

# Gulf of Mexico Oyster Genetics and Breeding Research Consortium (SALT project)

project update 10/17/2022

E. Saillant, K. Lucas, L. Sturmer, H. Yang,  
C. Hollenbeck, J. Stoeckel, B. Callam

# SALT consortium

*Assist industry and state agencies by developing a breeding program to improve production and market value traits as directed by industry needs, and a germplasm repository for management of restoration programs and/or dissemination of genetic progress.*

## *Business Advisory Council*

Mississippi	Jennifer Jenkins
Alabama	Lane Zirlott John Webster
Florida	Reid Tilley Brandon Smith
Louisiana	Boris Guerrero Nathan Herring
Texas	William Balboa Nasir Kureshy

## *Research team*

Eric Saillant Kelly Lucas	University of Southern Mississippi
Jim Stoeckel	Auburn University
Leslie Sturmer Huiping Yang	University of Florida
Brian Callam	Louisiana State University/LA Seagrant
Christopher Hollenbeck	Texas A&M University



# SALT Consortium

Job 1: Define project objectives to address industry needs in the Gulf of Mexico

Job 2: Develop breeding program to produce Eastern oyster with improved genetic values for characters of interest to industry

Job 3: Develop germplasm repository to include founders and selected parents during successive generations of breeding

Job 4: Test improved diploid lines for triploid performance in cross with tetraploid stocks

# Job 2

## Objectives

- Improve growout traits in Gulf environments (at least two salinity environments anticipated)
- Survival, disease resistance in the hatchery and reproductive traits (fecundity, timing of reproduction) monitored

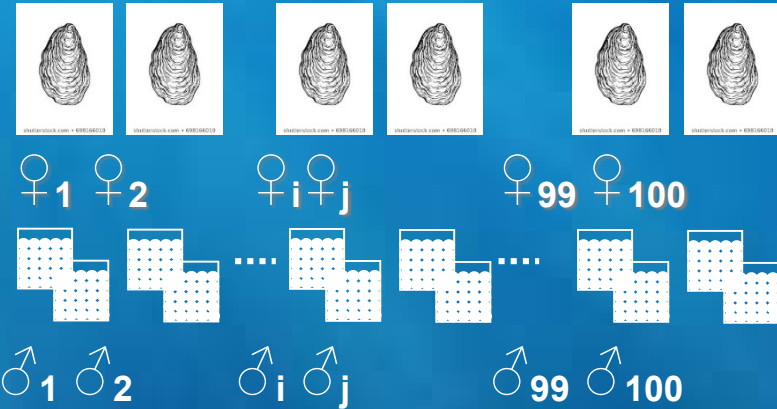
## Base population

- F1 bred as a mixture of regional genotypes (FL, MS/AL, LA, TX East of Corpus Christi) from 17 collection sites equally represented
- Additional populations (south Florida, selected lines at Auburn and LSU) incorporated in F2

# Project timeline

Funding yrs	Inclusive dates	Milestones
Yr1	06/19-09/20	Define objectives (survey), Collect F0, breed F1
Yr2	10/20-12/21	Grow F1, measure phenotypes, temperature and salinity tolerance
Yr3	01/22-01/23	Estimate genetic parameters and BV F1, breed F2, initiate growout F2
Yr4	02/23-02/24	Growout F2 measure phenotypes, estimate BV
Yr 5	03/24-03/25	Breed F3, test as 3n, deploy and growout

# Common garden design testing and walk-back selection



Mix equal # of fertilized embryos from each family

Growout challenge

Trait measurements, genotyping and pedigree analyses

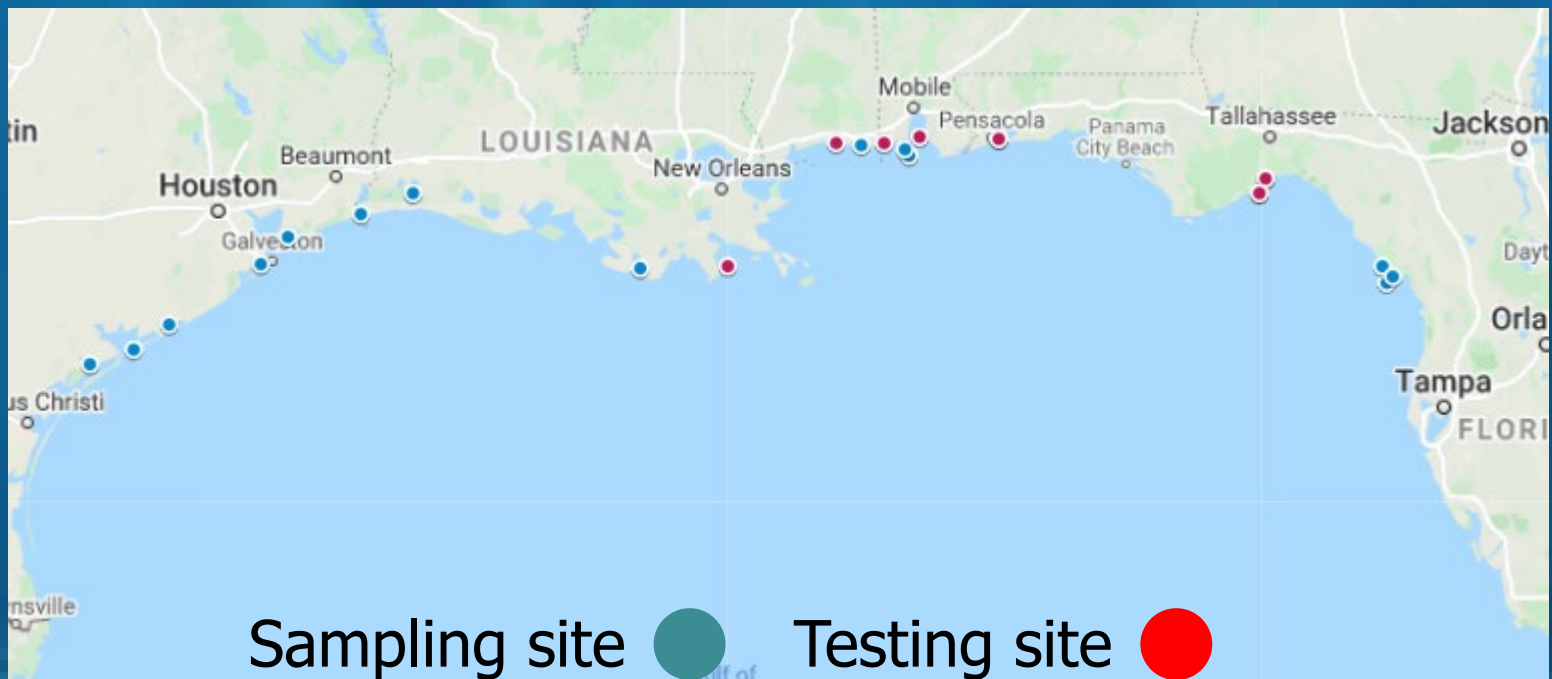
Estimate breeding values and select parents for next generation





