Can DNA-metabarcoding of multi-species samples from routine egg surveys be used to monitor the ichthyoplankton assemblage?

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Government of South Australia
Department of Primary Industries and Regions

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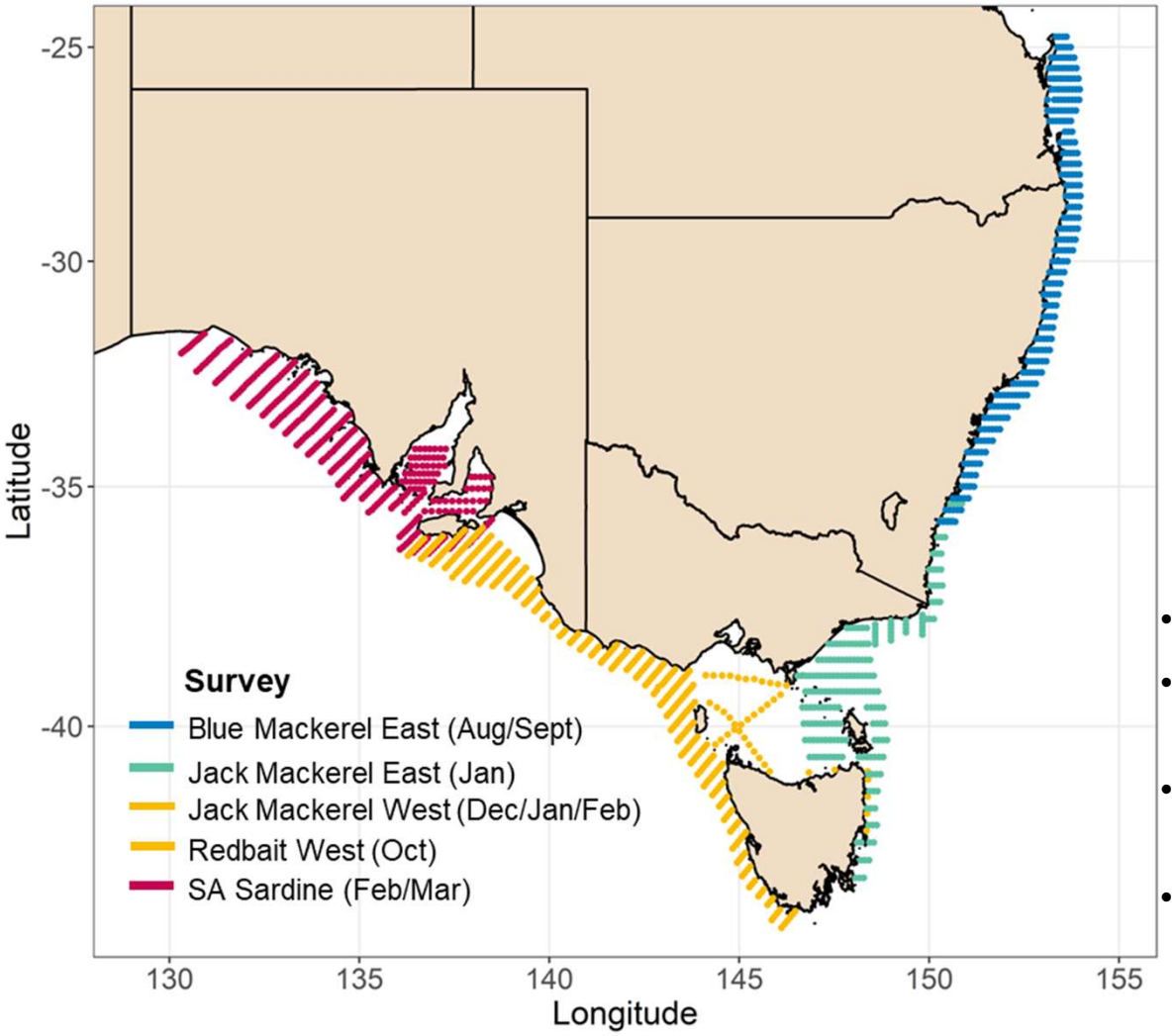
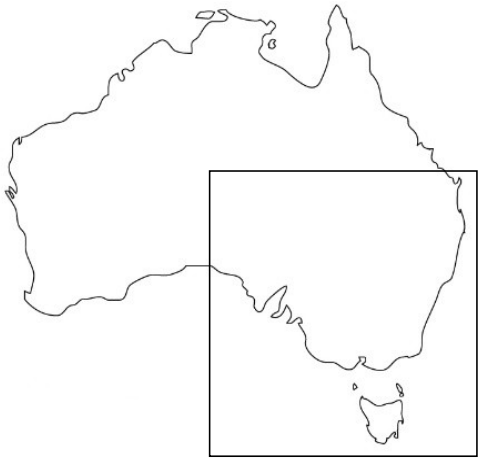
Broad-scale Egg Surveys





- ~45 surveys of SE Australia since 1995
- Each survey covers 60,000 to 150,000 km^2 and includes hundreds of samples
- sardine, jack mackerel, redbait, blue mackerel
- Used to set TACs in SA Sardine Fishery & Commonwealth SPF (TACs~80,000 t)
- **<u>Can we value-add?</u>**

