

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

project update 10/17/2022

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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

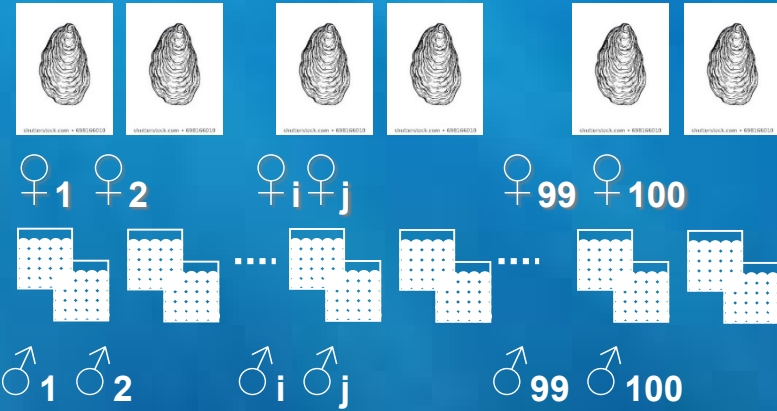
Base population

- 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

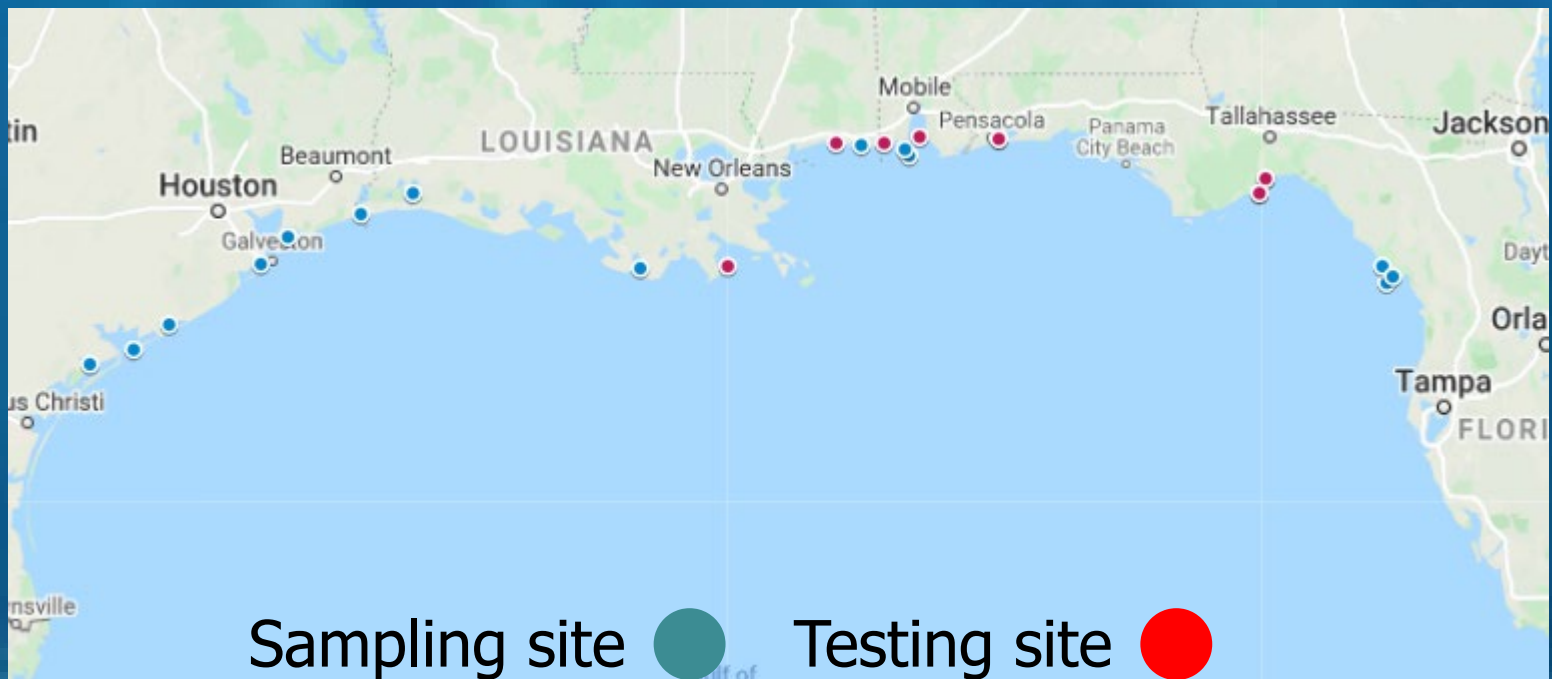
Trait measurements, genotyping and pedigree analyses