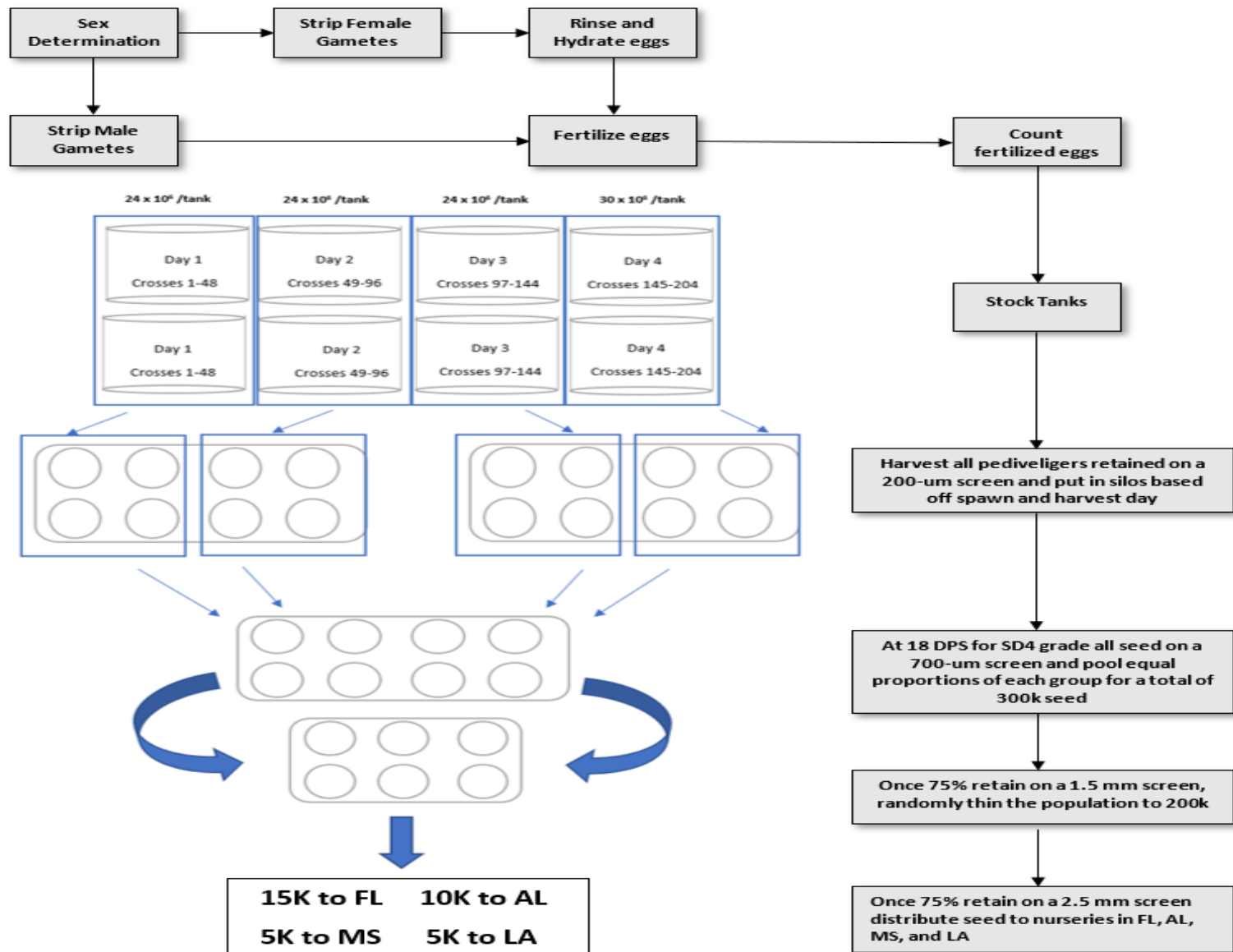
# Founding population

- Founders from different geographic populations incorporated in a mosaic base population (17 sampling sites characterized by different salinity conditions)
- Target 100 males and 100 females contributing each generation



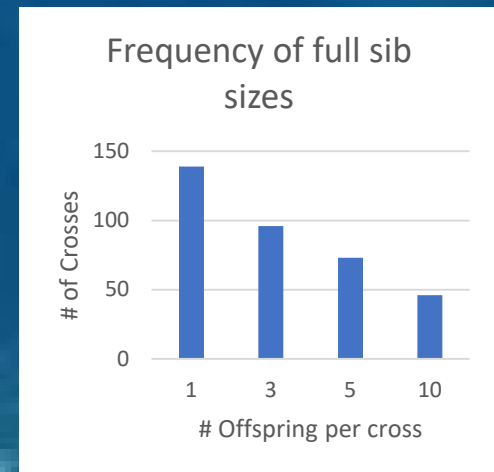
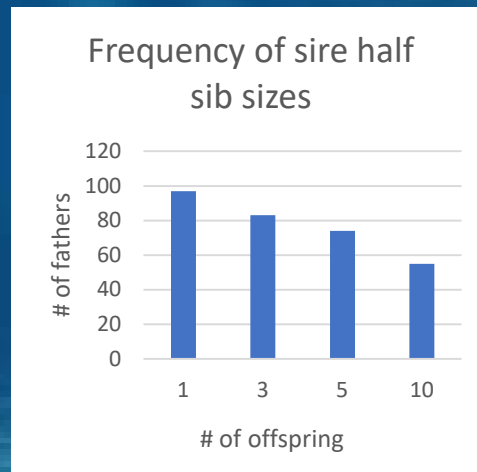
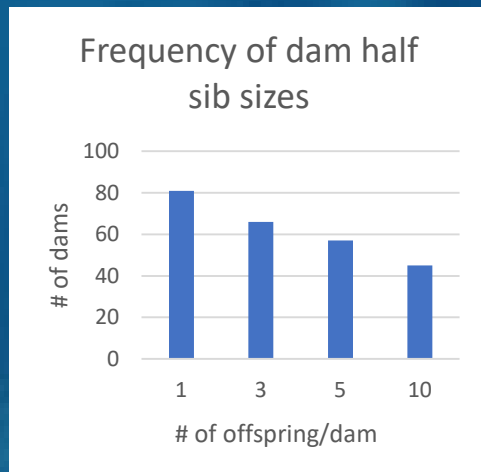


# Culture prior to growout



# Spawning results (F1 generation)

- Spawning at Auburn University Shellfish Laboratory (Aug 31-Sept 3 2020)
  - 204 crosses produced during in vitro fertilization, av 275,950 embryos per cross, 35.91% fertility
- Effective number of dams, sires and brooders at the end of setting were 37, 56, and 89



# Growout and harvest of F1

- Oysters stocked on growout sites in April 2021
- Harvest between October 18 and November 2 2021  
(harvested sites: Alligator Harbor, Mobile Bay, Deer Island, Grand Isle selected based on salinity patterns)
- High mortality during early summer in Alligator Harbor, some bags lost due to storm damage in Mobile Bay
- Heavy spatting of oysters at Alligator Harbor site

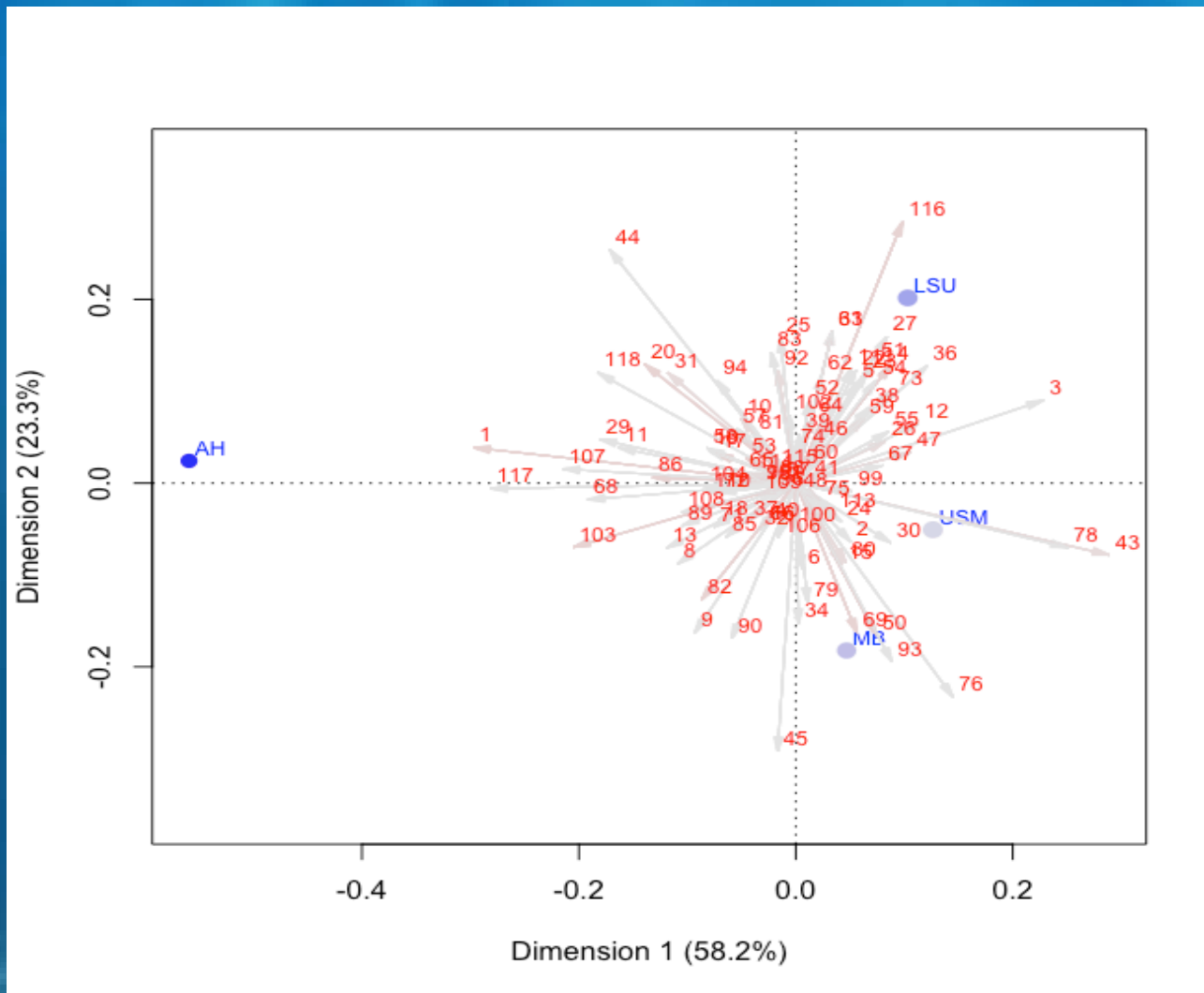
Site	Salinity	Harvest date	# survivors (%)	Height (mean $\pm$ SD)	% back bend
Alligator Harbor	High	10/25	997 (33)	55.3 $\pm$ 8.2	74
Mobile Bay	Low	10/18	1,950 (78)*	43.2 $\pm$ 5.7	99
Deer Island	Medium	10/20	2,659 (89)	44.5 $\pm$ 5.8	98
Grand-Isle	Medium	11/02	2,779 (93)	79.8 $\pm$ 10.1	70

