Clam Breeding Projects

Improving Florida Hard Clam Production by Hybridization, Backcrossing F1 Hybrids, Thermal Selection, and Use of Wild Stocks

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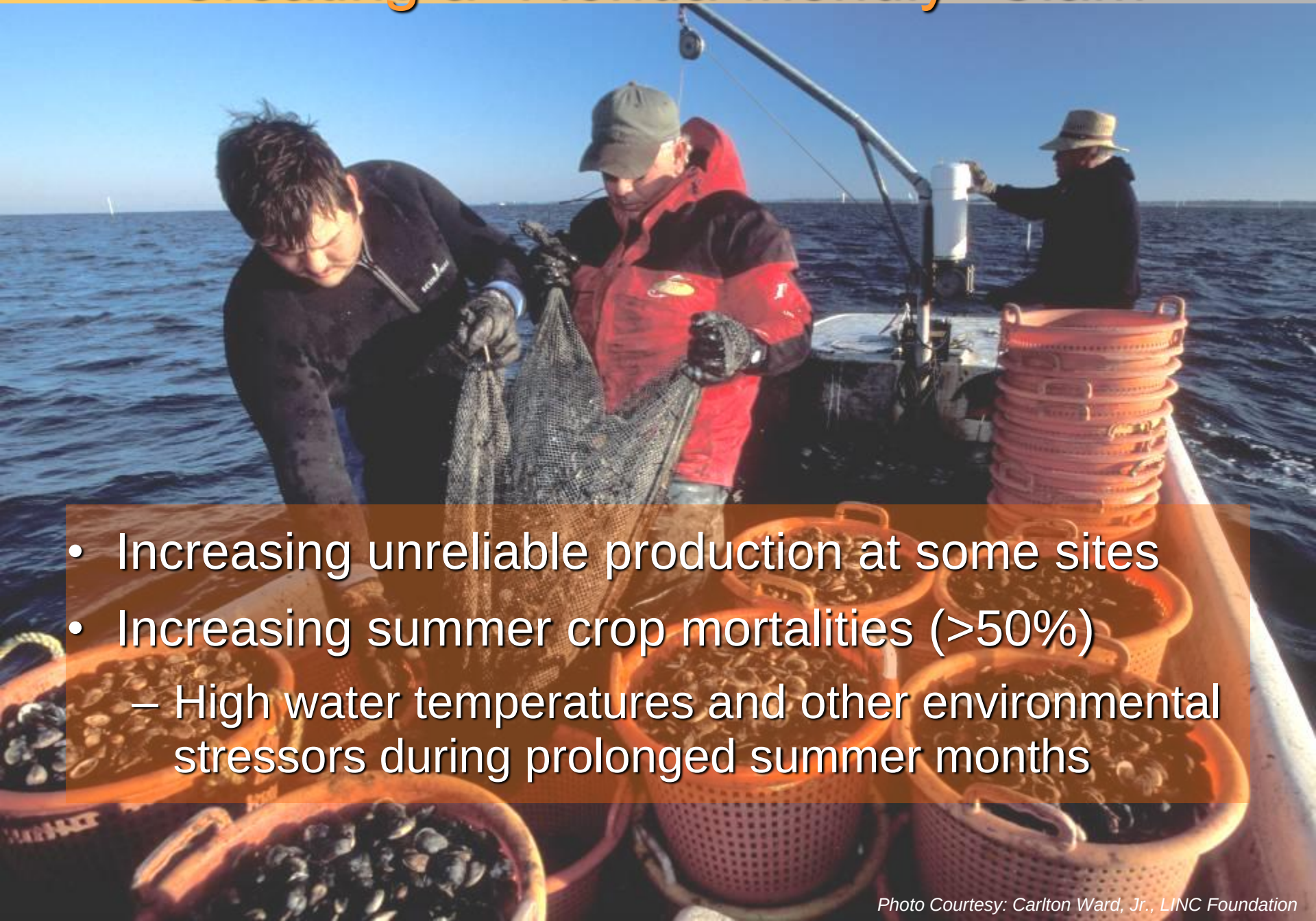
HARBOR BRANCH
FLORIDA ATLANTIC UNIVERSITY

Creating a “Florida-friendly” Clam



- Increasing unreliable production at some sites
- Increasing summer crop mortalities (>50%)
  - High water temperatures and other environmental stressors during prolonged summer months

Photo Courtesy: Carlton Ward, Jr., LINC Foundation

Improvement of Cultured Clam Stocks through Hybridization, 2007-9*

- Hybridization is a common breeding technique
- Hybrids have superior traits to either parent species
- The use of clam hybridization for “mariculture” potential was examined in the 1960-70s by Winston Menzel at Florida State University
  - Showed hybrids had improved growth, shelf life
  - Little data reported on merit of hybrids for improved survival
- A rigorous examination of clam hybridization was conducted
  - To improve production
  - To assure product quality

* Scarpa, J., Sturmer, L.N., Arnold, W., Geiger, S. and Baker, S.M. 2009. Culture of hard clam hybrids (*Mercenaria mercenaria*, *M. campechiensis*): Hatchery to field-nursery. Journal of Shellfish Research 28(3): 727-728.

Clam Species

- Northern hard clam
  - Gulf of St. Lawrence to Florida
  - Supports aquaculture and fishing industries
- Southern quahog
  - North Carolina to Caribbean
  - Supports recreational fishery
  - Traits for resisting environmental stressors
  - Gapes in refrigerated storage
- *Mercenaria* species normally separated by environmental tolerances
  - Hybridize where they do co-occur and under hatchery conditions
  - Produced and tested 3 families (A,B,C)
    - Parental stocks (♀X♂: MxM, CxC)
    - Reciprocal hybrids (♀X♂: **MxC**, **CxM**)

Northern hard clam
Mercenaria mercenaria notata

Southern quahog
Mercenaria campechiensis

Summary*

- Hybridization may offer improved clam production performance
  - **MxC**, ↑ SW and DryMtWt
- Genetic background played a significant role in responses
  - Family A, **MxC** ↑ SW,TW, DMtWt, Yield
  - Family C, **CxM** ↑ SW,TW, DMtWt, Yield
- Shelf life acceptable
  - 10 days for **MxC** (88%)
  - 8 days for **CxM** (98%)
- Gaping in storage problematic
  - By day 8 for **MxC** (47%)
  - By day 4 for **CxM** (63%)

* Sturmer, L.N., Scarpa, J. and Baker, S.M. 2010. Culture of hard clam hybrids (*Mercenaria mercenaria*, *M. campechiensis*): Results of growout production trials. Page 966, Book of Abstracts, Aquaculture 2010, San Diego, CA.

Improvement of Stocks by Backcrossing F1 Hybrids with Hard Clams, 2009-11*

- Mating of a hybrid with its parental species
- F1 Hybrids (**MxC** and **CxM**) backcrossed to hard clams (**MxM**) as female or male
- Objectives:
  - Improve product quality
  - Maintain improved growth and survival

* Sturmer, L.N., Scarpa, J., White, W., and Baker, S.M. 2012. Improving hard clam production in Florida through culture of backcrossed hybrids (*Mercenaria mercenaria*, *M. campechiensis*). Journal of Shellfish Research 31(1): 351.

