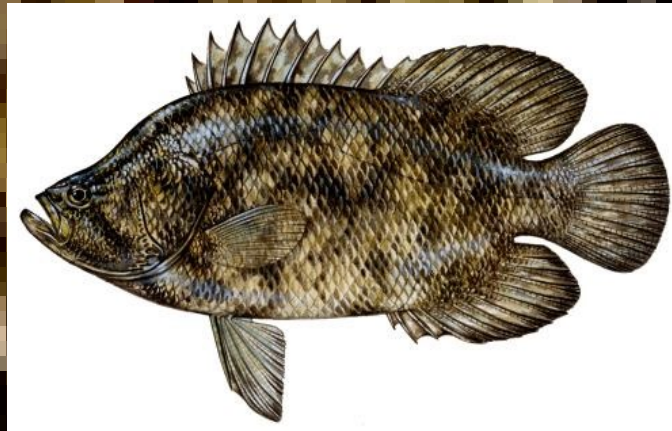
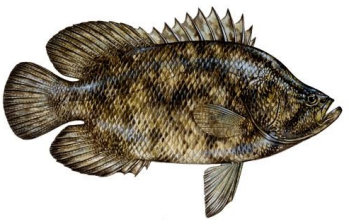


Genetic studies of Tripletail, *Lobotes surinamensis*

Eric Sallant, Steve Vanderkooy, Pearce Cooper, James Franks,
Pavel Dimens, Brooke McPeak, Sean Powers

University of Southern Mississippi, Gulf States Marine Fisheries Commission,
University of South Alabama





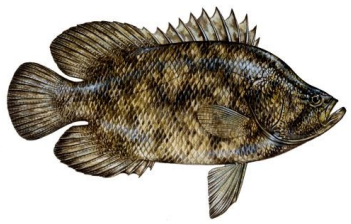
Tripletail *Lobotes surinamensis*

Circumglobal distribution

Warmwater

Not gregarious, associated with floating structures (e.g. Sargassum)



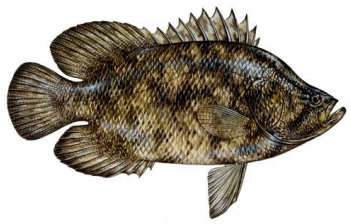


Tripletail US fisheries

- US fishery primarily recreational
- Average annual U.S. dockside value \$25,000 (GSMFC, 2016)
- Markets supplied by imports from South America (landings ~3 Million lbs/y GSMFC, 2016)
- Species managed with minimum size and bag limits

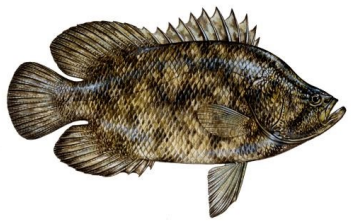


FL 15 inch (1996), 2/rec angler, 10/com angler, AL 18 inch (2012) 3/angler, MS 18 inch (2014) 3/angler, LA 18 inch (2014) 5/rec angler 100 lb/com angler, TX 17 inch (2006) 3/angler



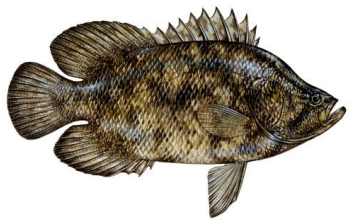
Tripletail Life-History

- Adult found around flotsam
- Summer spawner (June-Aug)
- Multiple batch spawner (Brown-Peterson, Franks, 2001)
- Larvae pelagic in surface waters (Ditty, 1994)
- Juveniles in coastal shallow waters



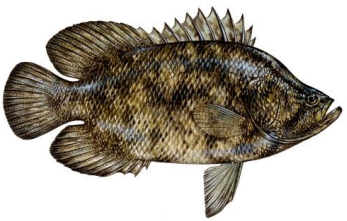
Aquaculture programs

- Interest related to flesh quality and anticipated fast growth rate
- USM pilot projects 2005, 2010-2014, 2017-2018, 2020-22
- Oceanus seafarms, horsecreek aquafarms