\* 2 bags lost due to storm damage

# Sampling and parentage analysis

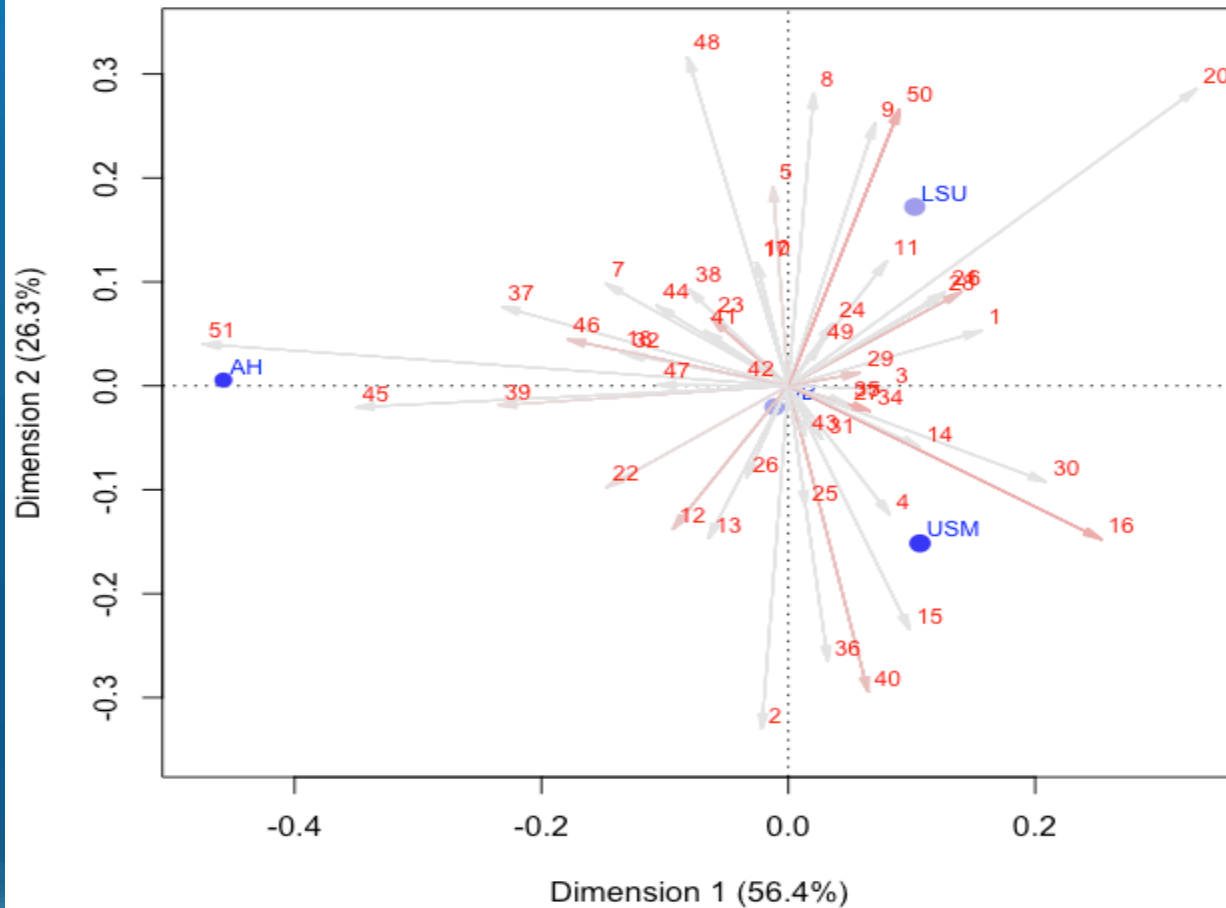
- F0 parental broodstock sampled and typed in 2020
- 2400 F1 oysters (600 per harvested site) pit-tagged and non-lethally sampled
- Another 5,536 oysters frozen and processed post mortem
- SL, SW, and SH measured and back-bend deformity (presence/absence) scored for all samples
- Parents and all offspring genotyped at 192 SNPs at the Center for Aquaculture Technologies using a previously developed 192 SNP fluidigm array assay
- Assignment of offspring to parental pairs using a LOD score approach in Cervus software.

