

Giant Breeding Projects

Results of Growout Production Trials

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HARBOR BRANCH

FLORIDA ATLANTIC UNIVERSITY

Why improve upon a good thing?

- 
- Increasing unreliable production
 - Growers accept 50-70% survival rates
 - Increasing summer crop mortalities
 - High water temperatures and other environmental stressors during prolonged summer months
 - Need to increase production efficiency and cost-effectiveness
 - Clam prices stagnant as costs increase

Why selectively breed hard clams?

- Need a heat tolerant clam for Florida
 - Northern quahogs, *Mercenaria mercenaria*, are “living on the edge”
 - Near southern limit of their natural distribution
 - Summer water temperatures in growing areas regularly exceed 90°F
 - Temperature related mortalities observed in lab and field



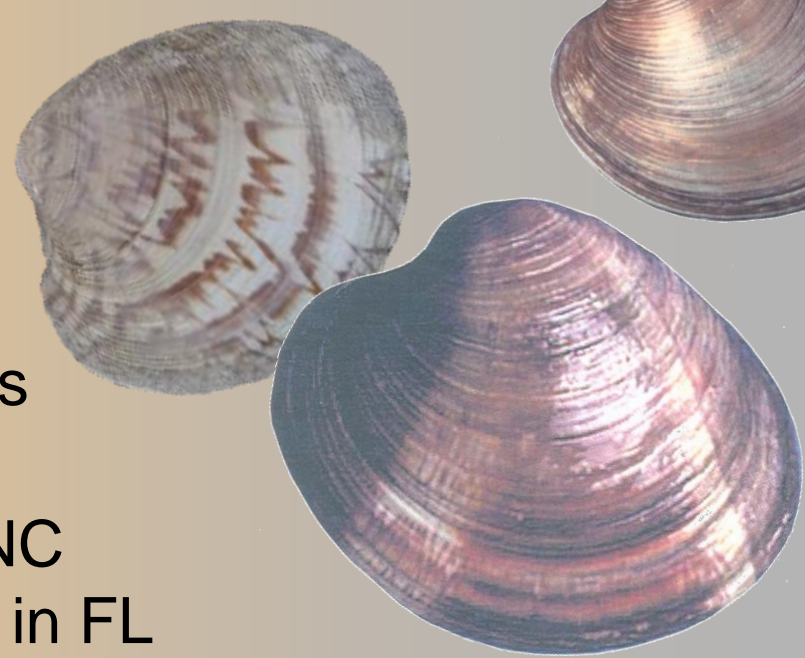
Improvement of Cultured Clam Stocks through Hybridization

- Hybridization is a common breeding technique
 - Used in commercial agriculture and finfish aquaculture
- Hybrids have superior traits to either parent species
 - For example, improved growth or environmental resistance
- The use of clam hybridization for “mariculture” potential was examined by Winston Menzel at Florida State University in the 1960-70s
 - Showed hybrids had improved growth, shelf life
 - Little data reported on merit of hybrids for improved survival
- This project allows for a rigorous examination of clam hybridization
 - To improve production
 - To assure product quality



Clam Species

- The northern hard clam supports fisheries and aquaculture industries along Atlantic coast from MA to FL
- The southern quahog found from NC to Caribbean, recreationally fished in FL
 - May have production traits for resisting environmental stressors
 - Not cultured because of their tendency to gape in refrigerated storage
- *Mercenaria* species are normally separated by environmental tolerances, but readily hybridize where they do co-occur or under hatchery conditions



Northern hard clam
Mercenaria mercenaria
notata



Southern quahog
Mercenaria campechiensis

Hatchery Production

- Northern hard clams obtained from a Florida hatchery
- Southern quahogs obtained from the wild (Sarasota), where highly pure populations are known to exist
- Single parent crosses utilized
- Multiple spawns accomplished with different sets of parents, October-December, 2007
- Stock verification by allozyme method
 - Arnold and Geiger, FWC FWRI



Nursing Hybrid Seed

- Standard hard clam protocols used
- Land-based nursing
 - Downwellers
 - **March-June 2008**
 - Cedar Key, FL
- Field nursing
 - Bottom bags, 4 mm
 - **June-September 2008**
 - Cedar Key, FL



Nursing Hybrid Seed

- Growth differences negligible
- Survival rates not statistical different
- About 600,000 seed from three families nursed for growout evaluation



Stock	Survival (%) Average \pm SD
M x M	72.9 \pm 11.5
M x C	82.4 \pm 16.9
C x M	79.5 \pm 4.6
C x C	86.1 \pm 4.9



Growout Trials

Stock Comparison

- Replicated plants - Parental stocks and reciprocal crosses from 3 families
 - 146K seed
 - Cedar Key, FL
 - Sept 2008-Sept 2009
- Standard planting procedures
 - Bottom bags, 9 mm
 - Net coated and covered with wire
 - Stocked at 1150/bag (72/ft²)

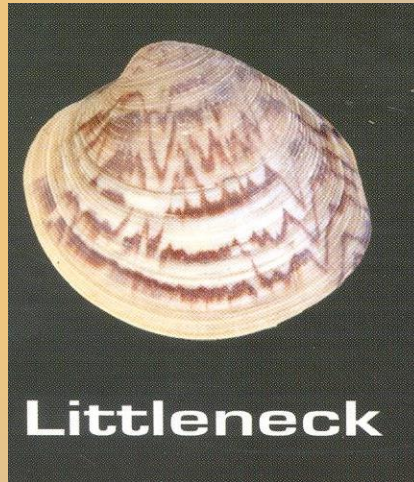


Comparison of Production Characteristics

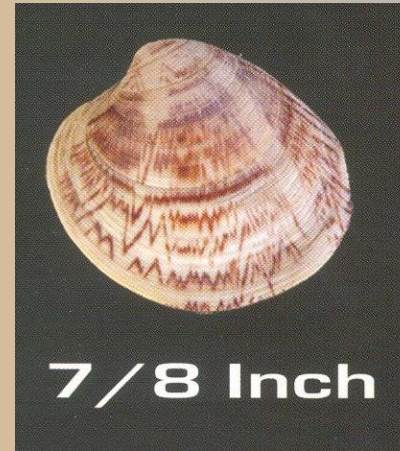
- Sampling every 4 months and at harvest (12 months)
- Growth – SL, SW, total and dry meat weight
- Survival
- Condition index – measure of degree of fattening or nutritive status
- Histology – determine gonadal stage and reproductive potential



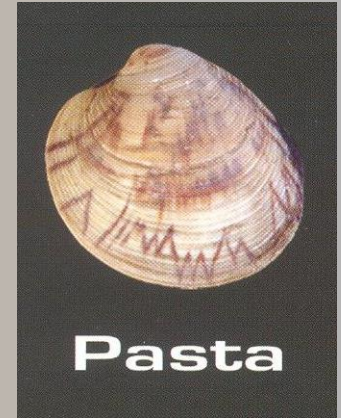
Commercial Market Sizes



Littleneck



7/8 Inch



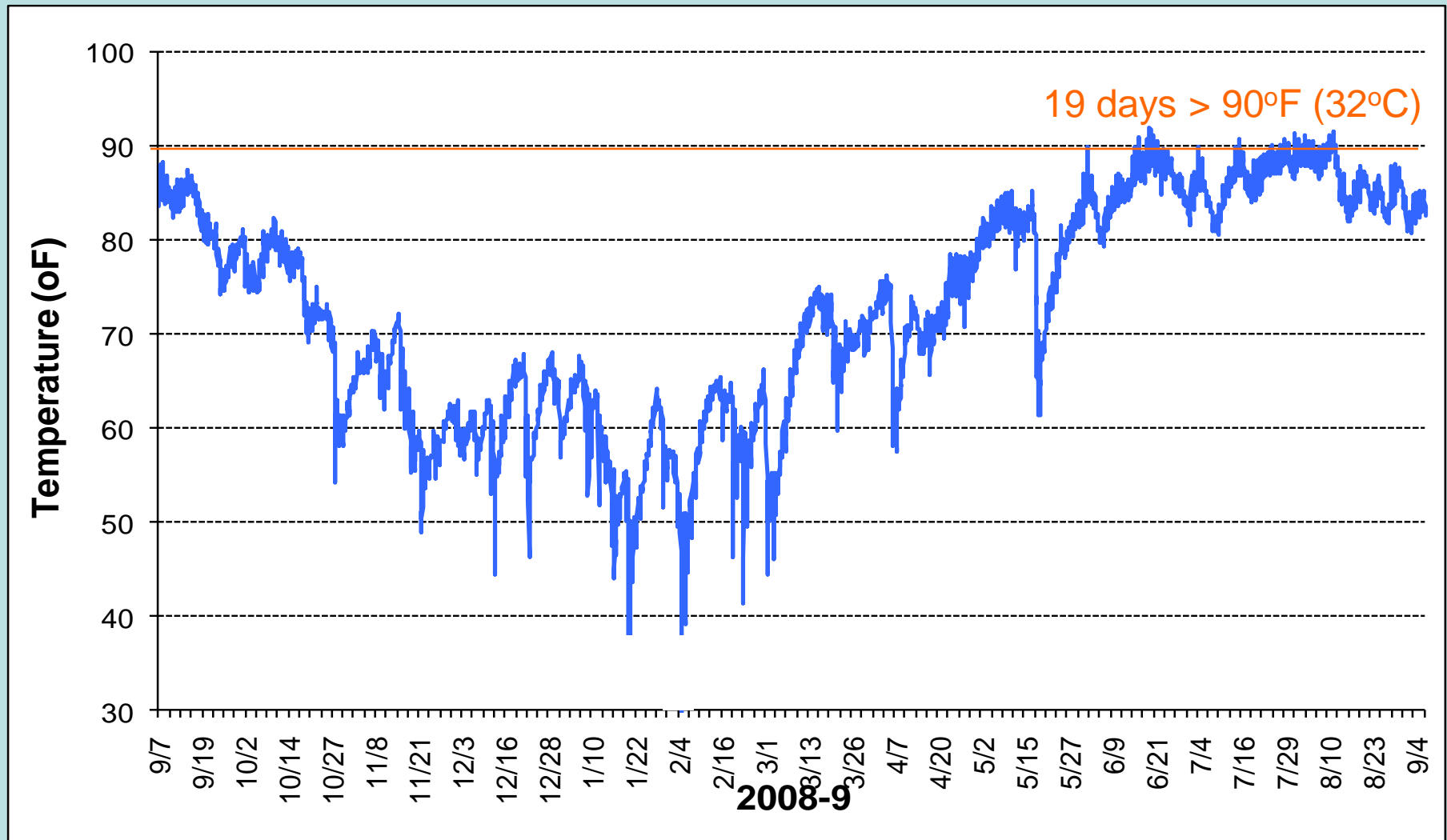
Pasta



Common Name	Shell Width/ Hinge Size (inches)	Number per Pound
Littleneck	1"	10-13
7/8 inch	7/8"	14-18
Pasta	3/4"	18-25

Water Temperature (°F)