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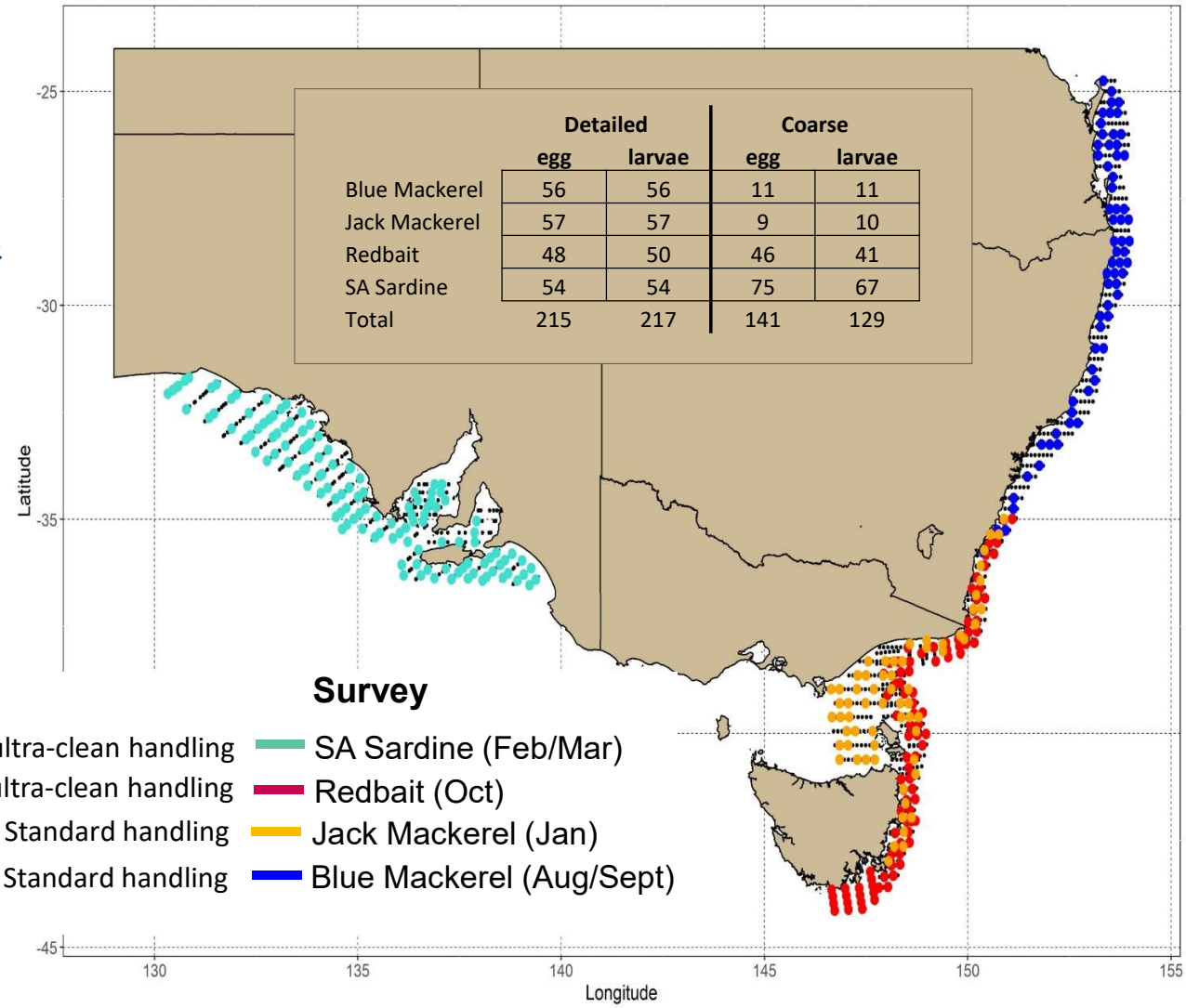
Can we use DNA metabarcoding to identify fish species in a plankton sample?

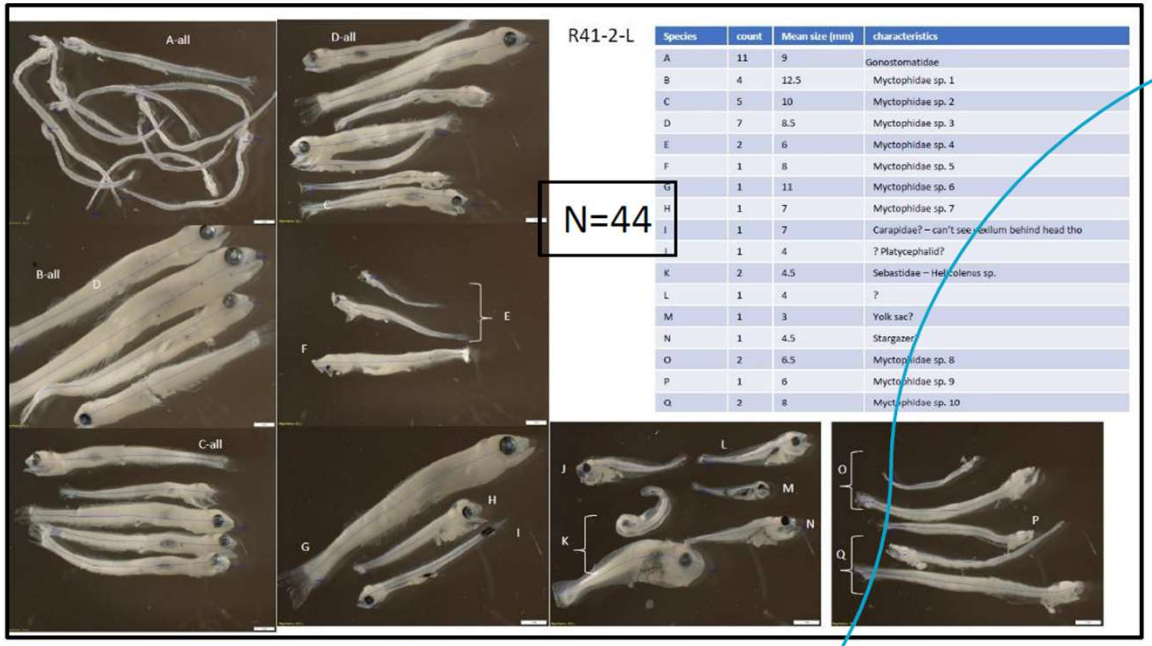
- Samples from 4 surveys
  - different handling methods
  - different species assemblages (seasons & depths)
- Fish larvae and eggs separated
- Morphological identification
- Tested 2 mtDNA-metabarcoding assays
  - Assays target different ends of COI gene
  - Cytochrome oxidase sub-unit I (COI)
    - COI_E (Shokralla et al. 2015):
      - LH (5') end
    - COI_L (Leray et al. 2013):
      - RH (3') end

	Detailed		Coarse	
	egg	larvae	egg	larvae
Blue Mackerel	56	56	11	11
Jack Mackerel	57	57	9	10
Redbait	48	50	46	41
SA Sardine	54	54	75	67
Total	215	217	141	129



Bulked Larval Libraries

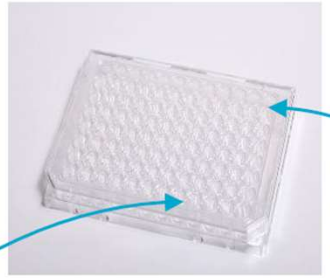
Detailed morpho



R41-2-L

Species	count	Mean size (mm)	characteristics
A	11	9	Gonostomatidae
B	4	12.5	Myctophidae sp. 1
C	5	10	Myctophidae sp. 2
D	7	8.5	Myctophidae sp. 3
E	2	6	Myctophidae sp. 4
F	1	8	Myctophidae sp. 5
G	1	11	Myctophidae sp. 6
H	1	7	Myctophidae sp. 7
I	1	7	Carapidae? – can't see maxillum behind head tho
J	1	4	? Platycephalid?
K	2	4.5	Sebastidae – Helicolenus sp.
L	1	4	?
M	1	3	Yolk sac?
N	1	4.5	Stargazer
O	2	6.5	Myctophidae sp. 8
P	1	6	Myctophidae sp. 9
Q	2	8	Myctophidae sp. 10