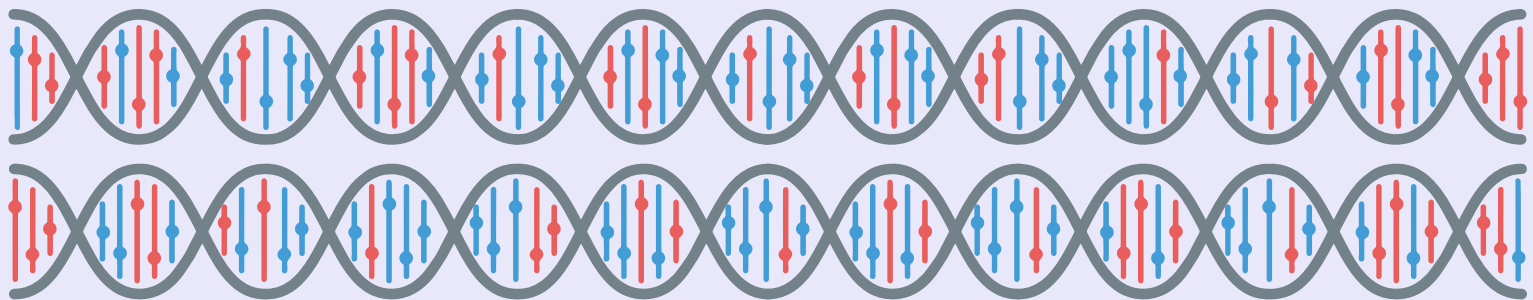
Study objectives

- Develop genomic resources (draft reference genome)
- Survey wild populations of tripletail in the US and abroad to assess stock structure in US waters and connectivity with other stocks



Genome sequencing

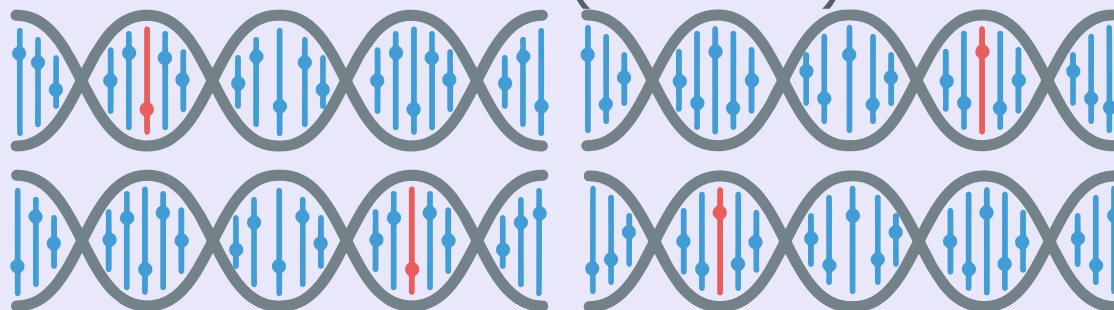
Sequencing of one tripletail specimen

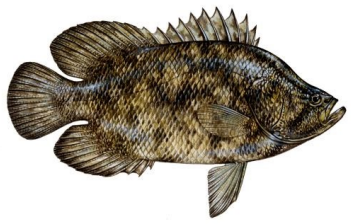


Long reads (PacBio)

+

Short reads (Illumina)



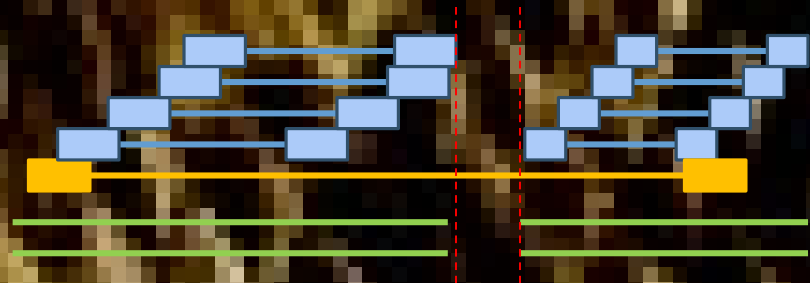


Draft Genome Assembly

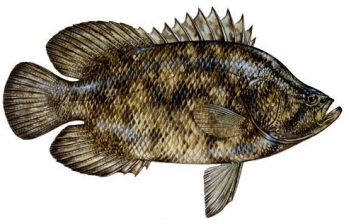
Sequencing reads assembled to generate contigs and scaffolds



