

# eDNA metabarcoding and upwelling in the Pacific Coast

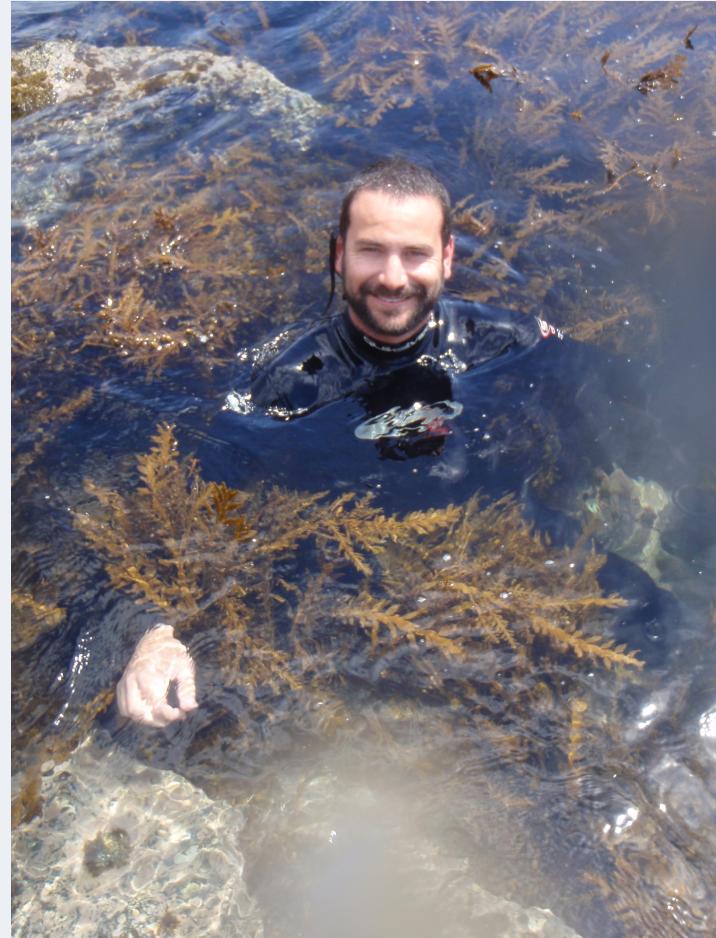


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Ramón Gallego Simón

# Introductions

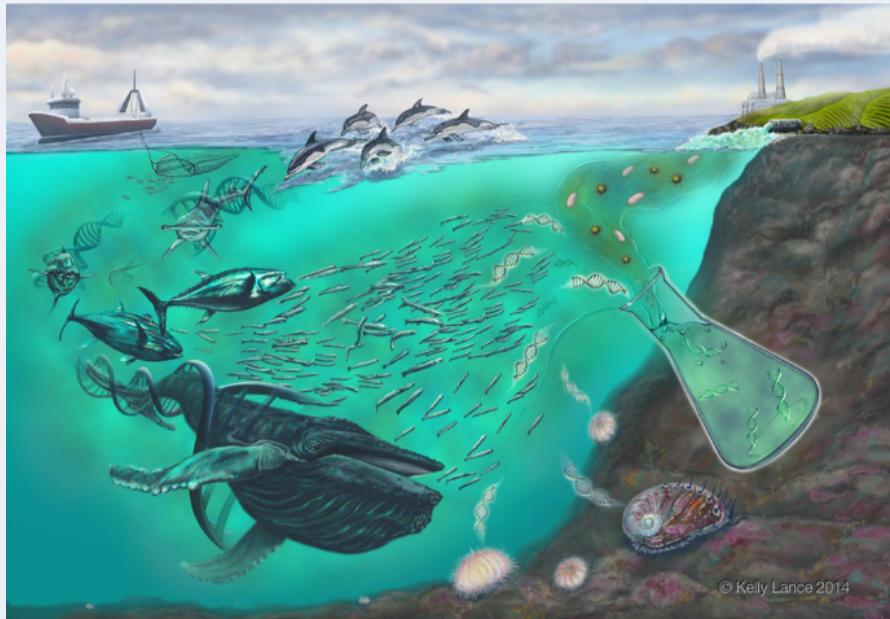
## Ramón Gallego



# Introductions

## environmental DNA

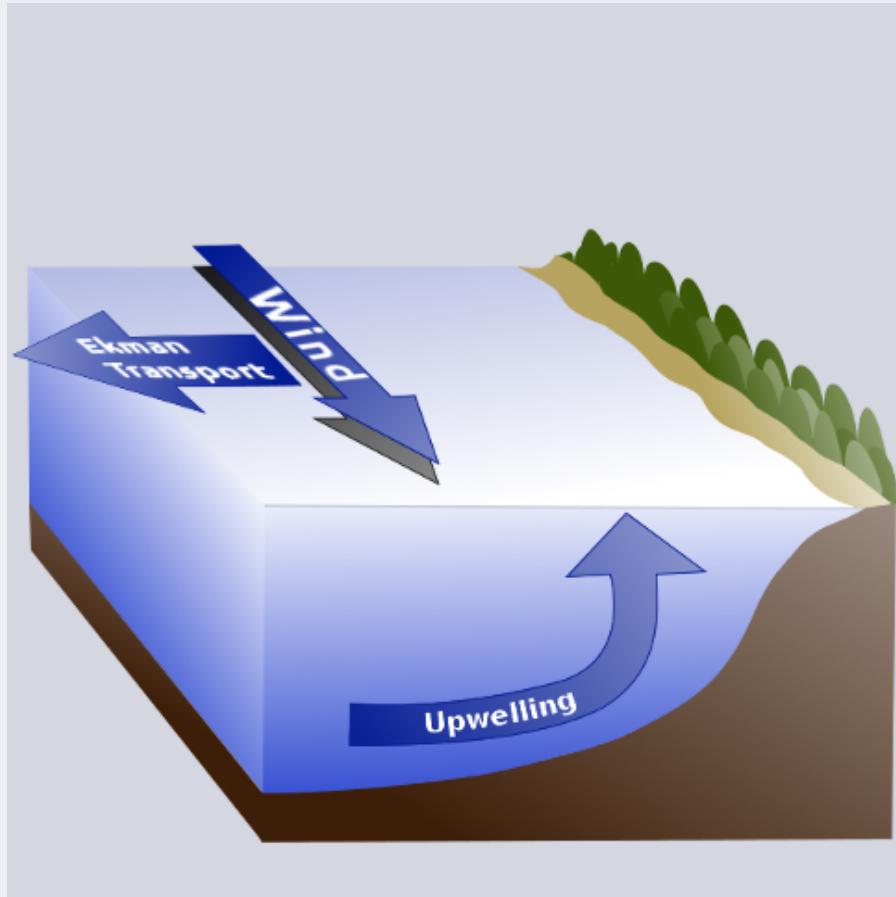
- DNA that can be extracted from environmental samples (such as soil, water or air), without first isolating any target organisms, Taberlet et al. 2012



