Enhancing Stress Resistance of Cultured Clams Through Triploidy: Final Report on Field Trials and Laboratory Challenges

John Scarpa, Harbor Branch Oceanographic Institute @FAU
Shirley Baker, UF, SFRC, Fisheries and Aquatic Sciences
Leslie Sturmer, UF Cooperative Extension Service
Chuck Adams, UF Department of Food and Resource Economics

HARBOR BRANCH
FLORIDA ATLANTIC UNIVERSITY

Sea Grant
Florida

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Hypothesis

- Mortalities from summer stressors can be reduced by creating sterile clams through triploidy
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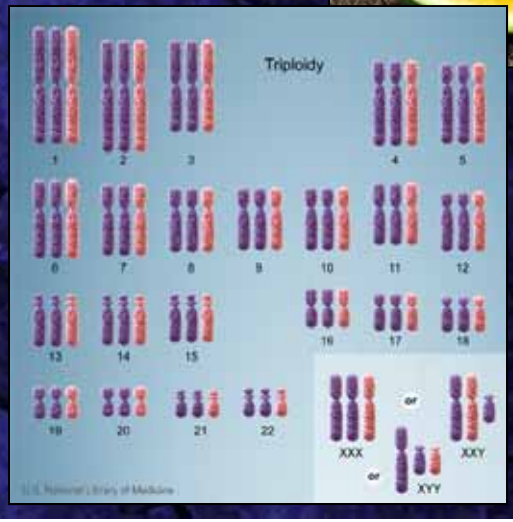
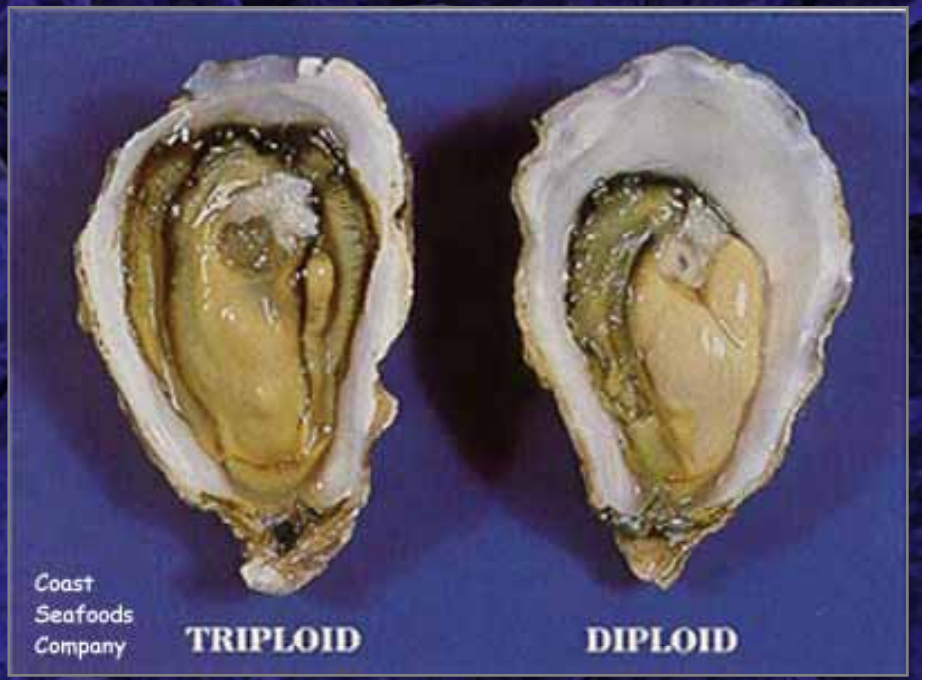
Triploidy = 3 sets of chromosomes



Why triploidy?

- Triploids divert energy from reproduction to storage and growth
- Used in PNW oyster aquaculture
- Need for hardier clam strain in SE

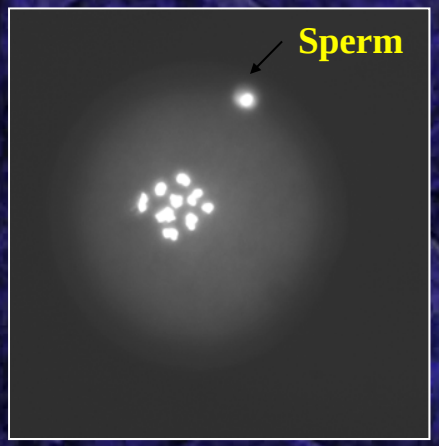


Specific objectives

- Create replicate diploid/triploid families
- Compare growth & survival during grow-out
- Compare physiological responses to stress
- Examine economics

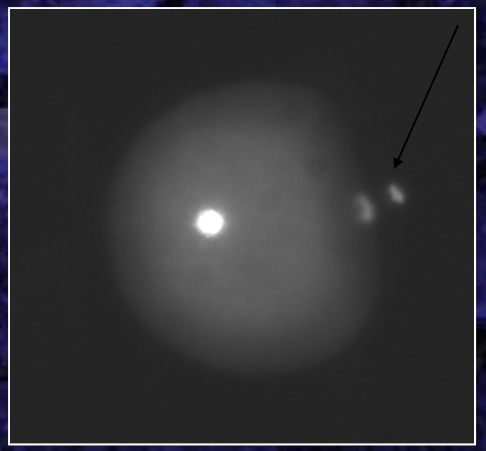
Meiotic and Mitotic Stages in Eggs of *Mercenaria mercenaria*