Contig: contiguous sequence data

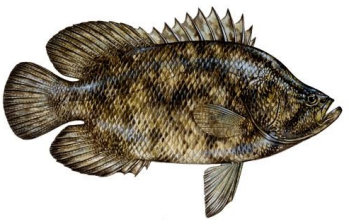


Scaffold: multiple contigs joined by gaps



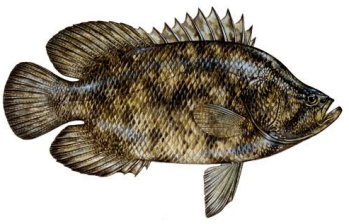
Draft reference genome

- 8 PAC Bio SMRT cells 43.1 Gb (estimated 73X coverage)
- Illumina short reads 206,582 Gb (345X coverage)
- Linkage map to anchor contigs 92 parents and 280 larvae



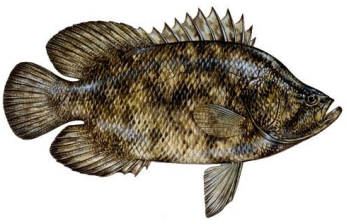
Draft reference genome assembly

- SparseAssembler - assemble illumina reads into contigs
- DBG2OLC - assemble pacbio reads and illumina contigs
- Blasr/pbdagcon - consensus using trimmed illumina reads
- Pilon - polishing using trimmed + aligned illumina reads



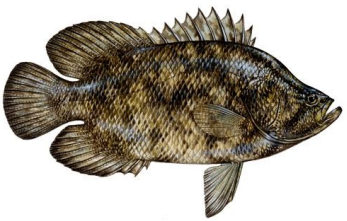
Draft reference genome

- Genome size estimate (k-mer)
585,001,442 bp
- Draft genome hybrid assembly
- 322 contigs > 50,000 bp (98.9%
assembly), N50 3,392,625, L50: 51
- Linkage map to scaffold contigs in
linkage groups
- Population structure study across the
range