# Contribution of sires

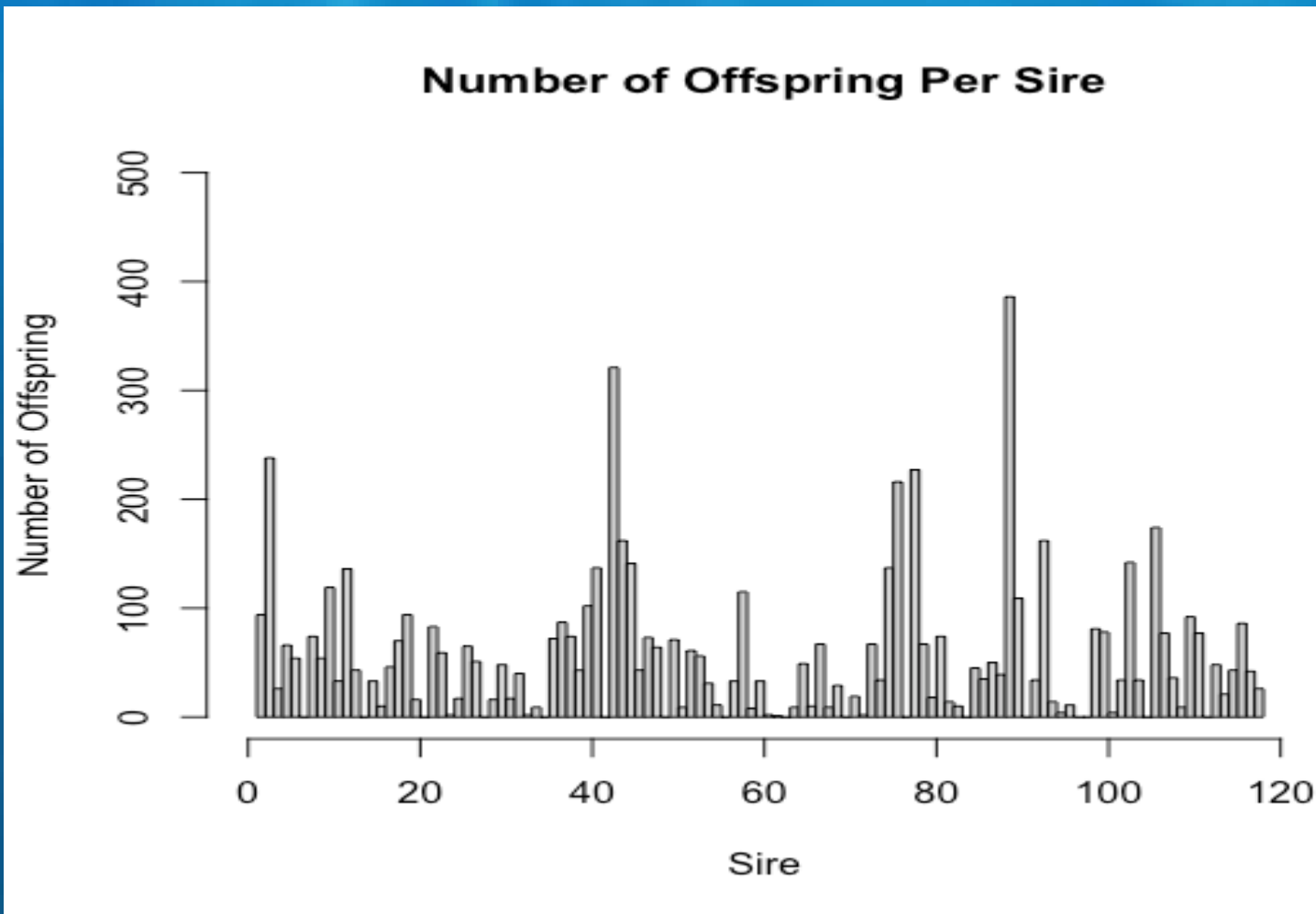




# Contribution of factorials



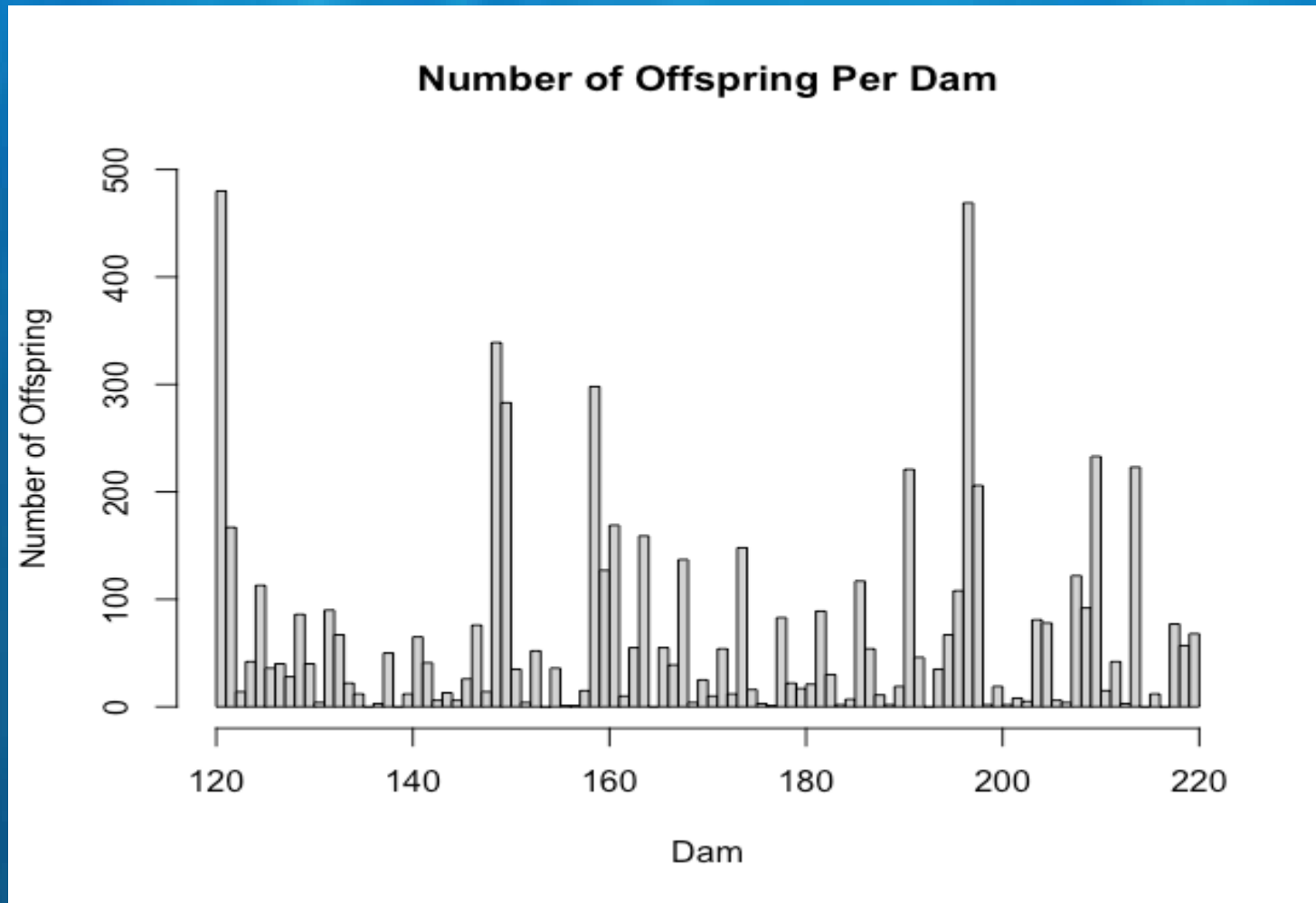
# Contribution of families



$$N_{em-AH} = 52, N_{em-LS} = 47$$

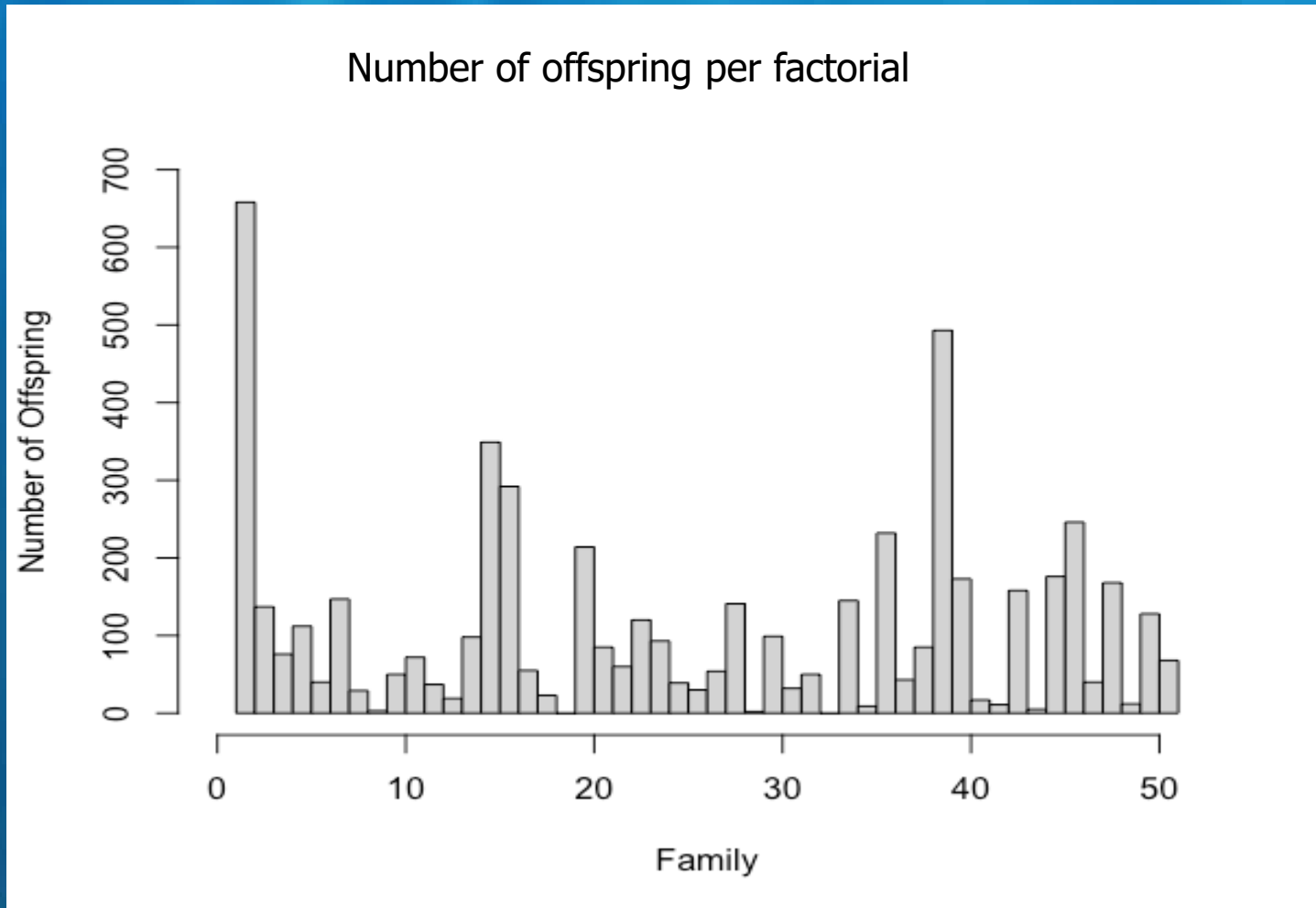


# Contribution of families



$$N_{em-AH} = 39, N_{em-LS} = 35$$

# Contribution of families



# Genetic parameters and breeding values

- Analysis model for growth in a multi-environment trial model:

$$y = \mu + Xb + Z_1a + Z_2f + Z_3r + e$$

b vector of fixed effects of site, a vector of random additive breeding values, f vector of dominance (non additive genetic effects), r random effects of bag(Cage) and measurement date(Site)

- Variance components estimated in ASREML 4
- Residual of length and width were re-analyzed with height as a covariate to describe variation in shape
- Back bend analyzed using a generalized linear model (link function logit)

