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

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HARBOR BRANCH
FLORIDA ATLANTIC UNIVERSITY

Sea Grant
Florida

UNIVERSITY OF FLORIDA
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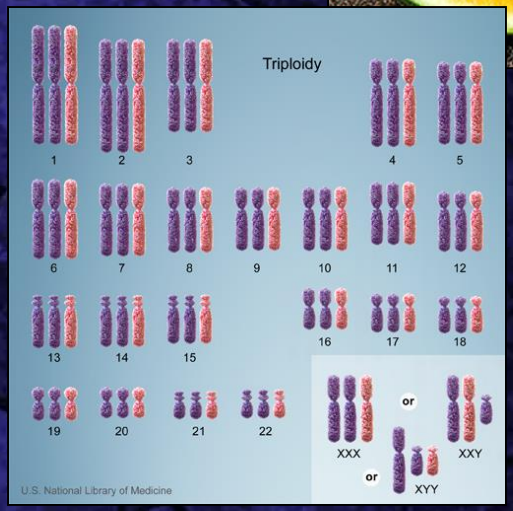
USDA

Hypothesis

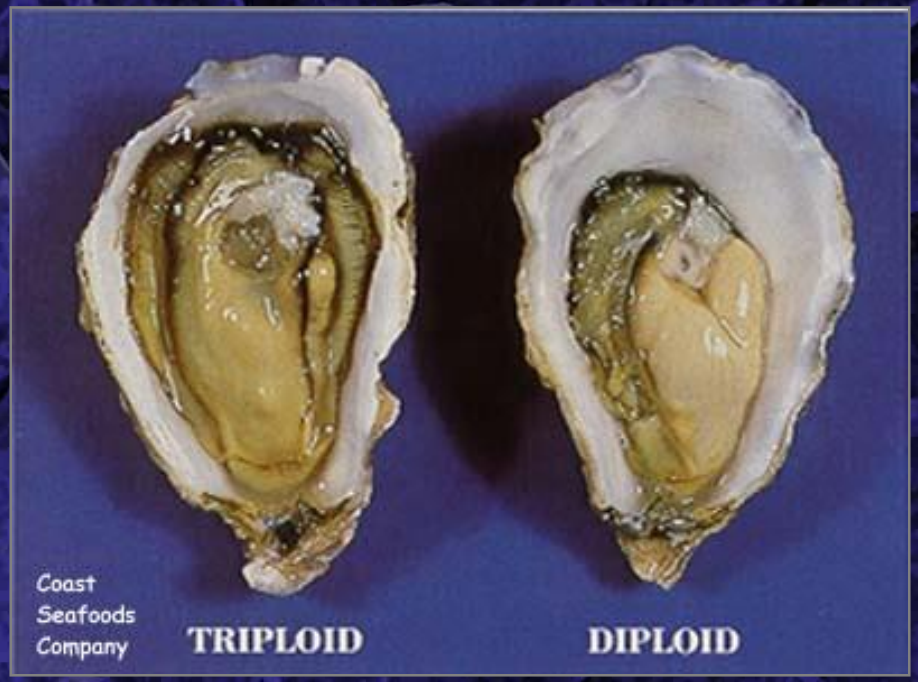
- Mortalities from summer stressors can be reduced by creating sterile clams through triploidy
-

Triploidy = 3 sets of chromosomes



Why triploidy?

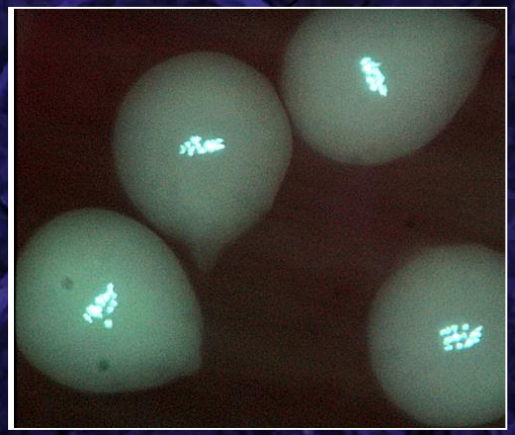
- Triploids divert energy from reproduction to storage and growth
- Used in PNW oyster aquaculture
- Need for hardier clam strain in SE