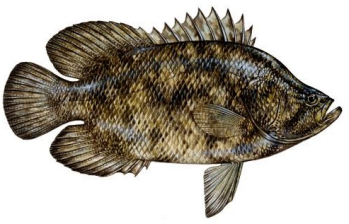
Population study - Sampling





Sampling

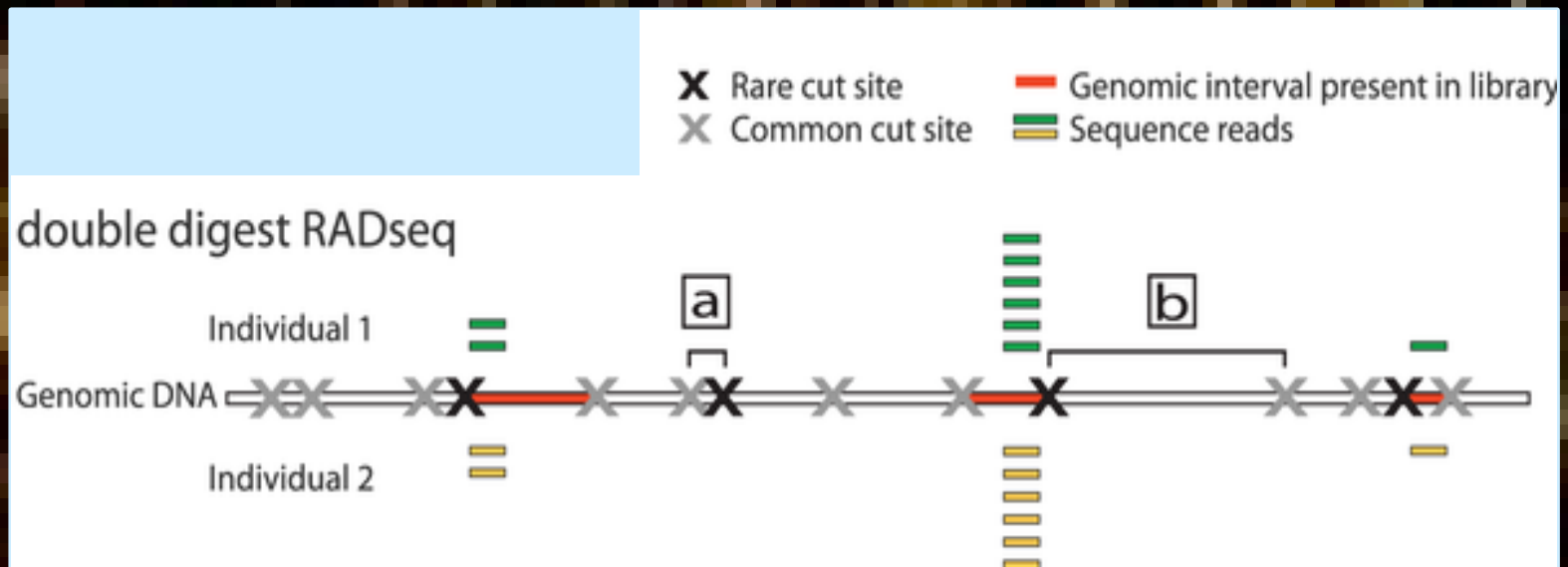
Location	Collection Year	Number of Samples	Total # Samples from Location	Samples Used
Georgia	2012	15	56	56
	2014	22		
	2019	19		
Mississippi	2017	68	221	118
	2018	89		
	2019	64		
Australia	2016/17	30	50	50
	2018	20		
Florida EC	2018	32	32	32
Florida GC	2018	59	59	59
Florida Keys	2019	77	77	77
Texas	2019	10	10	10
Peru	2019	50	50	50
Brazil	2019	18	18	18
Senegal	2020/21	62	62	62
Benin	2021	62	59	59
Malaysia	2019	11	11	0
Turkey	2021	27	27	27
Total			641	609

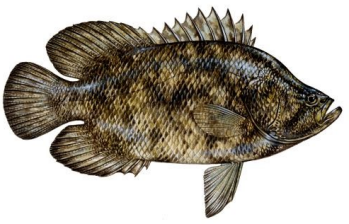


ddRAD-Tag Sequencing

DNA digested by restriction enzymes Eco-RI and MspI
Illumina adapters and sample-specific barcodes ligated to fragments

100 samples pooled and sequenced together





SNP Discovery and Filtering

ddRAD-Seq reads analyzed in the dDocent-FB pipeline (Puritz et al. 2014)

SNPs filtered to include:

SNPs with a minimum quality score of 30
Individual genotypes covered by 10 or more reads

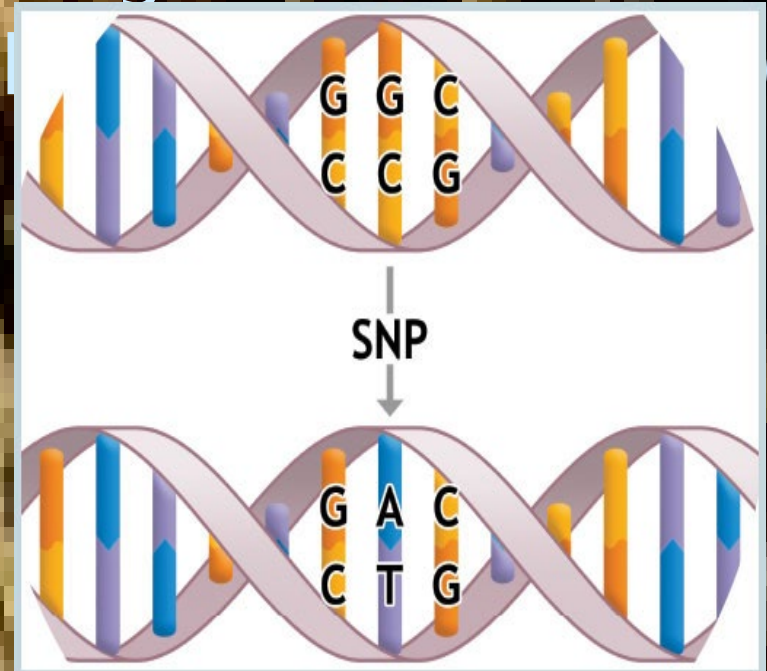
Individual genotypes with a genotype probability > 0.99