Estimate breeding values and select parents for next generation

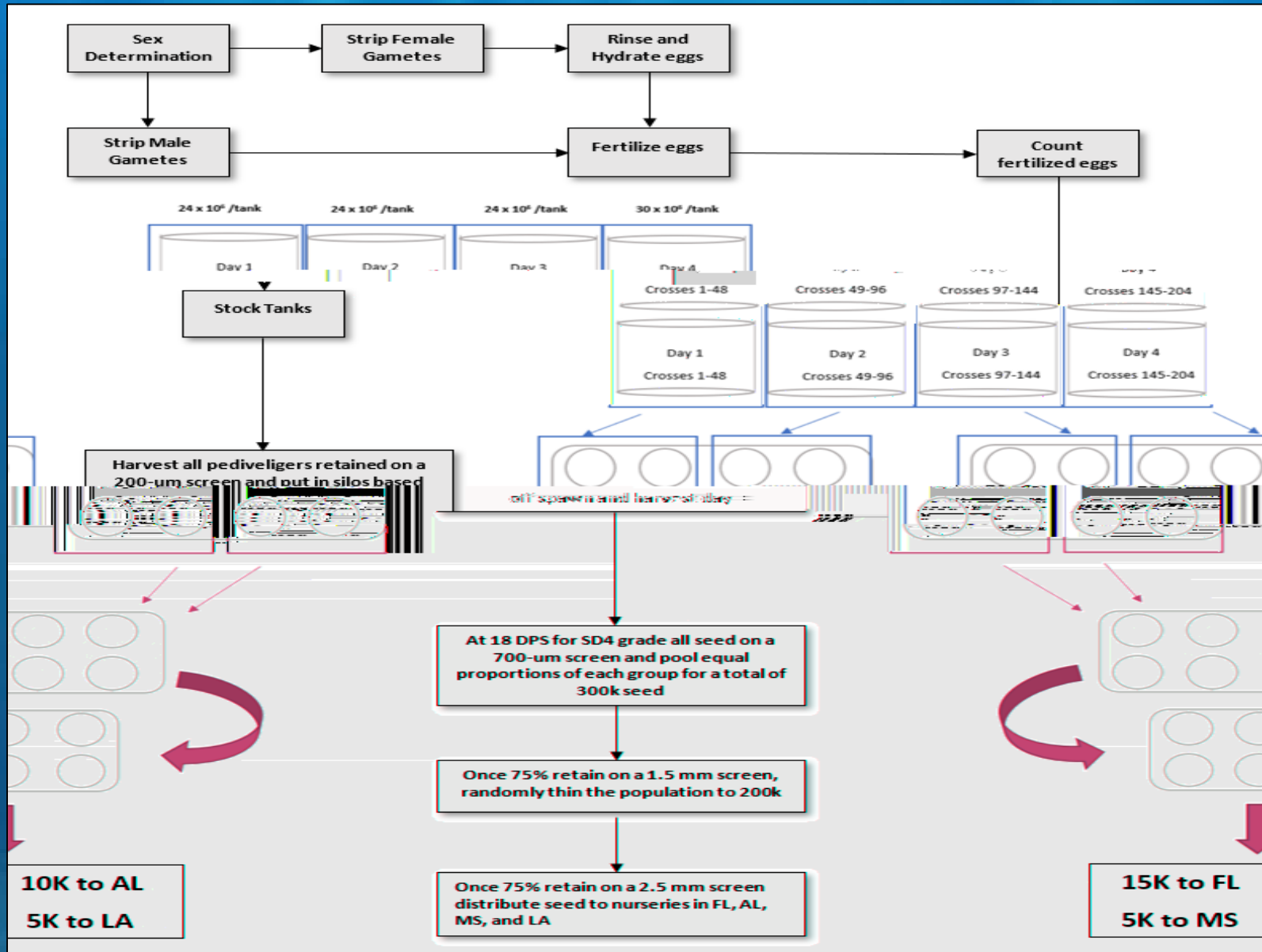


Founding population

- 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



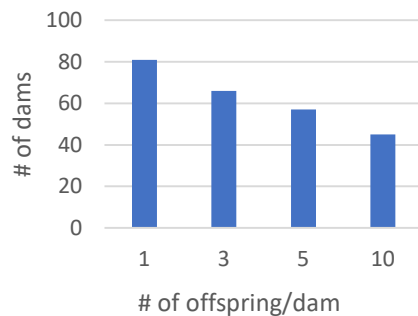
Culture prior to growout



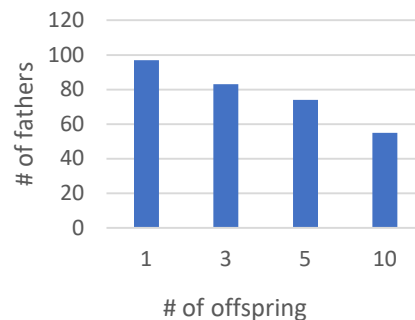
Spawning results (F1 generation)

- 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

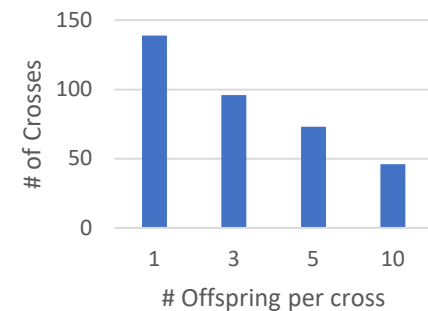
Frequency of dam half sib sizes



Frequency of sire half sib sizes



Frequency of full sib sizes



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

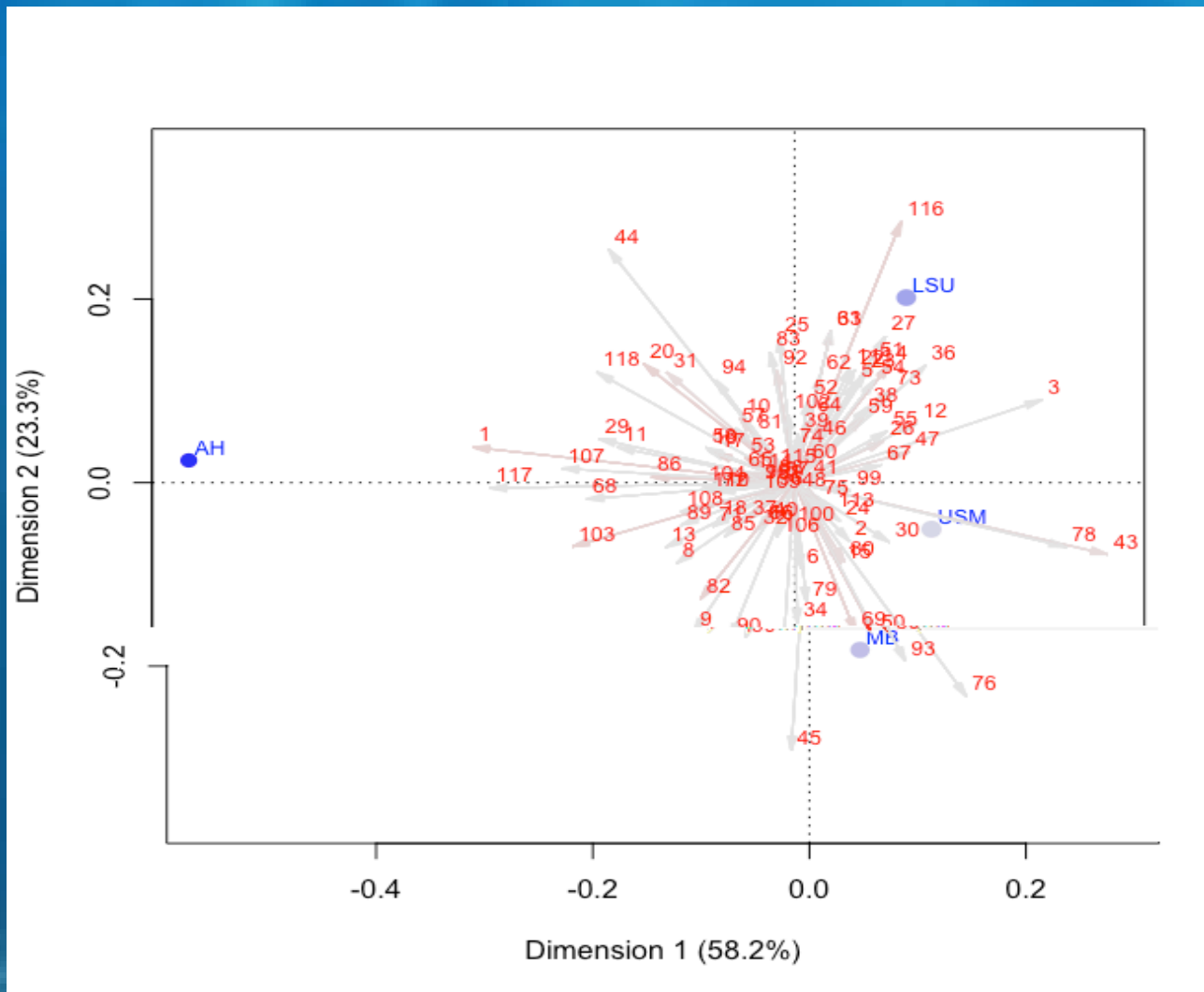
Site	Salinity	Harvest date	# survivors (%)	Height (mean \pm SD)	% back bend
Alligator Harbor	High	10/25	997 (33)	55.3 \pm 8.2	74
Mobile Bay	Low	10/18	1,950 (78)*	43.2 \pm 5.7	99
Deer Island	Medium	10/20	2,659 (89)	44.5 \pm 5.8	98
Grand-Isle	Medium	11/02	2,779 (93)	79.8 \pm 10.1	70