Metaphase I

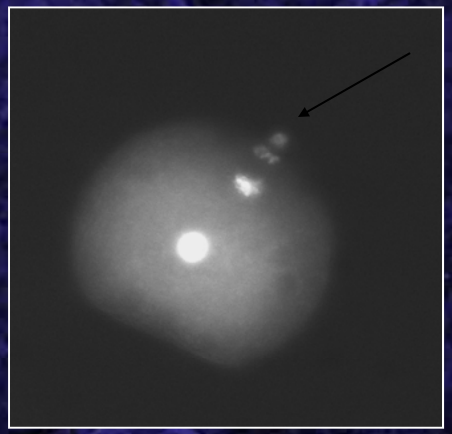


Early anaphase I

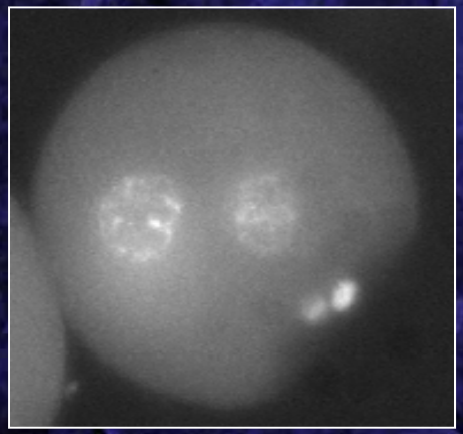
Late anaphase I



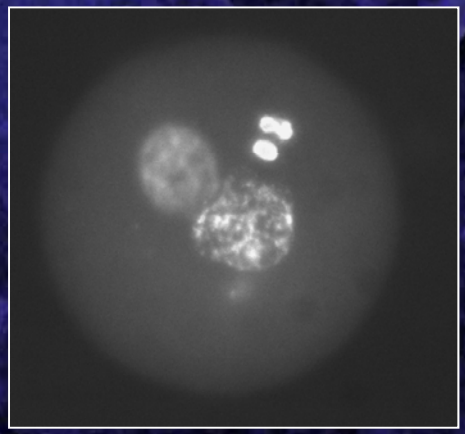
PB 1



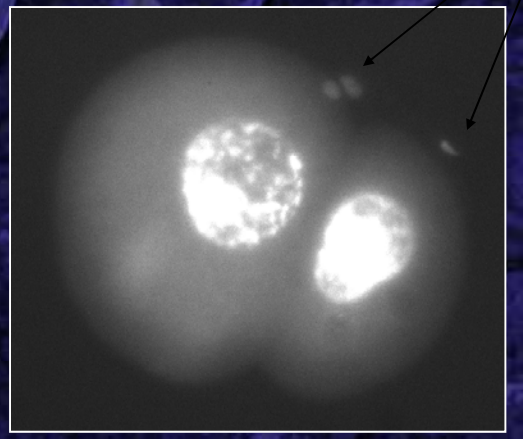
PB 2



Pronuclei

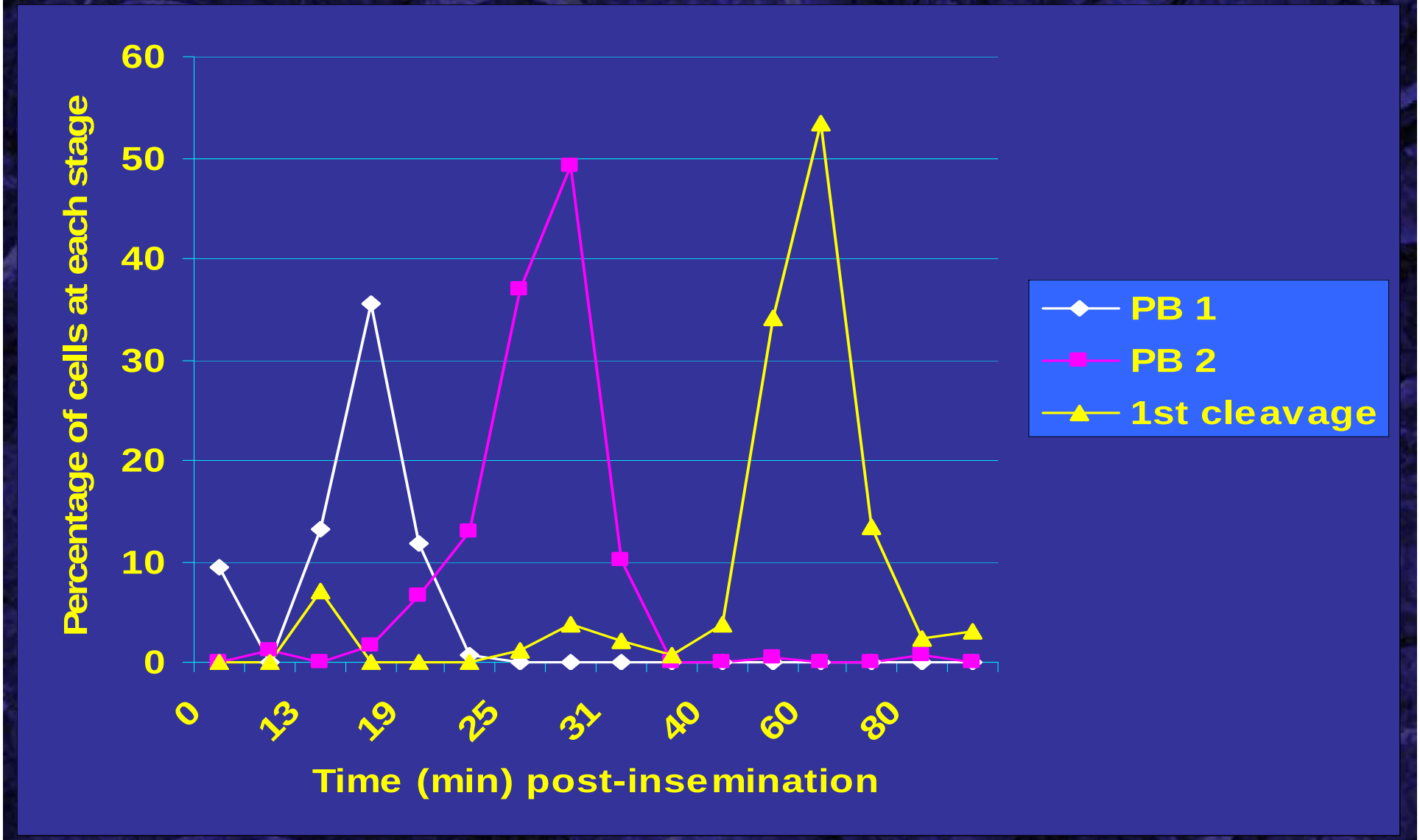


Syngamy

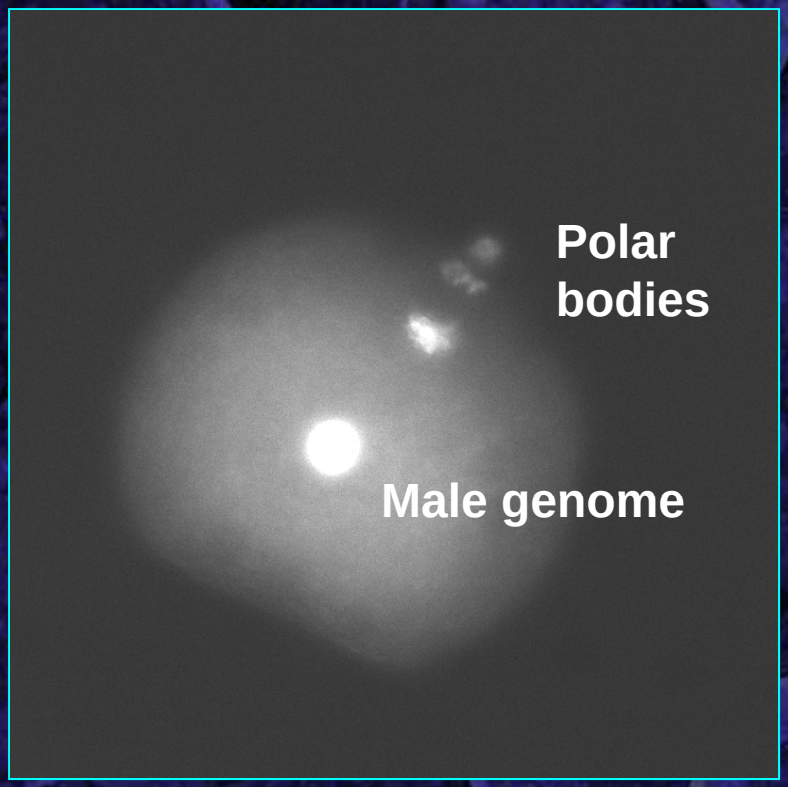


1st Cleavage
(2-Cell stage)

Mean Time to Formation of First Polar Body (PB 1), Second Polar Body (PB 2), and First Cleavage in Hard Clam Eggs