# Introductions

## Coastal Upwelling

- Prevailing winds displace surface waters
- Deep water comes to surface
- Brings up nutrient rich, low oxygen water



# Hake survey

Integrated ecosystem acoustic and trawl survey

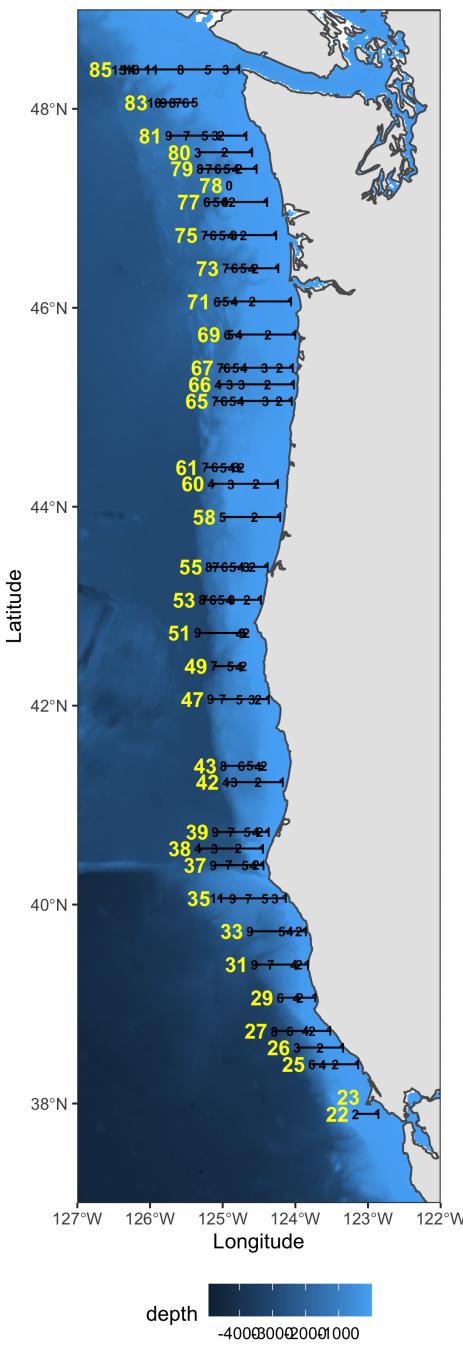
# Hake

*Merluccius productus*

- semi-pelagic fish
  - bottom during the day, up the water column during the day
- Largest fishery on the West Coast by volume
  - 440,942 t in 2017