* 2 bags lost due to storm damage

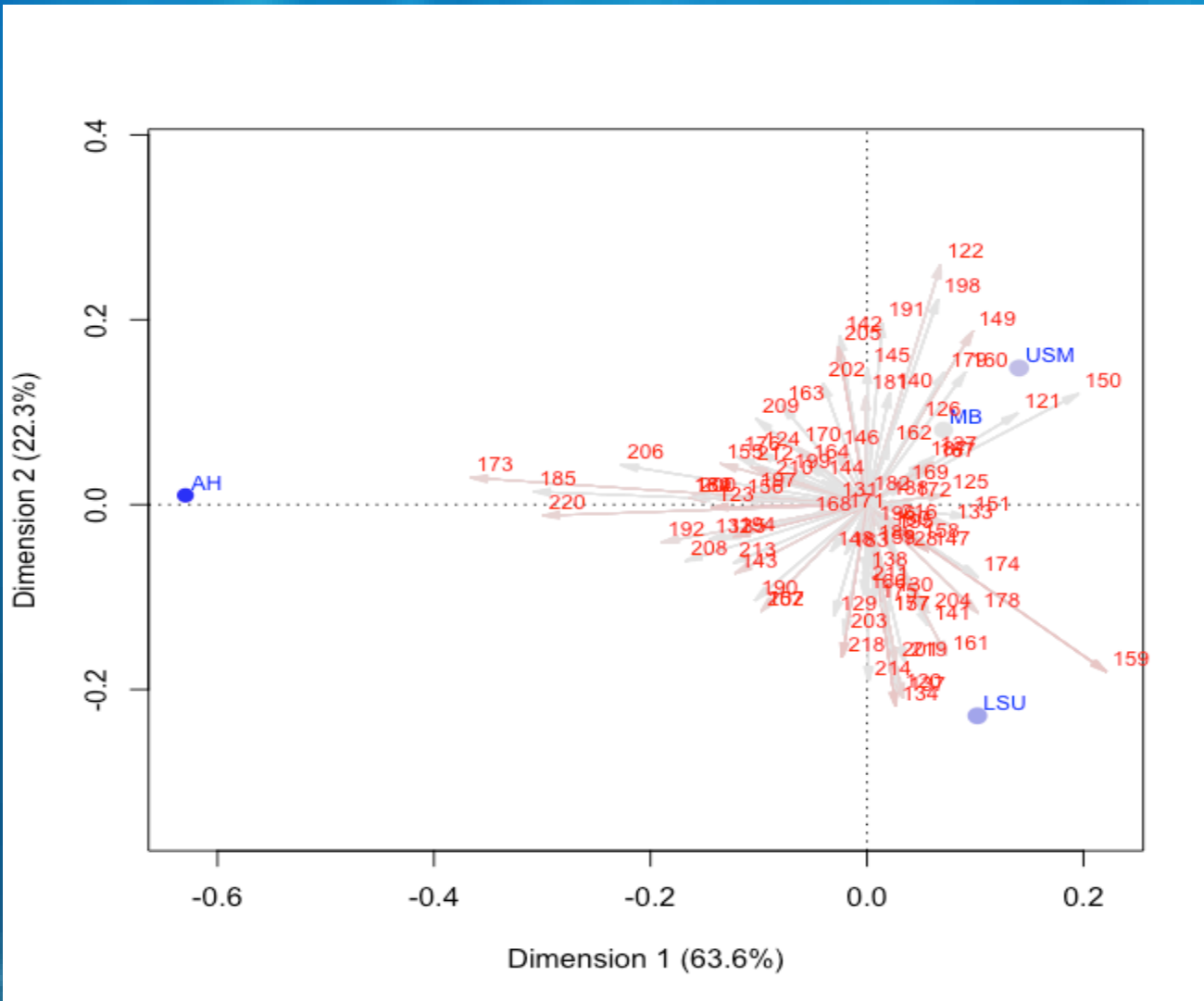
Sampling and parentage analysis

- 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.