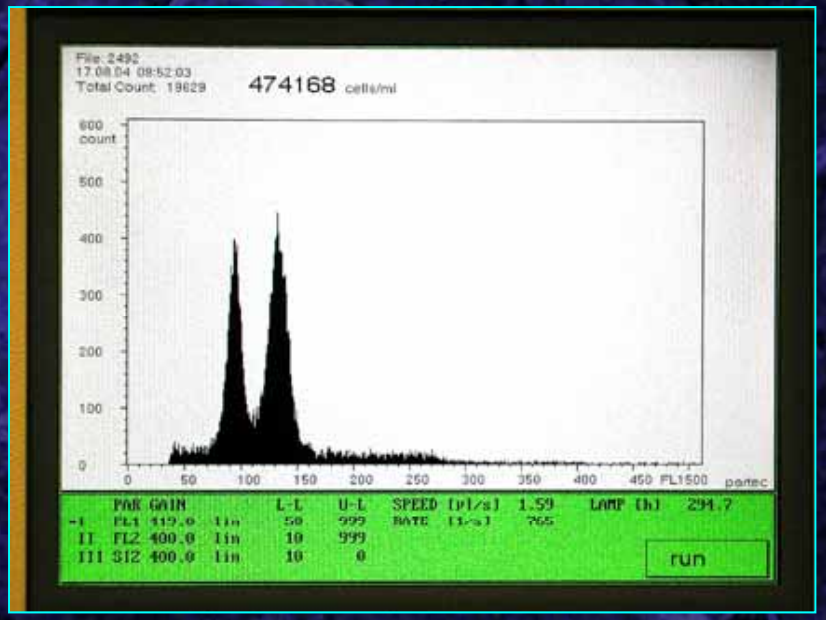


Flow Cytometry



Polar Body 2 release

Flow Cytometry output

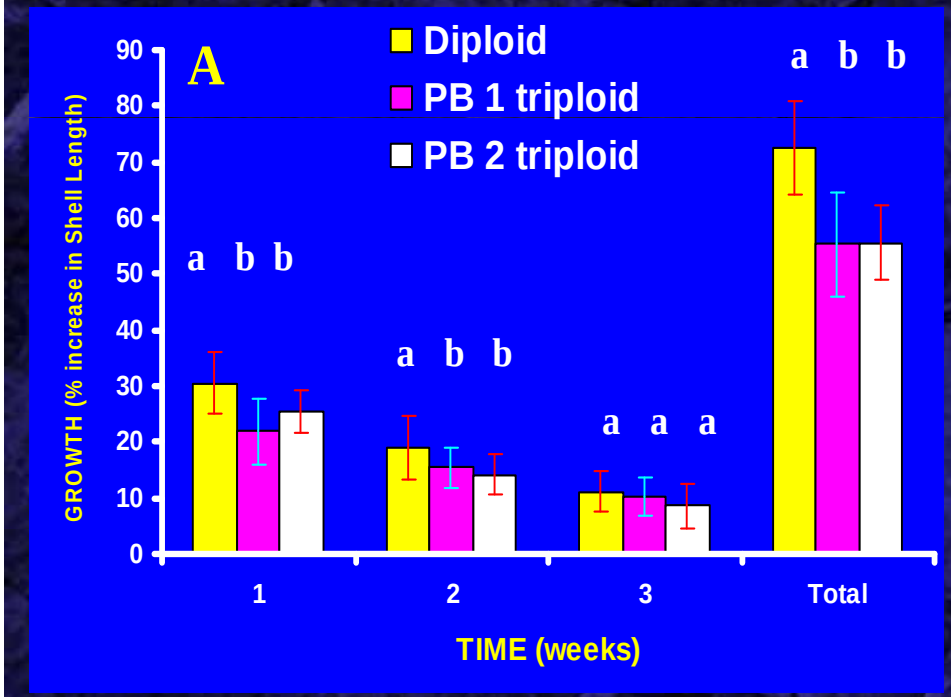


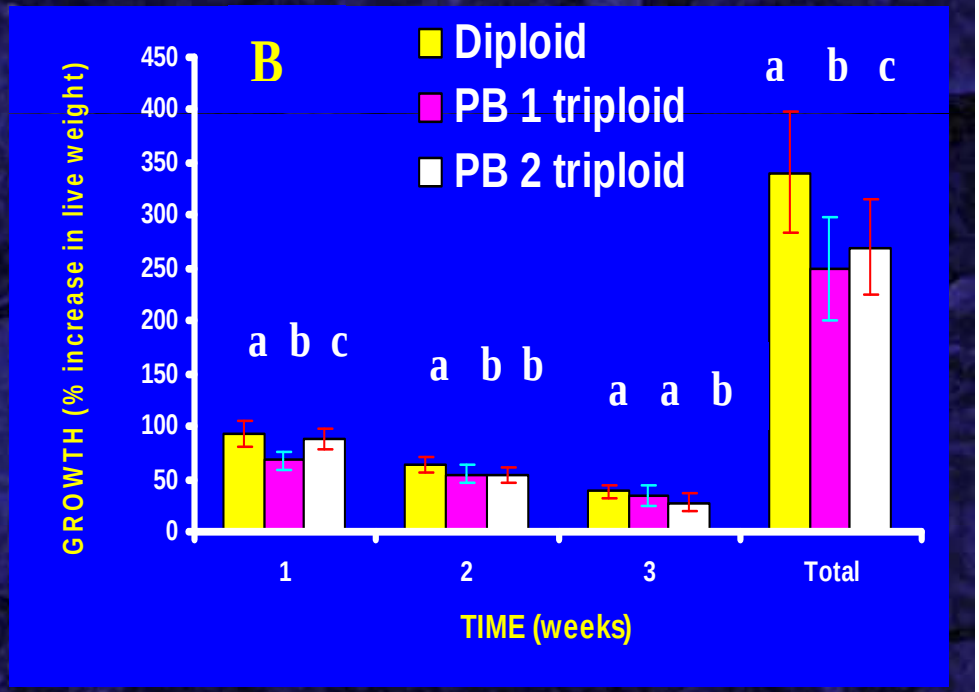
Cytological and Flow-Cytometric Data from Triploid Induction Experiments

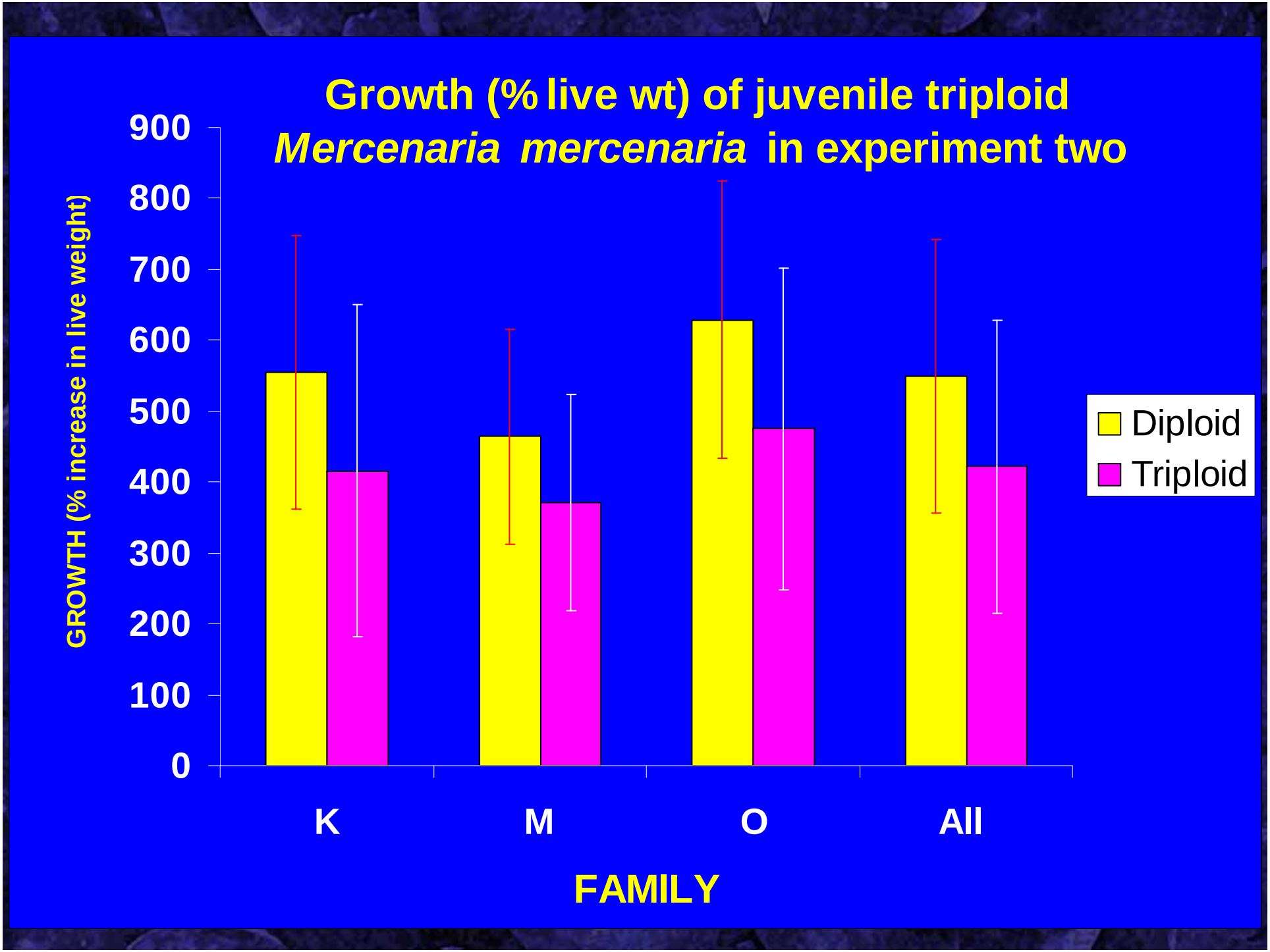
Trial #, treatment		Cytological Data (% of fertilized eggs)				Triploid (%)
		Pre-fertilized	Pre-PBI	Pre-PBII	Post-PBII	
1	PBI	55	50	7	43	39
	PBII		3	30	67	0
2	PBI	5	83	2	3	0
	PBII		23	77	0	0
3	PBI	1	100	0	0	0
	PBII		0	100	0	0
4	PBI	5	90	10	0	93
	PBII		0	44	56	33
5	PBI	0	85	0	0	33
	PBII		28	72	0	83
6	PBI	6	55	0	21	77
	PBII		56	39	5	86
7	PBI	5	41	0	1	48
	PBII		66	26	8	57
8	PBI	4	69	2	8	26
	PBII		36	48	13	69

Juvenile Growth Studies

Growth (%) in shell length (A) and live weight (B) of juvenile triploid *Mercenaria mercenaria*