Hatchery Production*

- Hatchery techniques modified for control of gamete collection
- Multi-parental spawns
- Five families produced
  - Dec 2009 – Feb 2010
  - 19 individual stocks

*Scarpa, J., Sturmer, L.N. and Baker, S.M. 2011. Hatchery and field-nursery culture of backcrossed hybrid (*Mercenaria mercenaria*, *M. campechiensis*) hard clams. J. Shellfish Research 30(2):551.

Backcross Parents

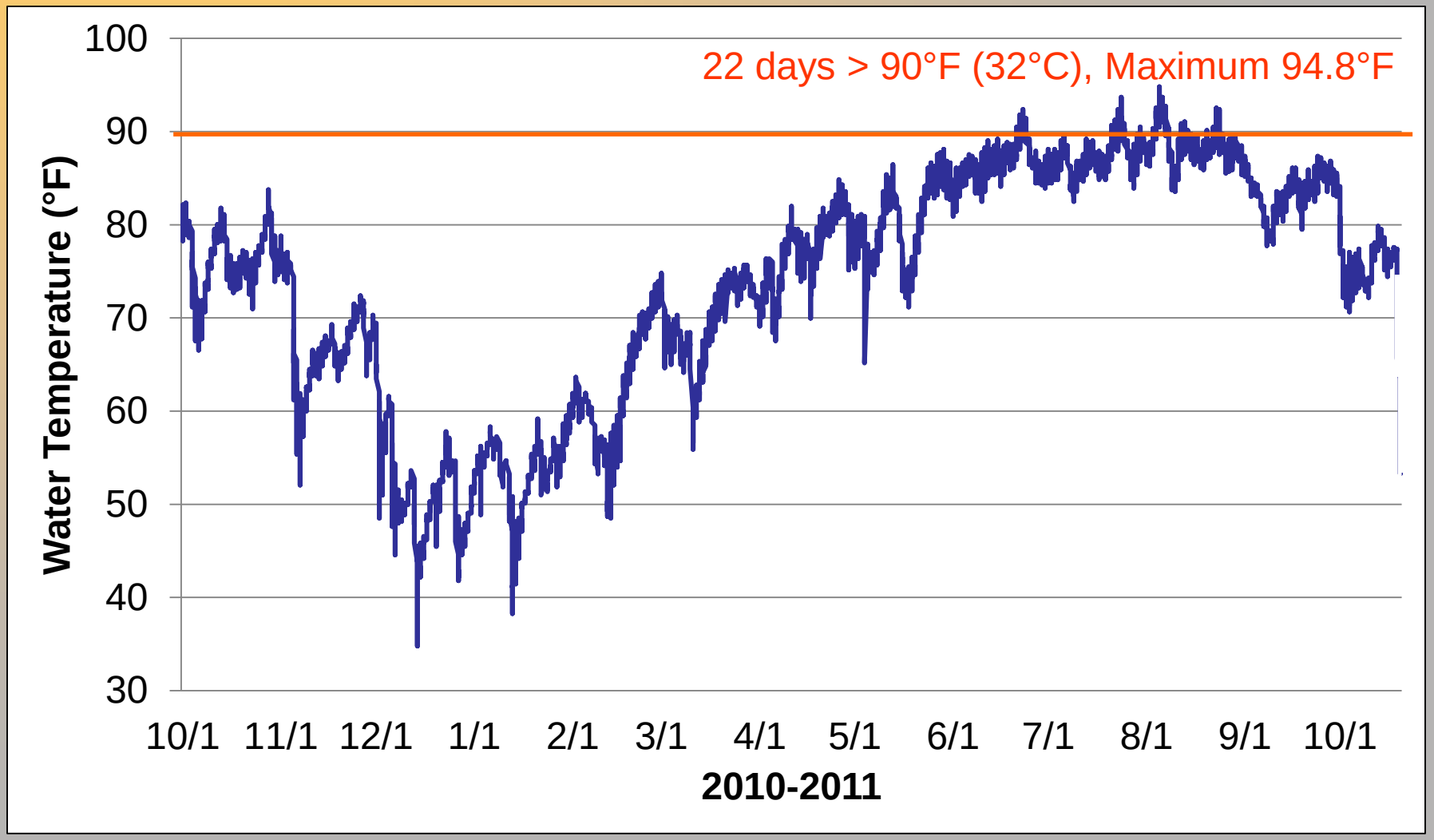
Backcross Families	Female ♀	Hybrid Family	x	Male ♂	Hybrid Family	=	Stock
F	M	C		M	C		M x M
G*	M	C		MxC	A		M x MC
H	M	C		CxM	C		M x CM
D	M	C		M	C		M x M
E	MxC	A		M	C		MC x M
	CxM	C		M	C		CM x M