Dog Island Lease Area, Cedar Key
September 2008- September 2009



Water temperature measured every 30 minutes with YSI 6600 data sonde

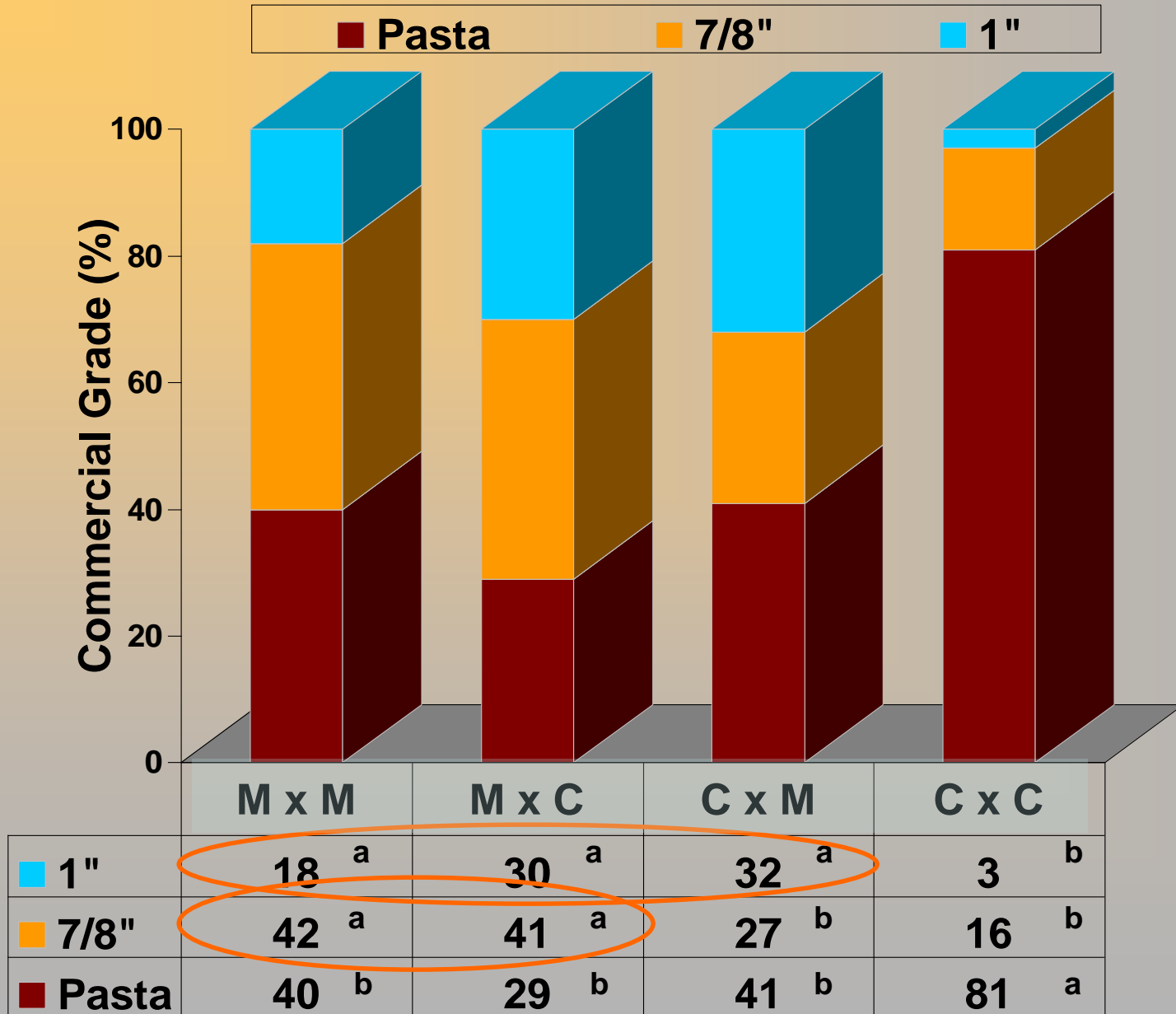
Harvest Results (12 months)–All Families

Average \pm Standard Deviation

Stock	Shell Width (mm)	Total Weight (g)	Dry Meat Wt. (g)	Survival (%)	Production (lbs/bag)
M x M	23.1 $\pm 1.2^b$	26.8 $\pm 3.7^a$	0.59 $\pm 0.09^{bc}$	93.3 $\pm 15.4^a$	62.8 $\pm 9.6^a$
M x C	24.3 $\pm 0.8^a$	30.2 $\pm 2.7^a$	0.73 $\pm 0.08^a$	99.5 $\pm 8.3^a$	76.1 $\pm 8.2^a$
C x M	23.3 $\pm 3.1^{ab}$	27.5 $\pm 9.1^a$	0.68 $\pm 0.20^{ab}$	90.9 $\pm 23.5^{ab}$	67.2 $\pm 33.0^a$
C x C	20.4 $\pm 1.3^c$	17.3 $\pm 3.0^b$	0.52 $\pm 0.10^c$	72.3 $\pm 25.3^b$	32.5 $\pm 13.9^b$

Note: ANOVA were performed using the PROC GLM procedure of SAS. Treatment means were considered significantly different when $p \leq 0.05$. Tukey's test groupings are displayed

Harvest Results (12 months) – All Families



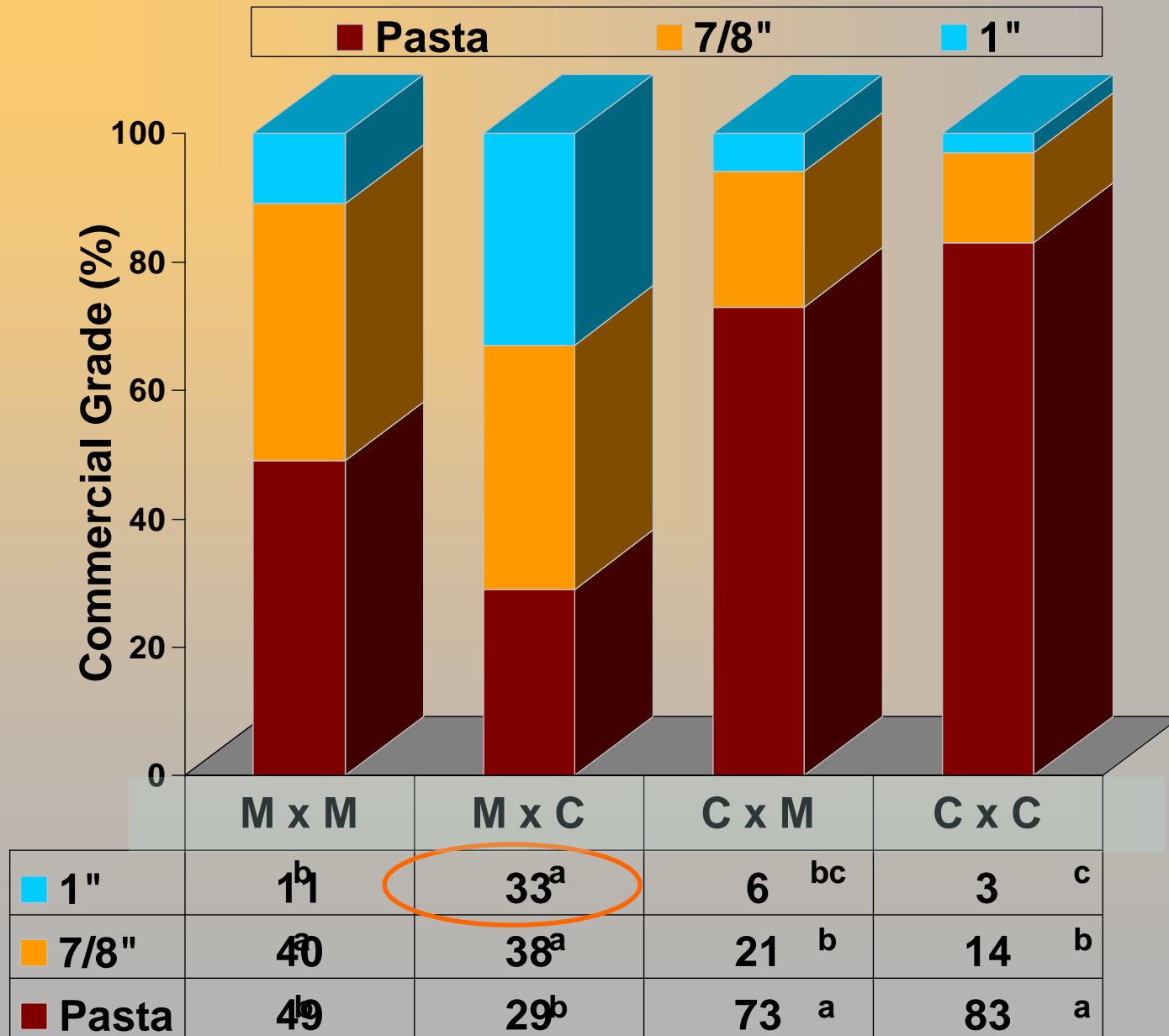
Harvest Results (12 months) – Family A

Average \pm Standard Deviation

Stock	Shell Width (mm)	Total Weight (g)	Dry Meat Wt. (g)	Survival (%)	Production (lbs/bag)
M x M	22.6 $\pm 1.2^b$	25.8 $\pm 3.6^b$	0.58 $\pm 0.08^b$	81.8 $\pm 11.3^{ab}$	53.0 $\pm 6.6^b$
M x C	24.5 $\pm 0.7^a$	31.0 $\pm 2.2^a$	0.76 $\pm 0.05^a$	96.8 $\pm 9.9^a$	75.8 $\pm 5.4^a$
C x M	20.7 $\pm 2.2^{bc}$	19.4 $\pm 5.6^c$	0.56 $\pm 0.13^{ab}$	68.4 $\pm 7.6^b$	34.4 $\pm 13.0^c$
C x C	20.1 $\pm 1.5^c$	16.5 $\pm 3.2^c$	0.50 $\pm 0.10^b$	72.5 $\pm 26.7^{ab}$	32.1 $\pm 16.3^c$

Note: ANOVA were performed using the PROC GLM procedure of SAS. Treatment means were considered significantly different when $p \leq 0.05$. Tukey's test groupings are displayed.