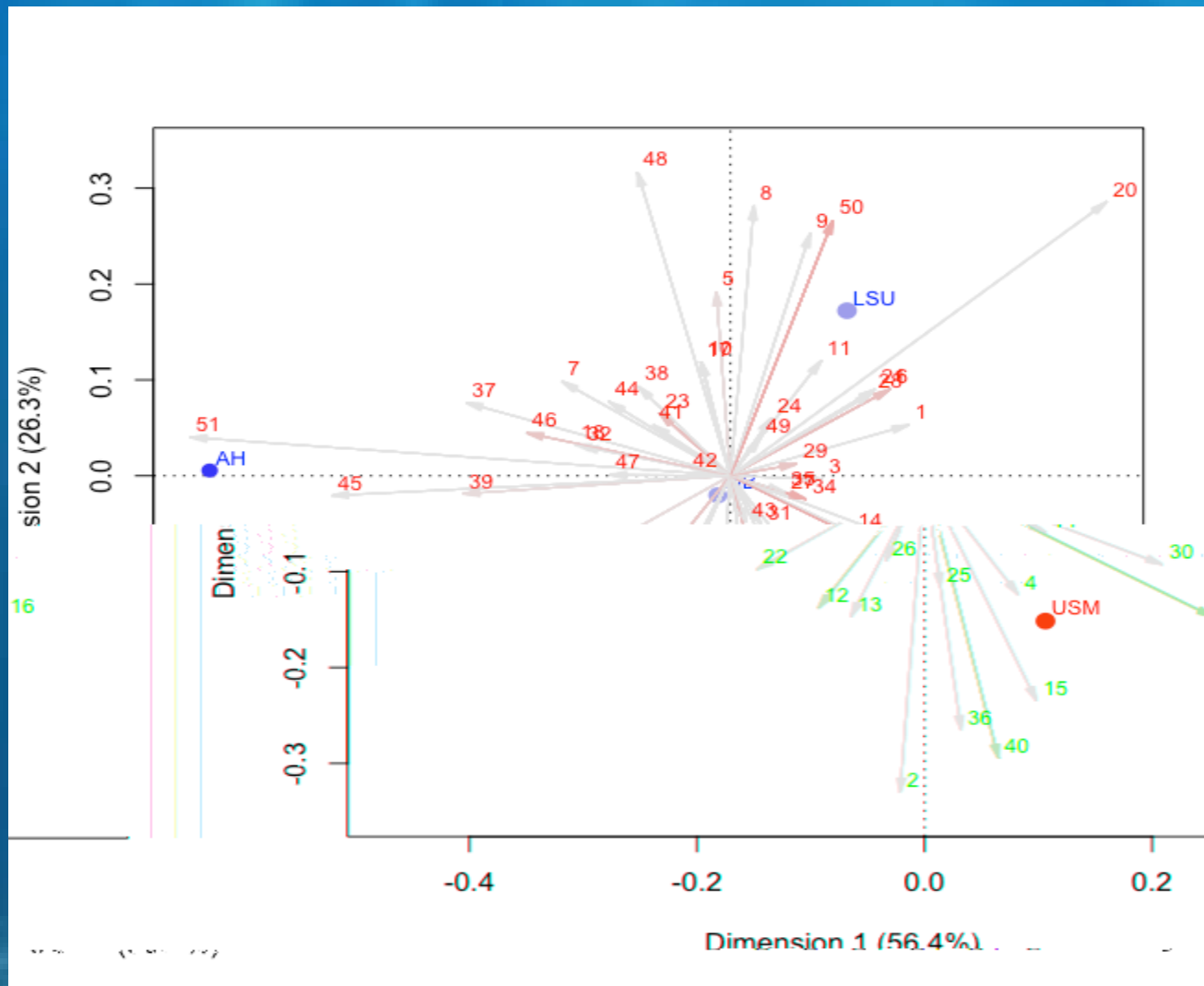
Contribution of sires



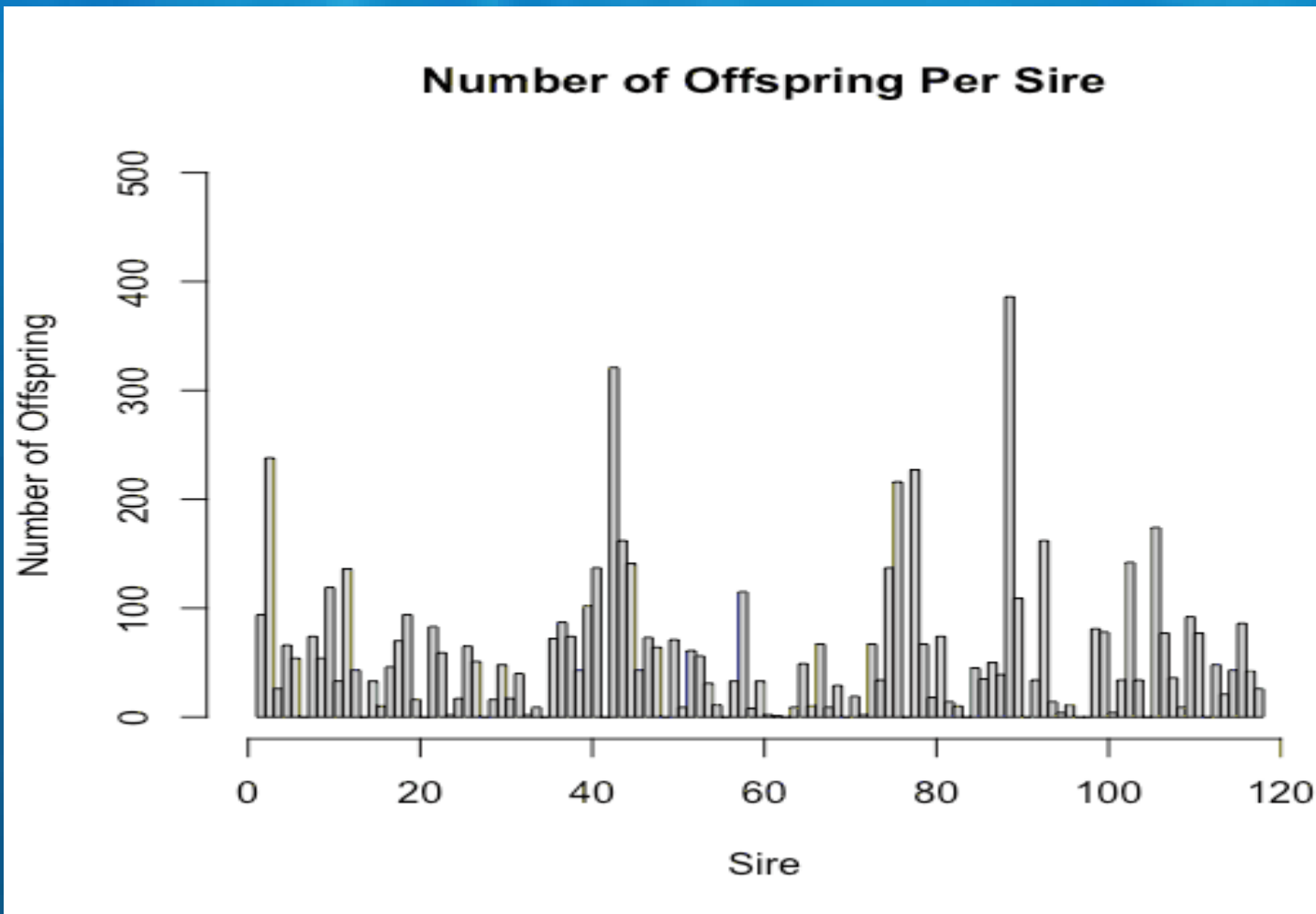
Contribution of dams



Contribution of factorials

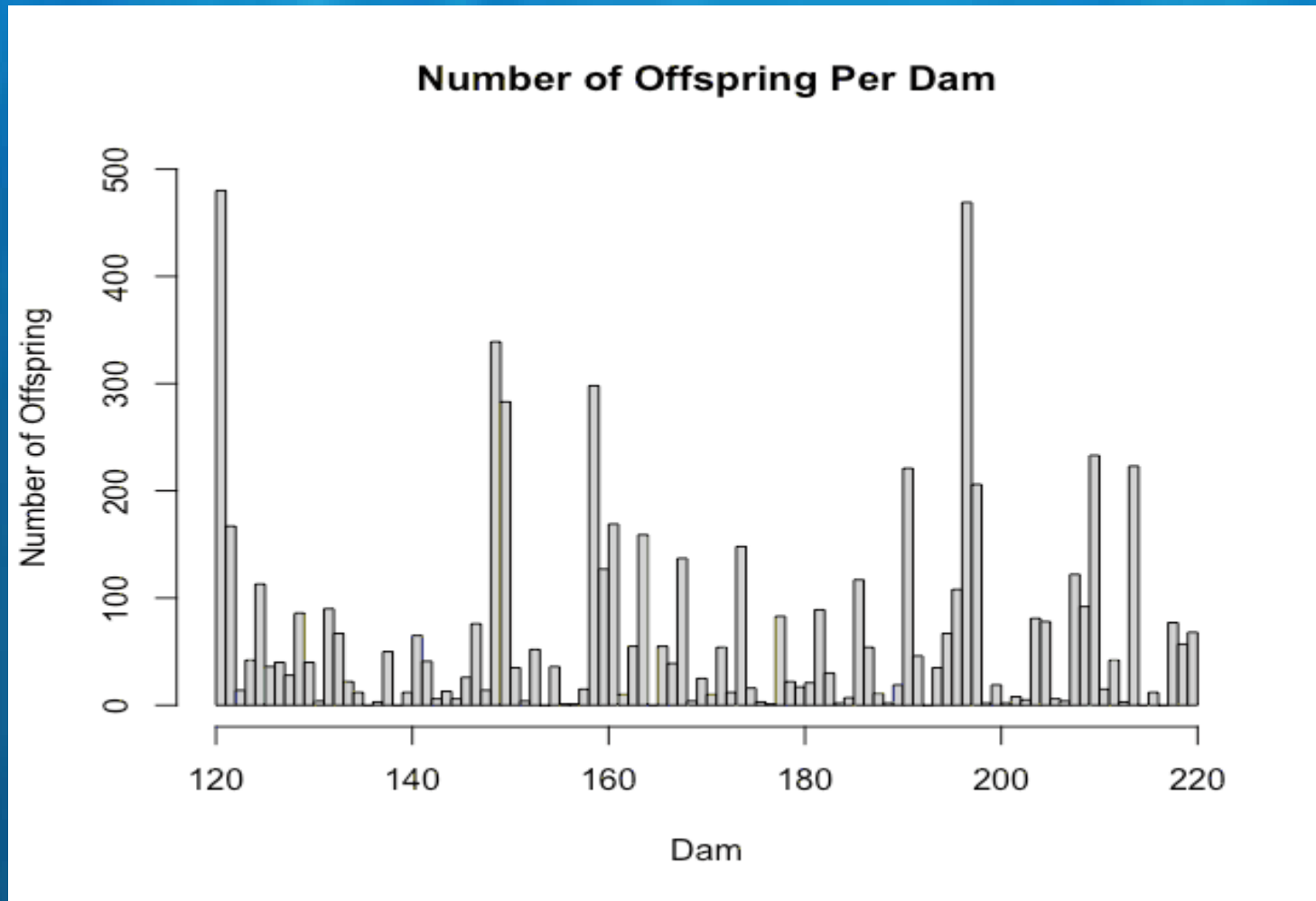


Contribution of families



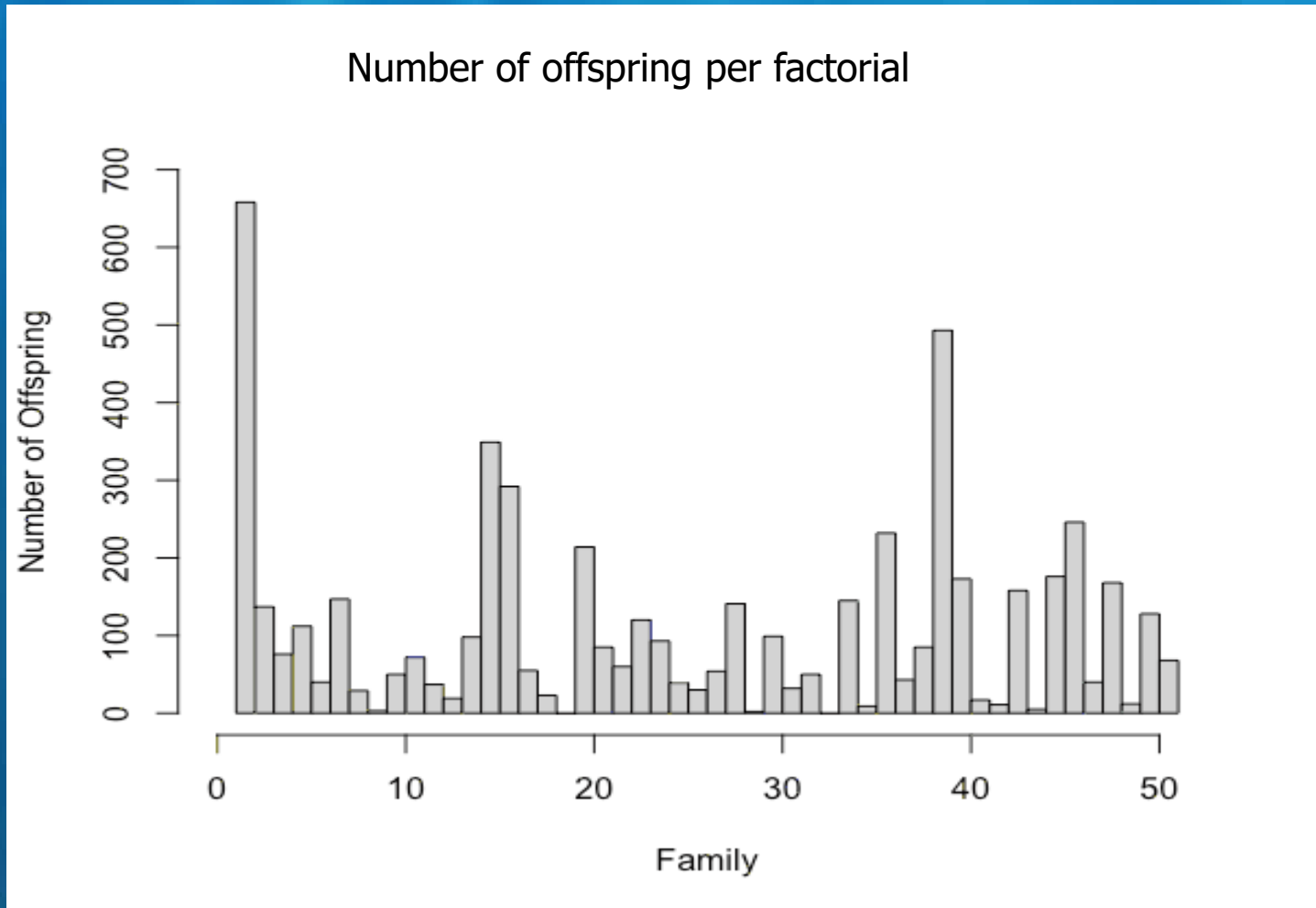
$$N_{em-AH} = 52, N_{em-LS} = 47$$

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 ± 0.05	0.11 ± 0.05
MB, DI, GB	0.50 ± 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 07/11-07/13 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