# Genetic parameters for growth (height)

## Heritability

	$h^2$	$d^2$
AH	$0.38 \pm 0.05$	$0.11 \pm 0.05$
MB, DI, GB	$0.50 \pm 0.01$	$<0.01$

## Genetic correlations between sites

	AH	MB	DI
MB	0.835		
DI	0.785	0.996	
GI	0.862	0.871	0.847

# Conformation traits

- Analysis of residuals of allometric regression of length or width on height: low heritability (AH and LSU site) and/or low genetic correlation with height: Average cup and fans ratios not expected to change in response to selection for growth
- Heritability for back bend was high ( $>0.8$ ) but phenotypic variance was very low (issue of very high prevalence of the deformity at all sites)

# Breeding F2

- High salinity pool: AUSL 06/06-06/10, based on Alligator Harbor survivors top 80% BVs for growth in AH ( $S = 2.3\%$ ): 64 males and females 128 crosses
- Low-salinity pool: USM 06/13-06/17, based on LSU, MB and USM F1s, top 50% BVs for growth ( $S = 6.5\%$ ); 102 males and females 204 crosses
- Control line: USM 06/20 50 randomly selected males and females
- Additional founders: USM 07/11 3 selected lines at Auburn, O'Boy line at LSU and wild oysters from the area of Port Marcos FL; 6 males and 6 females each

## Breeding F2 – High Salinity pool

- Progeny were reared in 4,000 L common garden tanks at AUSL until metamorphosis, harvested over 5 days and then set on cultch in nursery systems
- Equal proportions from each spawn day and set day were retrieved from each silo and placed out in the field for growout once they achieved R2 size
- Seeds were shipped to Cedar Key FL on 07/26/2022
- Stocked on 09/21 (Aligator Harbor) and October 5 (Indian Pass) for growout as R12 (selected oysters) and R6 (controls)

# Breeding F2 – Low Salinity

- Progeny were reared in 200 L common garden tanks (sets of 24 families in duplicates) at USM until metamorphosis, harvested over 5 days
- Equal proportions from each spawn day and set day were retrieved from each silo and nursed to R2-R6 until deployment
- Control and low salinity pool shipped to LSU and AUSL on 08/17 2022 and are deployed on growout sites on target testing sites (USM, Mobile Bay, Grand Bay, Grand Isle)



# Additional traits

- Additional traits potentially contributing to survival and fitness in Gulf environment are being evaluated by the consortium for selective breeding
- Tolerance to high temperature stress during late summer following reproduction
- Tolerance to low salinity events occurring in the northcentral Gulf (e.g. opening of spillways)
- Resistance to disease (dermo disease in growout, vibrio in hatchery)

# Thermal tolerance

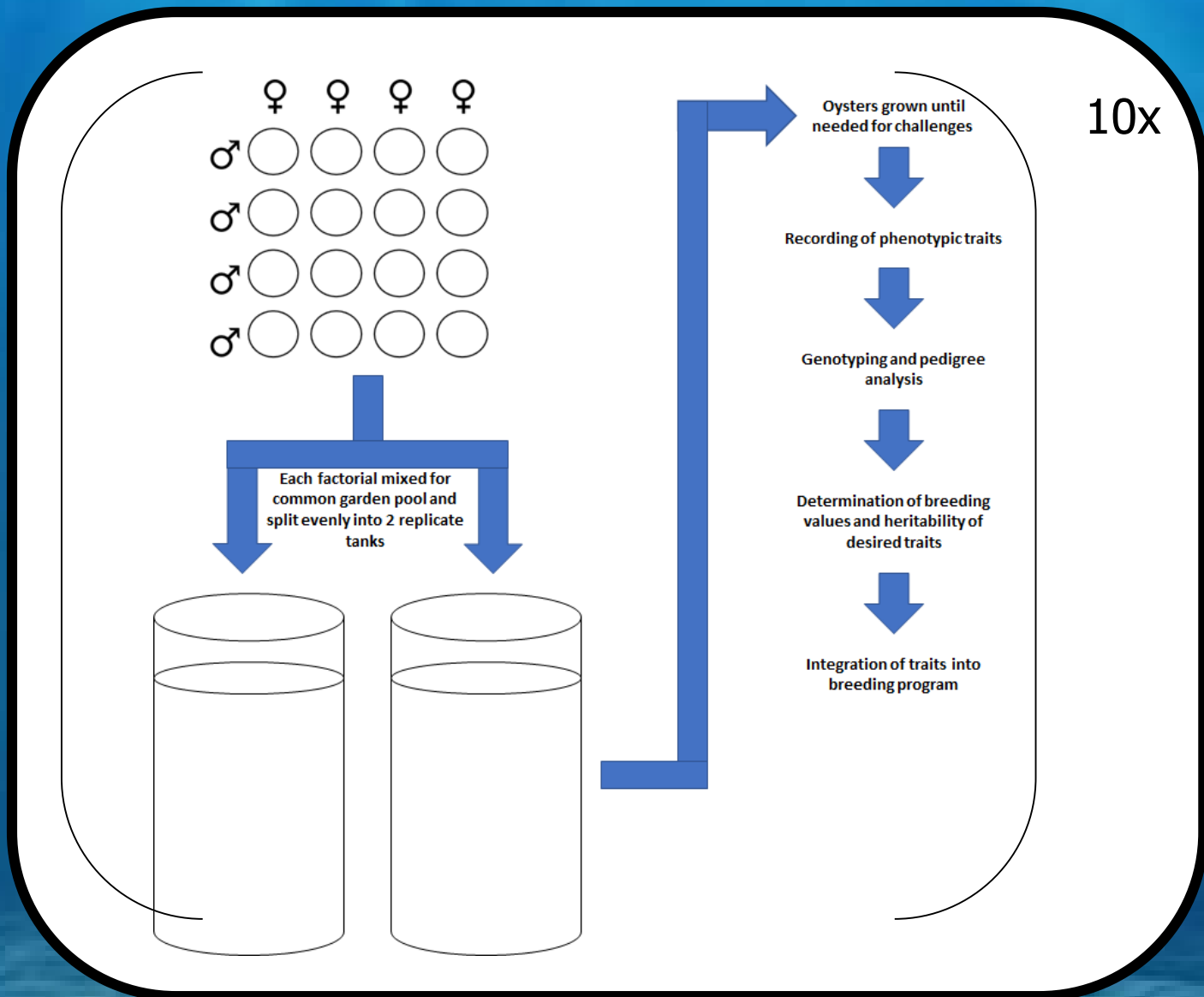
- Thermal tolerance most important in 1+ oysters (second summer post spawn) but measurement of phenotype at 1+ would lengthen generation time
- Estimation of tolerance of juveniles and adults from the same families to estimate heritability and genetic correlation between the two traits
- Experiment includes 160 families (10 minifactorial crosses  $4♀ \times 4♂$ )
- Challenge involves maintaining families close to the upper tolerance limit and apply daily heat challenges to simulate a summer heat wave. Juvenile challenge completed in fall 2021, adult challenge in progress (September 2022)

# Crossing design

- Full sib and half sib families generated by crossing 40 males and 40 females
- This design will produce 160 full sib and 80 half sib families

		Females																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Males	1	X	X	X	X																
	2	X	X	X	X																
	3	X	X	X	X																
	4	X	X	X	X																
	5					X	X	X	X												
	6					X	X	X	X												
	7					X	X	X	X												
	8					X	X	X	X												
	9									X	X	X	X								
	10									X	X	X	X								
	11									X	X	X	X								
	12									X	X	X	X								
	13													X	X	X	X				
	14													X	X	X	X				
	15													X	X	X	X				
	16													X	X	X	X				
	17																	X	X	X	X
	18																	X	X	X	X
	19																	X	X	X	X
	20																	X	X	X	X

# Common garden assessment of heritability and breeding values



# Challenge design

- 10 common garden silos within the same recirculating system
  - 16 randomly selected oysters from each 4x4 factorial in each silo
- Challenge simulated a summer heat wave
  - Acclimation
  - Increase to 38C and sun heat 6 hrs/day
  - Challenge lasted 18 days (juveniles)
  - Mortality and biometrics recorded during challenge and tissue preserved for genotyping and parentage analysis



# Resistance to low salinity stress and *P. marinus*

- Salinity challenge
  - Low salinity challenge simulates a rapid decrease of salinity following spring freshwater events
  - Crossing design and other challenge aspects follow the design of the thermal challenge
  - In progress (October 2022)
- Estimation of breeding values for *P. marinus*
  - Challenge could not be completed in 2021
  - Challenge will be implemented in 2023

