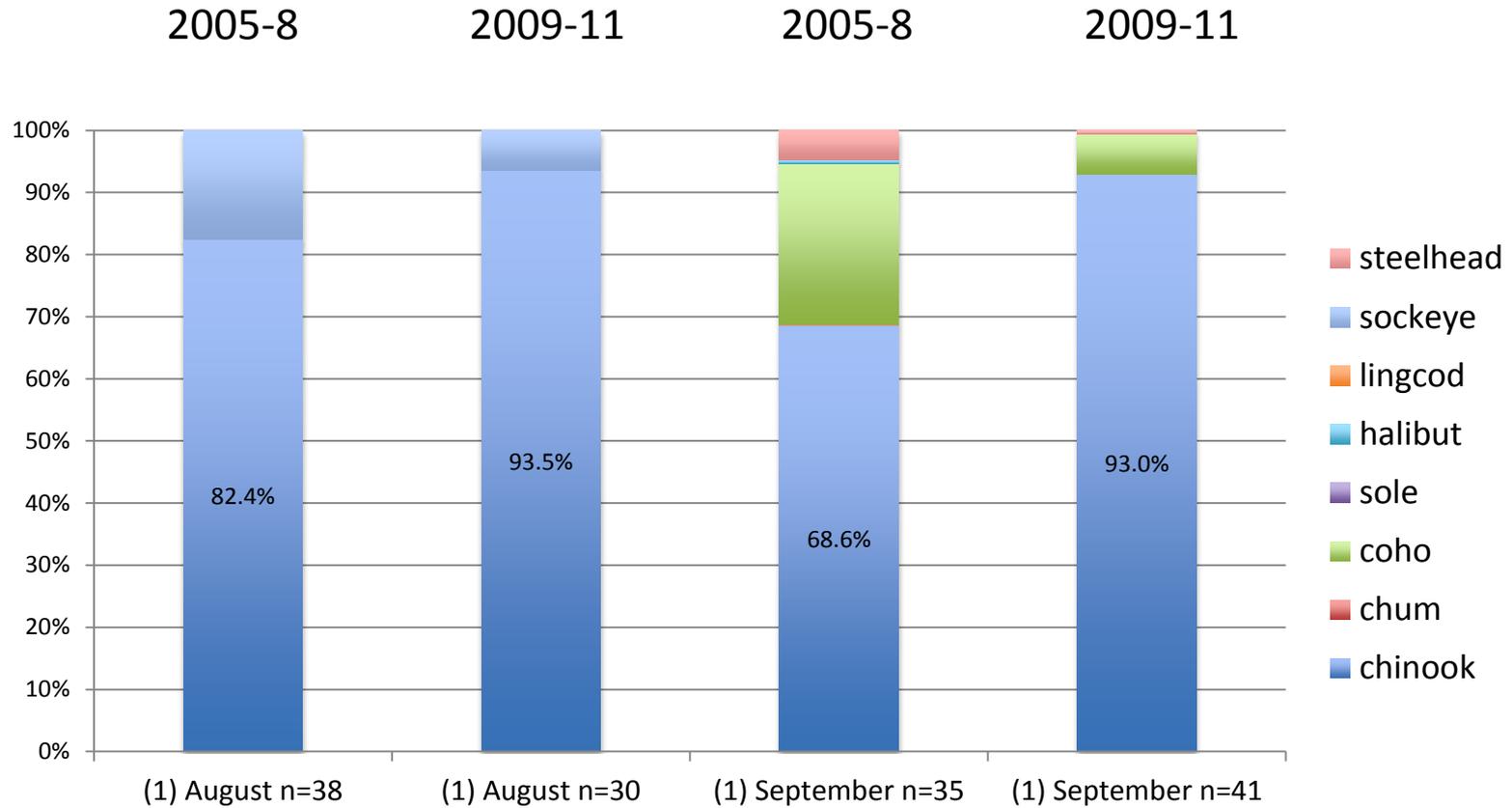
Sequences reads / month ranged from 3822 – 8758 = 46,940 sequences