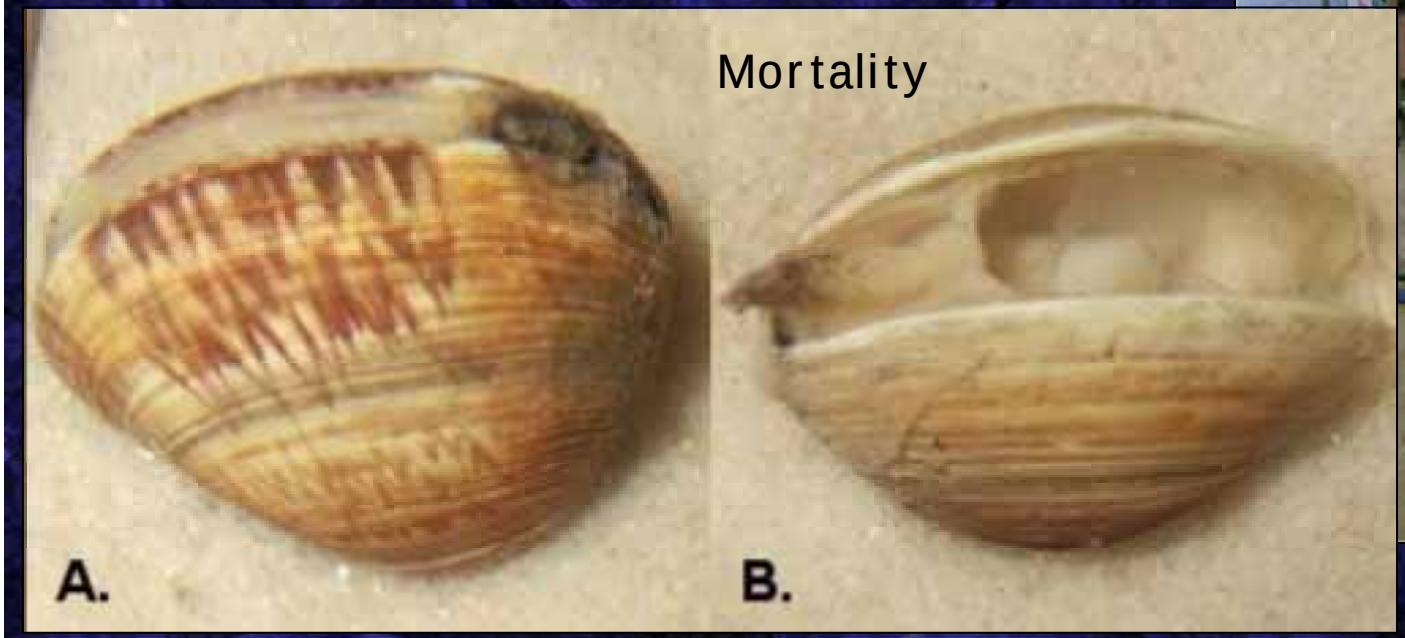





Growth (% live wt) of juvenile triploid
Mercenaria mercenaria in experiment two
GROWTH (% increase in live weight)
900
800
700
600
500
400
300
200
100
0
K
M
O
All
FAMILY
Diploid
Triploid

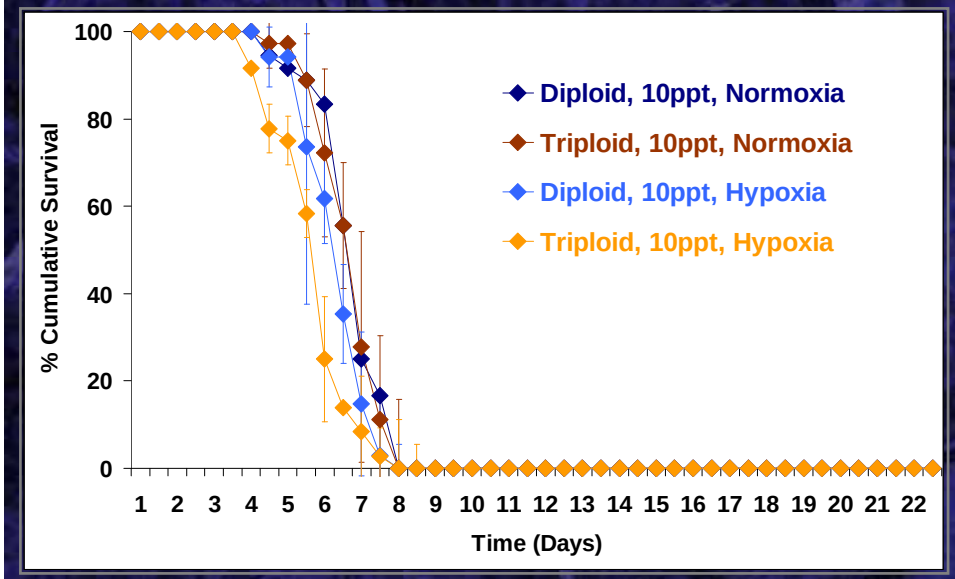
Survival under stress

- 45 mm SL
- Temperature: 90°C
- Salinity: 10, 25, 40 ppt
- Oxygen: Normoxia or hypoxia