N=44



DNA extraction

'Rules' developed iteratively for filtering and acceptance of presence

- 98-100% pairwise identity
- >20 reads unless

R44-1-L

Species	count	Mean size (mm)	characteristics
A	1	9	Myctophidae

N=1

Shokralla

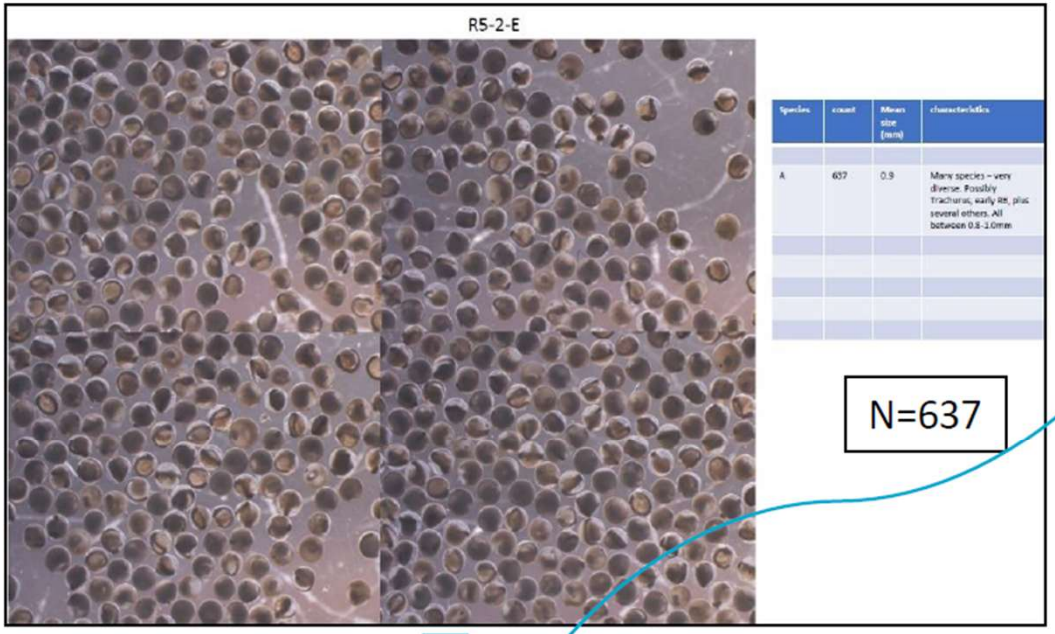
Leray

DNA extraction

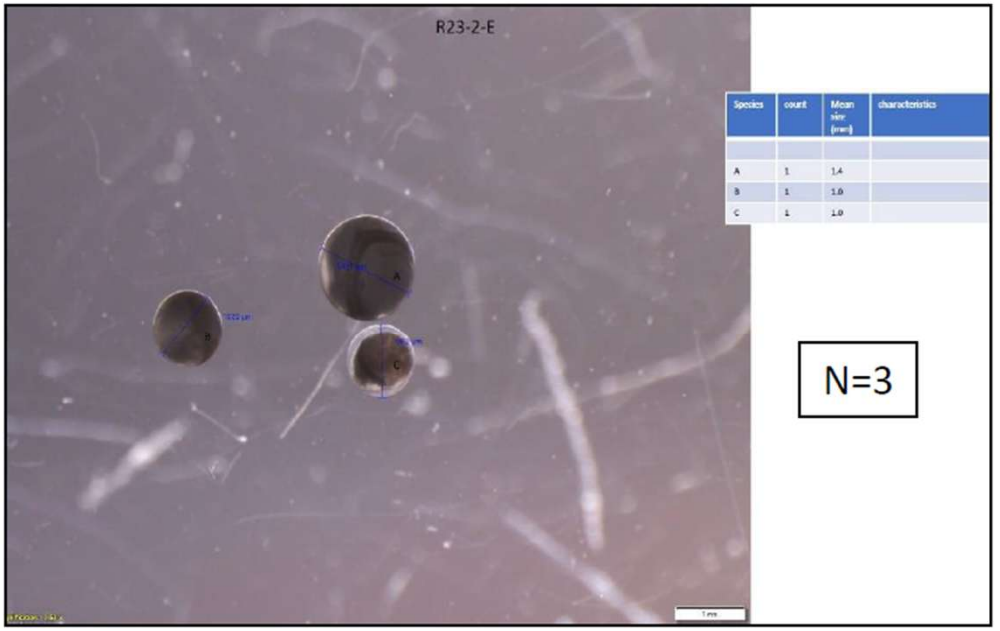
- Different numbers of individuals bulked in sample

Bulked Egg Libraries

Detailed morpho



DNA extraction



Shokralla

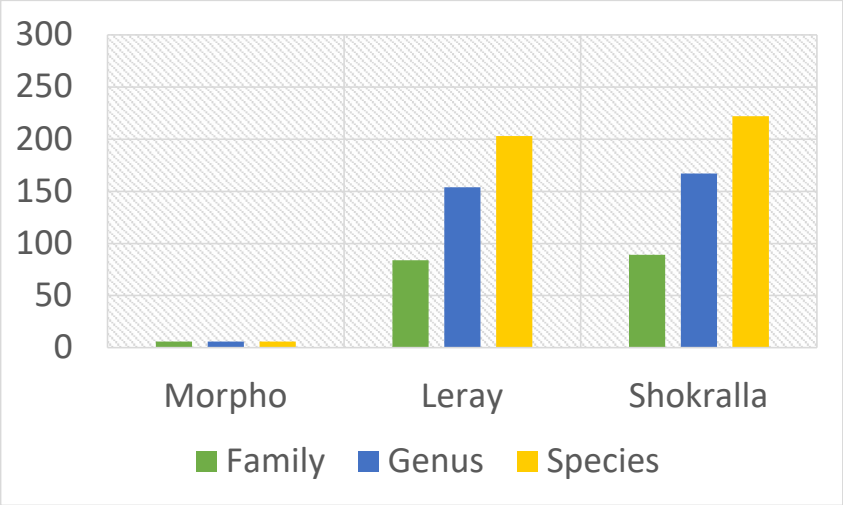
Leray

DNA extraction

- Different numbers of individuals bulked in samples

Comparisons: Morphology vs Leray & Shokralla

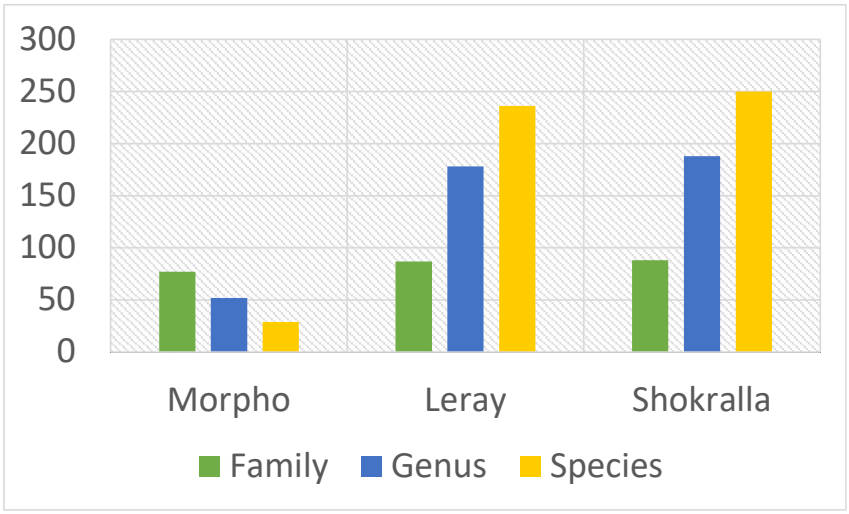
Eggs



	Family	Genus	Species
Morpho	6	6	6
Leray	84	154	203
Shokralla	89	167	222
TOTAL (L + S)	**91**	**177**	**248**

All Families, Genera & Species identified by MORPHO were ID'd by both assays

Larvae



	Family	Genus	Species
Morpho	77	52	29
Leray	87	178	236
Shokralla	88	188	250
TOTAL (L + S)	**90**	**198**	**275**

Assays detected ~200 species > morphology

Why?