Harvest Results (12 months) – Family A



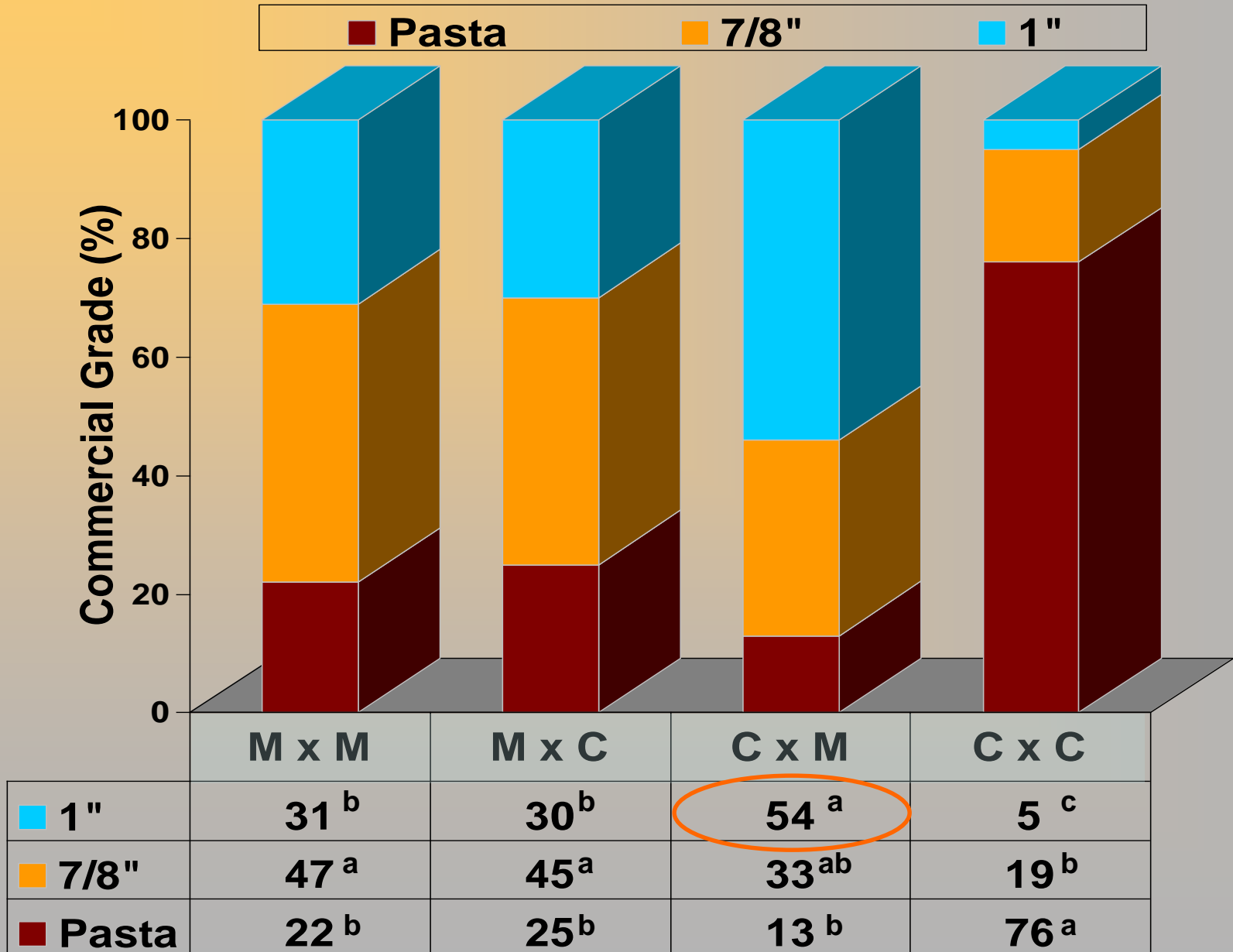
Harvest Results (12 months) – Family C

Average \pm Standard Deviation

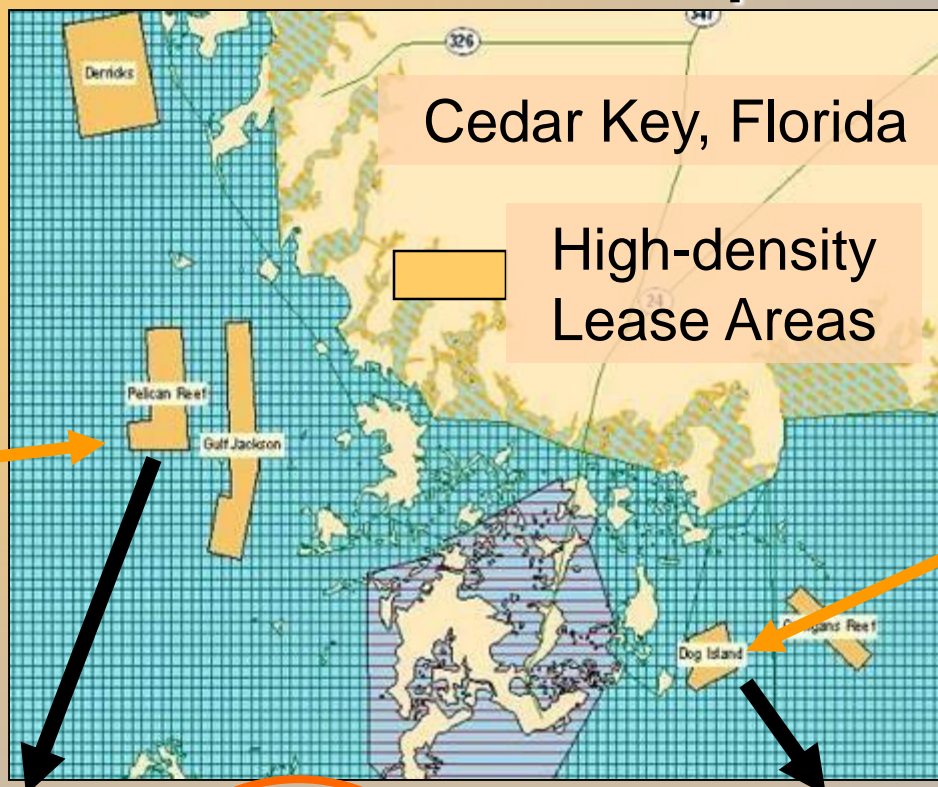
Stock	Shell Width (mm)	Total Weight (g)	Dry Meat Wt. (g)	Survival (%)	Production (lbs/bag)
M x M	24.1 ± 0.6 ^{bc}	29.8 ± 1.7 ^b	0.67 ± 0.09 ^b	92.9 ± 3.0 ^a	70.1 ± 3.3 ^b
M x C	24.3 ± 0.7 ^{ab}	30.5 ± 2.3 ^{ab}	0.77 ± 0.03 ^{ab}	104.1 ± 6.8 ^a	80.4 ± 7.7 ^b
C x M	25.6 ± 1.3 ^a	34.4 ± 4.4 ^a	0.89 ± 0.06 ^a	110.2 ± 10.9 ^a	95.3 ± 7.0 ^a
C x C	21.0 ± 1.3 ^c	18.4 ± 3.3 ^c	0.60 ± 0.11 ^b	59.0 ± 20.9 ^b	28.1 ± 12.8 ^c

Note: ANOVA were performed using the PROC GLM procedure of SAS. Treatment means were considered significantly different when $p \leq 0.05$. Tukey's test groupings are displayed.

Harvest Results (12 months) – Family C



Growers Site Comparisons



**Pelican Reef
Lease Area**

**Dog Island
Lease Area**

2009	Temp °F (\pm SD)	Salinity ppt (\pm SD)
June	85.3 (2.6)	19.5 (5.7)
July	86.2 (2.1)	18.8 (4.5)
August	86.8 (2.6)	26.3 (1.9)

2009	Temp °F (\pm SD)	Salinity ppt (\pm SD)
June	85.8 (2.5)	21.2 (3.1)
July	86.3 (2.1)	21.8 (2.8)
August	86.6 (2.5)	25.4 (1.6)

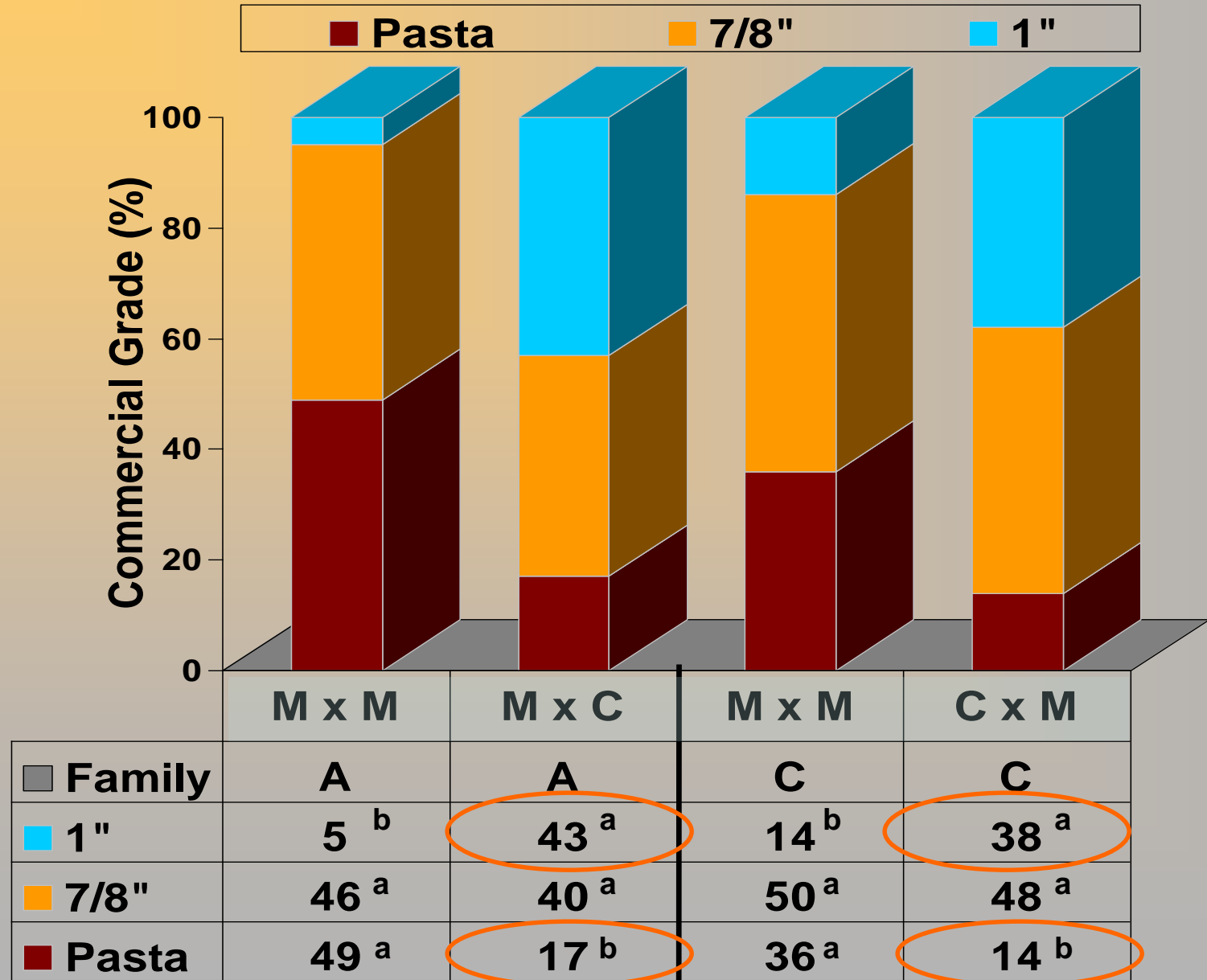
Harvest Results(12 months) – **Grower A**

Average \pm Standard Deviation

Stock	Shell Width (mm)	Shell Length (mm)	Total Weight (g)	Survival (%)	Production (lbs/bag)
M x M (Family A)	21.9 $\pm 0.2^b$	41.9 $\pm 0.5^b$	22.7 $\pm 0.7^b$	52.2 $\pm 8.5^b$	31.3 $\pm 5.0^b$
M x C (Family A)	25.7 $\pm 0.9^a$	46.9 $\pm 1.7^a$	34.5 $\pm 3.4^a$	90.2 $\pm 6.6^a$	82.0 $\pm 5.0^a$
M x M (Family C)	23.3 $\pm 0.4^b$	42.1 $\pm 1.0^b$	25.0 $\pm 1.6^b$	49.9 $\pm 7.3^b$	33.2 $\pm 6.8^b$
C x M (Family C)	24.6 $\pm 0.2^a$	43.5 $\pm 0.6^a$	28.8 $\pm 0.4^a$	86.2 $\pm 20.8^a$	65.1 $\pm 15.0^a$