SNPs called in at least 80% of individuals

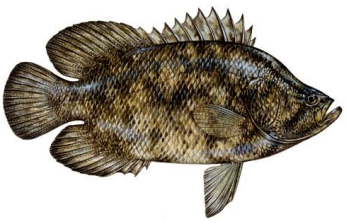
Minor allele frequency > 0.01

→ 25,293 SNPs in 363 individuals

Single Nucleotide



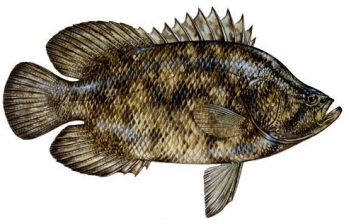
48 AUS, 15 BRA, 29 FLE,
45 FLG, 44 G, 128 MS,
47 PER, 7 TX



Population structure analysis

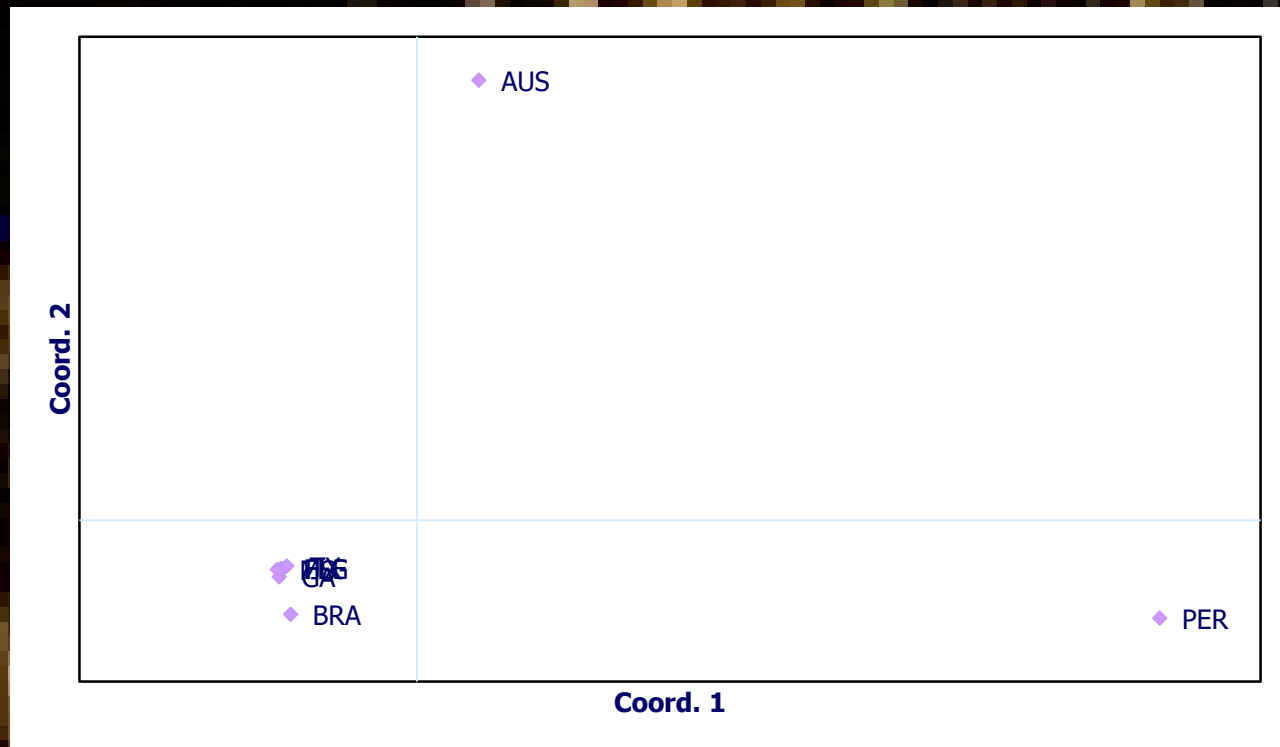
Analysis of molecular variance

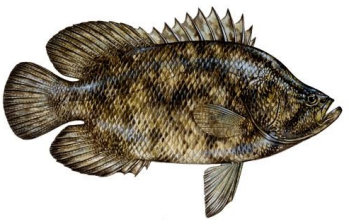
Source of variation	d.f.	Fstatistic	% variance	P
Among groups		4Va (FCT)	52	0.03
Among populations within groups		3Vb (FSC)	0.04	0.02
Among individuals w. populations		325Vc (FIS)	0.53	0.1
Within individuals		333Vd (FIT)	47.43	<0.001



Population structure analysis

Principal coordinates (PCoA) Axis 1 and 2 explain 71% and 26% of the variance respectively

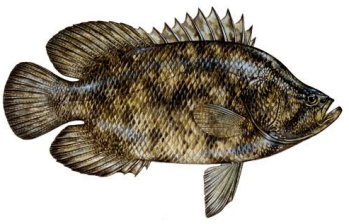




Population structure analysis

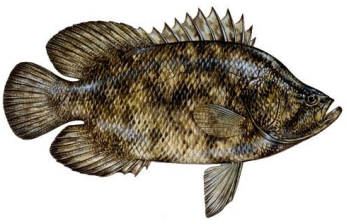
Pairwise F_{ST} (genetic distance) between sampled regions

	AUS	BRA	FLE	GA	FLG	MS	TX
BRA	0.33654*						
FLE	0.28089*	0.02558					
GA	0.28985*	0.02264	0.00136				
FLG	0.28077*	0.02394	0.00006	0.00132*			
MS	0.2834*	0.02413	0.00134	0.00171	0.00114		
TX	0.27836*	0.03115	0.00146	0.00237	0.00184	0.00217	
PER	0.7261*	0.71848*	0.71772*	0.72299*	0.72085*	0.72704*	0.71285*



Current steps

- Include recent samples in analysis (reruns and East Atlantic populations from Senegal, Benin and Turkey)