# Development of genomic tools and evaluation of progress as 3n

- Development of a medium-density genotyping platform for genomic selection
  - Collaborated with the AMFC consortium (X. Guo) to develop high density array platform (600k SNPs)
  - Broodstocks from additional wild (South Florida Marco Island) and captive (O'Boy LSU line and two selected lines from AUSL) populations assayed at the array
  - Medium genotyping by sequencing platform (5k SNPs) in development
  - Test planned in 2023 and incorporation of genomic selection in third generation
- Test of selected lines for performance as 3n in third generation (collaboration with 4C)

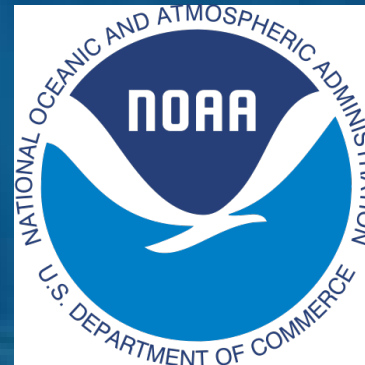
# Acknowledgements

Funding: Gulf States Marine Fisheries Commission  
and NOAA

Auburn, USM and UF teams for support during  
culture phases and sperm processing

Bio-West - Collections of Texas broodstock

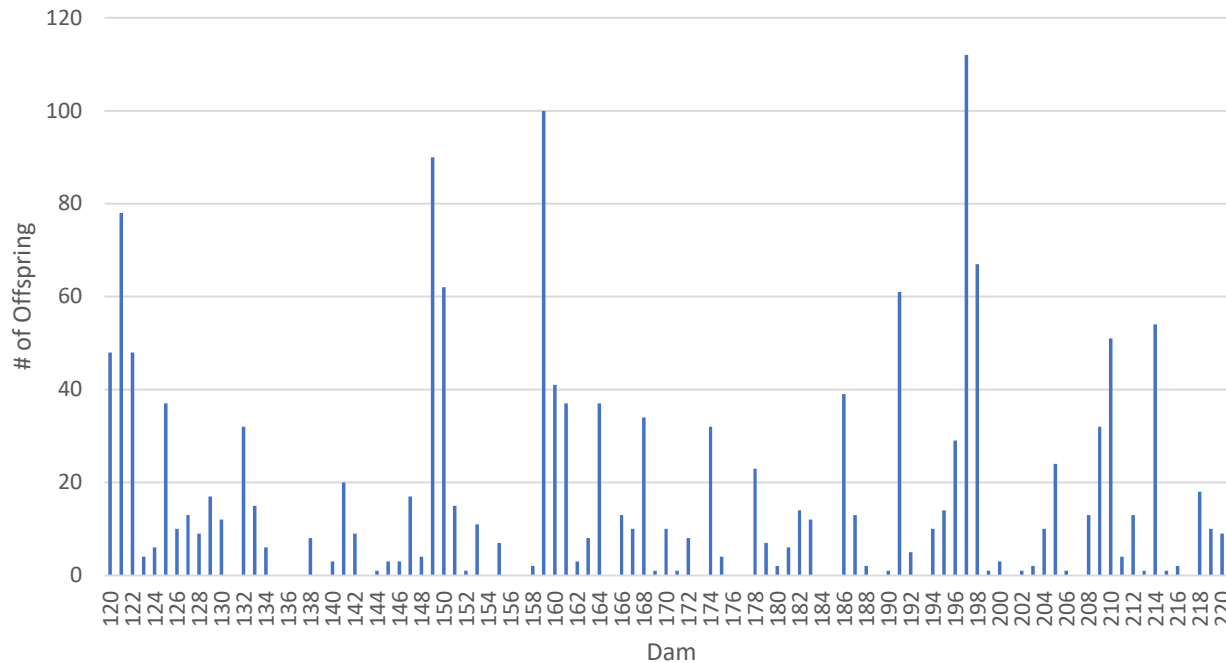
LSU – Collection of Louisiana Broodstock



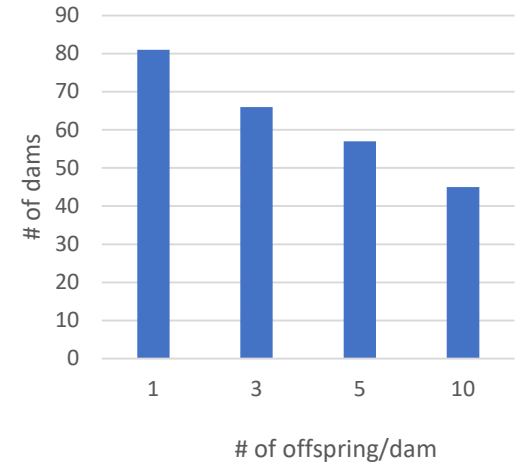


# Contribution of families

Offspring per Dam



Frequency of dam half sib sizes

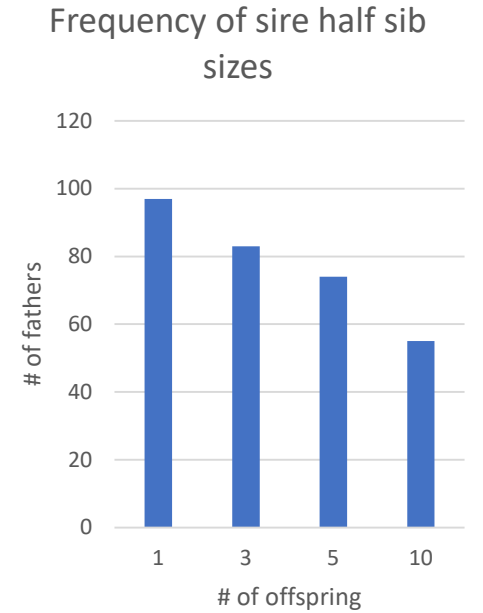
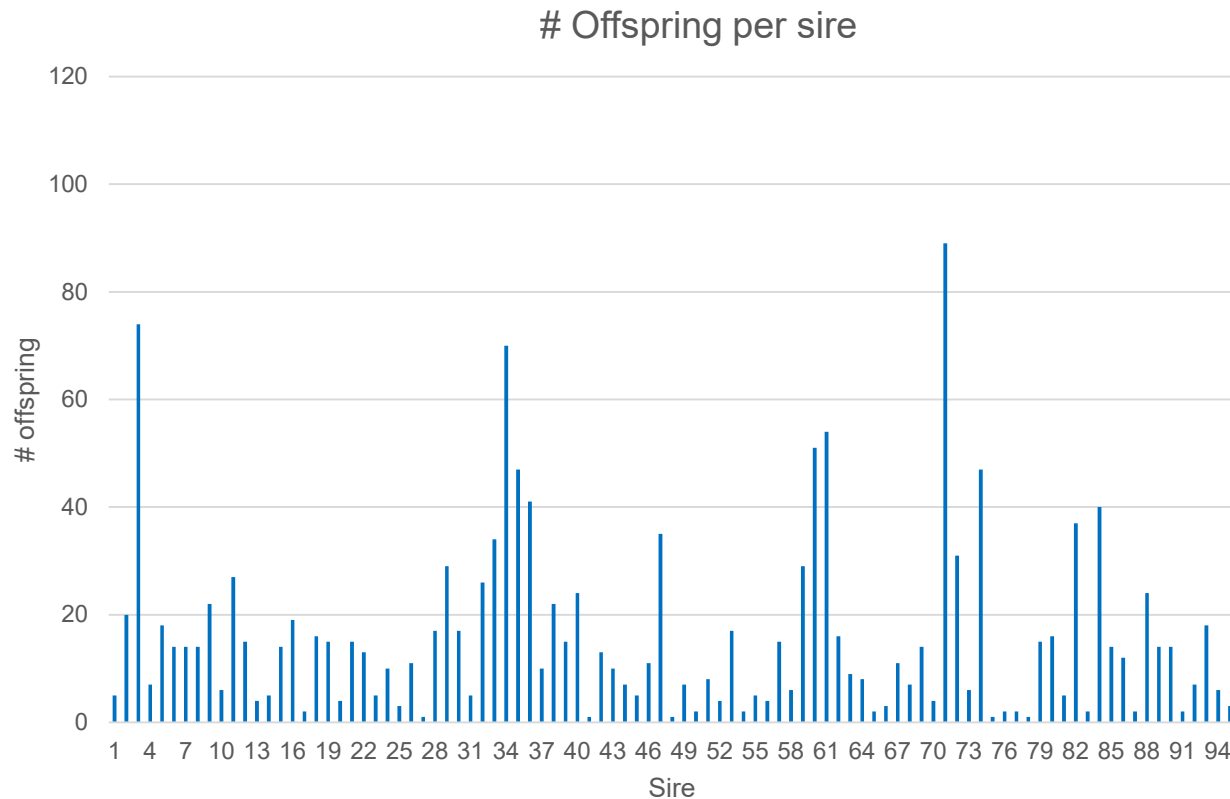