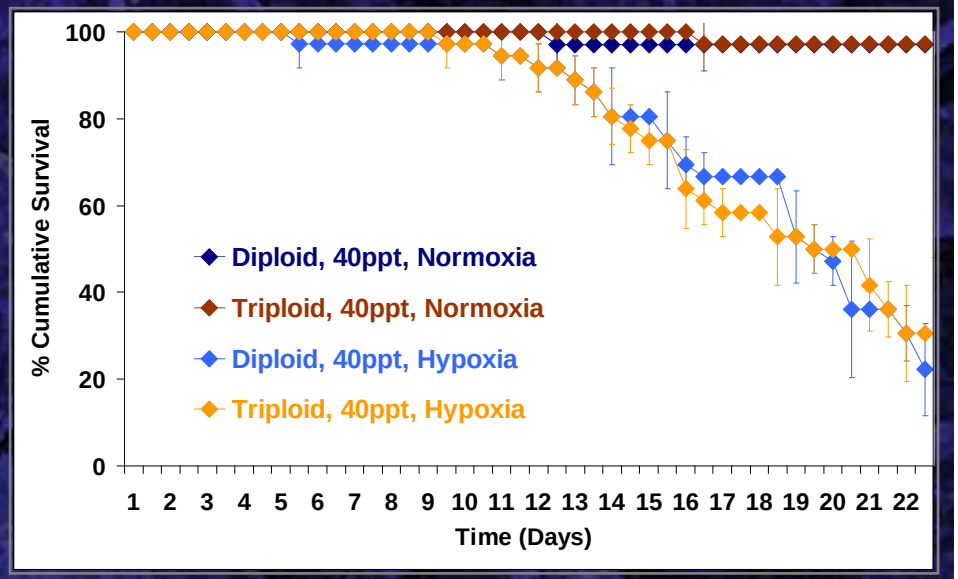


Survival – 10 and 40 ppt

10 ppt

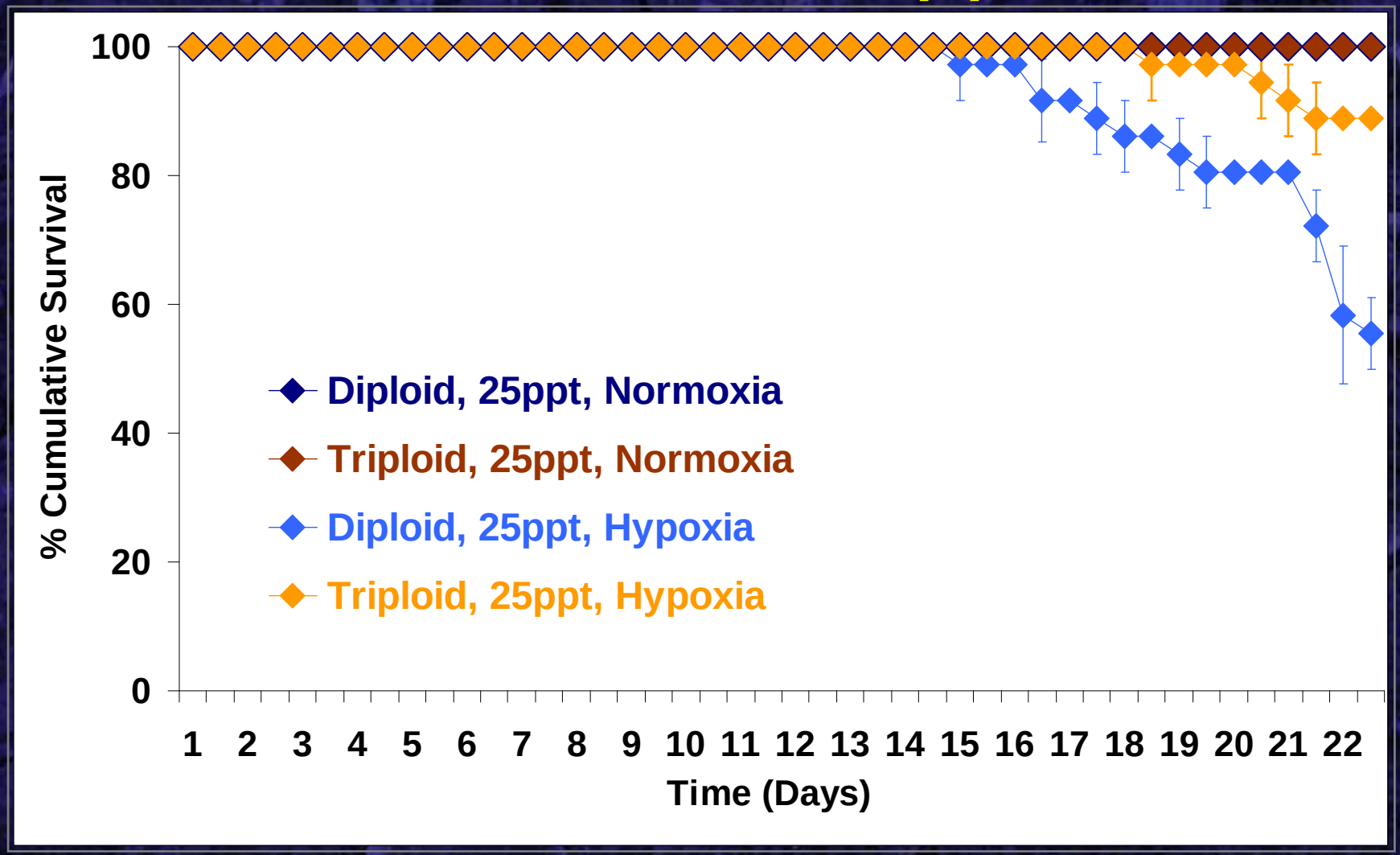


40 ppt



- At salinity extremes, triploid clams have no advantage over diploid clams

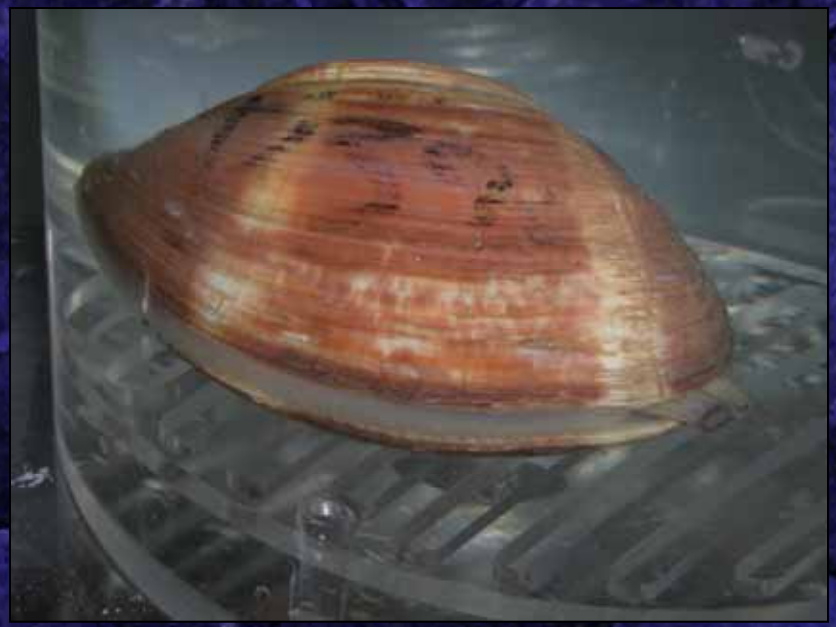
Survival – 25 ppt

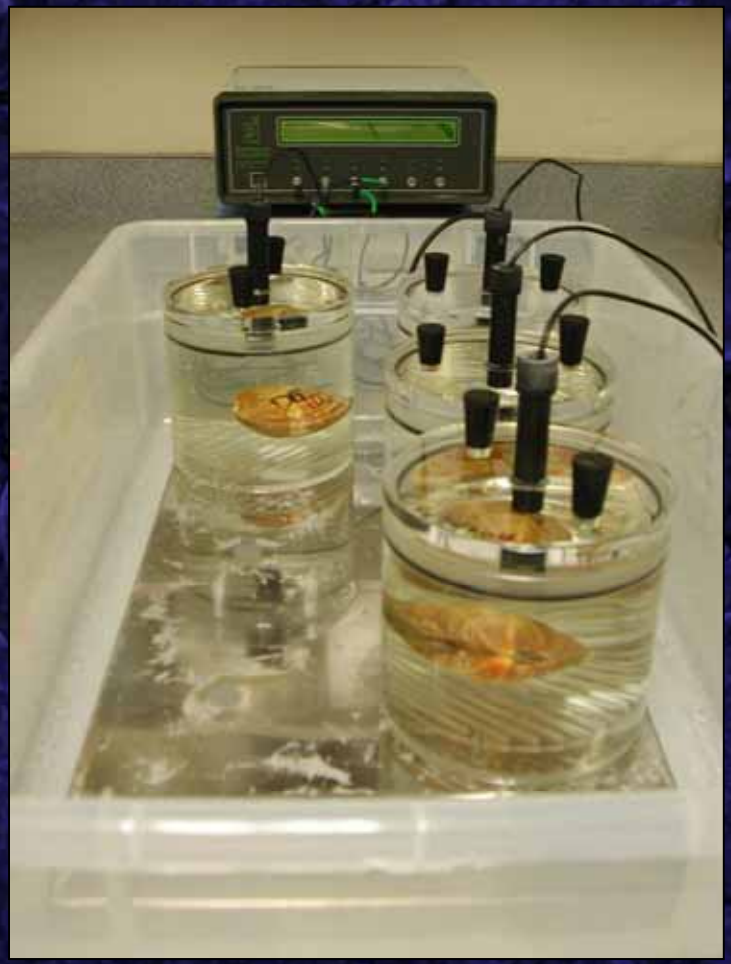


Triploidy may increase survival in hypoxia

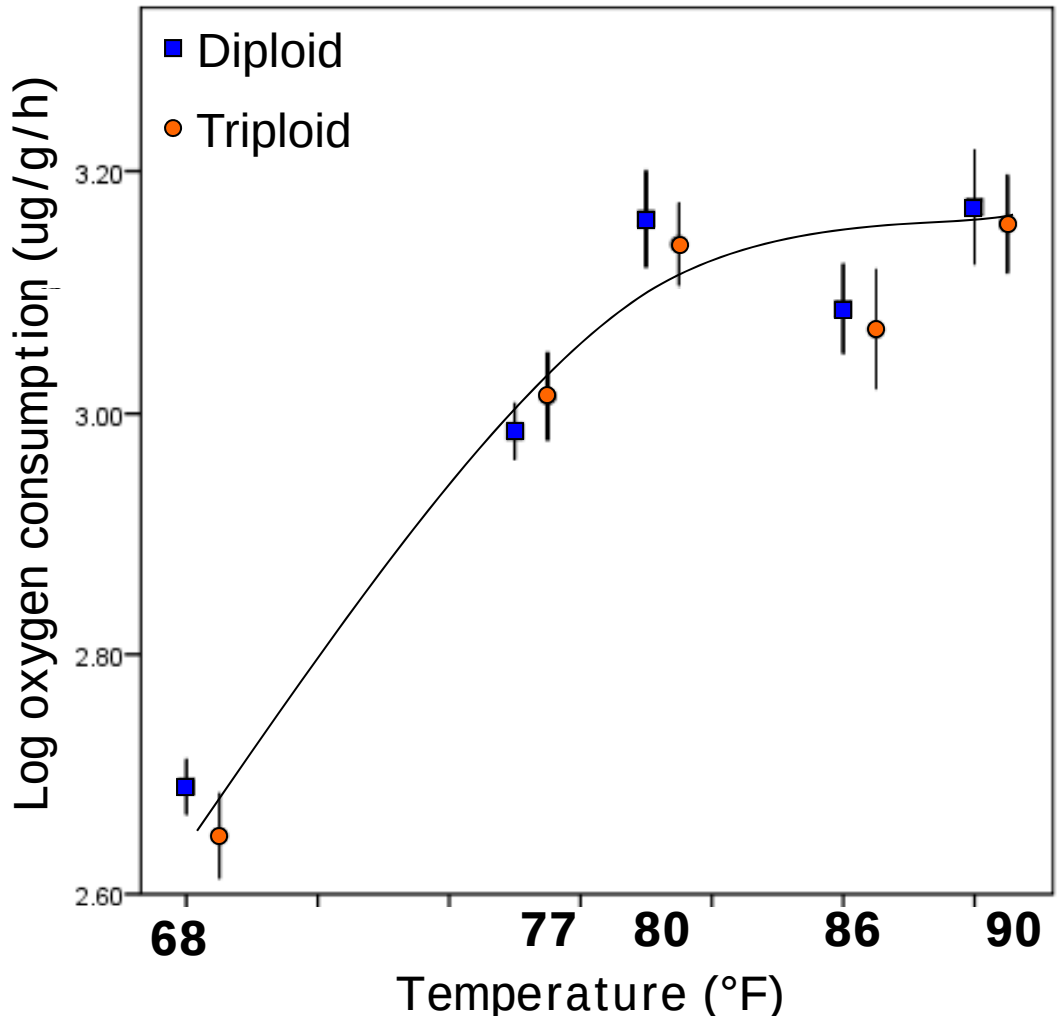
Physiological response to stress

- Oxygen uptake rates
- 50 mm SL
- 25 ppt or 15ppt
- 68, 77, 80, 86, and 90°F



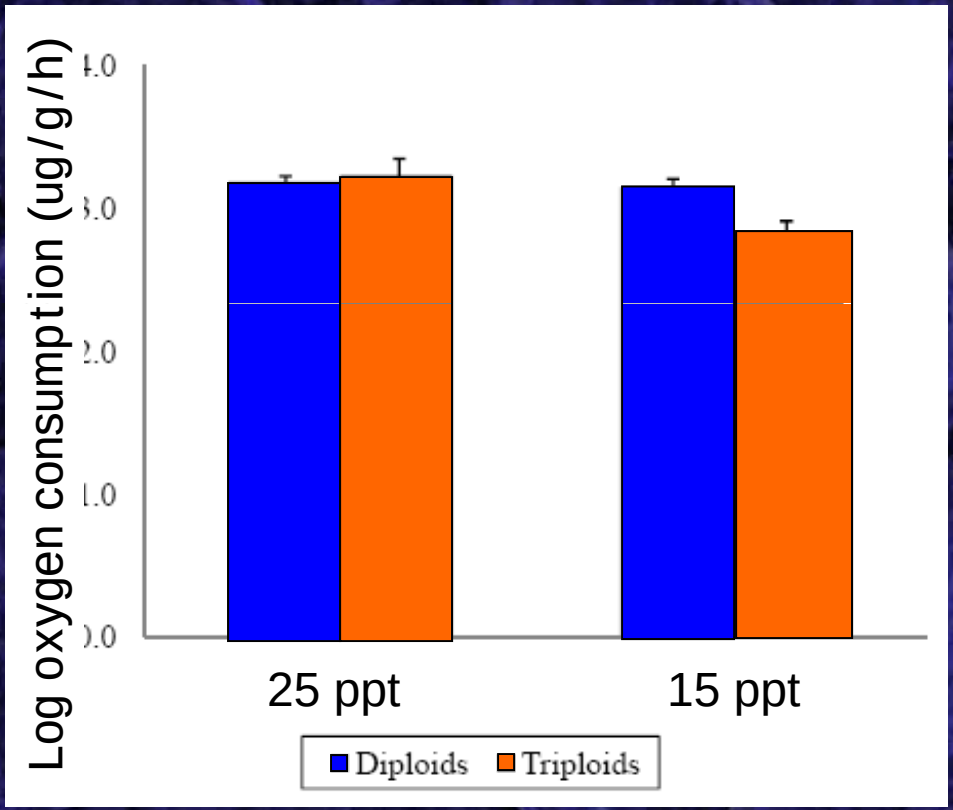