* M x CM replicate stock in Family G spawn was not viable

Water Temperature (ºF), Growout

Dog Island High-denisty Lease Area, Cedar Key

October 2010 - October 2011



Water temperature measured every 2 hours by HOBO Pendant

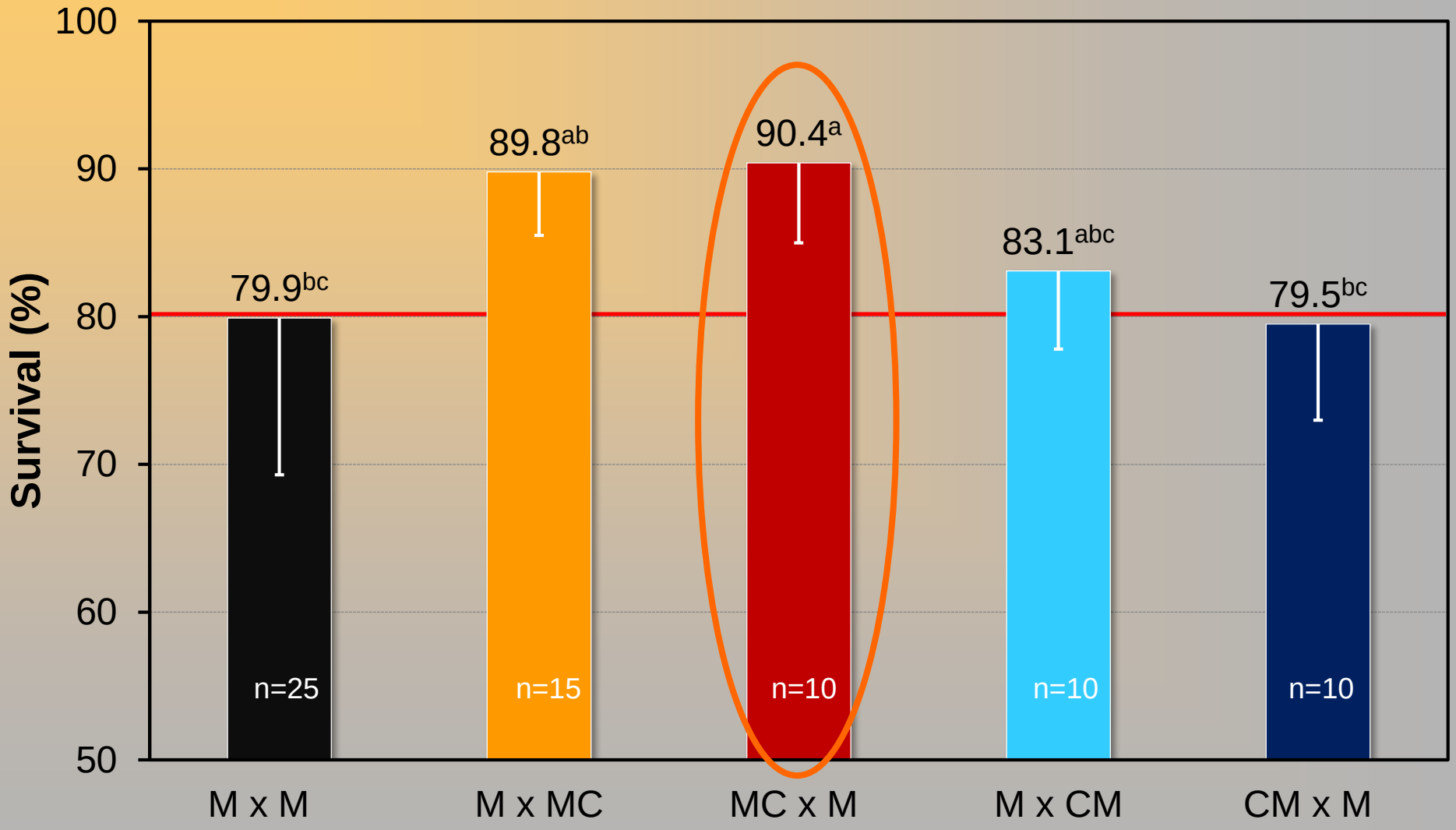
Harvest Results (13 months): All Families

Stock (♀ x ♂)	Shell Length (mm)	Shell Width (mm)	Total Weight (g)	Dry Meat Wt. (g)	Cond. Index (DM/DS x 100)
M x M	47.2 ± 2.9^{b}	25.5 ± 1.3^{ab}	34.0 ± 5.0^{b}	0.73 ± 0.11^{ab}	3.4 ± 0.28^{a}
M x MC	48.8 ± 2.1^{ab}	26.2 ± 0.90^{ab}	37.6 ± 4.3^{ab}	0.82 ± 0.11^{ab}	3.4 ± 0.21^{a}
MC x M	47.8 ± 2.1^{ab}	26.3 ± 0.86^{ab}	36.5 ± 4.7^{ab}	0.81 ± 0.14^{ab}	3.3 ± 0.25^{a}
M x CM	50.1 ± 1.7^{a}	26.8 ± 0.78^{a}	40.0 ± 3.4^{a}	0.85 ± 0.01^{a}	3.4 ± 0.23^{a}
CM x M	47.9 ± 2.9^{ab}	25.8 ± 1.8^{ab}	36.2 ± 6.7^{ab}	0.72 ± 0.11^{ab}	3.2 ± 0.40^{a}

Note: ANOVA were performed using the PROC GLM procedure of SAS. Treatment means were considered significantly different when $p \leq 0.05$.

Harvest Results (13 months): Survival

Average of All Backcross Families



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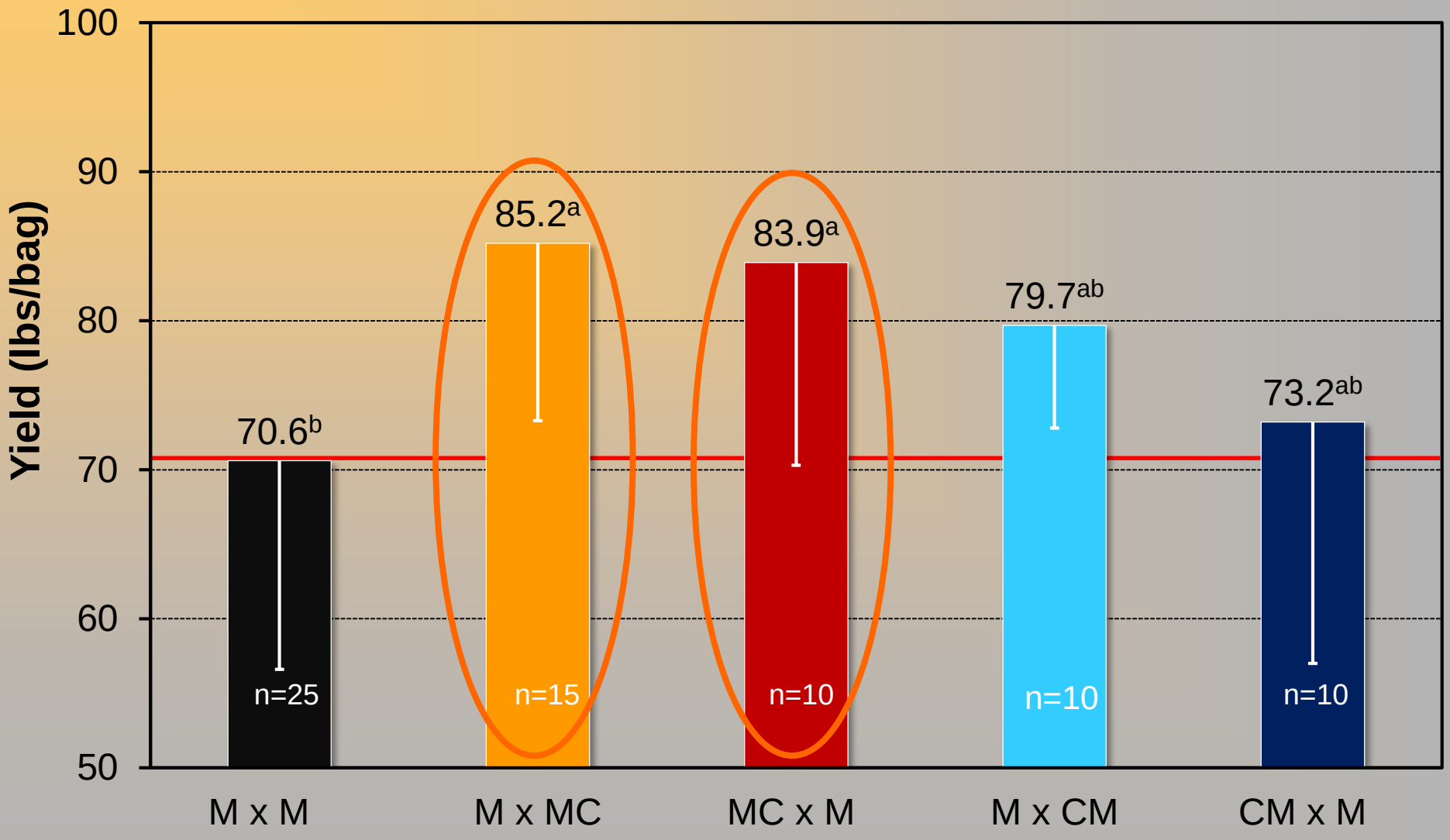
Harvest Results (13 months): Yield