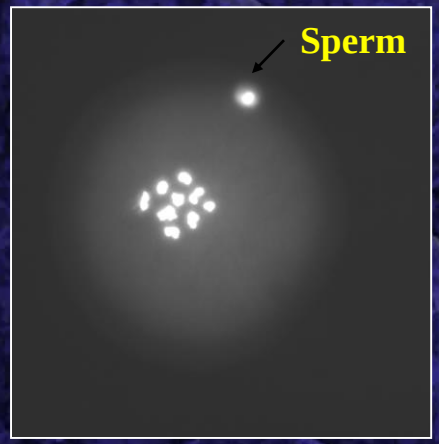
Specific objectives

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

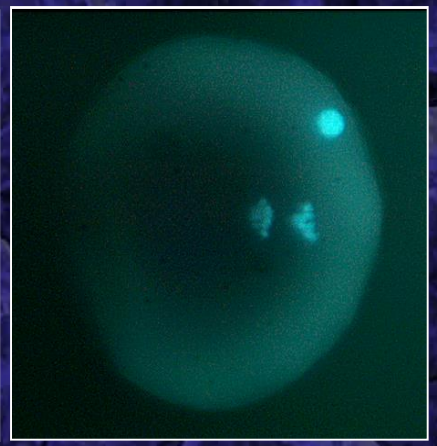
Meiotic and Mitotic Stages in Eggs of *Mercenaria mercenaria*