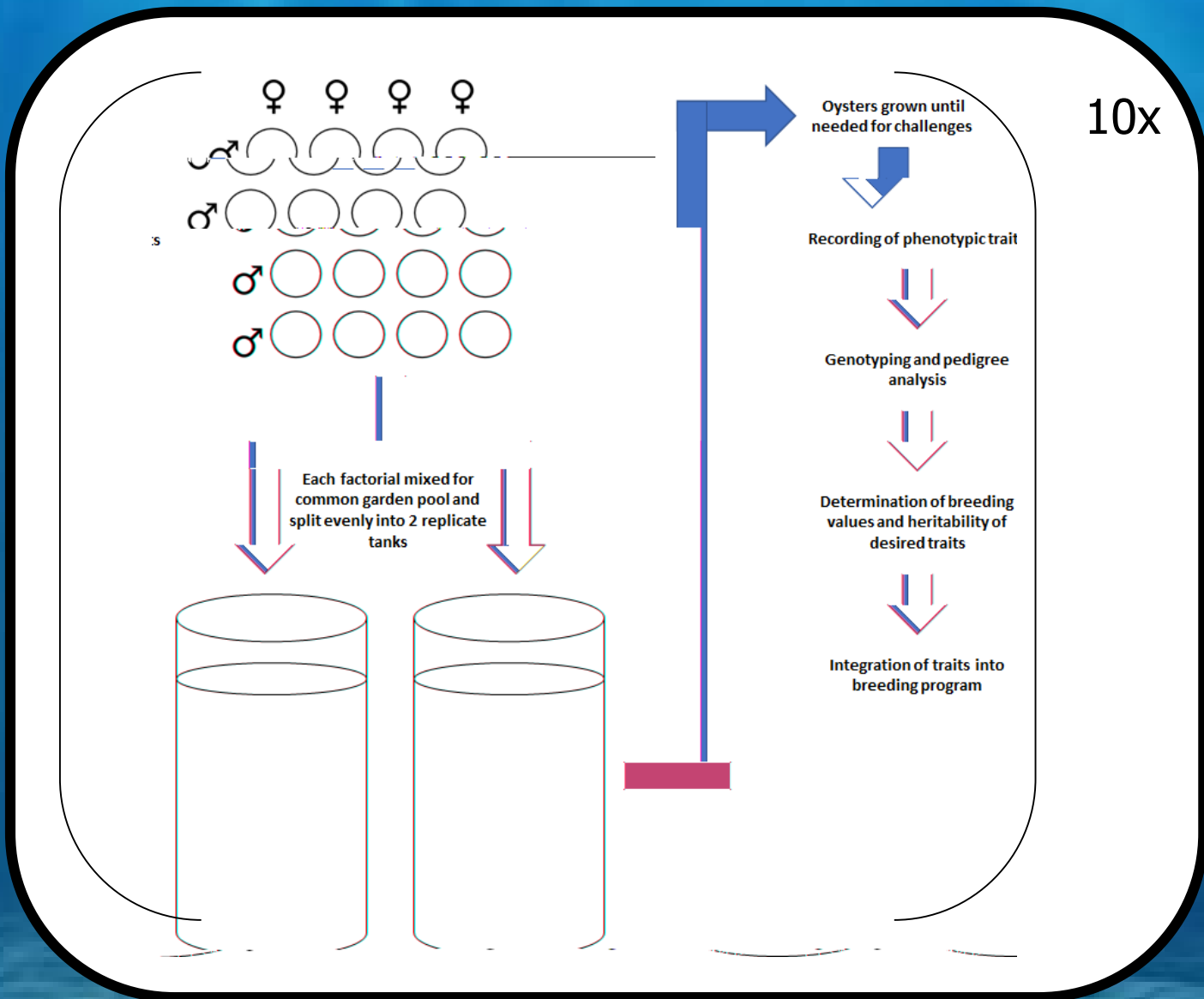
- Thermal tolerance most important in 1+ oysters (second summer post spawn) but measurement of phenotype at 1+ would lengthen generation time
- Estimation of tolerance of juveniles and adults from the same families to estimate heritability and genetic correlation between the two traits
- Experiment includes 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)