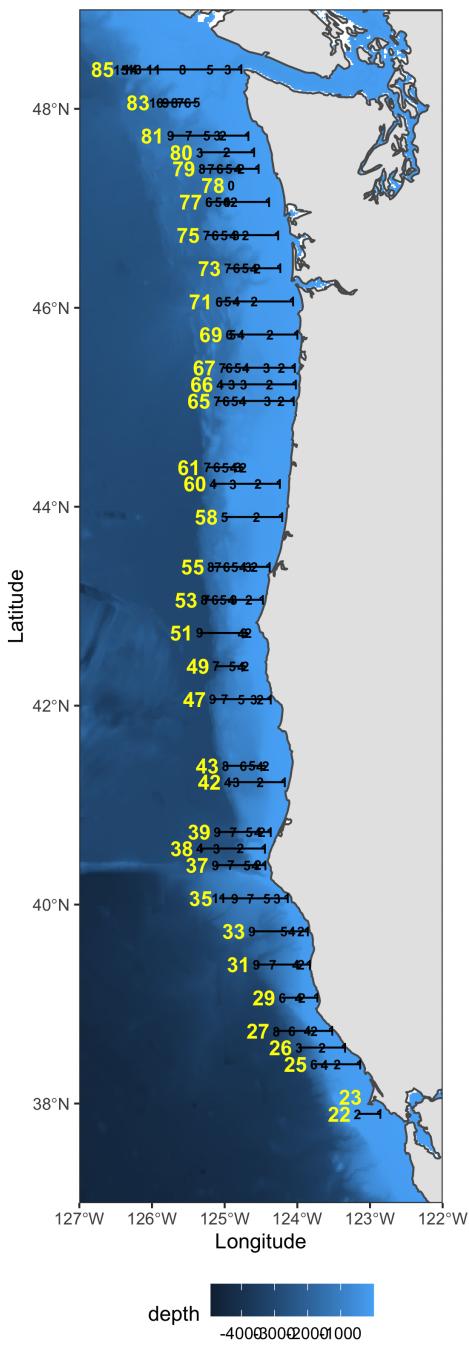


# Hake survey

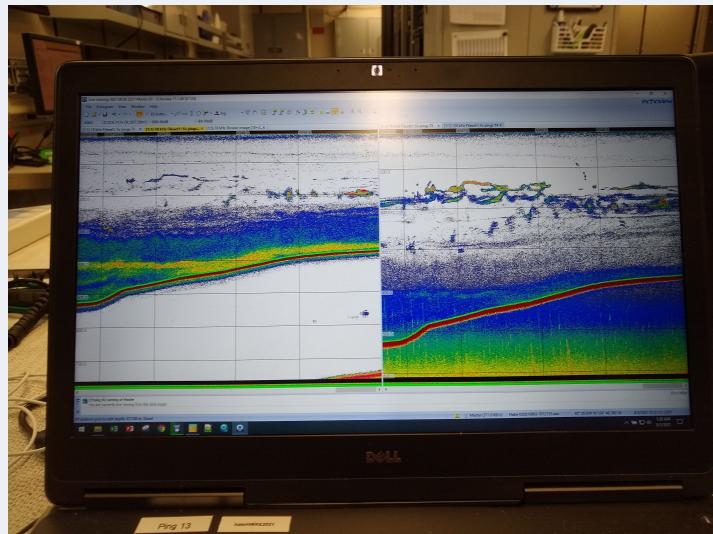


- Run every two years
- Covers onshore/offshore transects
- Estimates Hake abundance, age classes, maturity
- Uses acoustic signal + trawl
- In 2019 started an eDNA program
- Sampling spans ~ 2 months

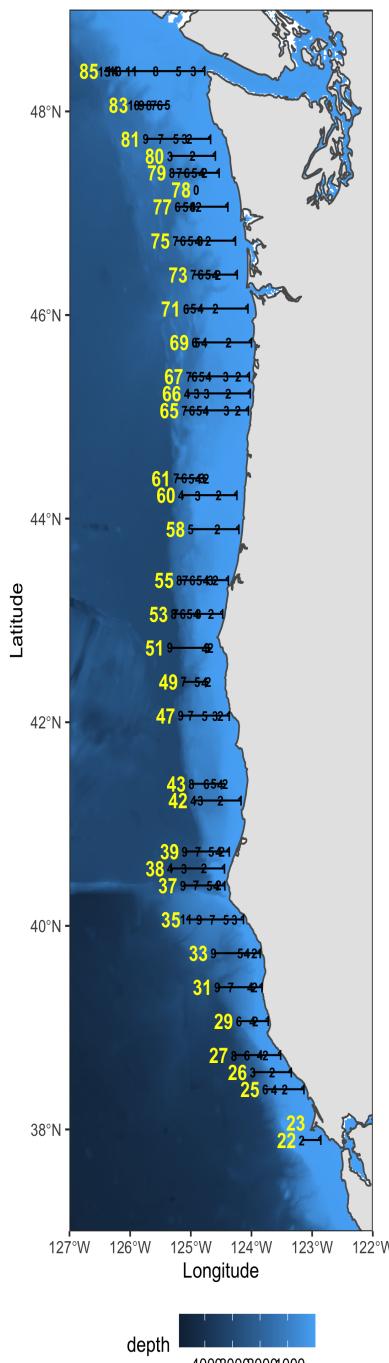
# Acoustics Component



- Distinct backscatter pattern
- Fish ID corroborated with trawls
- Stock biomass
- Age-class & maturity information