Relative frequency of half sib size (/102)

#o/par	≥1	3	5	10
Dam	79.41%	64.71%	55.88%	44.12%
Sire	95.10%	81.37%	72.55%	53.92%

$$N_{ef} = \frac{N_f \bar{k}_f - 1}{\bar{k}_f + \frac{\sigma_f^2}{\bar{k}_f} - 1} = 37$$

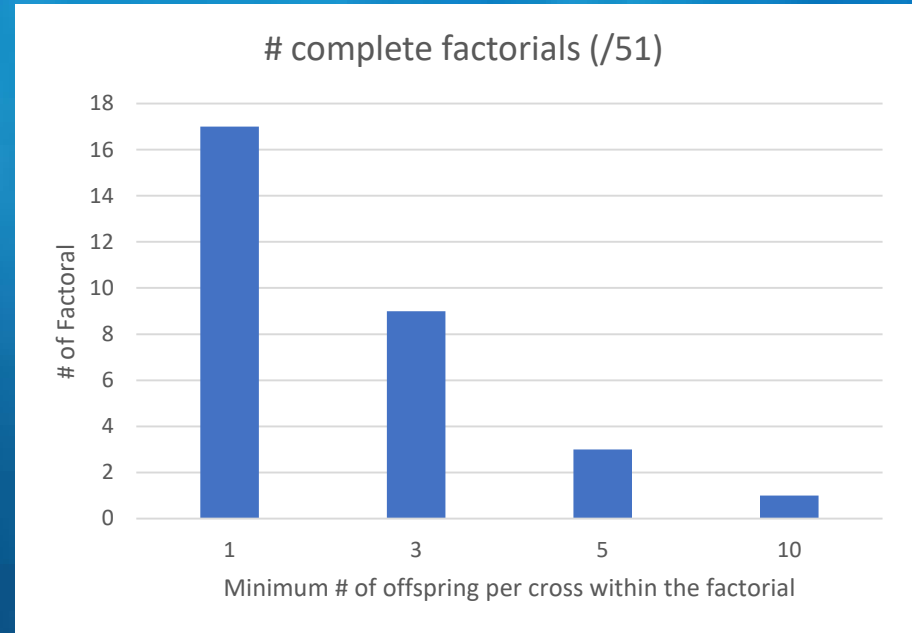
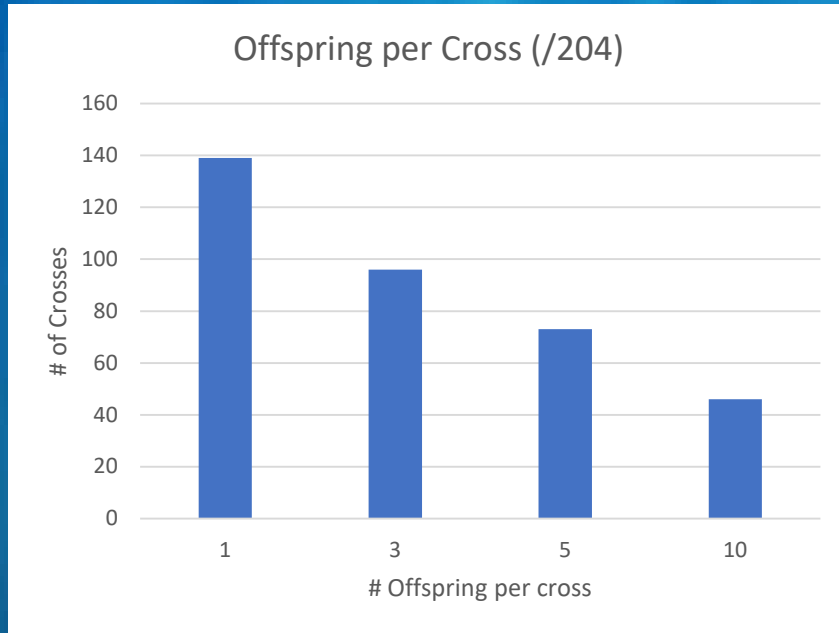
# Contribution of families



Relative frequency of half sib size (/102)				
#o/par	≥1	3	5	10
Dam	79.41%	64.71%	55.88%	44.12%
Sire	95.10%	81.37%	72.55%	53.92%

$$N_{em} = \frac{N_m \bar{k}_m - 1}{\bar{k}_m + \frac{\sigma_m^2}{\bar{k}_m} - 1} = 56$$

# Contribution of families



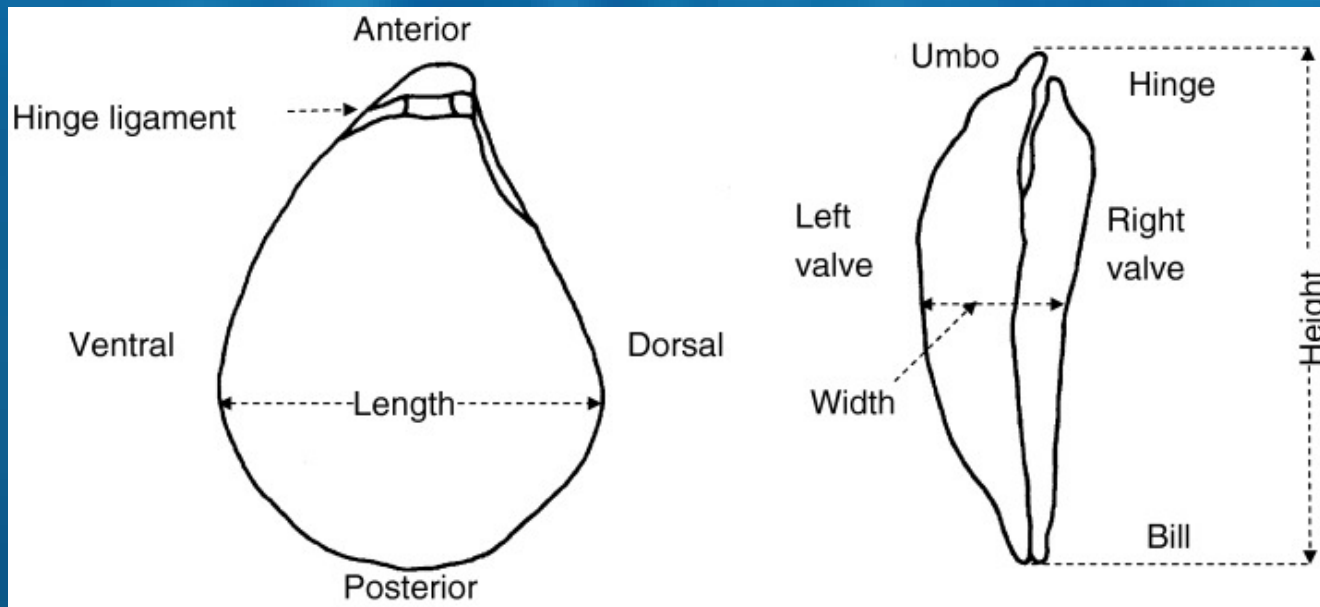
Relative frequency of family sizes				
# off/cross	≥1	≥3	≥5	≥10
Cross (/204)	68.14%	47.06%	35.78%	22.55%
Factorials (/51)*	33.33%	17.65%	5.88%	1.96%

$$N_e = \frac{4N_{em}N_{ef}}{N_{em} + N_{ef}} = 89$$

\* Proportion of 2Damsx2Sires factorials where all 4 families within the factorial are represented with at least 1, 3, 5 or 10 offspring