Identified by MORPHO but not by either Assay:

- **8** Families
- **10** Genera
- **8** Species

Checking Comparisons: Larvae Morphology vs Leray & Shokralla

8 Families identified by MORPHO but not by Assays

Family	Morpho	Leray	Shokralla
Carapidae	Y	-	-
Champsodontidae	Y	-	-
Chlorophthalmidae	Y	-	-
Howellidae	Y	-	-
Ipnopidae	Y	-	-
Lophiidae	Y	-	-
Nemichthyidae	Y ?	-	-
Syngnathidae	Y	-	-

10 Genera identified by MORPHO but not by Assays

Genus	Morpho	Leray	Shokralla
Bathylagus	Y	-	-
Champsodon	Y	-	-
Chlorophthalmus	Y	-	-
Gymnapistes	Y	-	-
Hime	Y	-	-
Howella	Y	-	-
Melanostomias	Y	-	-
Neosebastes	Y	-	-
Rhombosolea	Y	-	-
Stigmatopora	Y	-	-

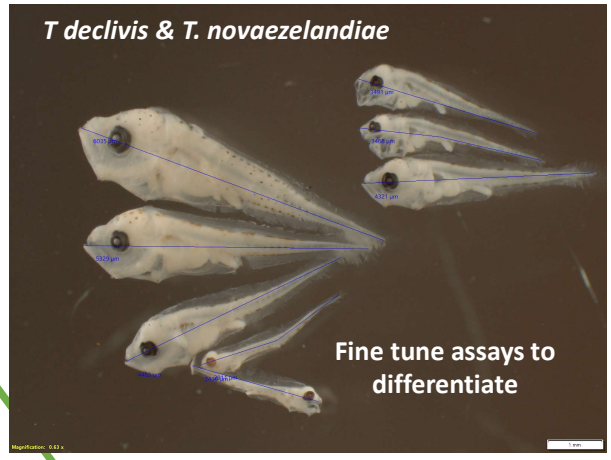
8 Species identified by MORPHO but not by Assays

Species	Morpho	Leray	Shokralla
Aldrichetta fosteri	Y ?	-	-
Centroberyx affinis	Y ?	-	-
Cepola australis	Y	-	-
Cynoglossus broadhursti	Y ?	-	-
Gaidropsarus novaezealandiae	Y ?	-	-
Gymnapistes marmoratus	Y	-	-
Rhombosolea tapirina	Y ?	-	-
Trachurus novaezelandiae	Y	-	-

Reasons:
Morpho: mis-ID's

Assays: <98% identity or low detections (<20)

Species record not in reference database



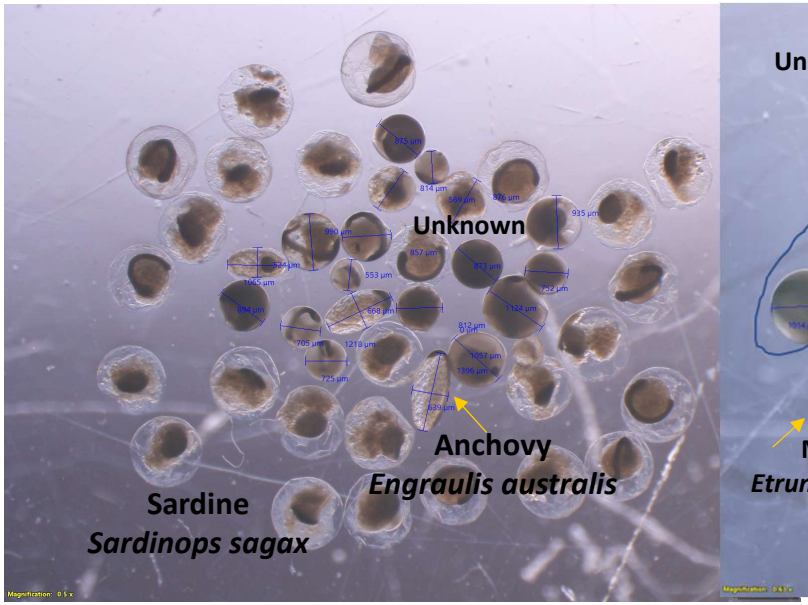
Checking Comparisons: Eggs -> Morphology vs Leray & Shokralla

All Families, Genera and Species ID'd by MORPHO ID'd by both Assays

Family	Genus	Species	Morpho	Leray	Shokralla
Bothidae			Y	Y	Y
Carangidae	*Trachurus*	*Trachurus declivis*	Y	Y	Y
Clupeidae	*Sardinops* *Etrumeus*	*Sardinops sagax* *Etrumeus teres*	Y	Y	Y
Emmelichthyidae	*Emmelichthys*	*Emmelichthys nitidus*	Y	Y	Y
Engraulidae	*Engraulis*	*Engraulis australis*	Y	Y	Y
Scombridae	*Scomber*	*Scomber australasicus*	Y	Y	Y