# eDNA Component

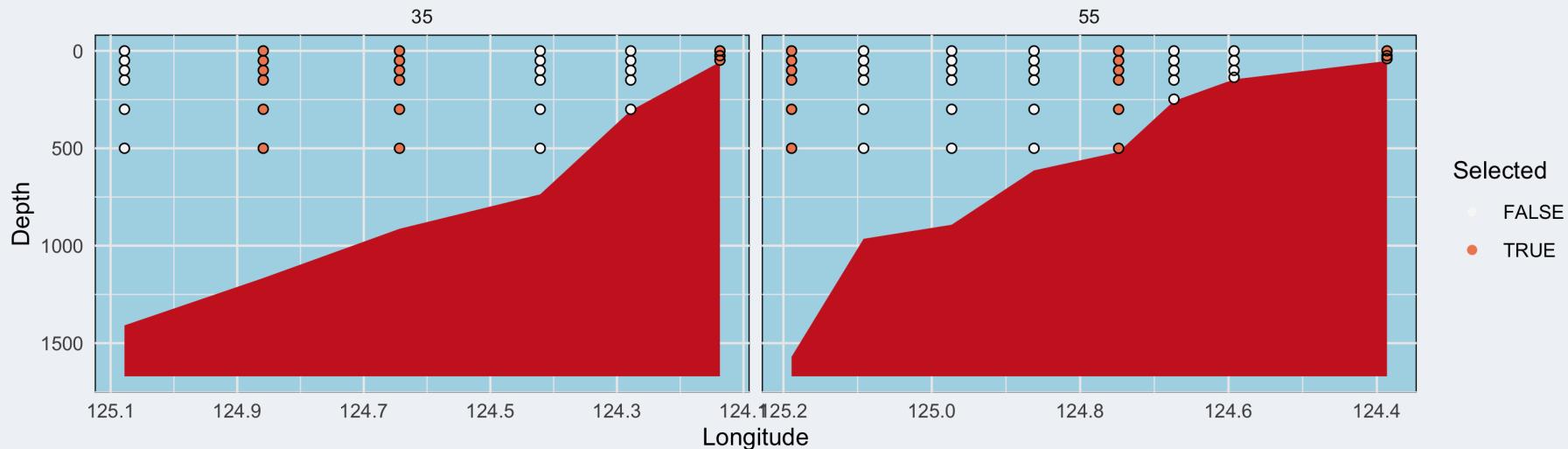


- 1930 eDNA samples
- From 36 longitudinal transects
- ~ 6 stops per transect
- ~ 5 depths per stop
- 2 Niskins bottles per depth (2.5L per Niskin = 1 eDNA sample)
- >350 samples from Flow-through system
- All working from sunset to sunrise



# Where do we sample?

- Near the surface
- Mid water
- Up to 500m depth
- So in some stations surface–midwater–near bottom
- in others quite far from there

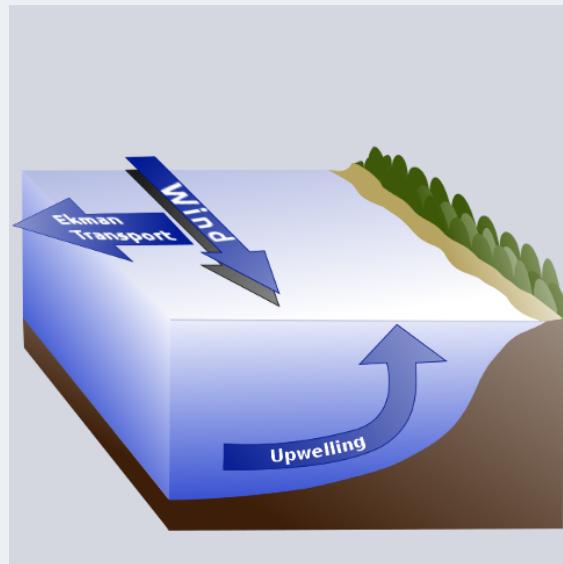


But where is this eDNA  
coming from?

# Where is eDNA coming from

Two interconnected questions:

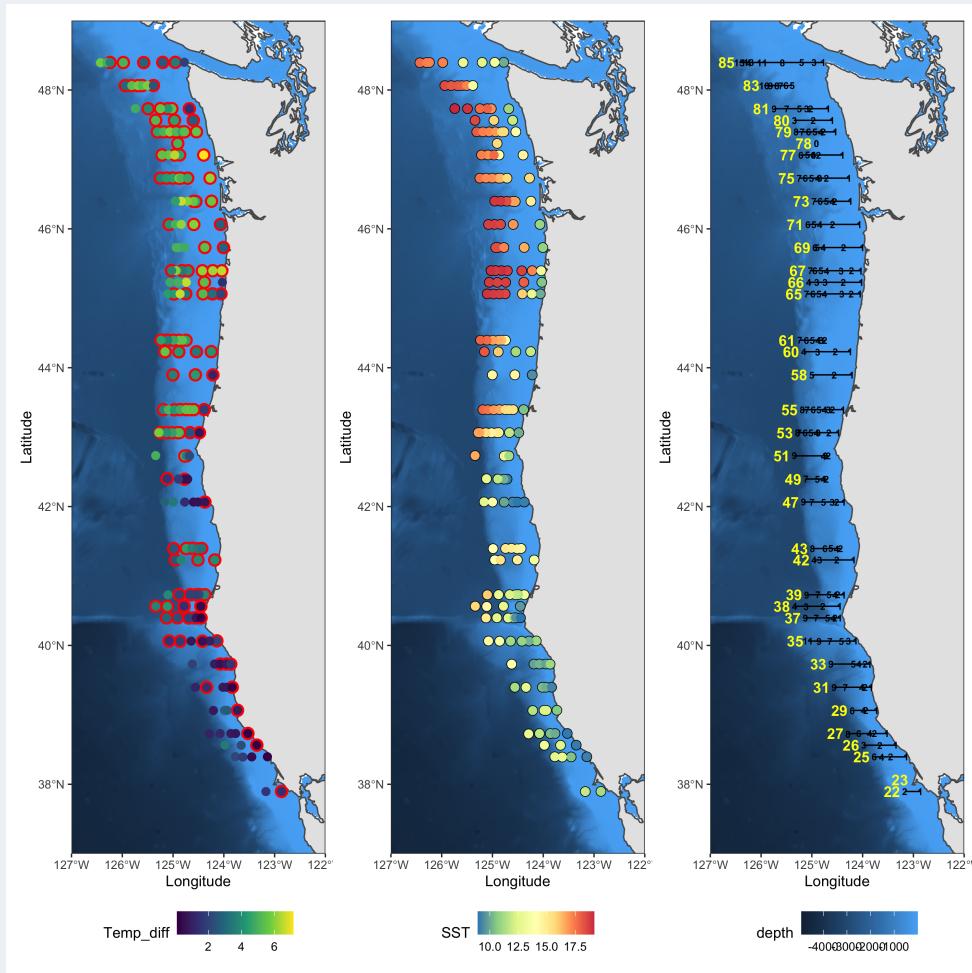
- Does upwelling bring eDNA from deeper water?
- Does an established Thermocline function as a barrier for eDNA flow?



# Experimental design

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- Choosing Transects with / without established Thermocline



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- Choosing Transects with / without established Thermocline
- Metabarcoding of eDNA targetting Fishes with 16S
  - Potentially amplifying cartilaginous and bony fishes
  - Target fragment ~ 350 bp
  - Sequencing on an Illumina MiSeq

# Experimental design

- Choosing Transects with / without established Thermocline
- Metabarcoding of eDNA targetting Fishes with 16S
- Decontamination, denoising and taxonomical assignment
  - cutadapt -> DADA2 -> Decontamination script -> insect

# Experimental design

- Choosing Transects with / without established Thermocline
- Metabarcoding of eDNA targetting Fishes with 16S
- Decontamination, denoising and taxonomical assignment
- Statistical framework
  - Community analysis:

$$\bar{Comm} \sim Transect + Depth + Upwelling$$

- Individual species analysis:

$$\bar{X}_i \sim Transect + Depth + Upwelling$$

# Results

# Results

## The numbers

- We sequenced 115 biological samples, 215 technical replicates
- After QC and taxonomical assignment: 16M reads from 138 taxa
- Identified 4 species of sharks, 4 Rays, 1 Chimaera
- 84 species of bony fishes, including 16 **Sebastes** spp.

# Results

Search:

Previous

1

2

3

4

5

...