# Growout results

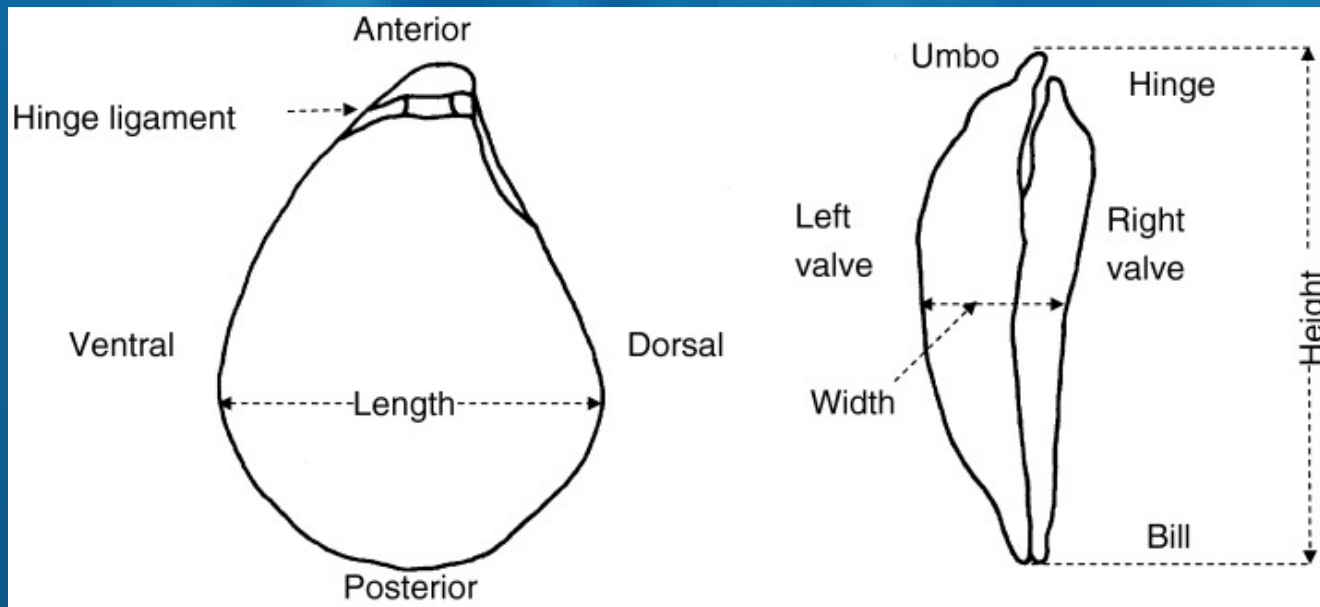
- Oysters were measured for Shell height, width and length



Martin and Gall, International Journal of Food Science and Technology 41(3)

# Larval growth

- Oysters were measured for Shell height, width and length



Martin and Gall, International Journal of Food Science and Technology 41(3)

# Larval growth results

- Analysis model

$$Y = Xb + Za + e$$

b vector of fixed effects of spawning day, a vector of random additive breeding values

- Variance components estimated in VCE 6.0
- Variables were log transformed to reduce scale effects on variance
- Length and width were re-analyzed with height as a covariate to describe variation in shape

# Larval growth results

- The estimate of heritability ( $h^2$ ) of (log transformed) height was  $0.26 \pm 0.04$ .
- Length and width were strongly correlated to height

Phenotypic ( $r_p$ ) and genetic ( $r_g$ ) correlations between height and length and width

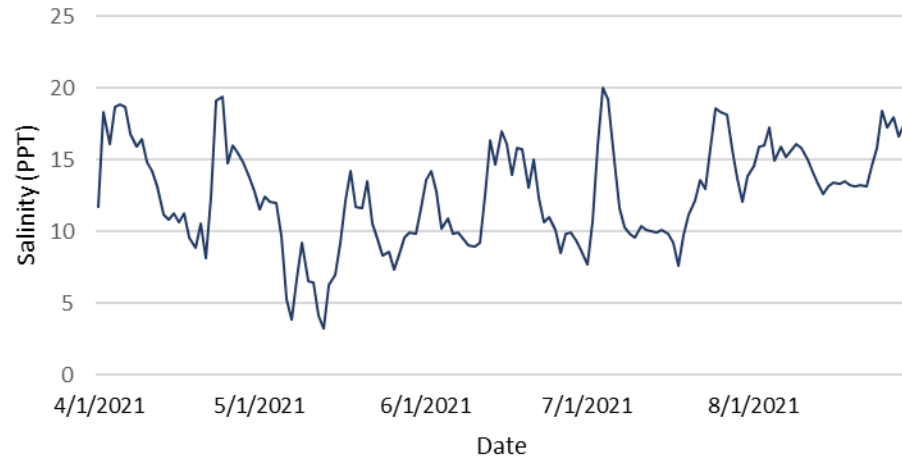
	$r_p$	$r_g$
Length	0.89	$0.92 \pm 0.02$
width	0.77	$0.89 \pm 0.04$

- The estimate of  $h^2$  of length and width analyzed with height as a covariate were  $0.36 \pm 0.07$  and  $0.21 \pm 0.04$
- Results may reflect differences in ontogenic stages
- Dominance could not be estimated accurately with the dataset

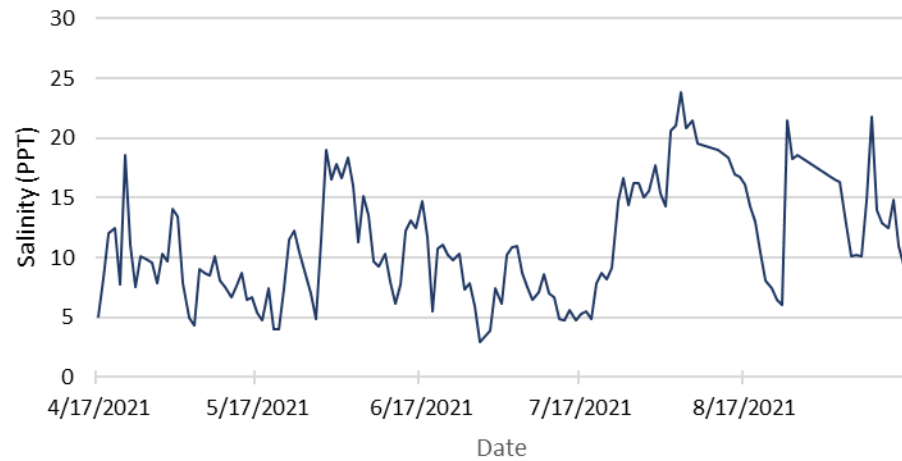




### Salinity at LSU April-August



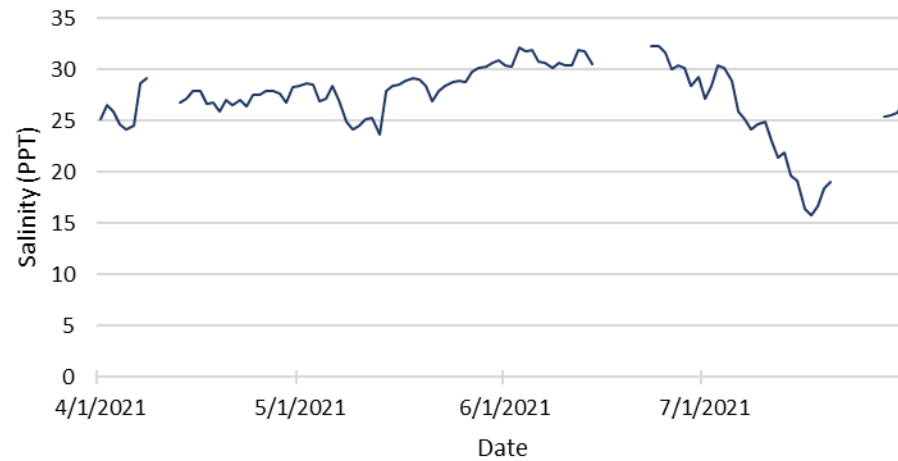
### Salinity at DITS April-September 2021



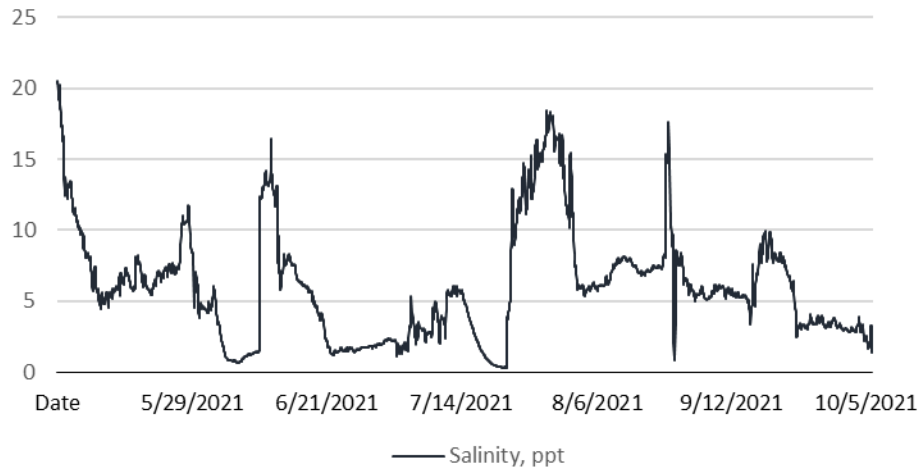
### Salinity at AH April-August 2021



### Salinity at CK April-July 2021



### Salinity at Mobile Bay May-October



### Salinity at OB April-July 2021