Average of All Backcross Families



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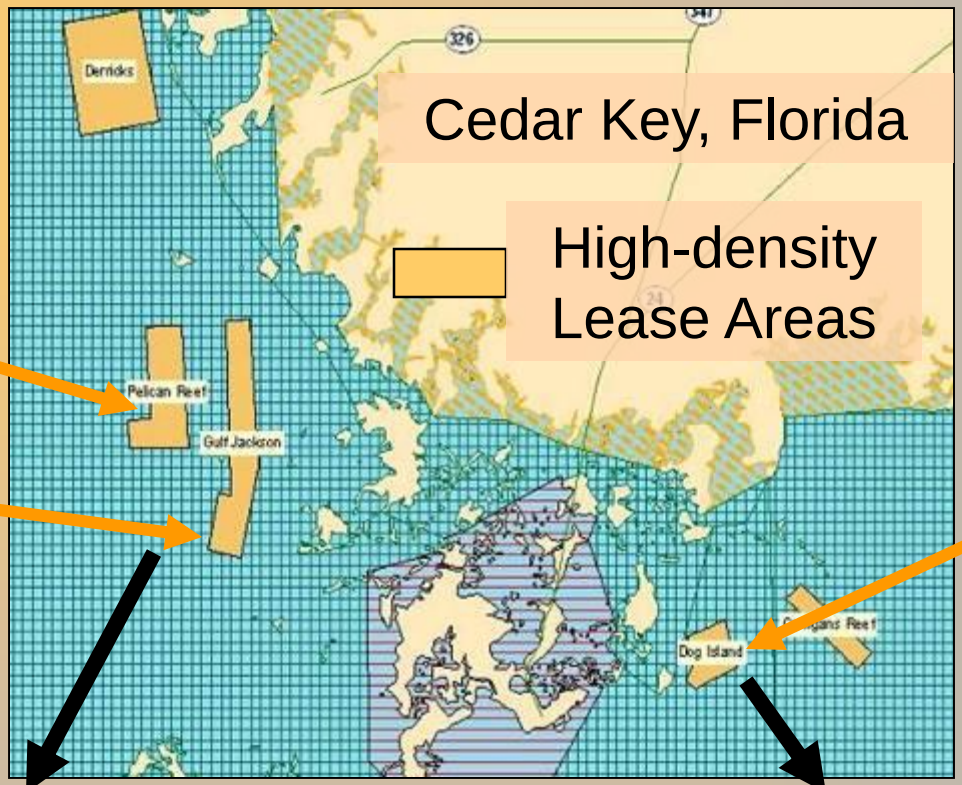
Growers Site Comparisons

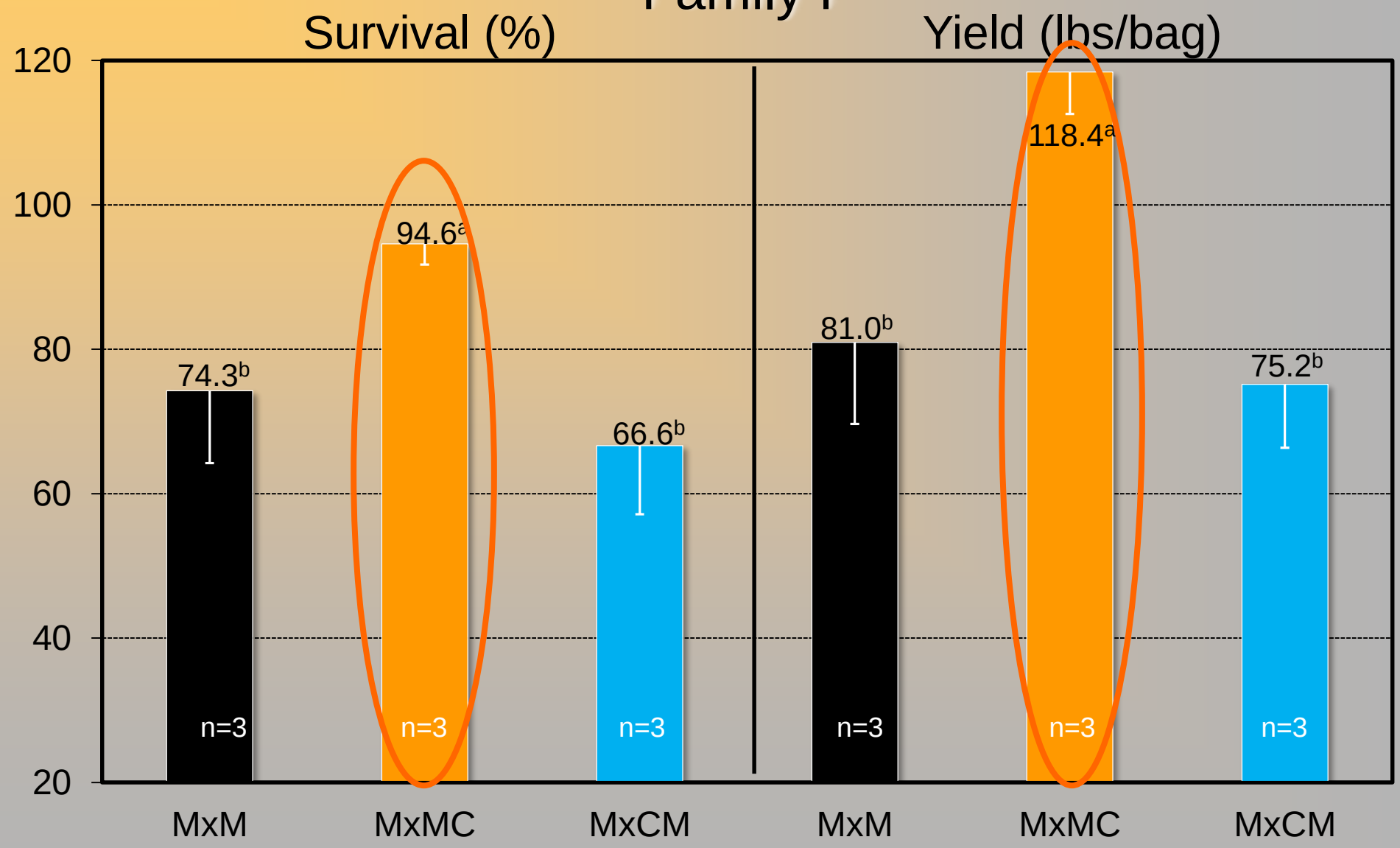


2011	Temp ºF (± SD)	Salinity ppt (± SD)
June	86.1 (2.1)	24.5 (3.2)
July	86.6 (2.1)	25.7 (2.7)
August	87.0 (2.1)	21.8 (4.4)