14

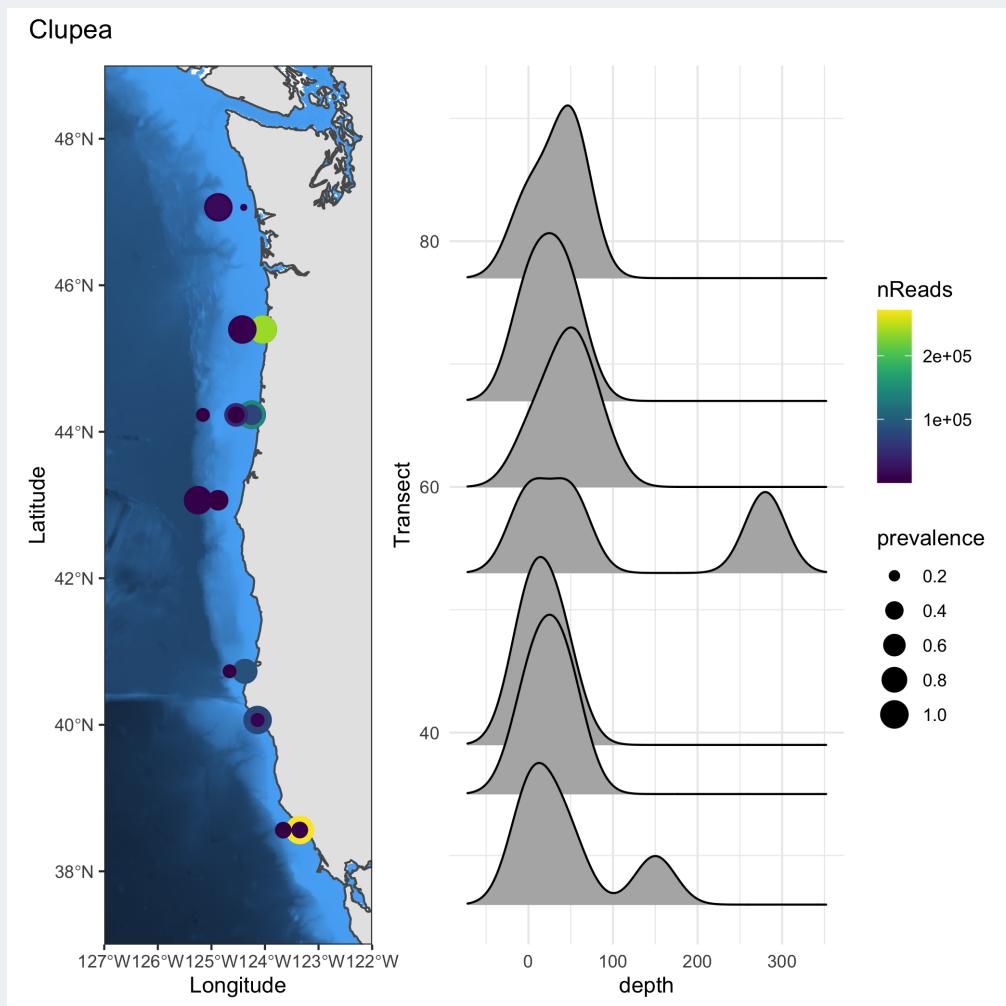
Next

Family	Species	#Sequences	#samples found
Agonidae	<i>Chesnonia verrucosa</i>	319	1
Agonidae	NA	1360	1
Alopiidae	<i>Isurus oxyrinchus</i>	1089	4
Alopiidae	<i>Lamna ditropis</i>	22409	7
Anarhichadidae	<i>Anarrhichthys ocellatus</i>	33082	4
Anarhichadidae	NA	541	1
Anoplopomatidae	<i>Anoplopoma fimbria</i>	83984	6
Anoplopomatidae	NA	28113	4
Atherinopsidae	NA	20	1
Bathylagidae	<i>Bathylagus stilbius</i>	74913	22 / 32

# Results

## Species by species exploration

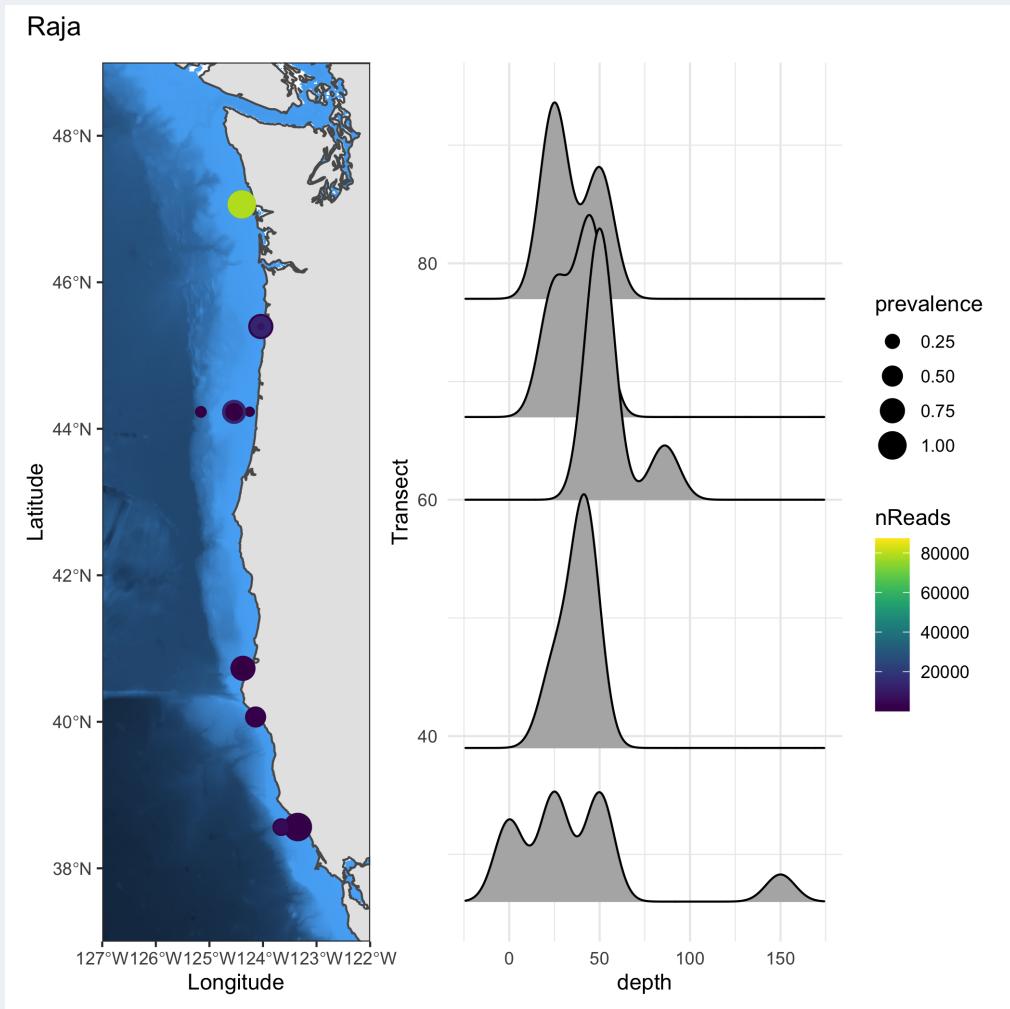
- Based on each species life-history, we can trace changes in relative abundance and prevalence