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

Grade (12 months) – Grower A



Product Quality



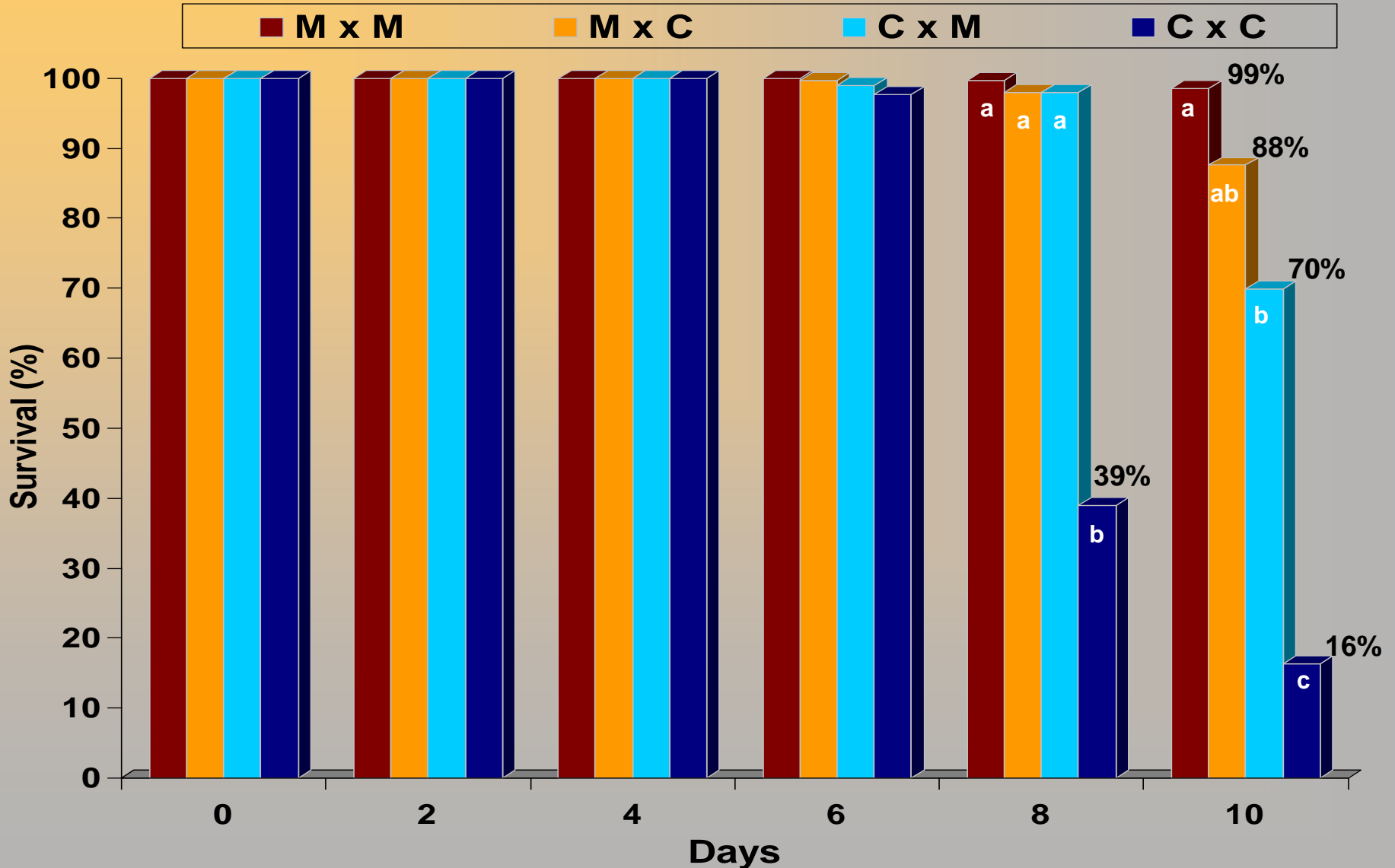
- Consumer acceptance
- Sensory evaluation and profiling

- Document shelf life
 - Survival in refrigerated storage (45°F)



Shelf Life: Survival in 45°F Storage

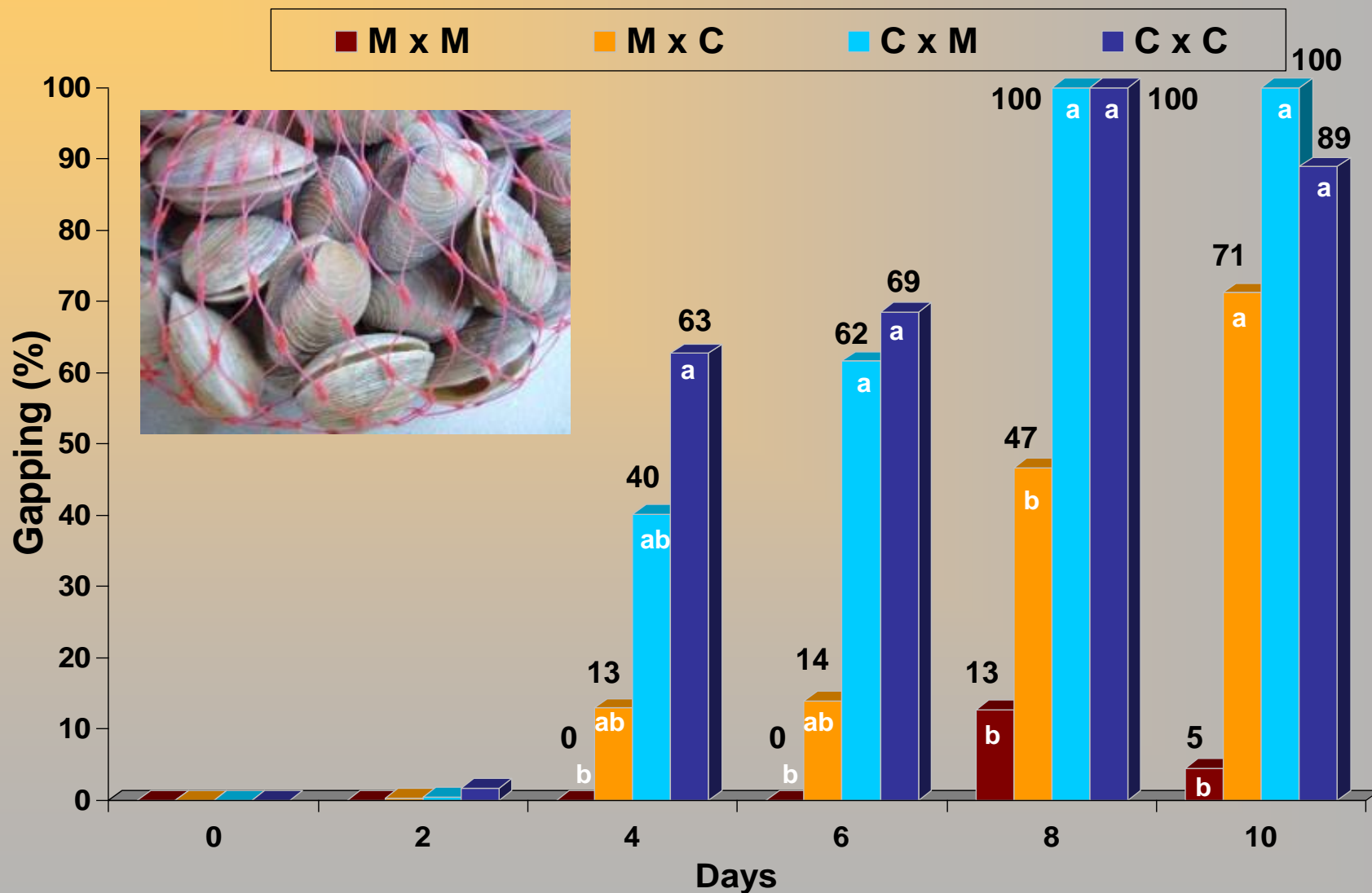
Average of Families A,B,C – Harvested at 84.6°F



Note: A repeated measures analysis (PROC GLIMMIX) was performed ($p \leq 0.0001$).

Shelf Life: Gapping in 45°F Storage

Average of Families A, B, C



Note: A repeated measures analysis (PROC GLIMMIX) was performed ($p=0.0237$).

Summary

- Hybridization may offer improved clam production performance
 - MxC \uparrow SW and DryMtWt
- Genetic background played a significant role in responses
 - Family A, MxC \uparrow SW, TW, DMtWt, Prod
 - Family C, CxM \uparrow SW, TW, DMtWt, Prod



Sunshine Clam (M X C)

TropiClam (C X M)



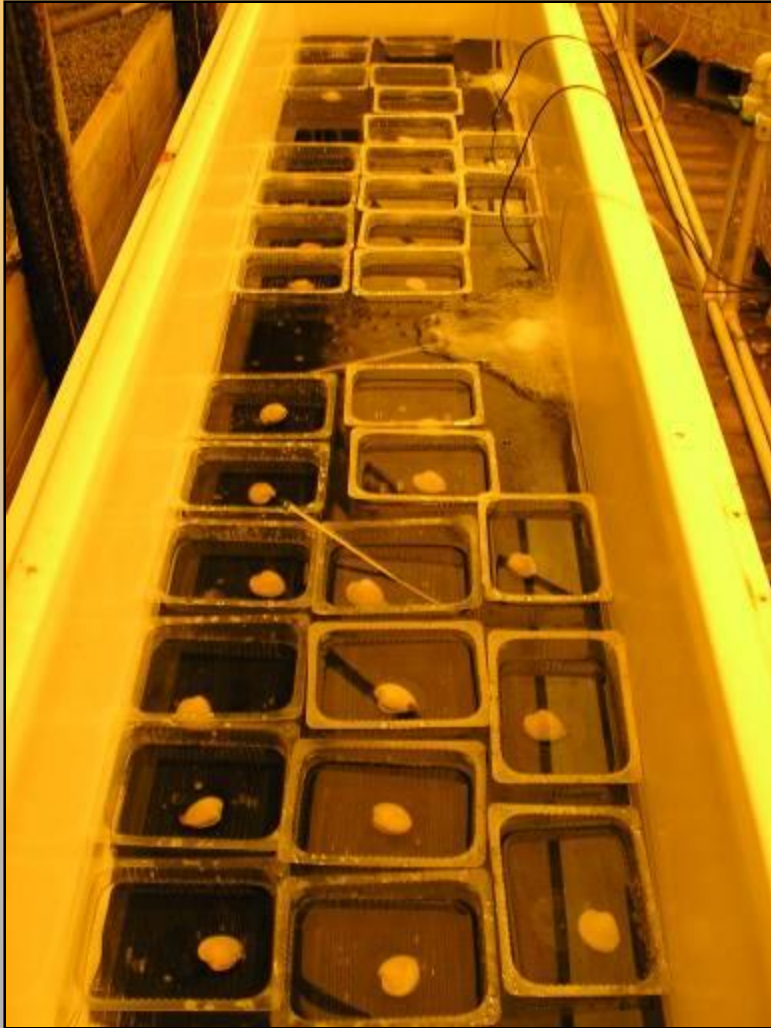
- Environmental conditions at growing sites also played a role in responses
 - Grower A, Hybrids $>$ MxM
- Shelf life acceptable
 - 10 days for MxC, 8 days for CxM
- Gapping in refrigerated storage problematic
 - By day 8 for MxC, day 4 for CxM

Backcrossing F1 Hybrids with Hard Clams

- Mating of a hybrid with one of its parents (or parental species)
- Hybrids (MxC and CxM) backcrossed to hard clams (MxM) as female or male
- Objective: Improve product quality, but maintain improved growth and survival



Hatchery Production



- Pure hard clams (MxM) crossed with F1 hybrids
 - MxC “Sunshine Clam”
 - CxM “Tropiclam”
- Multi-parental spawns
- Five families produced
 - Dec 2009 – Feb 2010
 - 19 individual stocks



Backcross Parents

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

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

Nursing Backcross Seed

- Standard hard clam protocols used
- Land-based nursing
 - Downwellers
 - **May—June 2010**
 - Cedar Key
- Field nursing
 - Bottom bags, 4mm
 - **June—Sept 2010**
 - Cedar Key