2011	Temp ºF (± SD)	Salinity ppt (± SD)
June	86.0 (1.8)	27.3 (1.3)
July	86.7 (1.9)	29.5 (2.4)
August	87.6 (1.9)	25.1 (1.9)

Harvest Results: Grower A, Pelican Reef

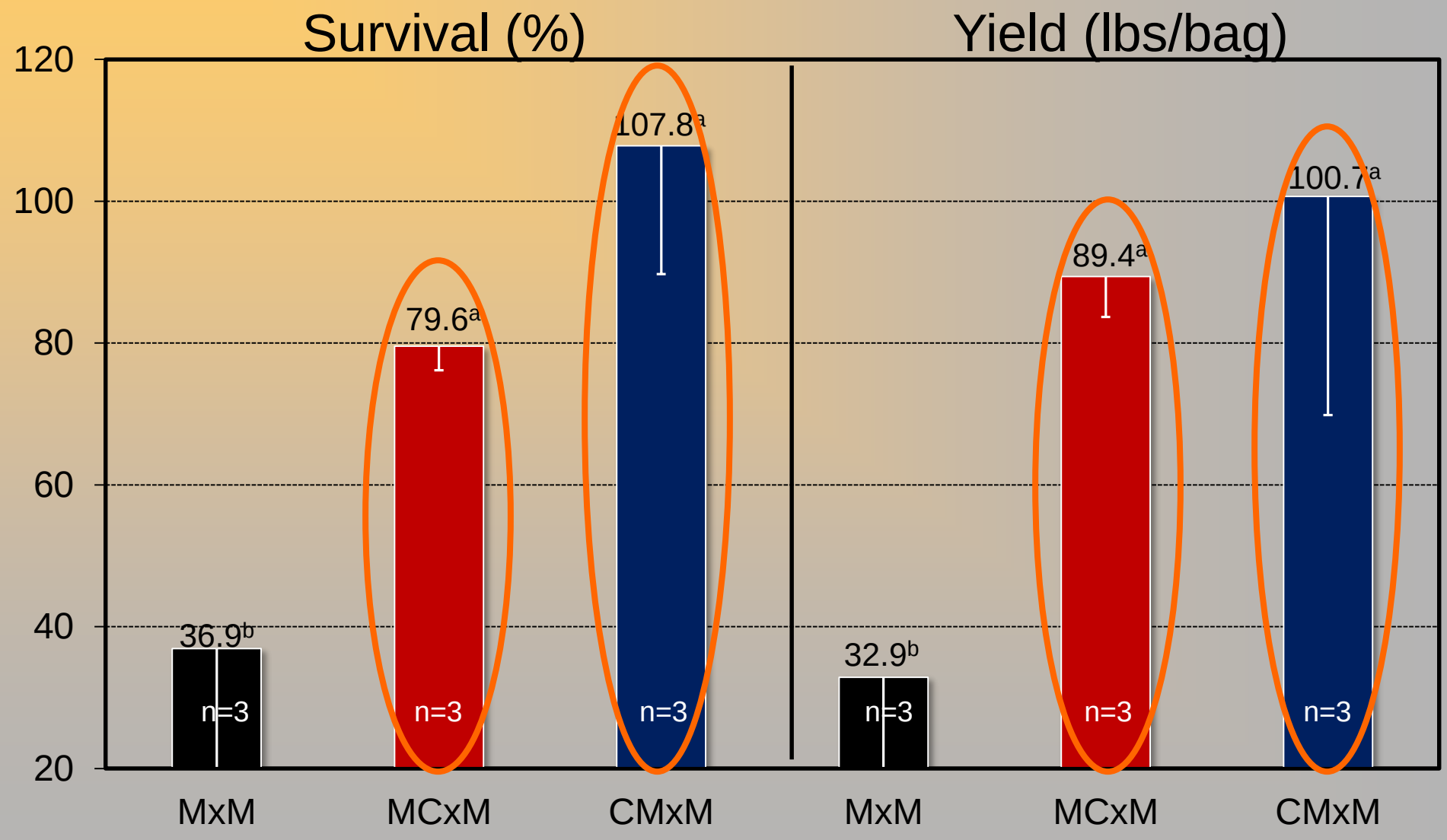
Family F



Note: ANOVA were performed using the PROC GLM procedure of SAS. Treatment means were considered significantly different when $p \leq 0.05$.

Harvest Results: Grower B, Gulf Jackson

Family D



Note: ANOVA were performed using the PROC GLM procedure of SAS. Treatment means were considered significantly different when $p \leq 0.05$.