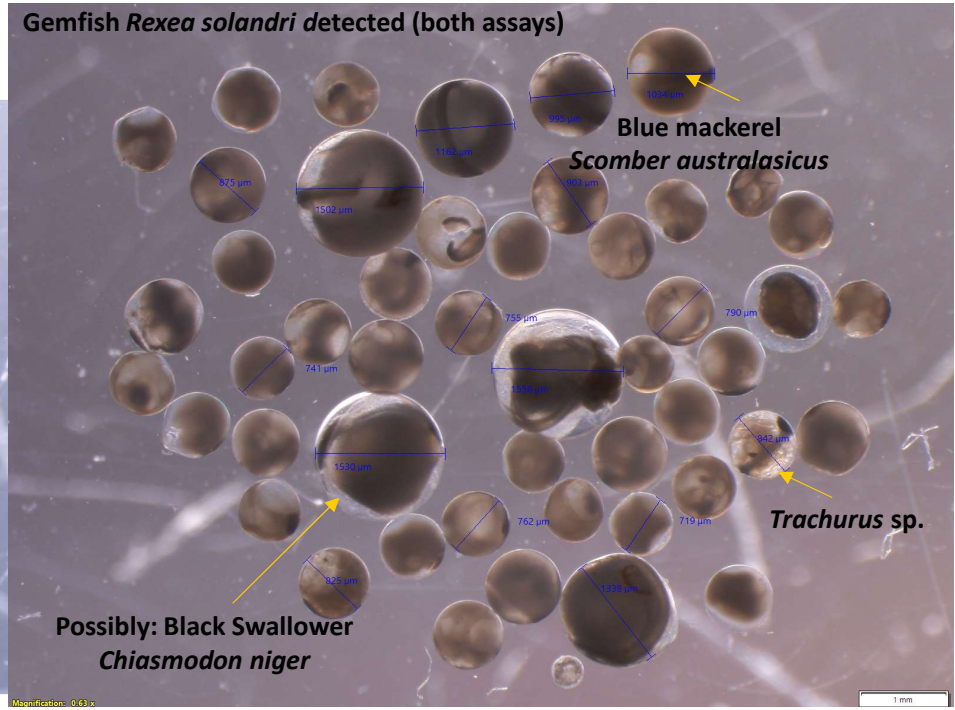
DNA good for eggs: majority of fish eggs really hard to identify morphologically and many undescribed