- Fine scale analysis of West Atlantic to assess
 - Spatial autocorrelation and isolation by distance
 - Temporal stability
 - Migrants from East Atlantic



Acknowledgments

People: Joecemar Mendonca

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Renan Alfredo Flores Garcia

Steve Qualia

Christopher Kalinowsky

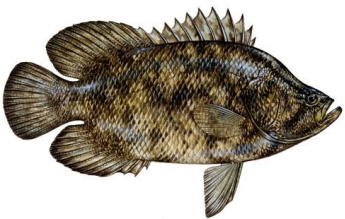
Fambaye Ngom Sow

Zacharie Sohoun

S. Schindler and Shore Thing charters



Funding: Gulf States Marine Fisheries Commission



Sampling

Location	Collection Year	Specific Location	Number of Samples	Total # Samples area	Samples Used
Georgia	2012	Sapelo Sound	1	37	37
	2012	Jekyl Island	14		
	2014	Ossabaw Sound	22		
Georgia	2019	Wassaw Sound	3	19	19
		Ossabaw Sound	2		
		Jekyl Island	14		
Mississippi	2017	unknown	68	68	50
Mississippi	2019	Bay St Louis,	64	64	18
		Chandeleur Island			
Mississippi	2018	Mississippi sound	89	89	50
Australia	2016/2017	Port Alma	15	30	30
	2016/2017	Weipa, QLD	15		
Australia	2018	Strange Bay	1	20	20
		Port Alma	14		
		Dampier Arch	5		
Florida EC	2018				32
Florida GC	2018				59
Florida Keys	2019		77	77	77
Texas	2019	Carancahua Bay	1	10	10
		Matagorda Bay	8		
		Aransas Bay	1		
Peru	2019	La Cruz	10	50	50
		La Jota	7		
		Playa hermosa	24		
		Punta malpelo	6		
		Plataforma la cruz	2		
		Cherrez	1		
Brazil	2019	Trincheira	8	18	18
		Prainha	8		
		Pereirinha	1		
		Hub	1		