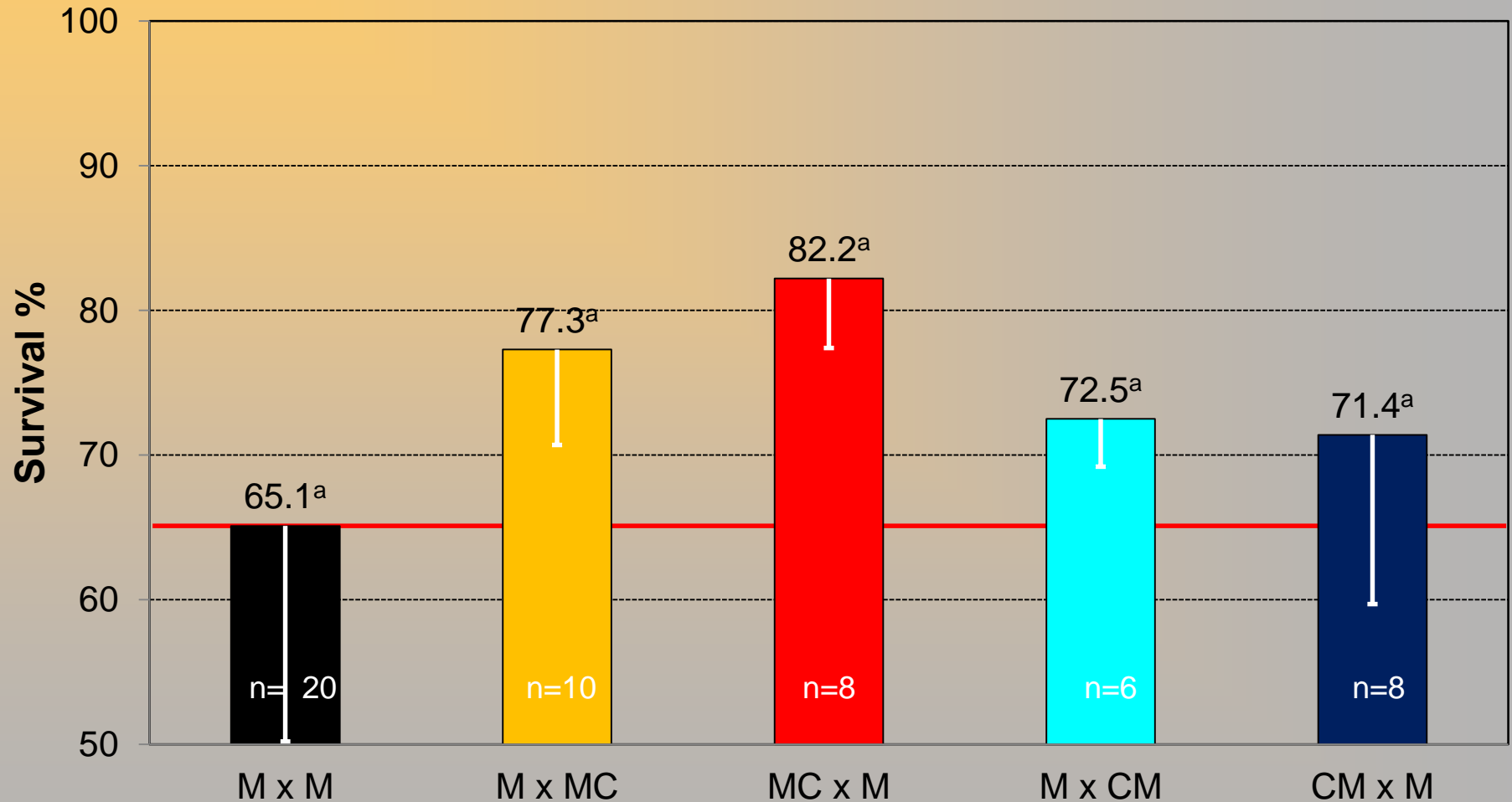
Nursing Backcross Seed: Field Results

Stock (♀ x ♂)	Shell Length (mm)	Shell Width (mm)	Total Weight (g)	Dry Meat Wt. (g)	Cond Index (DM/DS x 100)
M x M	20.0 ± 1.3	9.6 ± 0.73	2.4 ± 0.49	0.08 ± 0.018	4.8 ± 0.34
M x MC*	20.2 ± 1.1	9.7 ± 0.55	2.5 ± 0.39	0.09 ± 0.014	4.7 ± 0.32
MC* x M	20.8 ± 0.82	9.9 ± 0.46	2.6 ± 0.32	0.08 ± 0.017	4.7 ± 0.33
M x CM+	20.9 ± 1.2	9.8 ± 0.70	2.7 ± 0.45	0.08 ± 0.012	4.8 ± 0.45
CM+ x M	20.3 ± 0.97	9.7 ± 0.60	2.5 ± 0.34	0.07 ± 0.013	4.7 ± 0.43

* Results from one family (Family A), +Results from one family (Family C)

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

Field Nursing Backcross Seed: Survival



* Results from one parental stock family (Family A)

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



29 Sept 2010, Family D, Group 31, M x M



29 Sept 2010, Family H, Group 46, M x M



29 Sept 2010, Family D, Group 32, M-C x M



29 Sept 2010, Family H, Group 47, M x M-C



29 Sept 2010, Family D, Group 33, M-C x M



29 Sept 2010, Family H, Group 48, M x M-C



29 Sept 2010, Family D, Group 34, C-M x M



29 Sept 2010, Family H, Group 49, M x C-M

Growout Trials

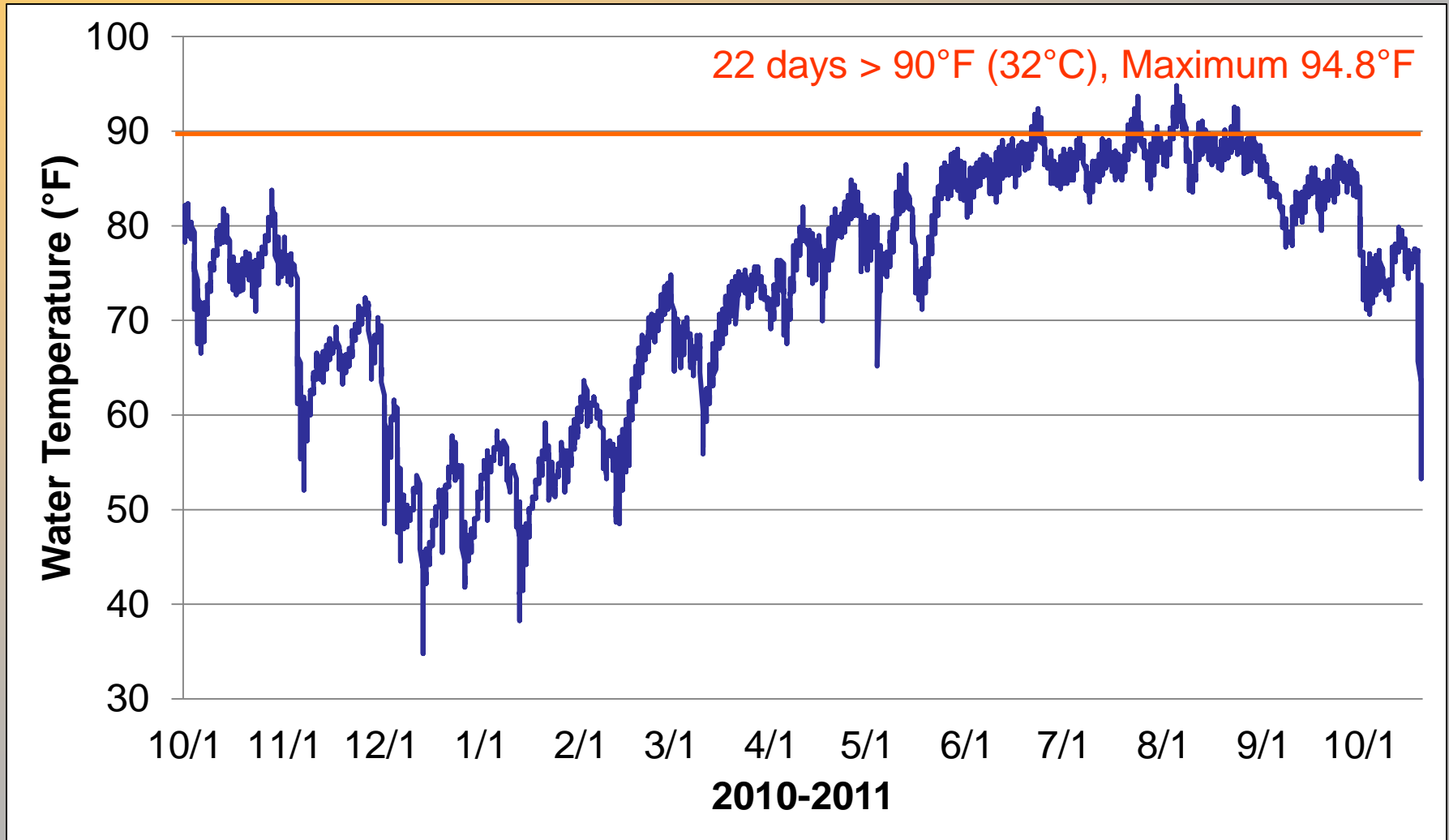
Stock Comparison

- Replicated plants -
Parental stocks and
reciprocal backcrosses
from 5 families
 - D, E, F, G, H
 - 109,000 seed
 - Cedar Key, FL
 - **Sept 2010-Oct 2011**
- Standard procedures
 - Bottom bags, 9 mm
 - Net coated and covered
with wire
 - Stocked at 1150/bag
(72/ft²)



Water Temperature (°F)

Dog Island Lease Area, Cedar Key
October 2010 - October 2011



Water temperature measured every 2 hours by HOBO Pendant

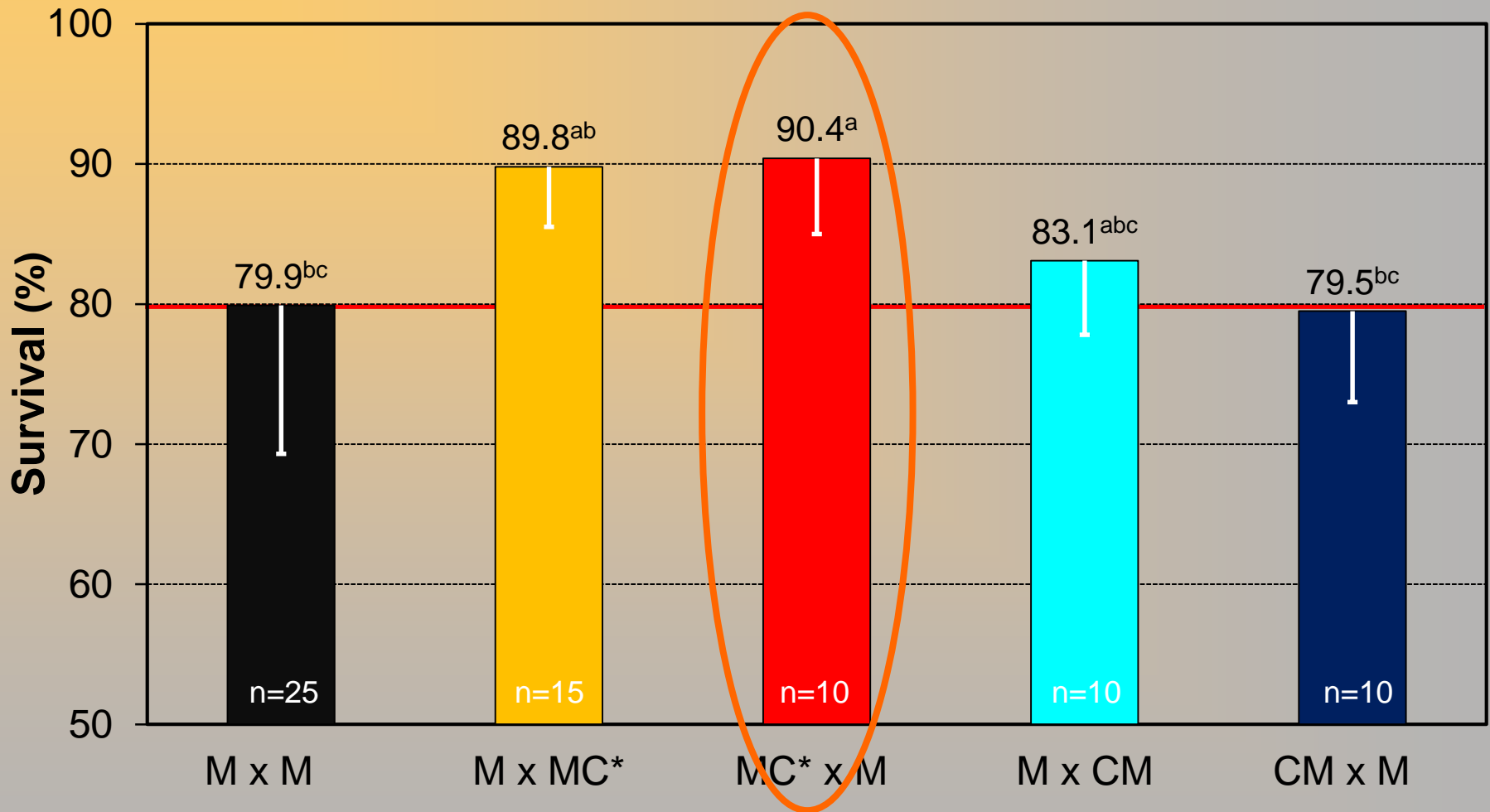
Harvest Results (13 months): All Families