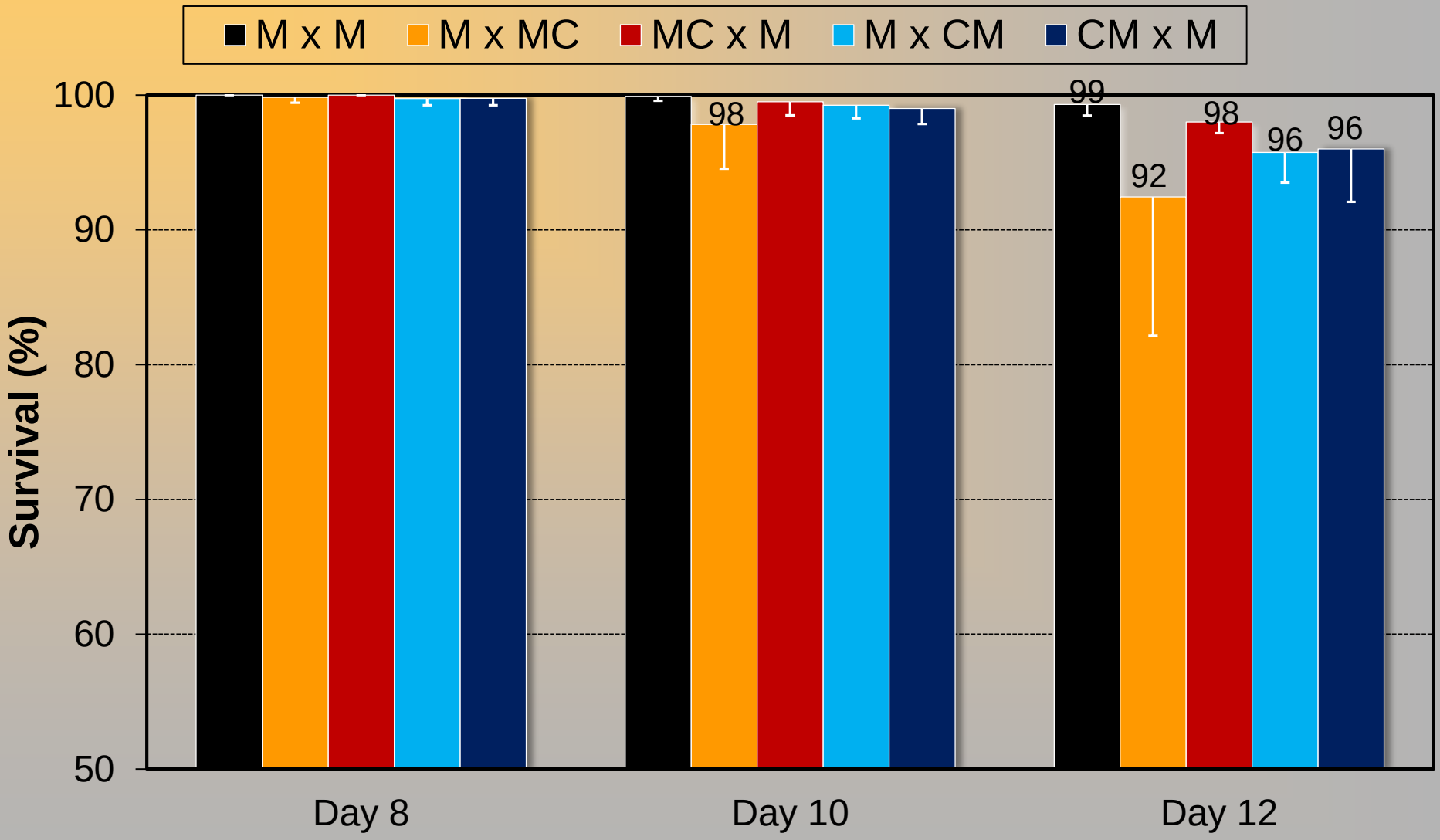
Product Quality

- Document shelf life in refrigerated storage, <45ºF
  - Survival over 12-day period
  - Gaping of product

- Water temperatures at harvest, 75-78ºF
- Temper at 65ºF for 6-12 hours

Shelf Life: Survival in 45ºF Storage

Average of All Families



Note: A repeated measures analysis (PROC GLIMMIX) was performed.

Shelf Life: Gaping in 45ºF Storage

Average of All Families

	Day 6	Day 8	Day 10	Day 12
M x M	1	2	2	3
M x MC	2	6	8	10
MC x M	1	5	3	7
M x CM	2	4	11	14
CM x M	1	3	5	7

Gaping (%)

Note: A repeated measures analysis (PROC GLIMMIX) was performed.

Summary

- Backcrossing F1 hybrids to hard clams offered improved survival and yield
  - MxMC and MCxM had ↑ survival, ↑ yield
- Genetic background (families) played a significant role in responses
  - For Family F, M x MC had ↑ survival, ↑ production
  - For Family D, MC x M had ↑ survival, ↑ production
- Shelf life commercially acceptable
  - At 10 days, 98-100% for all stocks
  - At 12 days, 92-96% for backcrosses vs 99% for hard clams
- Gaping in refrigerated storage acceptable
  - At 10 days, 3-11% for backcrosses vs 2% for hard clams
  - At 12 days, 7-14% for backcross stocks vs 3% for hard clams
- This breeding approach can increase summer survival and productivity, while maintaining product quality standards

Broodstock Made Available to Industry

- High performing broodstock lines provided to 90% of Florida hatcheries
  - Group 32, Family D: MC-A x M
  - Group 40, Family F: M x MC-A
  - Group 44, Family G: M x MC-A

"We spawn millions!"

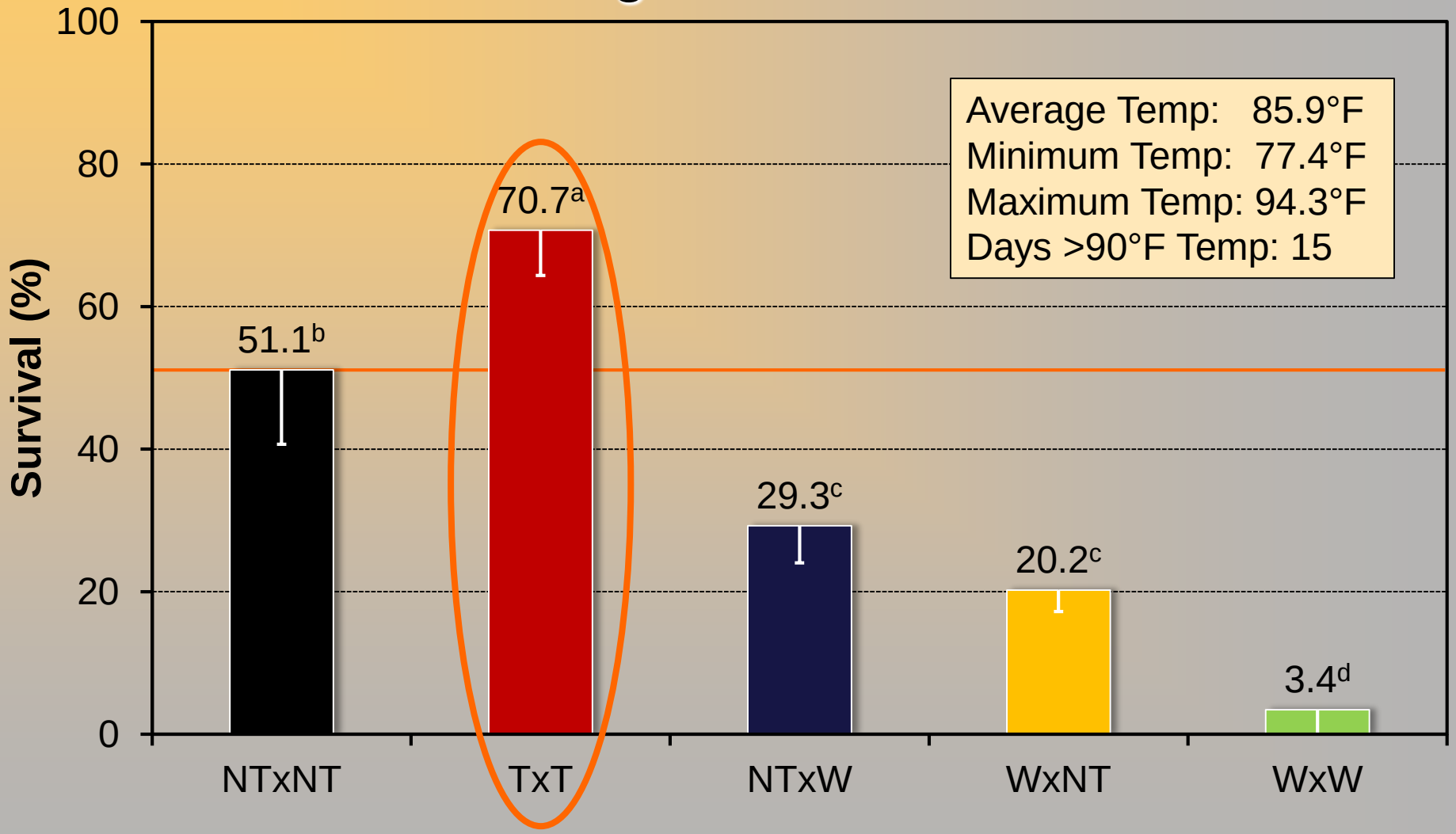
Development of Clam Broodstock for Seed Production Workshop
Harbor Branch Oceanographic Institute at FAU
December 2011