Oxygen uptake rate – temperature



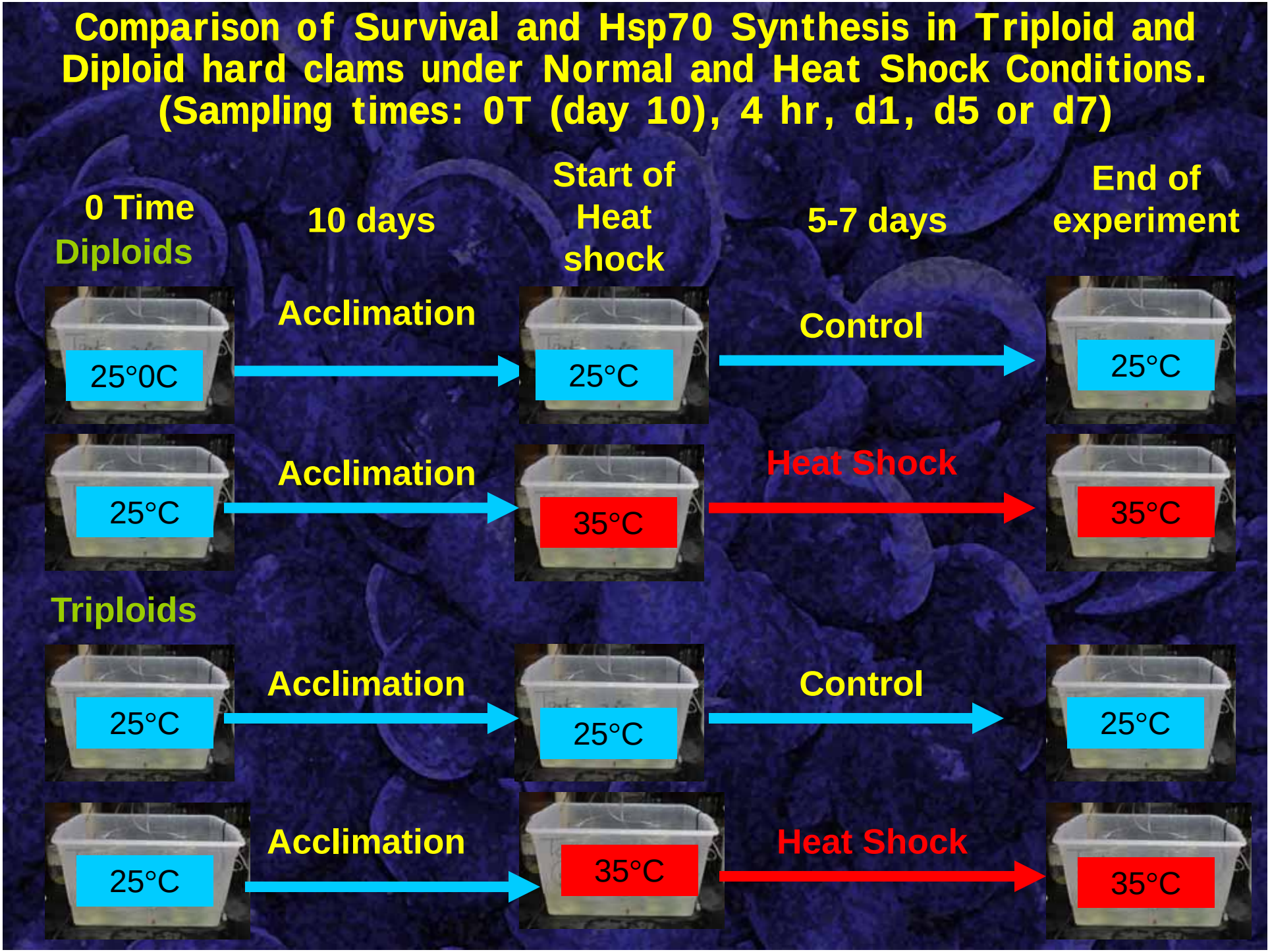
- Metabolic rate increases with temperature, but not above 80°F
- Triploid clams have no advantage over diploids

Oxygen uptake rate – salinity



- Triploid clams have lower metabolic rate at 15 ppt than at 25 ppt (80°F)
- Triploid clams may use less energy during stress

Comparison of Survival and Hsp70 Synthesis in Triploid and Diploid hard clams under Normal and Heat Shock Conditions. (Sampling times: 0T (day 10), 4 hr, d1, d5 or d7)