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 ± 0.096 ^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

* Results from one parental stock family (Family A)

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

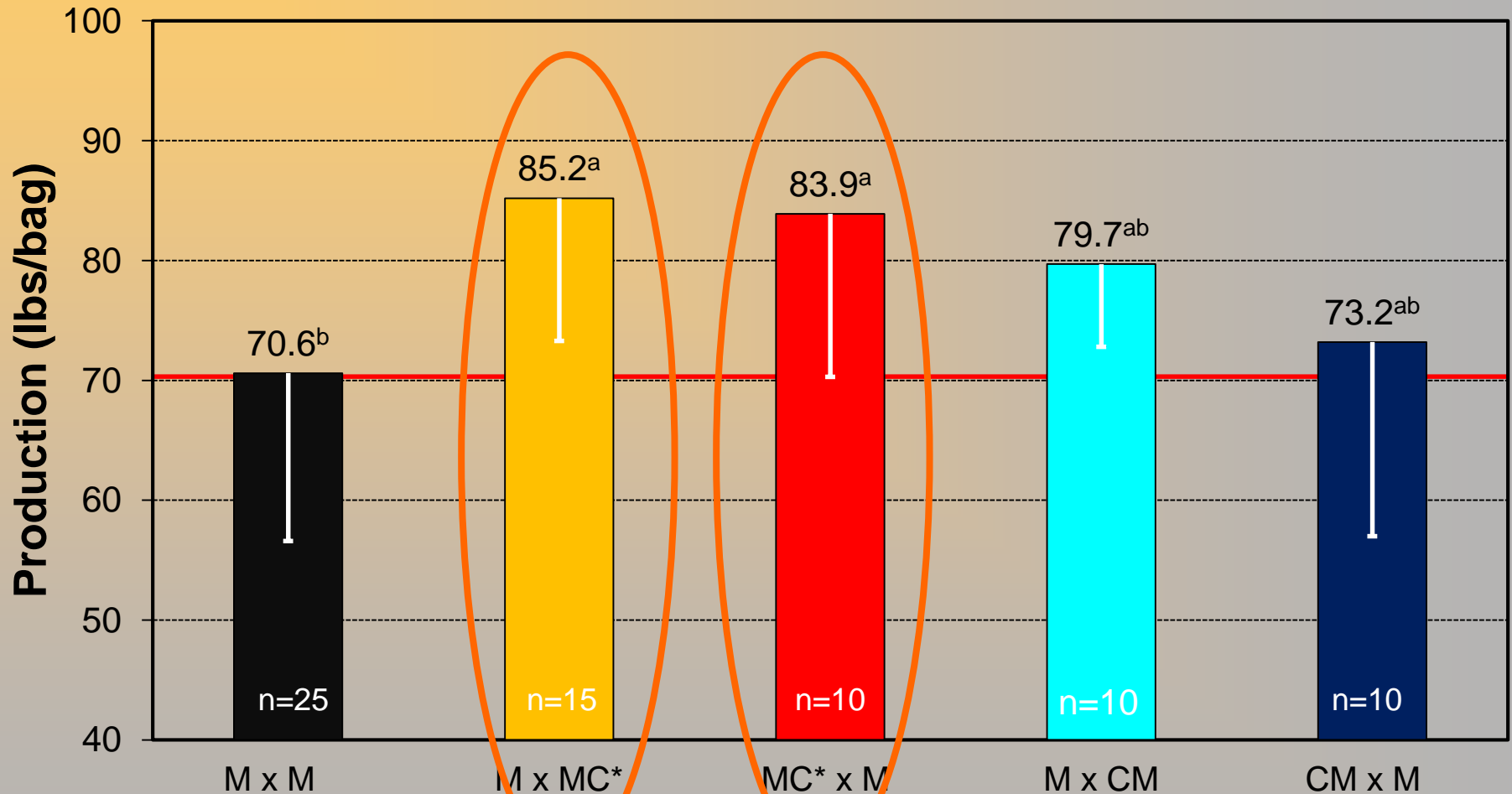
Harvest Results (13 months): Survival All Backcross Families



* Results from one parental stock family (Family A)

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

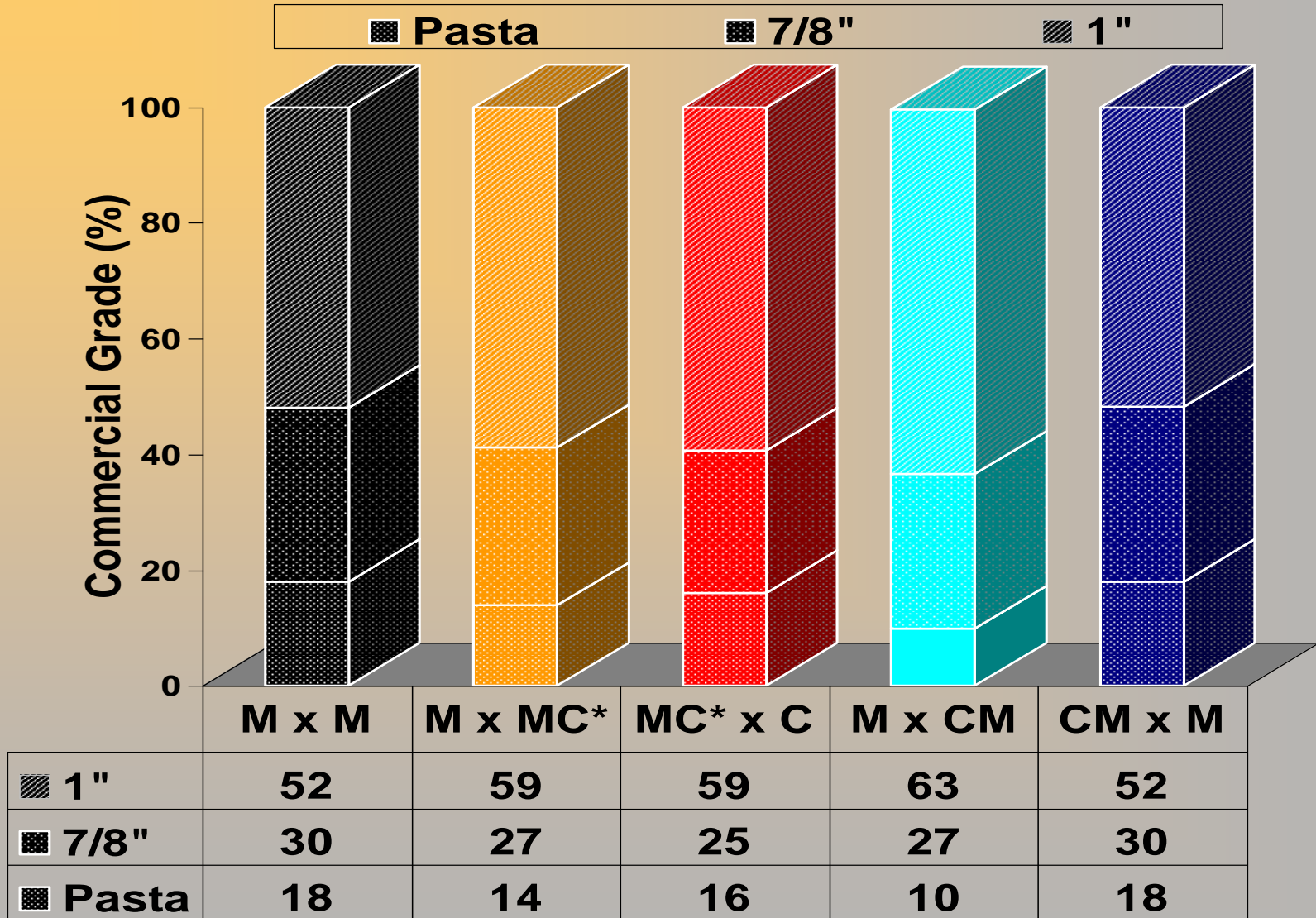
Harvest Results (13 months): Production All Backcross Families



* Results from one parental stock family (Family A)

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

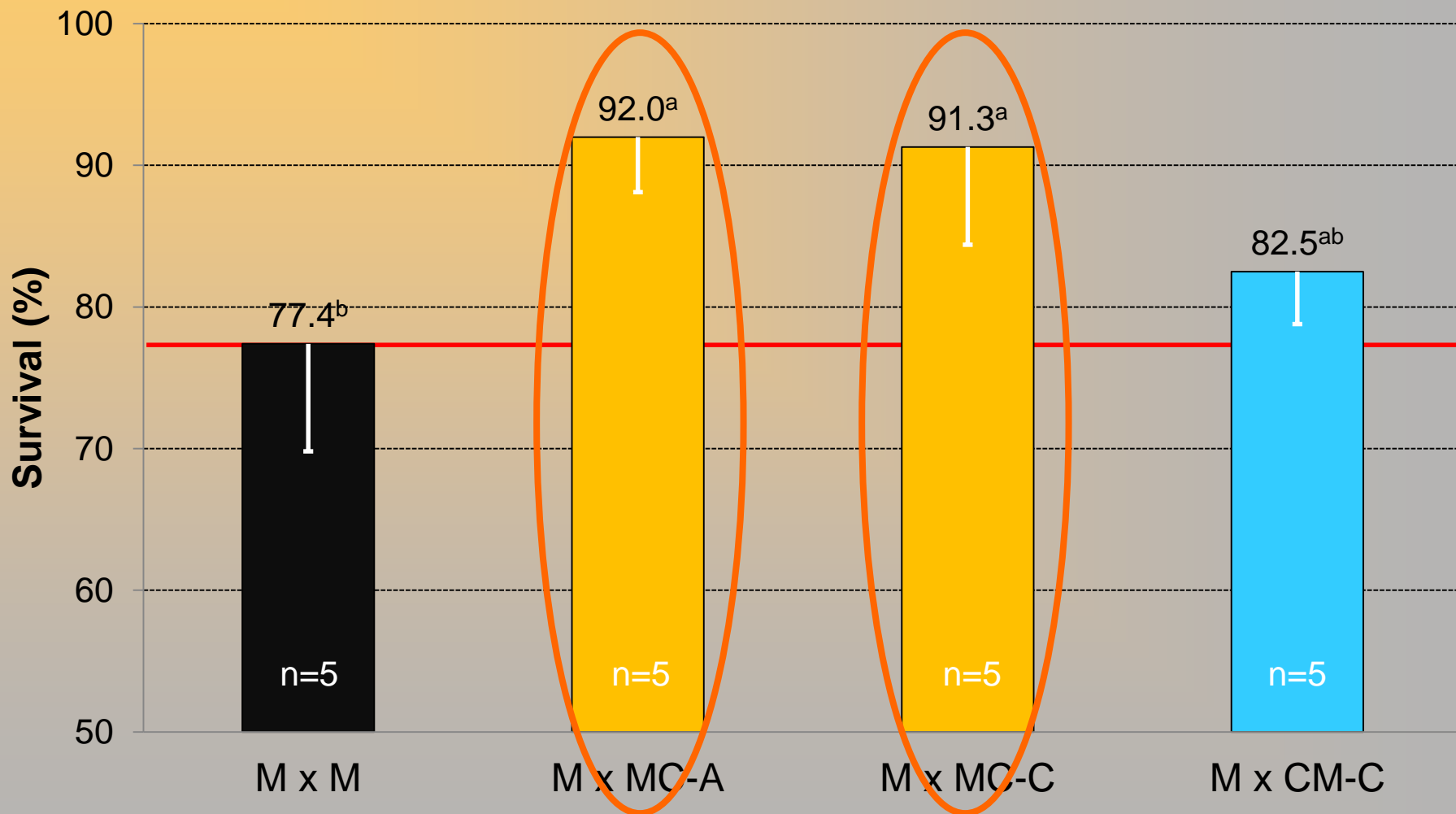
Harvest Results (13 months)