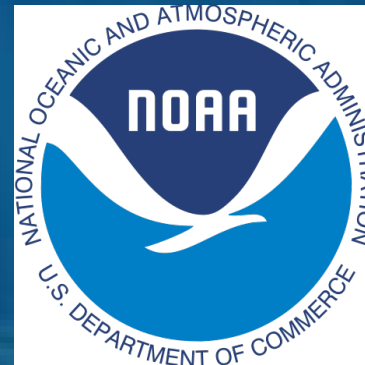
Acknowledgements

Funding: Gulf States Marine Fisheries Commission
and NOAA

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

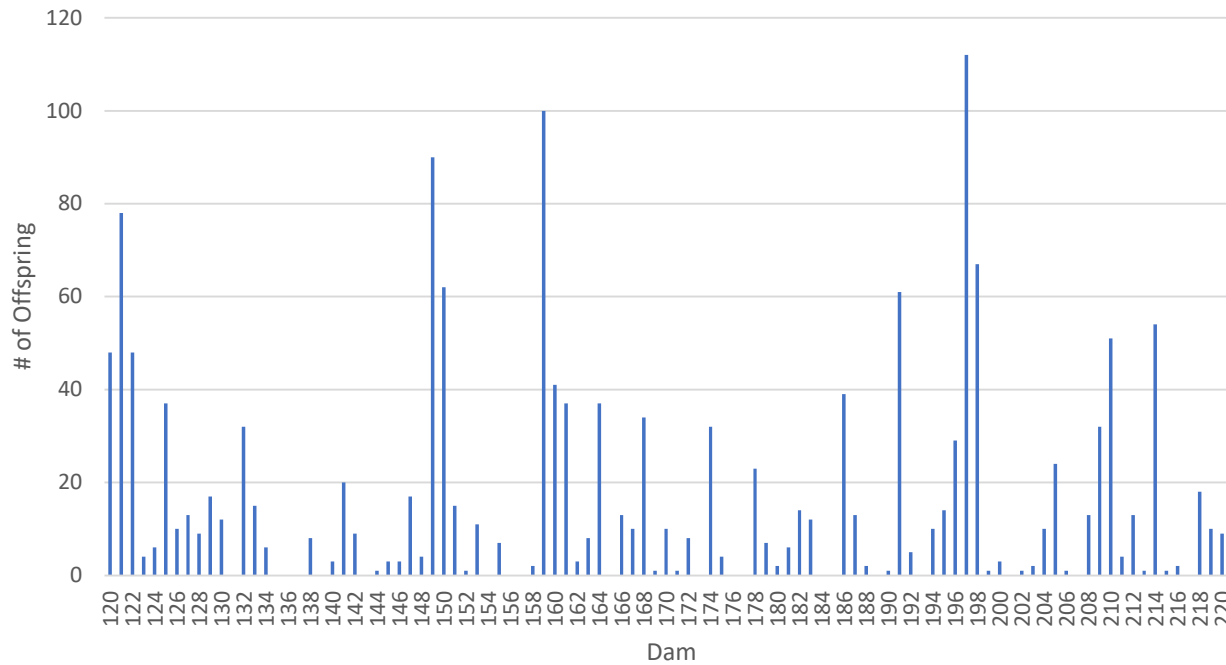
Bio-West - Collections of Texas broodstock

LSU – Collection of Louisiana Broodstock

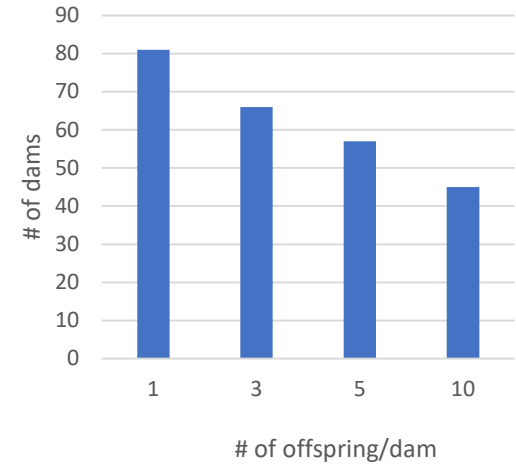


Contribution of families

Offspring per Dam



Frequency of dam half sib sizes



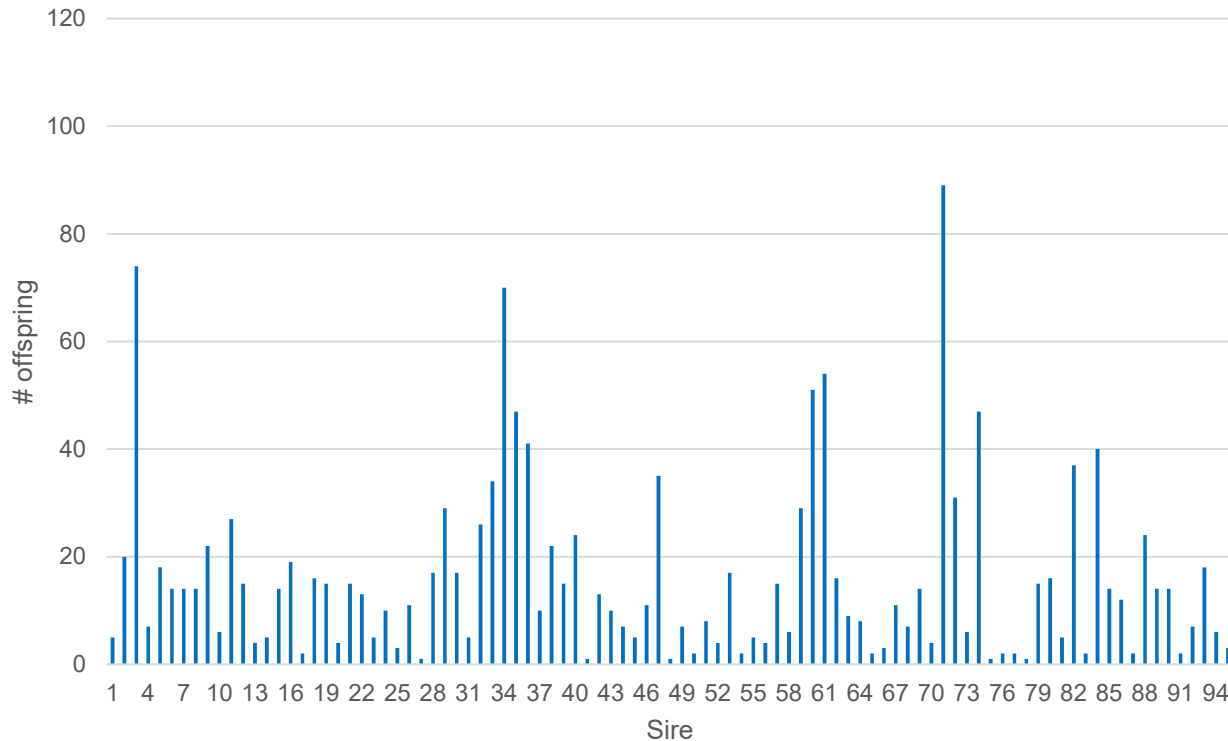
Relative frequency of half sib size (/102)

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

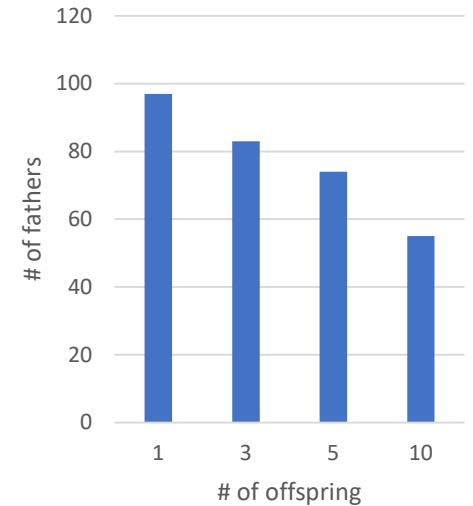
$$N_{ef} = \frac{N_f \bar{k}_f - 1}{\bar{k}_f + \frac{\sigma_f^2}{\bar{k}_f} - 1} = 37$$