SA Sardine Survey



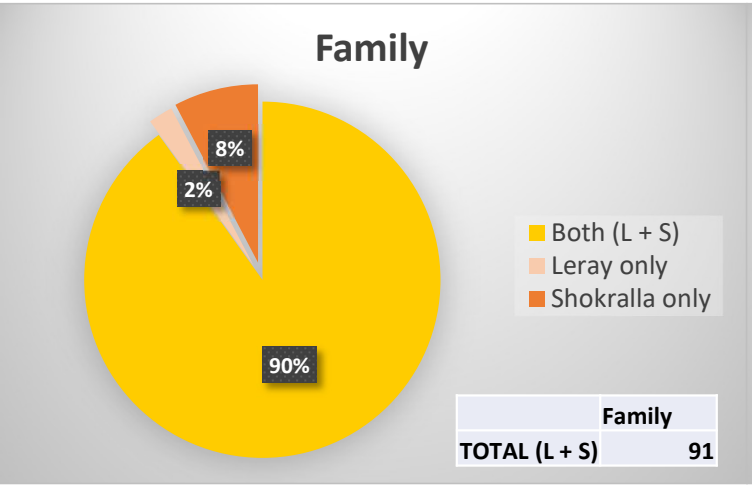
SA Sardine Survey

Blue Mackerel East Survey

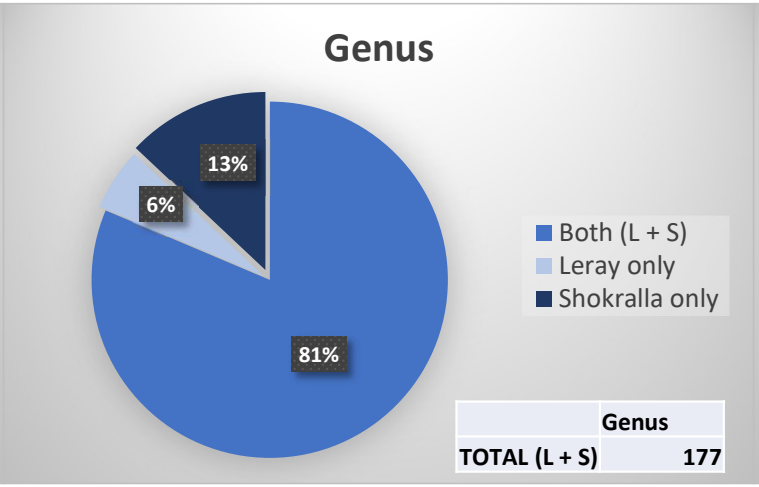


Comparisons: Leray vs Shokralla - % identification

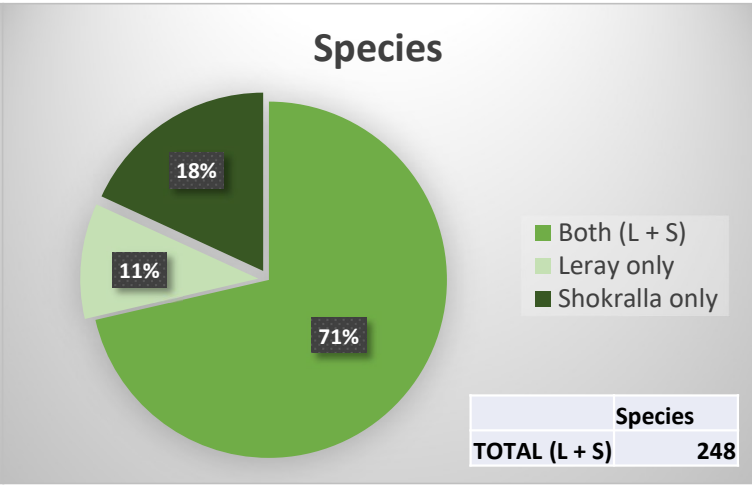
Eggs



	Family
TOTAL (L + S)	91

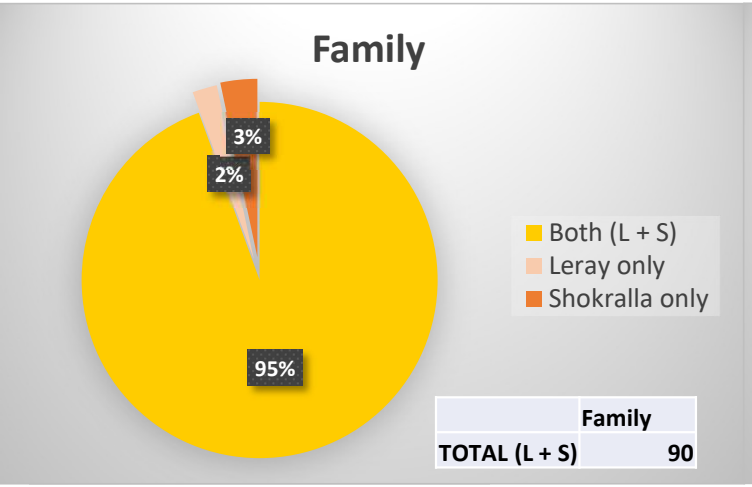


	Genus
TOTAL (L + S)	177

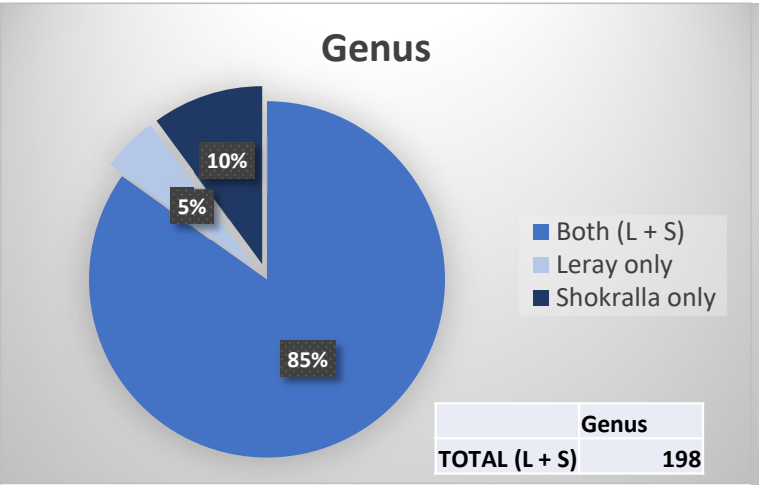


	Species
TOTAL (L + S)	248

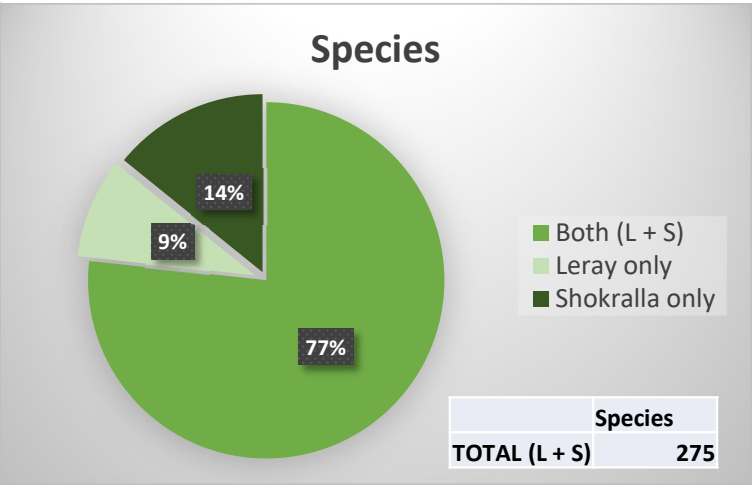
Larvae



	Family
TOTAL (L + S)	90



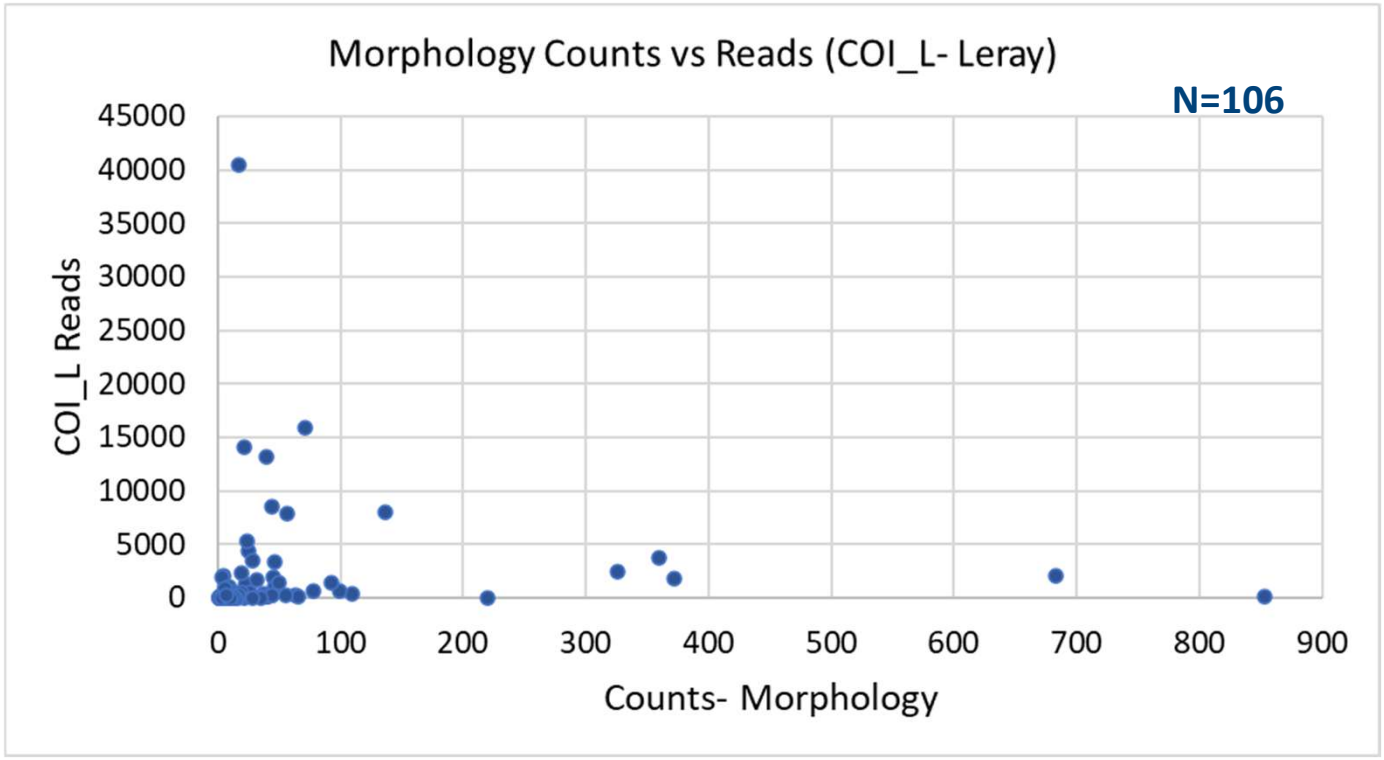
	Genus
TOTAL (L + S)	198

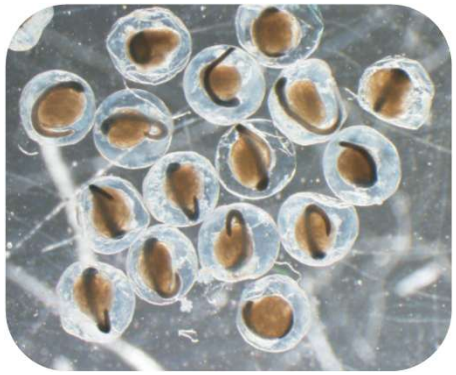


	Species
TOTAL (L + S)	275

Comparisons: Morphology Counts vs Reads (Leray)

Sardine (*Sardinops sagax*) - Eggs





Reasons:

- **Inhibition**
- **Swamping**

Mismatch between morphological counts and number of reads demonstrates approach is not quantitative