Improvement by Thermal Selection and Addition of Wild Stocks, 2011-12

- Thermally challenged hard clams
  - 2250 subjected to 95ºF for 48 hours
  - Spawned 45 survivors, or 2% (♀X♂: **TxT**)
  - Compared with non-thermally challenged hard clams (♀X♂: **NTxNT**)
- Incorporation of “wild” stocks
  - “Wild” hard clams obtained from St. Augustine
  - Spawned with hard clam stocks
  - Created reciprocals (♀X♂: **NTxW**, **WxNT**) and controls (♀X♂: **NTxNT**, **WxW**)

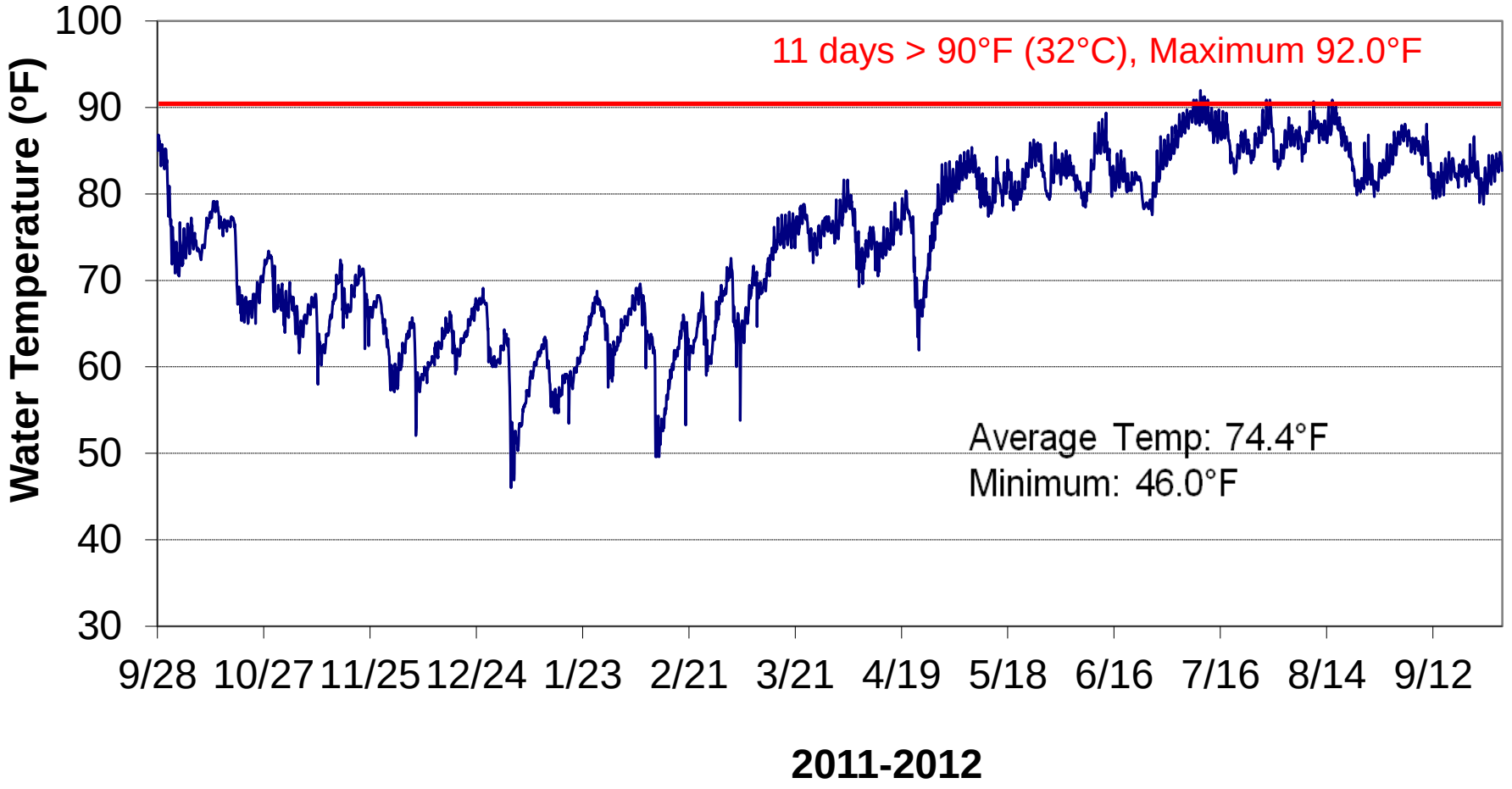
- Objectives:
  - Improve growth, survival, and genetic diversity
  - Reduce color variant – “notata”

Field Nursery Results (July-Sept 2011): Survival Average of Families



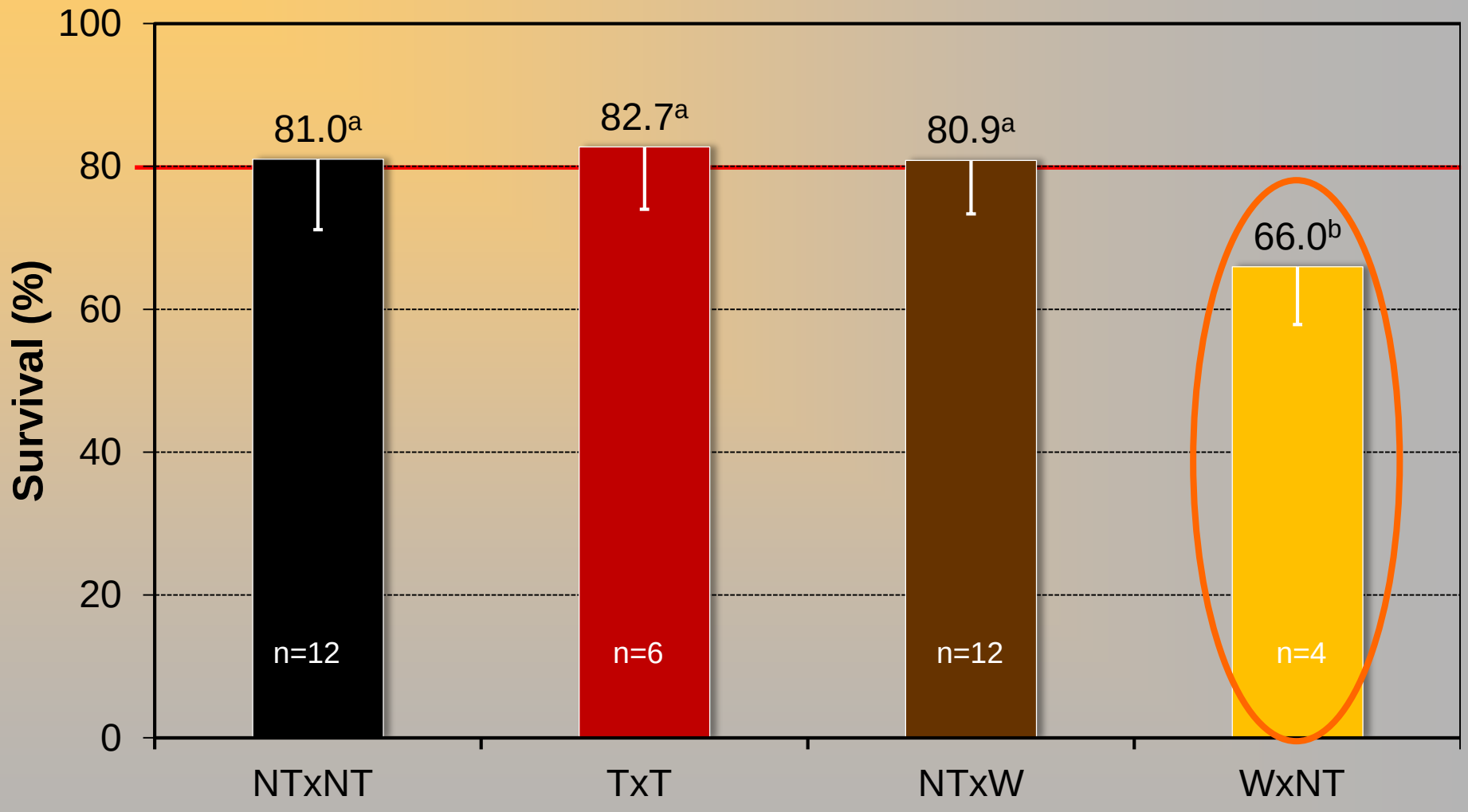
Note: ANOVA were performed using the PROC GLM procedure of SAS. Treatment means were considered significantly different when $p \leq 0.05$.