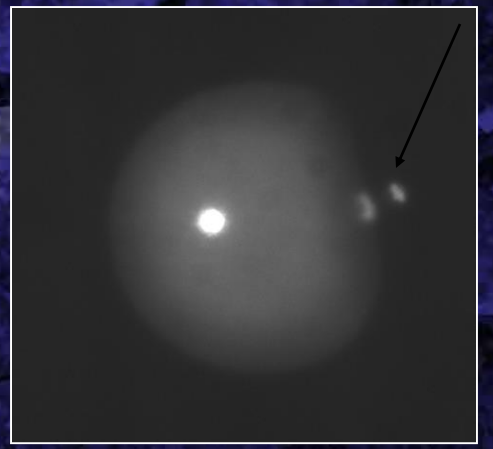
Metaphase I



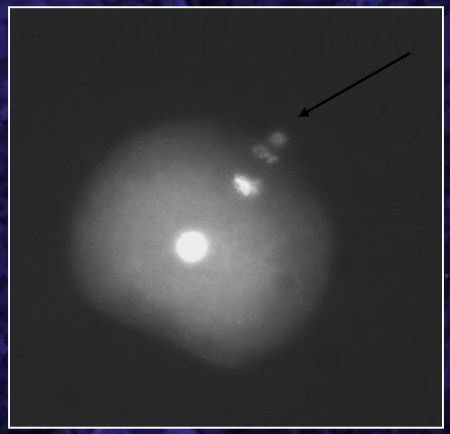
Early anaphase I



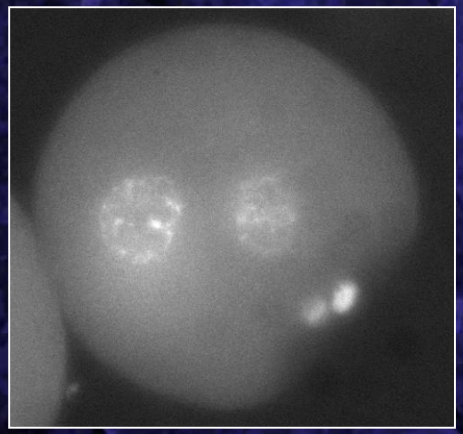
Late anaphase I



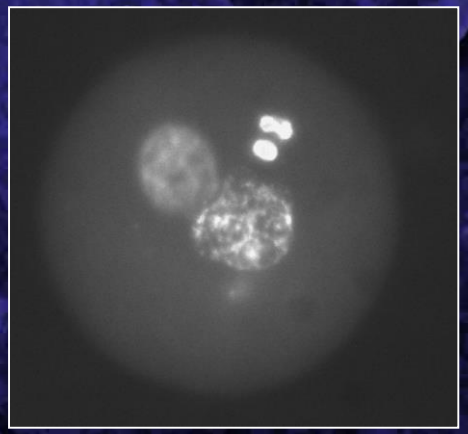
PB 1



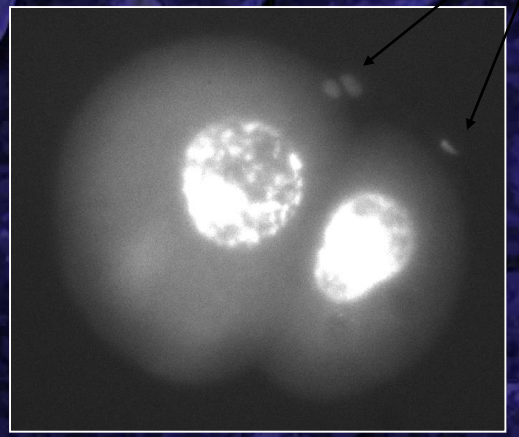
PB 2



Pronuclei