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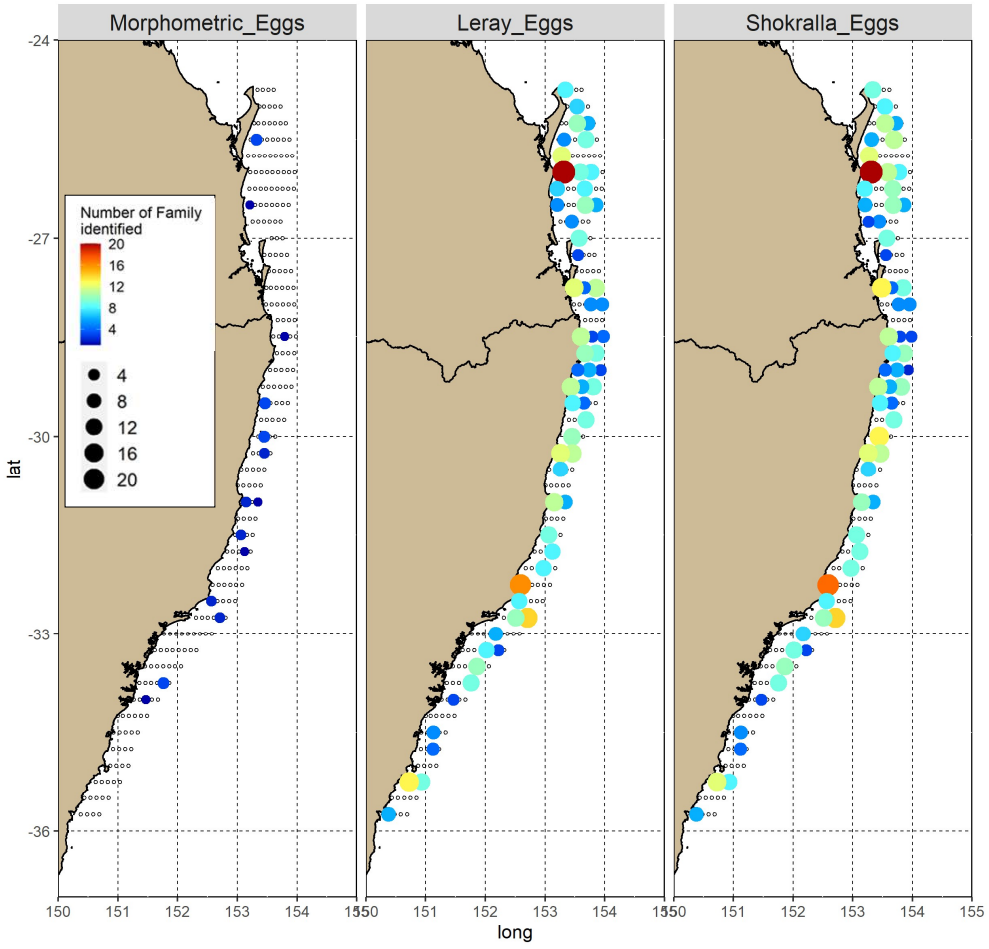
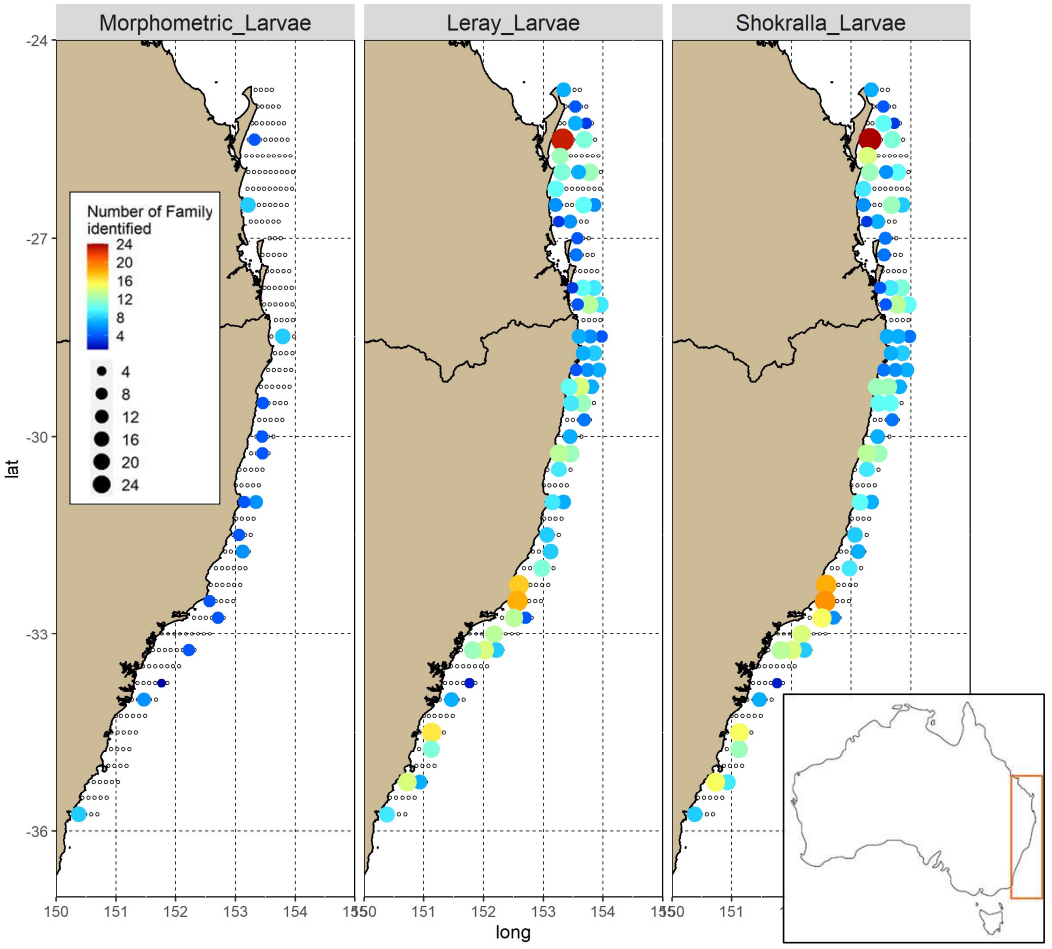
Practical applications: Species assemblages)

Both assays useful for identifying biodiversity hotpots and potential monitoring changes over time.