Contribution of families

Offspring per sire



Frequency of sire half sib sizes

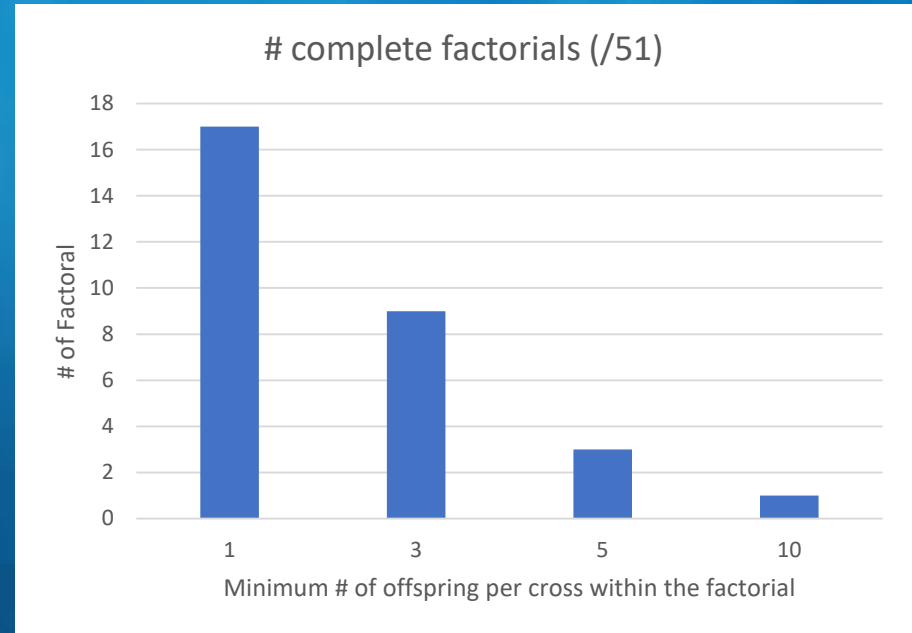
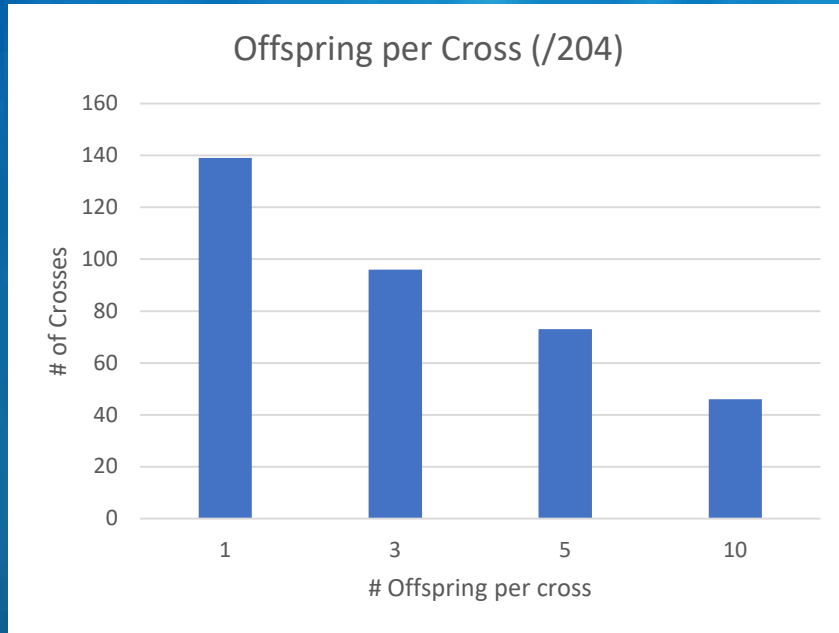


Relative frequency of half sib size (/102)

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

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

Contribution of families



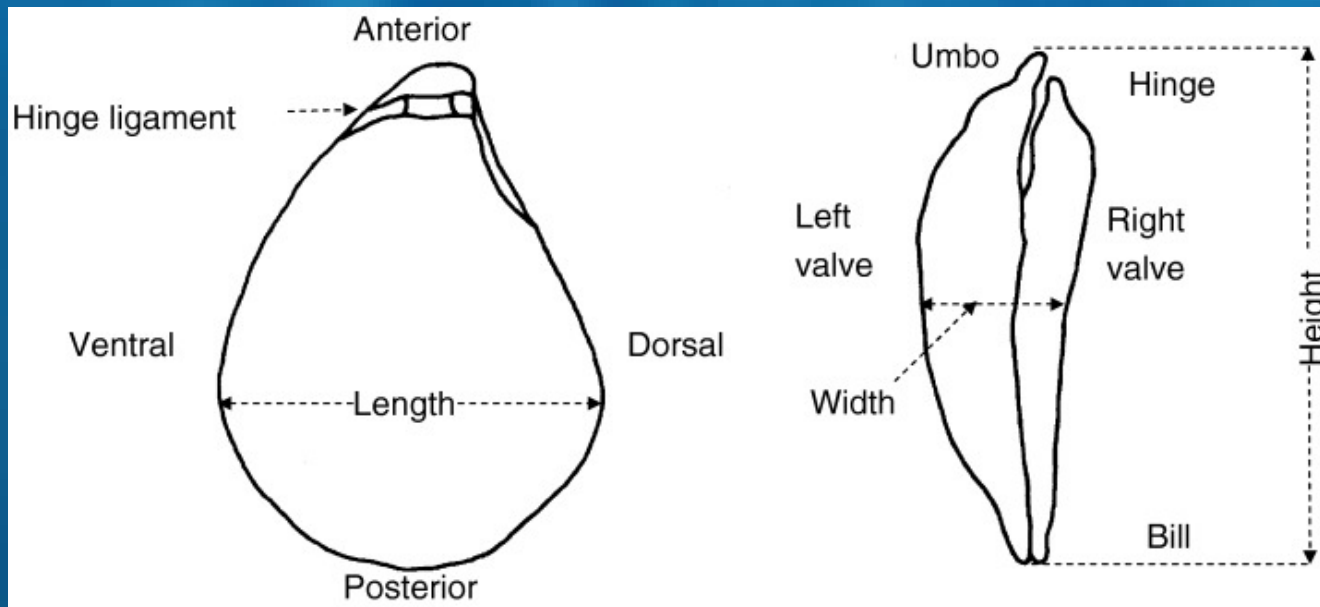
Relative frequency of family sizes				
# off/cross	≥1	≥3	≥5	≥10
Cross (/204)	68.14%	47.06%	35.78%	22.55%
Factorials (/51)*	33.33%	17.65%	5.88%	1.96%

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

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

Growout results

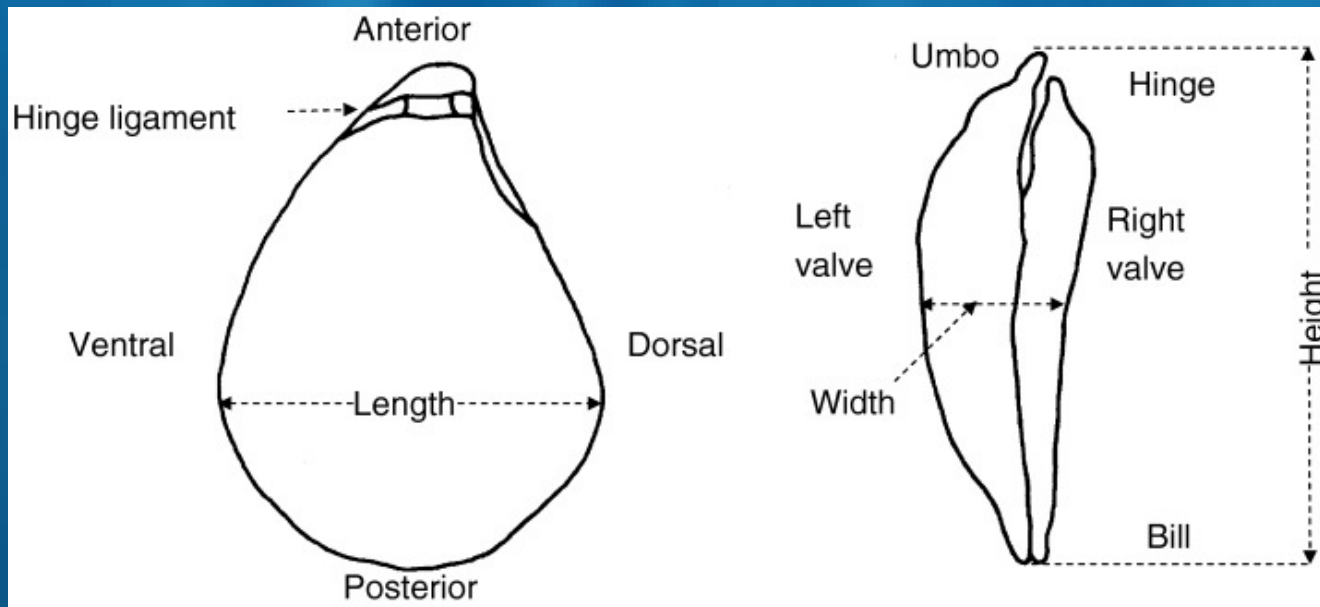
- Oysters were measured for Shell height, width and length