Overall summary

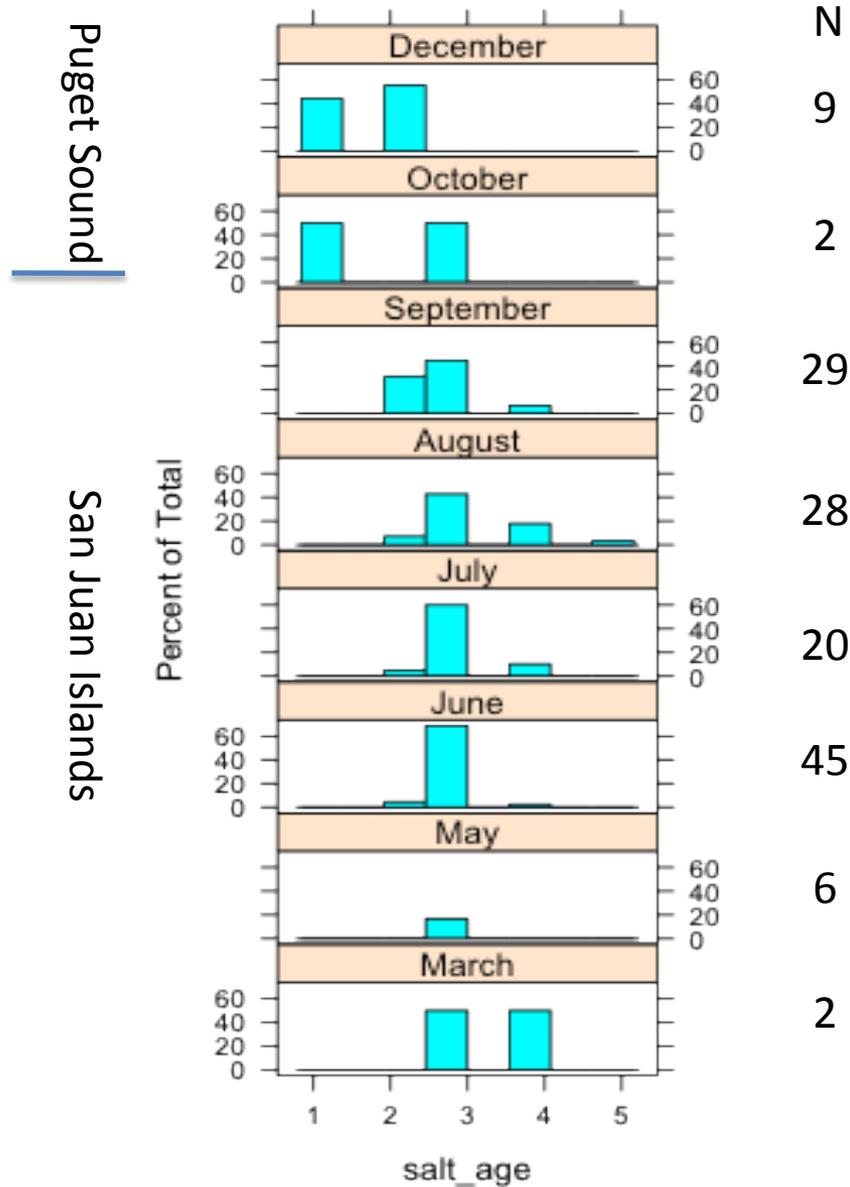


For scales/tissues Feb = Jan-March and May = April/May. For cloning Nov = Nov/Dec.

Sequencing – variation among years



Chinook ages by season



Summary

- Total sample size = >1000 predation events, >300 fecal samples
 - June – Oct dominate
- Overall (fecal samples, predation events)
 - May-July == Chinook
 - August-Sept == mostly Chinook plus others (sockeye, coho)
 - Oct – Dec == mostly Chum, some Chinook
 - Jan – Mar == Chinook (small sample size)
- SR and NR similar
 - Transition to chum maybe a bit earlier in NR (NR-Sept; NR-Oct)
- Different methods (scales vs fecal samples) provide similar results