* Results from one parental stock family (Family A)

Harvest Results (13 months): Survival

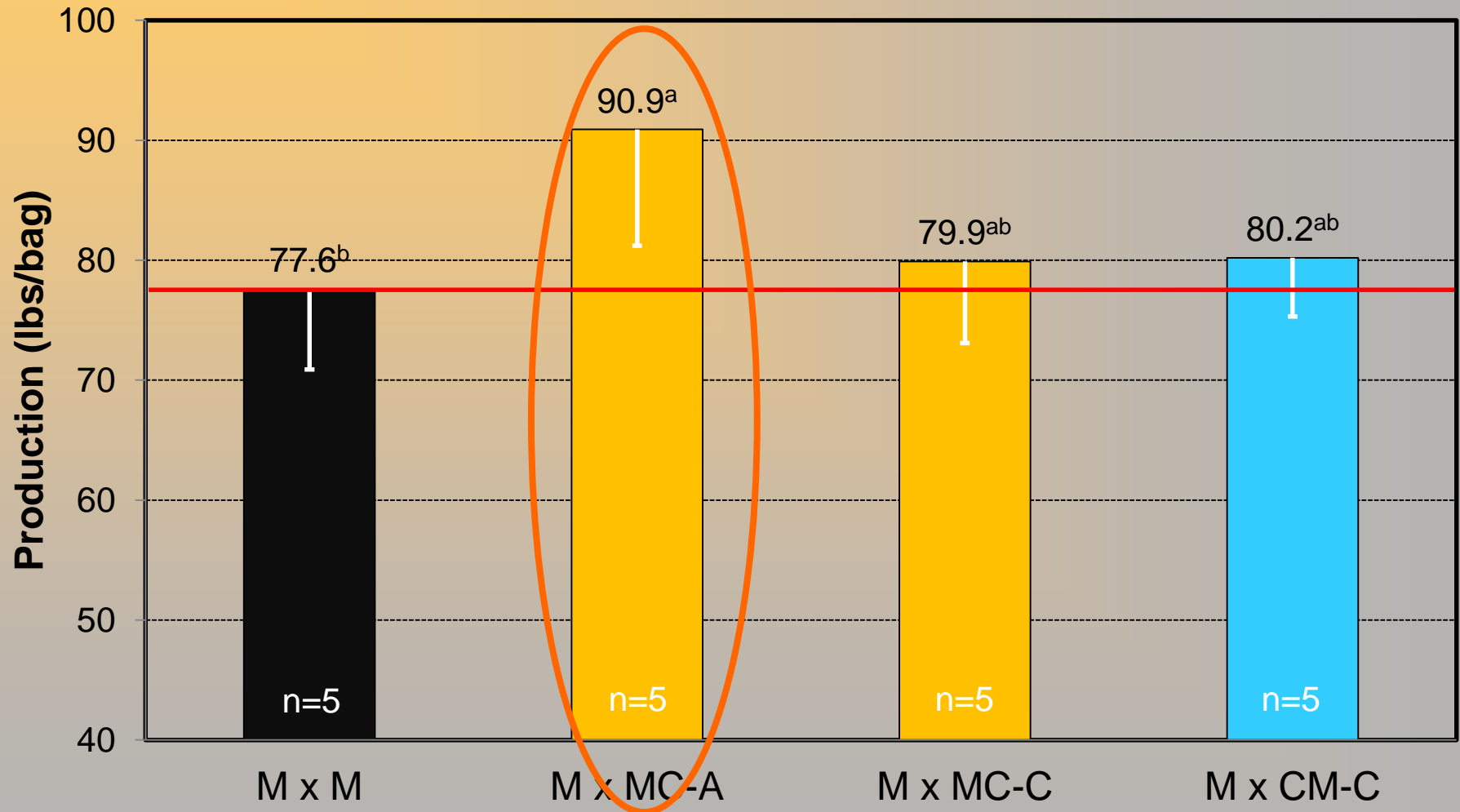
Family F: Hard Clam (M) ♀ x Hybrid ♂



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): Production

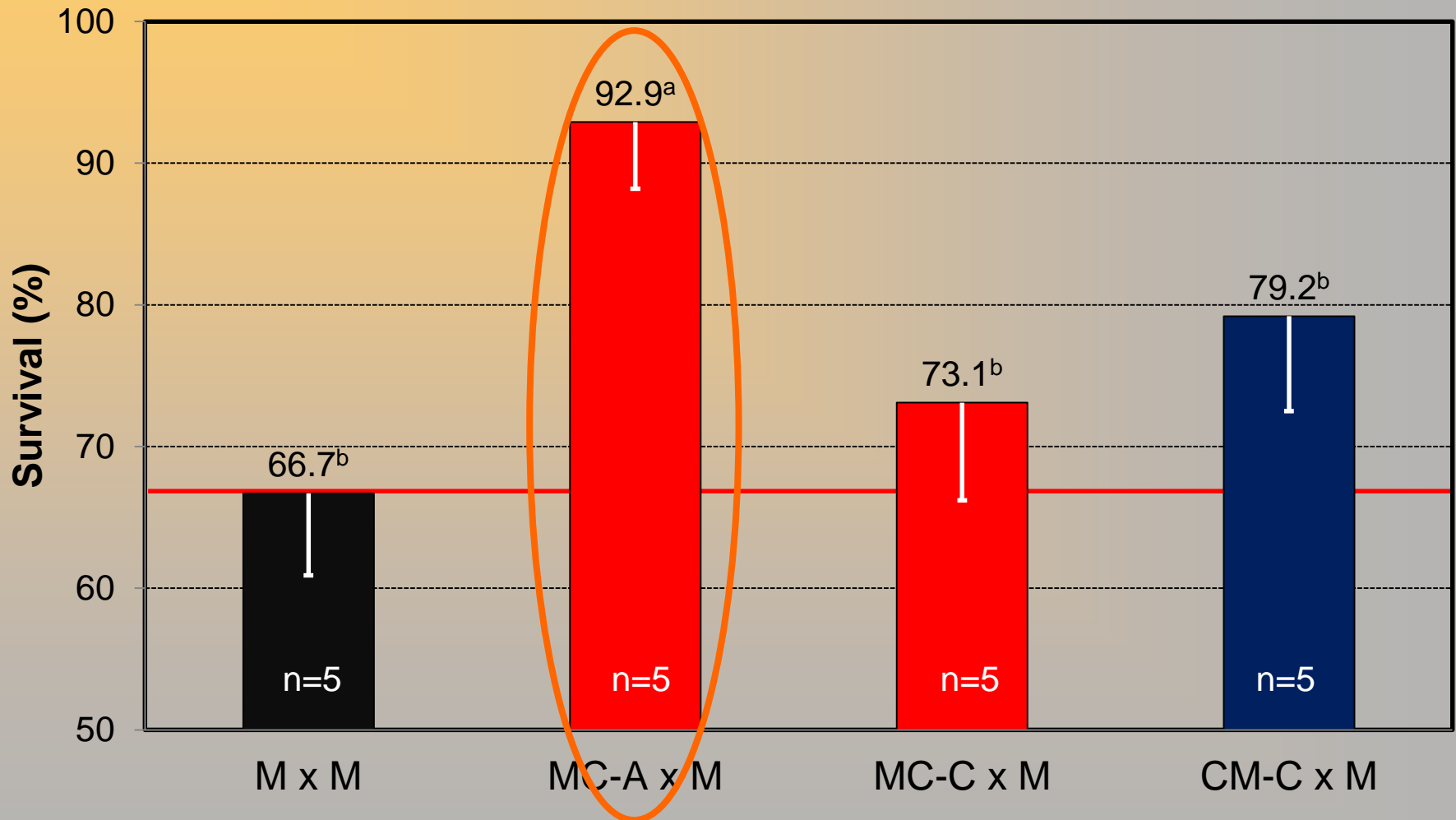
Family F: Hard Clam (M) ♀ x Hybrid ♂



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

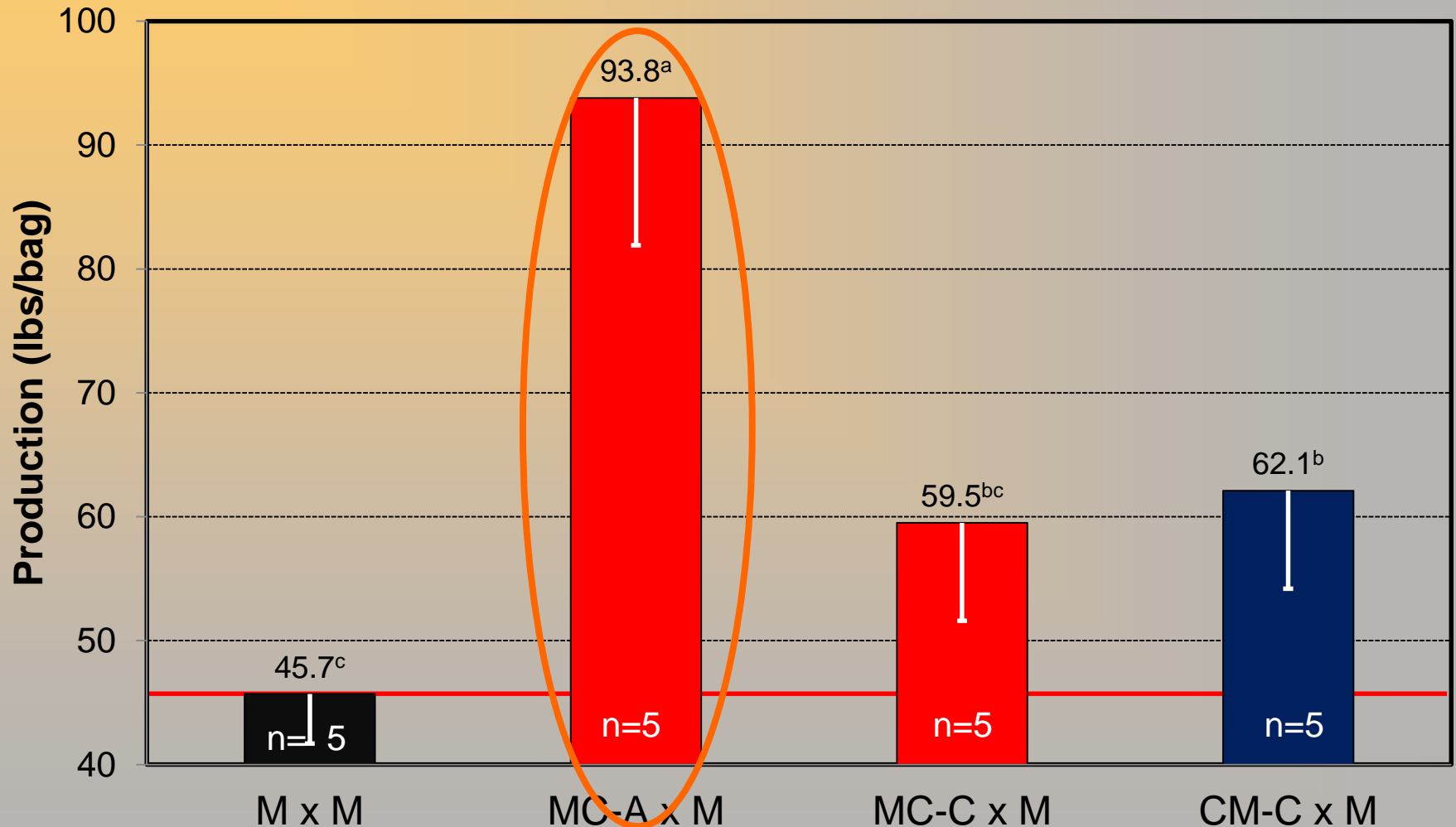
Family D: Hybrid ♀ x Hard Clam (M) ♂



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): Production

Family D: : Hybrid ♀ x Hard Clam (M) ♂

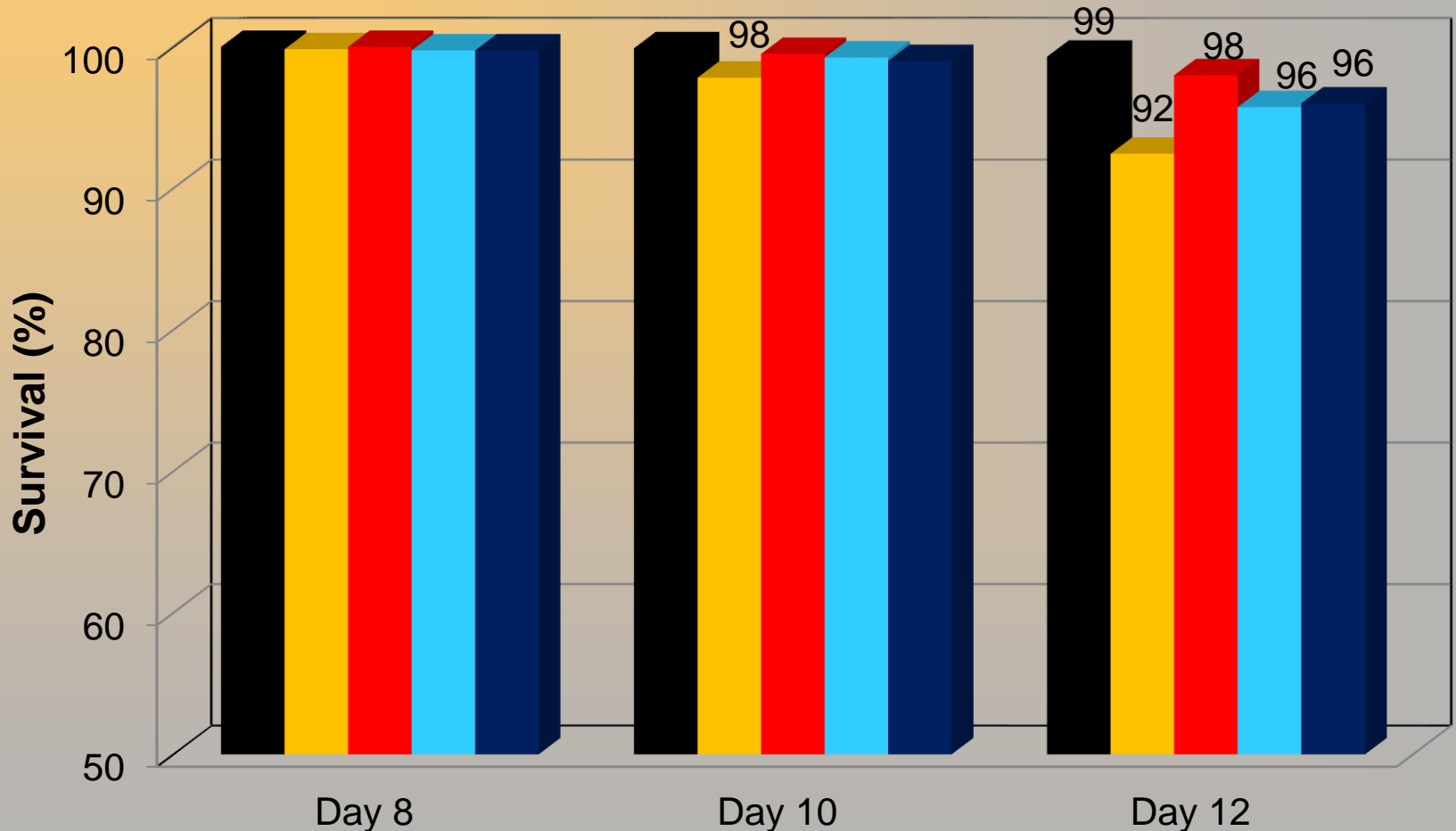


Note: ANOVA were performed using the PROC GLM procedure of SAS. Treatment means were considered significantly different when $p \leq 0.05$.

Shelf Life: Survival in 45°F Storage

Average of All Families

■ M x M ■ M x MC ■ MC x M ■ M x CM ■ CM x M

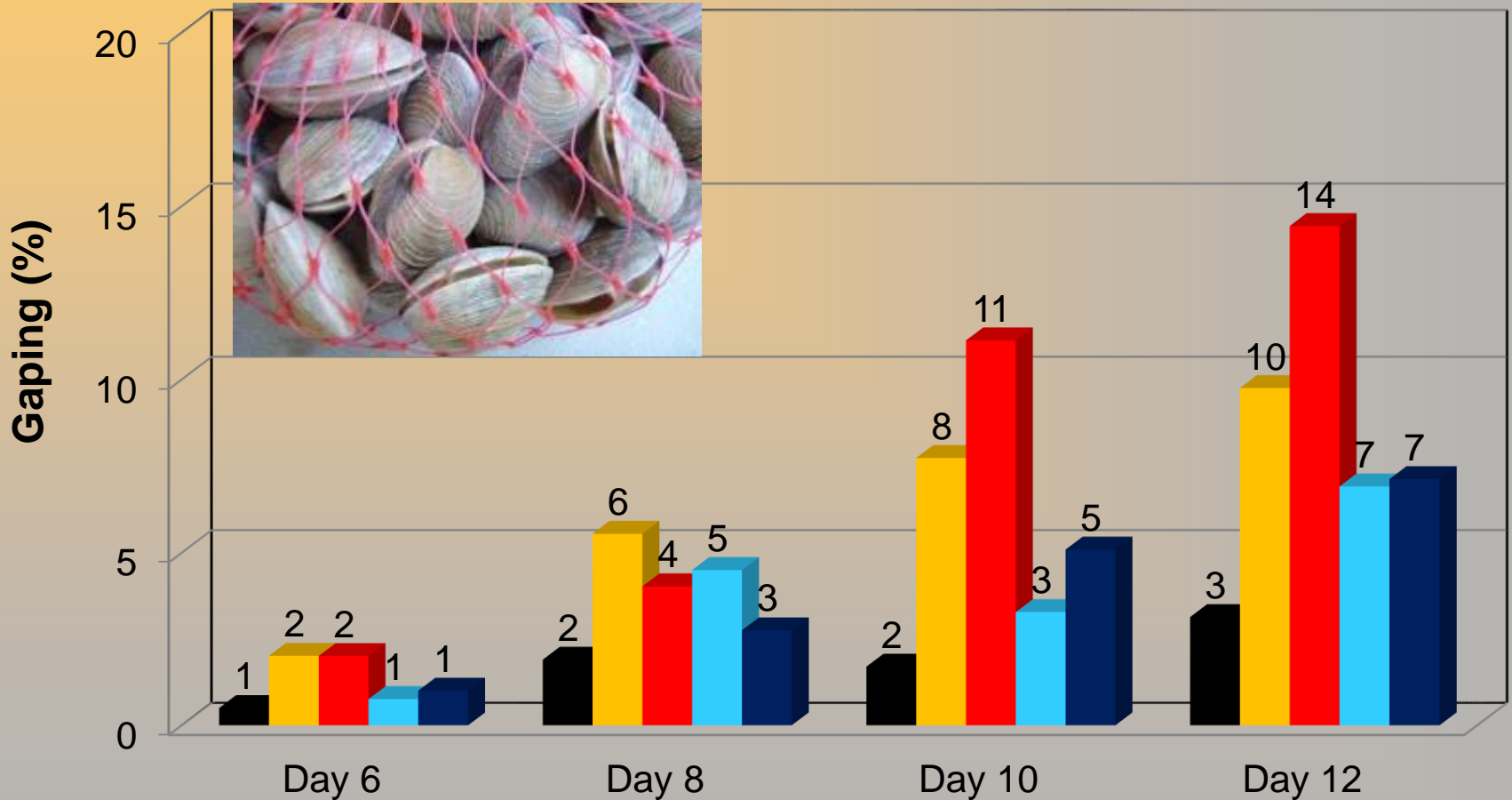


Note: A repeated measures analysis (PROC GLIMMIX) was performed.

Shelf Life: Gaping in 45°F Storage

Average of All Families