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 6.0.0
- 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

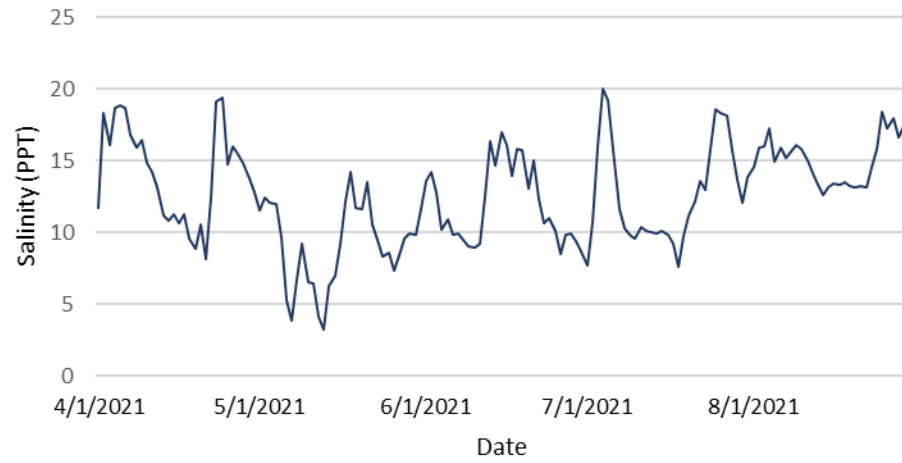
- The estimate of heritability (h^2) of (log transformed) height was 0.26 ± 0.04 .
- Length and width were strongly correlated to height

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

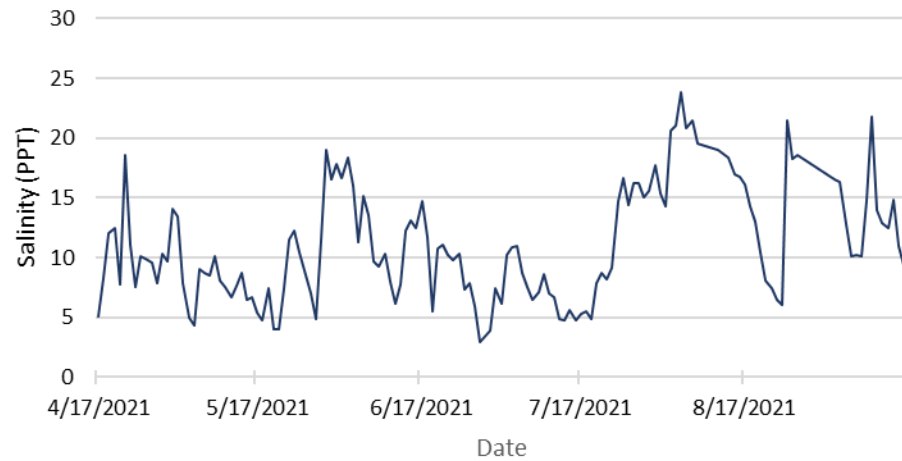
	r_p	r_g
Length	0.89	0.92 ± 0.02
width	0.77	0.89 ± 0.04

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

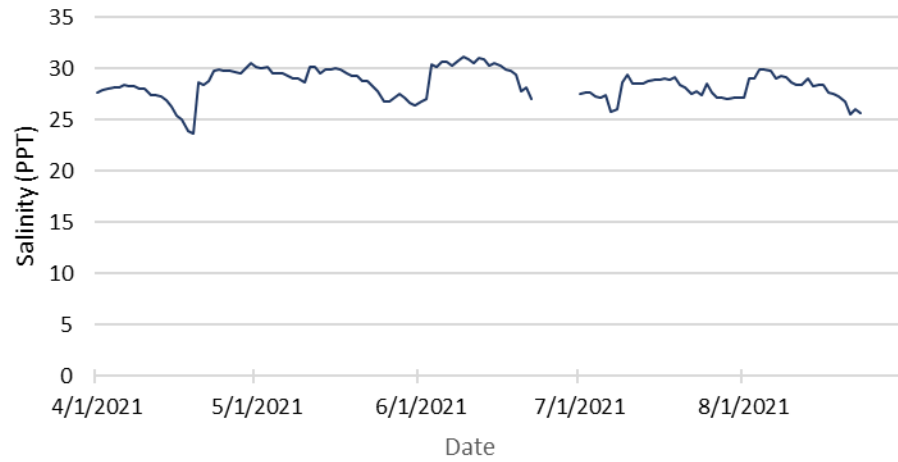
Salinity at LSU April-August



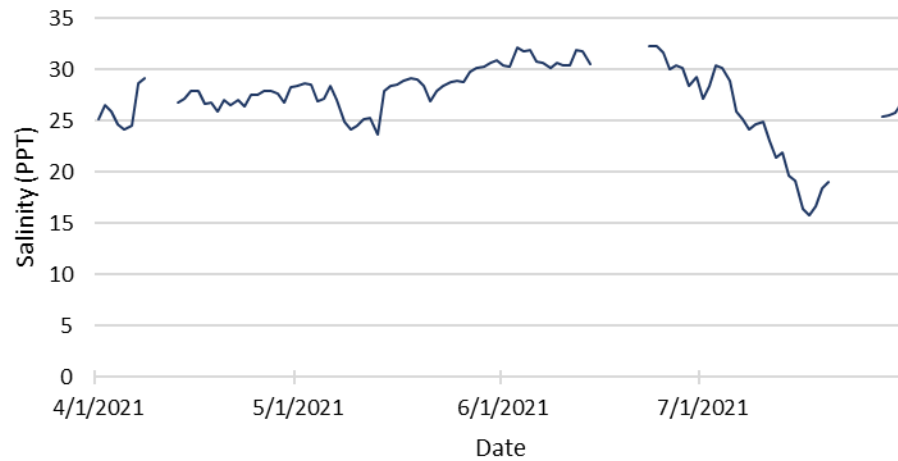
Salinity at DITS April-September 2021



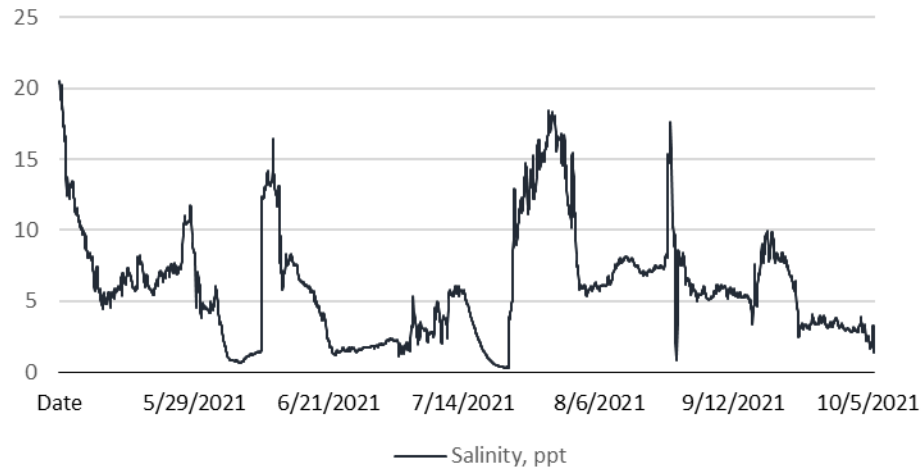
Salinity at AH April-August 2021



Salinity at CK April-July 2021



Salinity at Mobile Bay May-October



Salinity at OB April-July 2021