# Results

## Species by species exploration

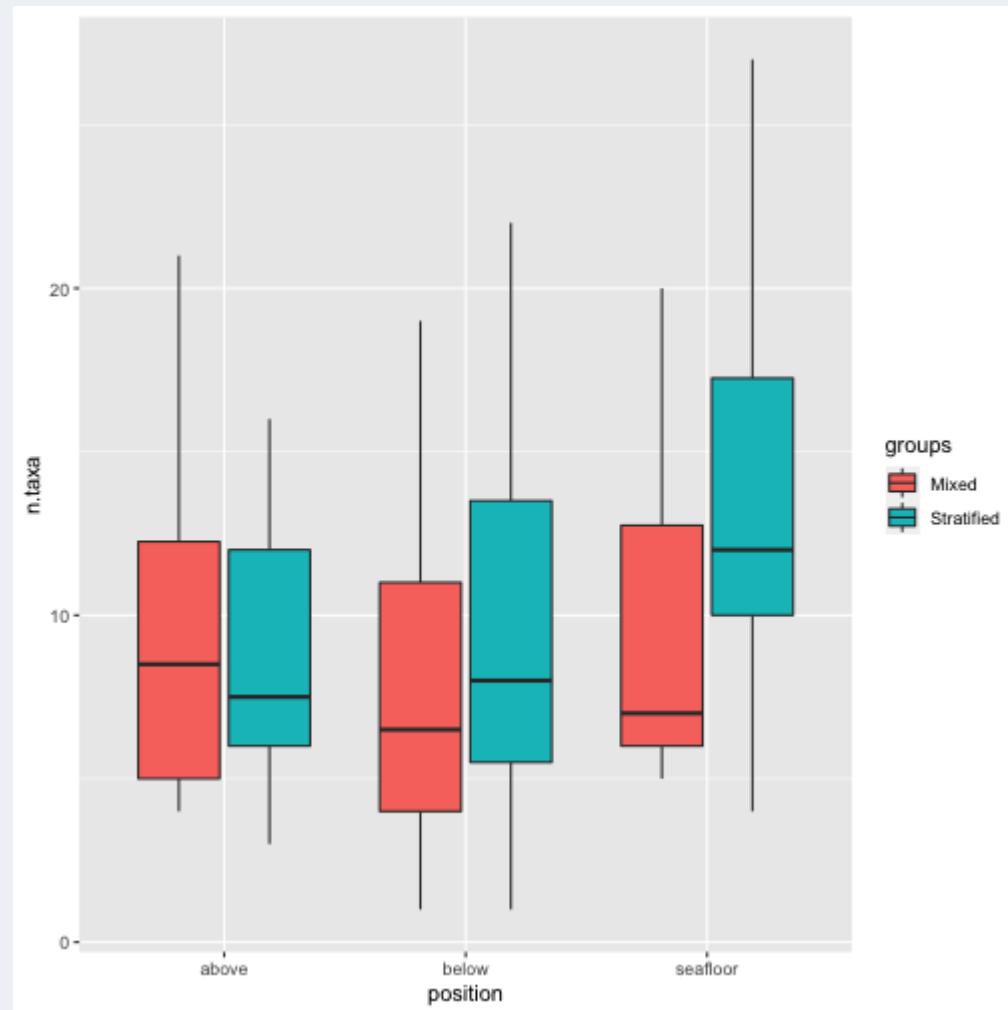
- Deep water species are close to the bottom, but also show up near surface