Water Temperature (ºF) during Growout
Dog Island Lease Area, Cedar Key
September 2011- October 2012



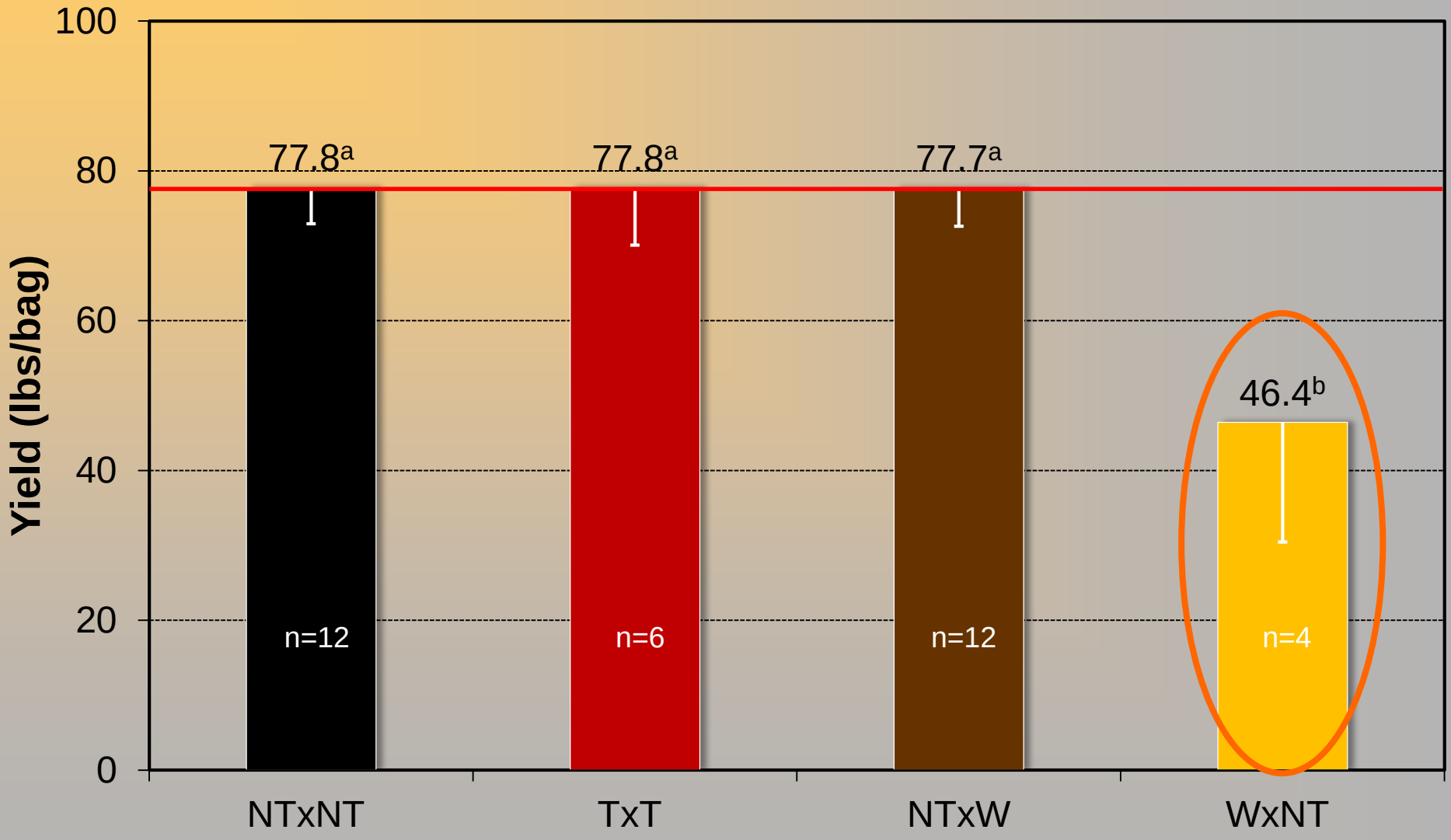
Water temperature measured every 2 hours by HOBO Pendant

Harvest Results (Sept 2011-Oct 2012): Survival Average of Families



Note: ANOVA were performed using the PROC GLM procedure of SAS. Treatment means were considered significantly different when $p \leq 0.05$.

Harvest Results (Sept 2011-Oct 2012): Yield Average of Families



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Shell Coloration of Harvested Clam Stocks, % Notata

NTxNT - 92%

- Rated on a scale of 1 to 5, where
  - 1 = 0%
  - 2 = 25%
  - 3 = 50%
  - 4 = 75%
  - 5 = 100%
- Rated 135 clams per group

NTxW - 58%

WxNT - 39%

What's Next ?

- Determine genetic diversity of hard clams stocks
  - Study conducted by Dr. Jim Austin, UF Dept. Wildlife Ecology and Conservation
  - Using microsatellite markers developed for hard clams by the UF Interdisciplinary Center for Biotechnology Research
  - Stocks being evaluated
    - Four "wild" stocks collected from FL east coast
    - Seven FL cultured stocks
- Genetic sequencing of hard clams and southern quahogs
  - Proposal submitted to DACS ARC for 2013-4 funding by Dr. Jim Austin, UF Dept. Wildlife Ecology and Conservation
  - Objectives
    - Identification of potentially important genetic variants (alleles)
    - Develop low cost PCR-based assays to screen stock and wild clams for alleles related to preferred traits

Acknowledgements

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