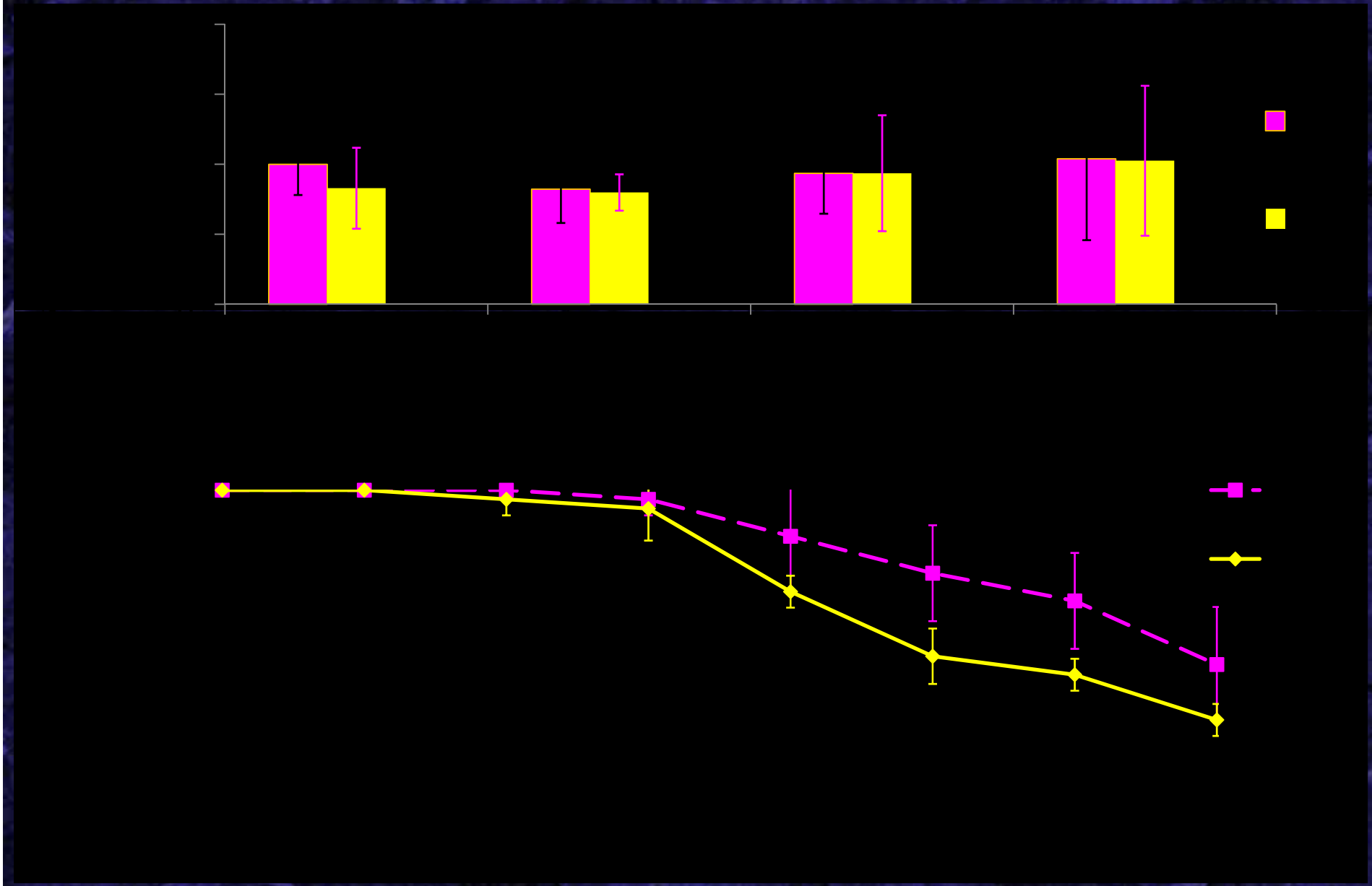
Experimental System

(1) Acclimation Unit : Sump connected to a chiller @ 25±1° C.

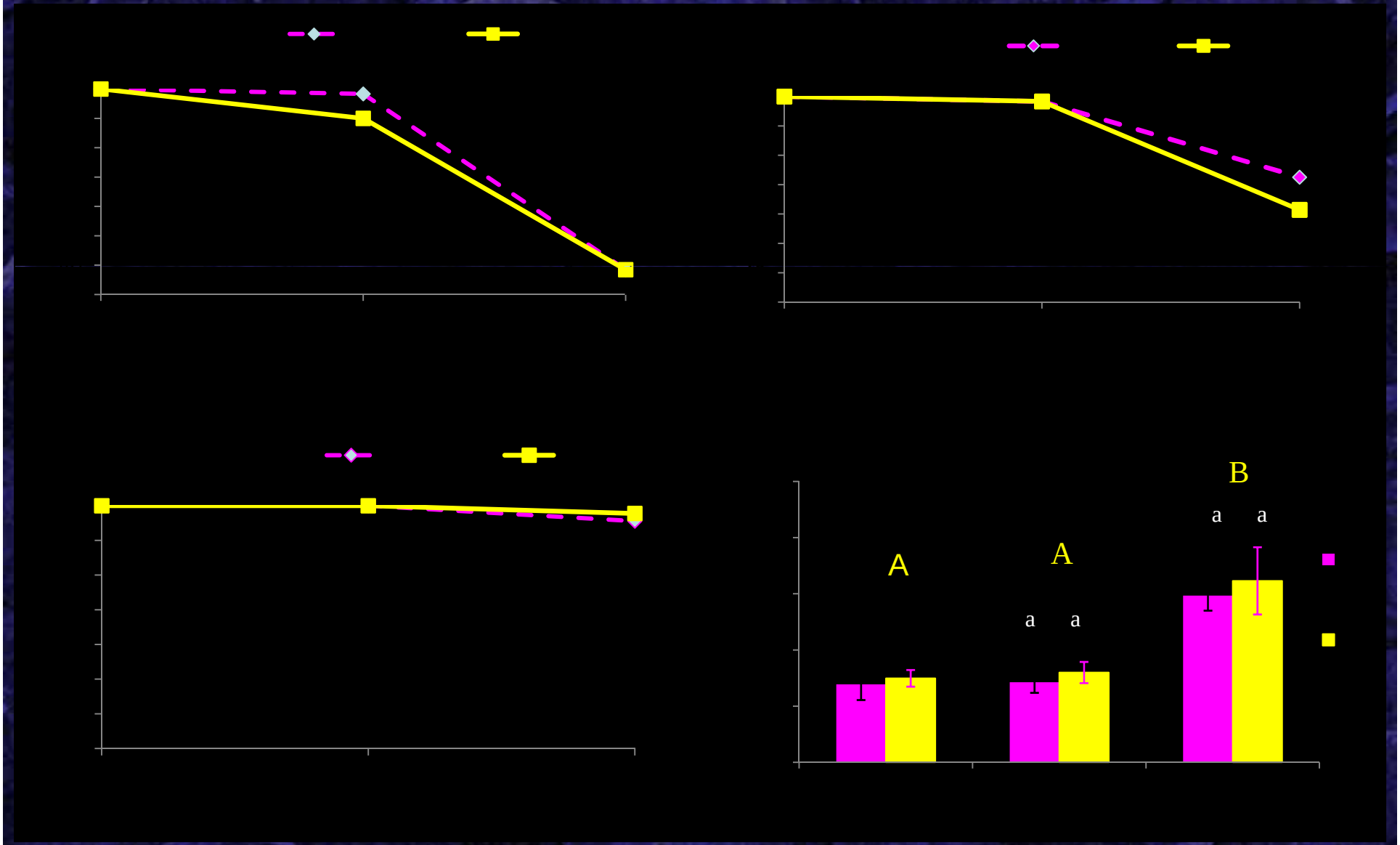
(2) Cold Treatment Unit: Sump connected to a chiller @ 25±1° C.

(3) Hot Treatment Unit: Sump with titanium Heater @ 35±1° C.

Hsp70 and Survival (± SD) in Triploid and Diploid Hard Clams (SL=31.6 ± 6.6) Following Severe Heat Shock (25-35ºC).



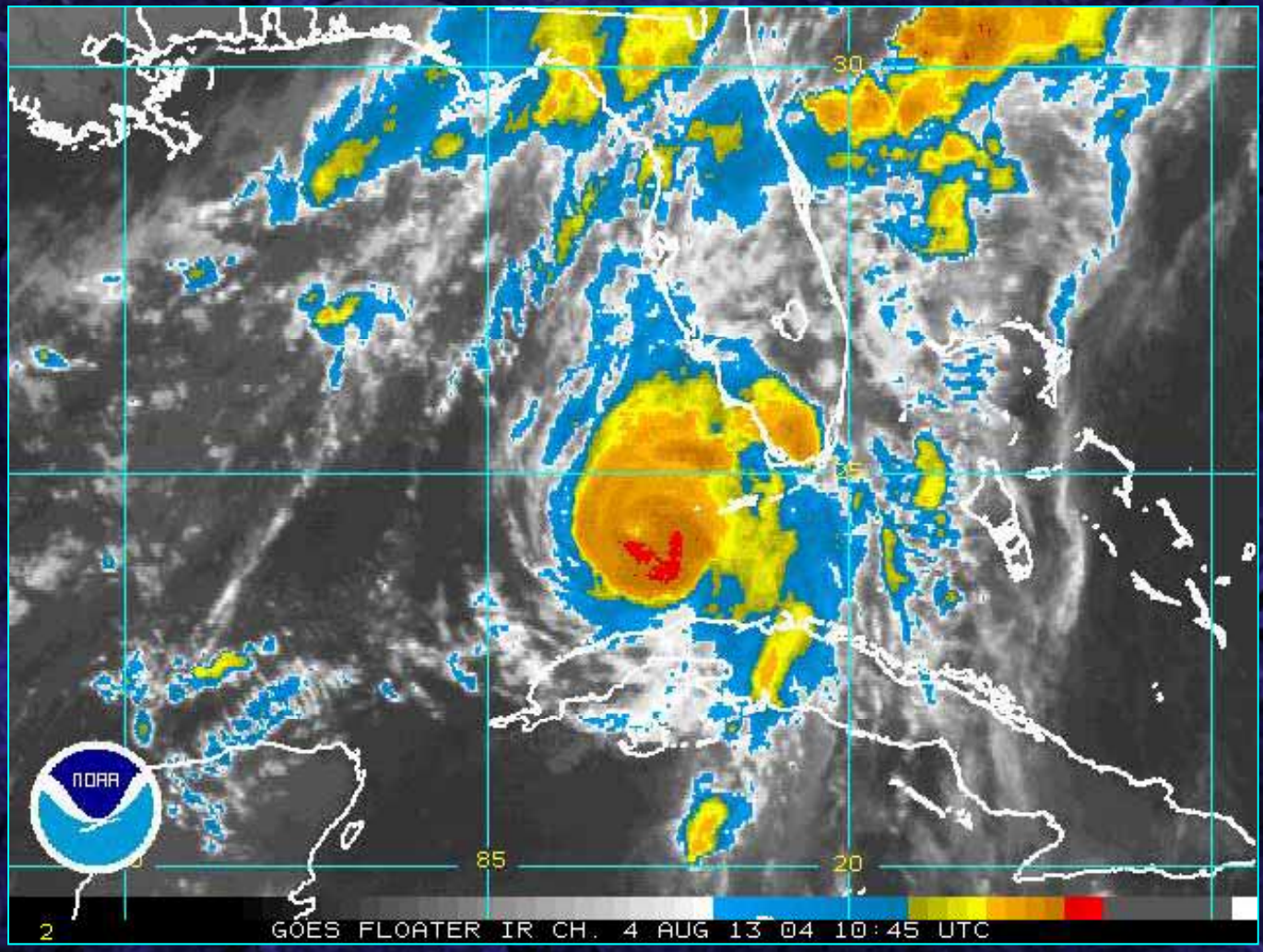
Survival (± SD) of hard clams (SL= 44.0± 3.3) exposed to severe heat shock (25-35°C) was influenced by genetic background represented by higher initial Hsp70 concentration (*P*= 0.042), than by ploidy (*P*= 0.184).