■ M x M ■ M x MC ■ MC x M ■ M x CM ■ CM x M



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

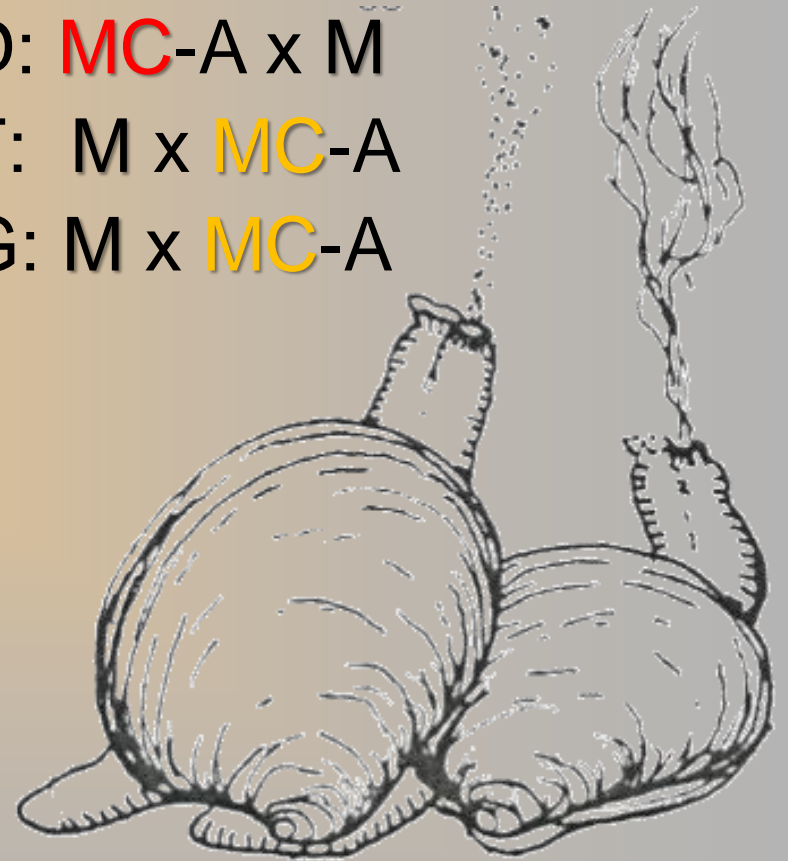
Summary

- Backcrossing F1 hybrids to hard clams offered improved survival and production
 - M x MC and MC x M had > survival and production
- Genetic background (families) played a significant role in responses
 - For Family F, M x MC-A had > survival and production
 - For Family D, MC-A x M had > survival and production
- Shelf life commercially acceptable
 - At 10 days, 98-100% for all stocks
 - At 12 days, 92-96% for backcrosses versus 99% for hard clams
- Gaping in refrigerated storage acceptable
 - At 10 days, 3-11% for backcrosses versus 2% for hard clams
 - At 12 days, 7-14% for backcross stocks versus 3% for hard clams



Available Broodstock

- 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!"

Acknowledgements

- University of Florida
 - Eric Cassiano, Reggie Markham, Barry Clayton, Russ Colson, Charles Mulligan
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