# Results

## Alpha diversity

- Not clear patterns



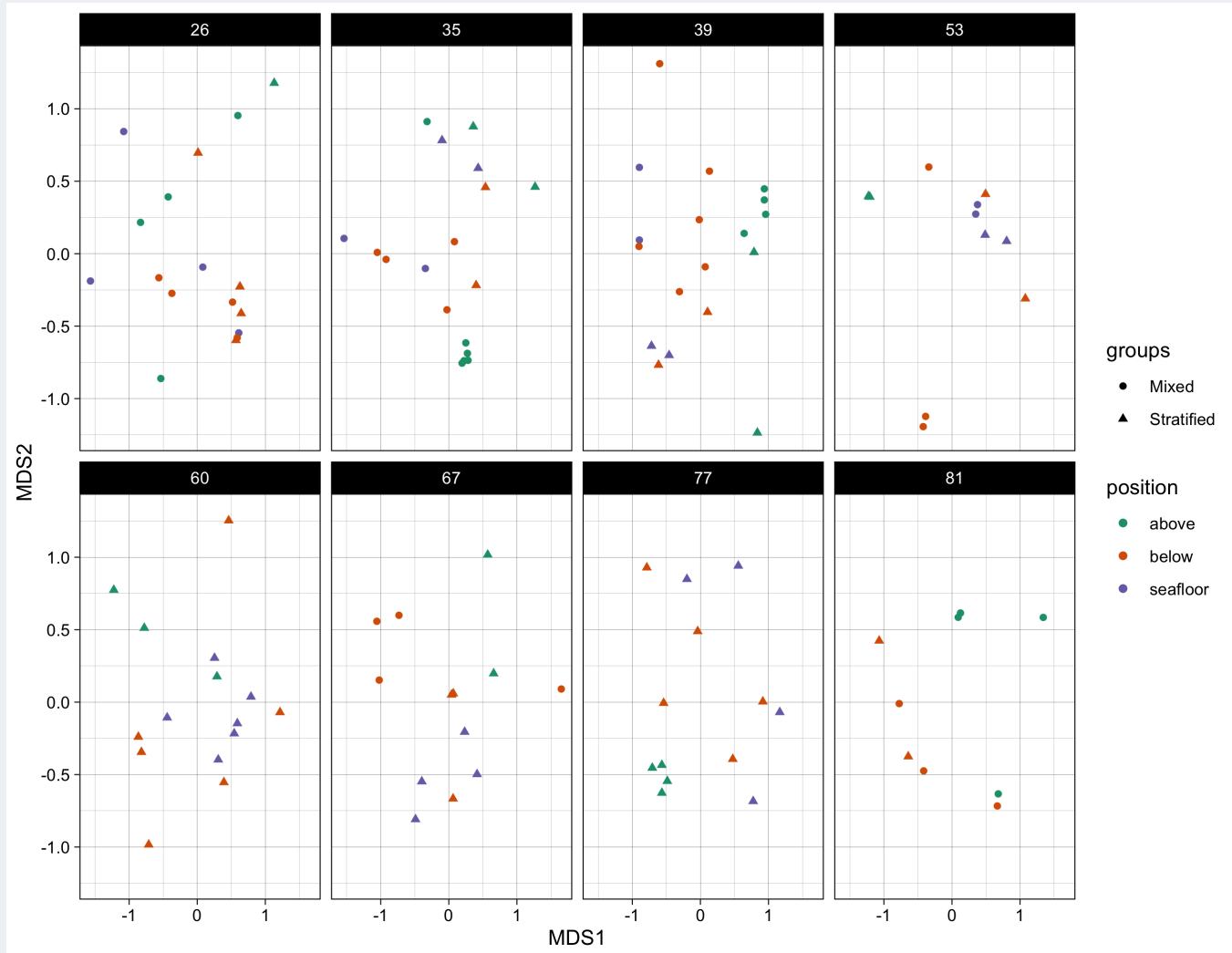
# Results

## Community Composition Analysis

- The building block is a calculation of sample dissimilarity
- No use of raw number of reads → usually a transformation
  - Balance between importance of rare vs dominant species
- Different algorithms from community ecology
  - Ditto plus different algorithms track different changes in community composition

# Results

## Community Composition Analysis



# Results

## Community Composition Analysis

- PERMANOVA: Using the Formula

$$\text{Bray} - \text{Curtis} \sim \text{Transect} + \text{Depth} + \text{Upwelling}$$

term	df	SumOfSqs	R2	statistic	p.value
Transect	7	5.56	0.11	1.95	0.0001
Position	2	3.03	0.06	3.71	0.0001
Upwelling	1	0.65	0.01	1.60	0.0255
Residual	103	42.00	0.82		
Total	113	51.24	1.00		

- PERMANOVA, and BC use one value to capture dissimilarity between two samples

# Results

## Community Analysis – Another way

- Our hypothesis can better be tested using a Dirichelet Regression:  
so our Community in the original

$$\bar{Comm} \sim Transect + Depth + Upwelling$$

is not constrained to one value per sample pair, but a matrix of proportions

Sample	Spp1	Spp2	Spp...	Sppn
S1	50	300	–	5000
S2	4000	250	–	300
...	0	0	–	0
Sn	0	400	–	350

# Results

## Community Analysis – Another way

- Our hypothesis can better be tested using a Dirichlet Regression:  
so our Community in the original

$$\bar{Comm} \sim Transect + Depth + Upwelling$$

is not constrained to one value per sample pair, but a matrix of proportions

Sample	Spp1	Spp2	Spp...	Sppn
S1	0.01	0.06	–	0.93
S2	0.88	0.05	–	0.07
...	0.00	0.00	–	0.00
Sn	0.00	0.53	–	0.47

# Results

## Community Analysis – Another way

- Dirichlet does not bode well with many zeroes
- Combining taxa into functional categories

Group	Number of taxa	Number of families
Marine; bathydemersal	2	1
Marine; bathypelagic	19	8
Marine; benthopelagic	2	2
Marine; brackish; benthopelagic	2	2
Marine; brackish; demersal	2	1
Marine; brackish; pelagic–neritic	3	1

# Thanks!

## Acknowledgments

- The Captain, crew and Scientists from the RV Shimada
- The NWFSC – Genetics Lab
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Slides created via the R package [xaringan](#).