Eggs

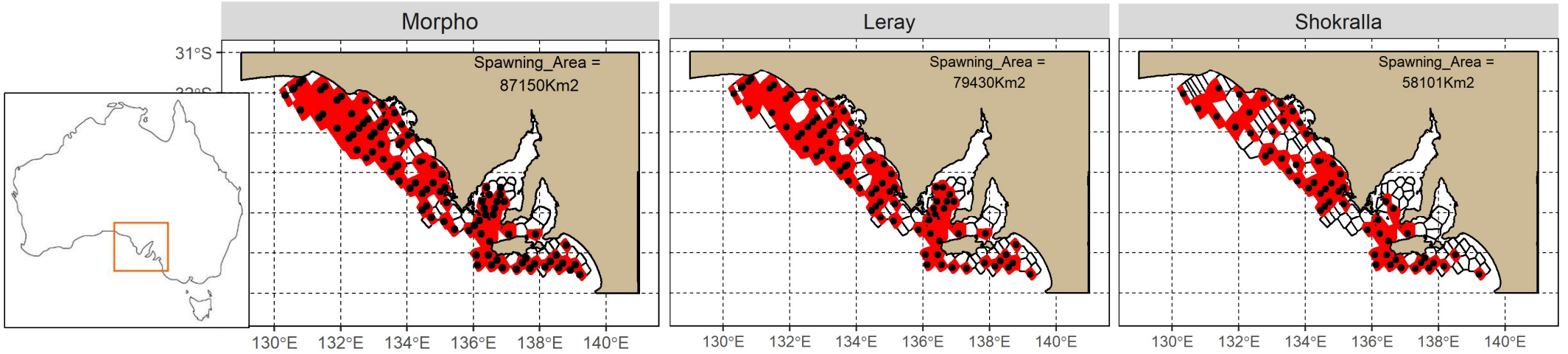


Larvae



Practical applications: Estimating Spawning Area for the DEPM and other specie

Sardinops sagax **Sardine**



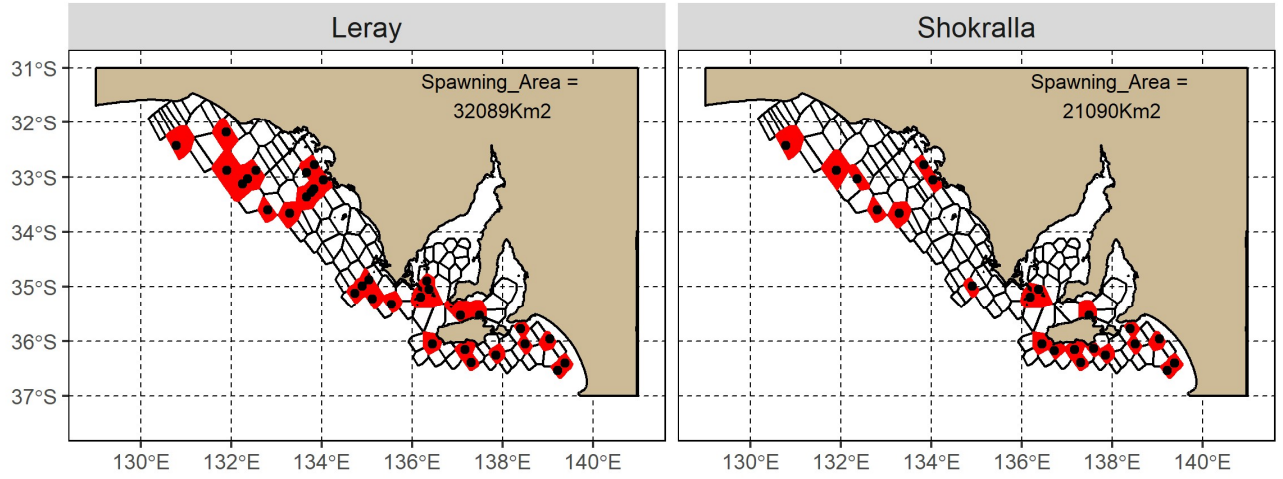
***A* in 2020 Sardine DEPM**

~82,000 km^2 (Ward et al. 2020)

Spawning area:

- **Voronoi natural neighbour method**
- **Presence/absence of eggs in grid cell**

Scomber australasicus **Blue Mackerel**

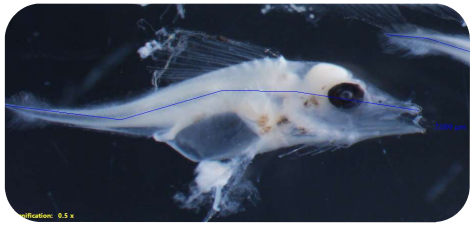


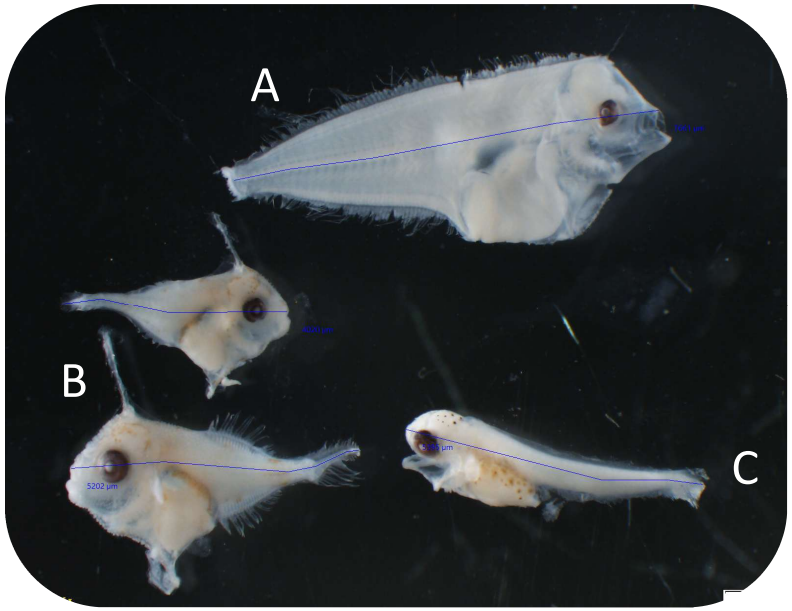
Eggs

***A* in 2005 Blue Mackerel DEPM**

~36,000 km^2 (Ward & Rogers 2007)

Key Findings



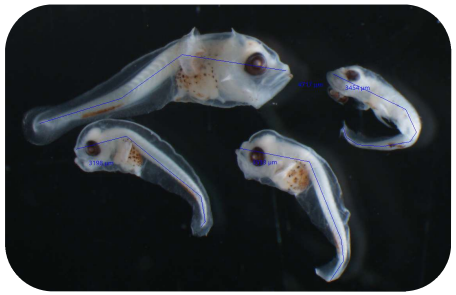


Morphological identification:

- Advantage: counts not presence/absence
- Disadvantages:
  - difficult, time consuming, expensive
  - can be prone to error
  - <50% identification of spp for larvae, much less spp ID for eggs

DNA-metabarcoding (Leray and Shokralla assays):

- Advantages:
  - 2 assays very similar results
    - Shokralla generally > number species detected,
    - BUT one assay maybe detect a target species better than other (e.g. Trachurus)
  - more spp and genera identified compared to morphology,
  - relatively cheaper
  - does not require (rare) high level taxonomic expertise
  - good for eggs – estimating spawning area
  - Handling protocol (ultraclean vs standard) -- less important than initially thought
- Disadvantages:
  - not quantitative **(reads not related to abundance)**
  - needs checking/calibrating for species in region prior to regular use



- **Hybrid approach (coarse morpho + DNA-mb) → Valuable**
- **Funding: despite surveys being cost-recovered, govt funding limited**