GROW - OUT

Leslie Sturmer

Clam samples



Hurricane Charley

Mean values of different parameters measured for PB2 triploid clams cultured in Cedar Key

	Diploids			Triploids			T-test
	N	Mean	SD	N	Mean	SD	Signif.
Shell Length	32	23.4	± 3.5	13	19.7	± 3.2	0.002
Shell Width	32	11.3	± 1.7	13	9.3	± 1.5	0.000
Live Weight	32	3.33	± 1.32	13	1.97	± 0.85	0.001
Dry Meat Weight	18	0.104	± 0.039	7	0.068	± 0.026	0.034
Condition Index	18	5.6	± 0.5	7	6.6	± 0.5	0.000

Four hurricanes hit Florida in 2004 and destroyed 80% of all clams planted for the study. Data presented is from only one group of clams cultured in Cedar Key and sampled in December 2004. Triploid clams were estimated at 42-70% before the hurricanes, but only 29% after. Triploid clams were significantly smaller for all parameters measured except condition index. Histological analysis indicated 50% of diploid clams had spawned, whereas 100% of triploids had no mature gonad.

Grow Out 2 (PBII triploids)

Cedar Key

Ploidy	Length (mm)	Weight (g)	Cond. Index	Survival (%)
2N	45.9	30.6	4.77	**80.1**
3N	45.9	29.0	**5.30**	69.4

Charlotte Harbor

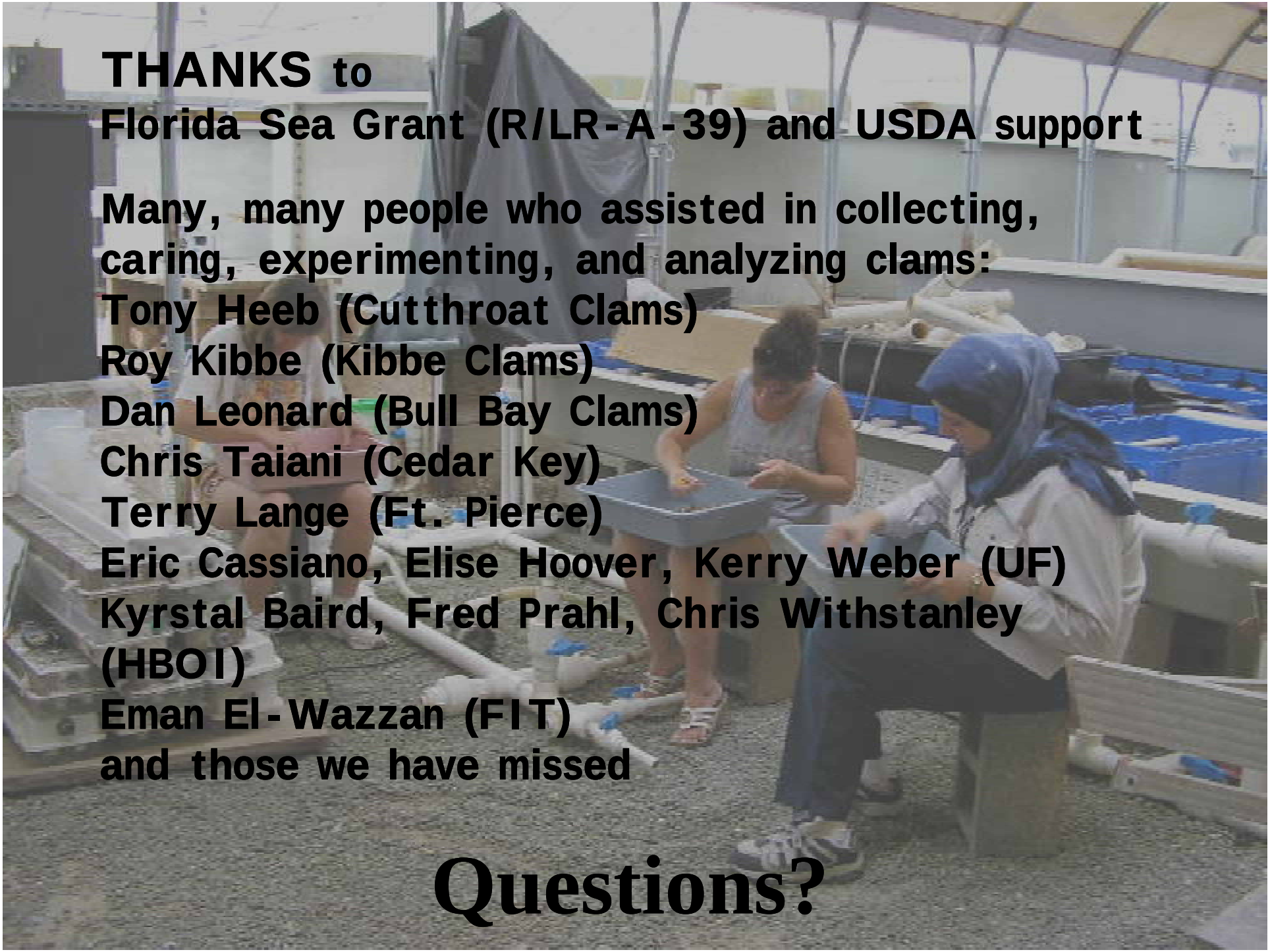
Ploidy	Length (mm)	Weight (g)	Cond. Index	Survival (%)
2N	50.8	**43.8**	4.65	**48.6**
3N	48.2	33.9	4.65	43.1

Economics (Cost Categories)

- Broodstock Conditioning: 2x # of clams (capital investment, not calculated)
- Spawning: increase area?, cost?
- Chemical Treatment: CB/DMSO ~$20.45/5M eggs (= $0.02-0.04/1K 1mm seed)
- Chemical Waste Disposal: ~$71.20/5M eggs (= $0.014-0.028/1K 1mm seed)
- Triploidy Verification: $100/5M eggs (= $0.10-0.20/1K 1mm seed)
- Larvae Culture: no sign. expense anticipated
- Setting: no sign. expense anticipated
- TOTAL = $0.14-0.27/1K 1mm seed or ~5-10% incr.

SUMMARY

- Produced triploid clams
- Growth of triploids in lab was lower
- Growth of triploids in field was similar/lower
- Survival/stress resistance of triploids in lab exhibited mixed results (Hsp70 selection)
- Survival of triploids in field was lower
- CI of triploids in field was similar/greater
- Cost of producing triploids is minimal
- **No apparent advantage of triploid clams for Florida culturists**



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Many, many people who assisted in collecting, caring, experimenting, and analyzing clams:
Tony Heeb (Cutthroat Clams)
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Terry Lange (Ft. Pierce)
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Eman El-Wazzan (FIT)
and those we have missed

Questions?