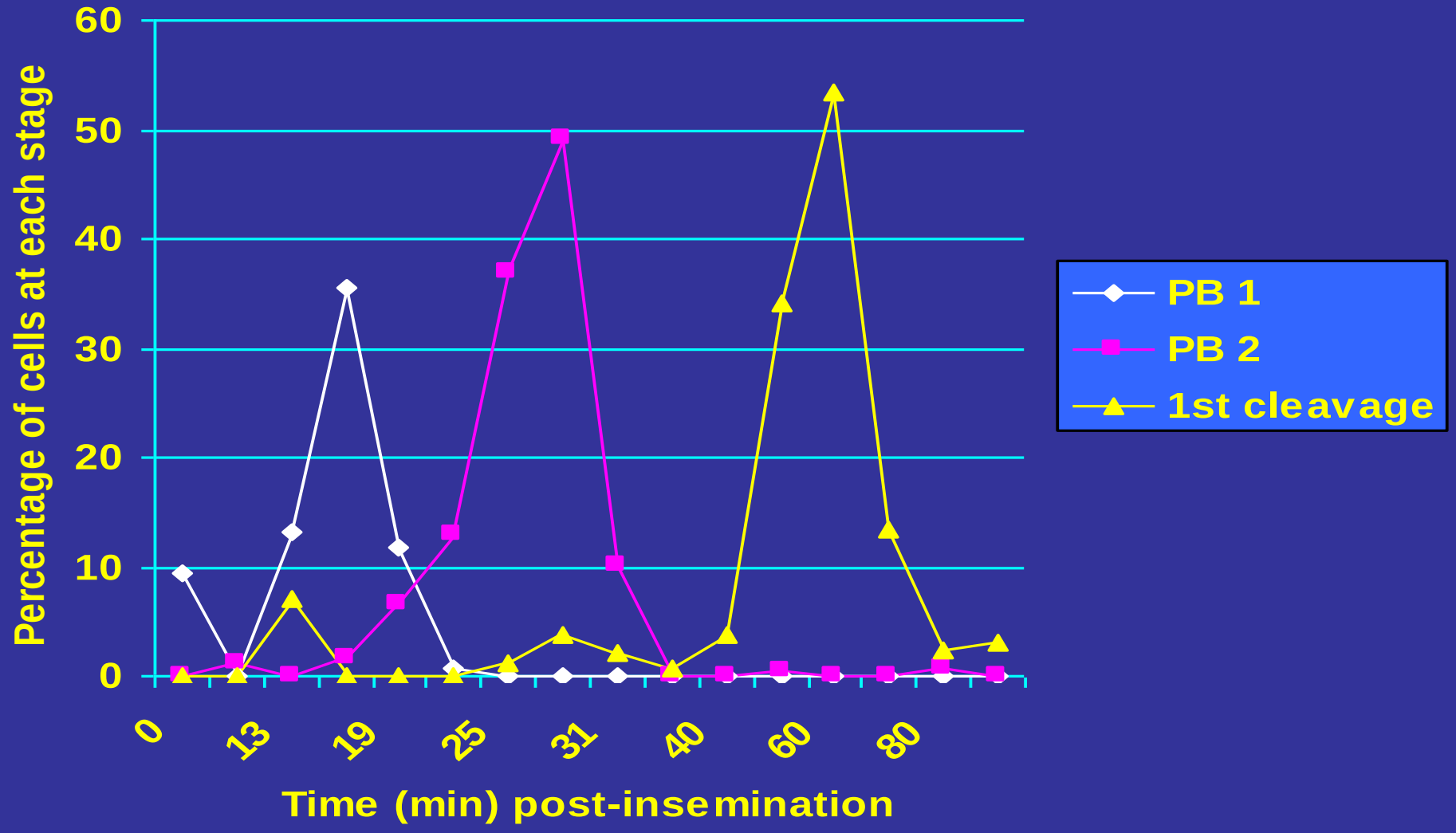


Syngamy

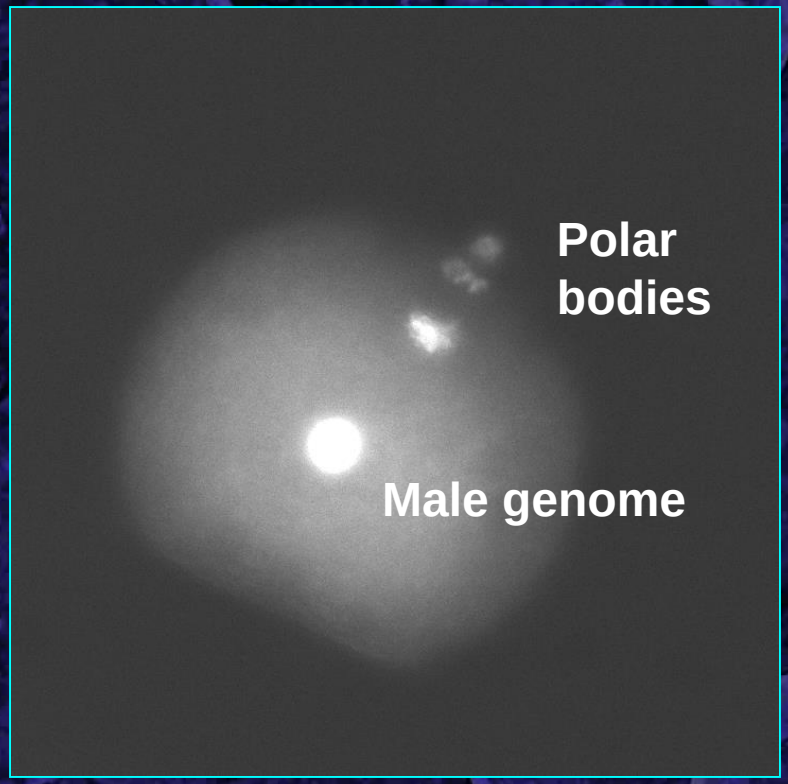


1st Cleavage
(2-Cell stage)

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

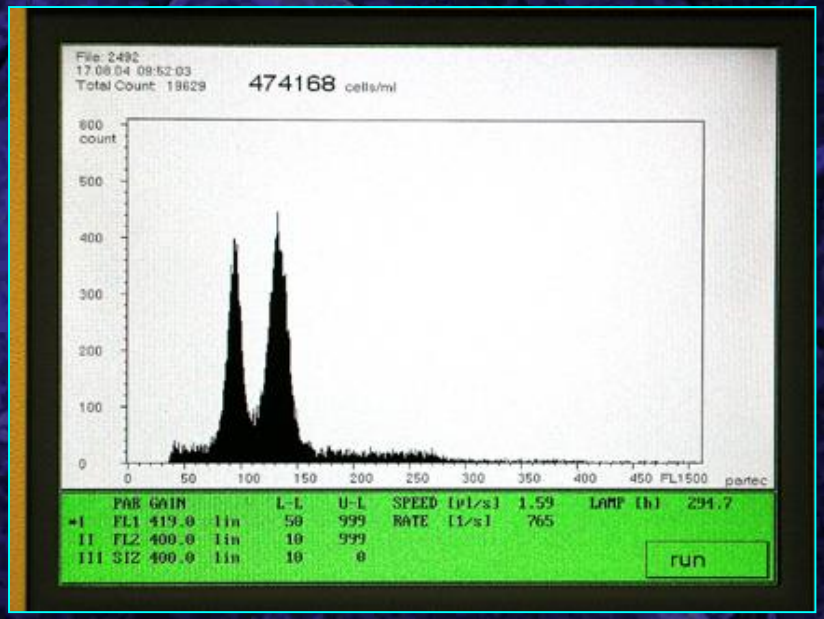


Flow Cytometry



Polar Body 2 release

Flow Cytometry output

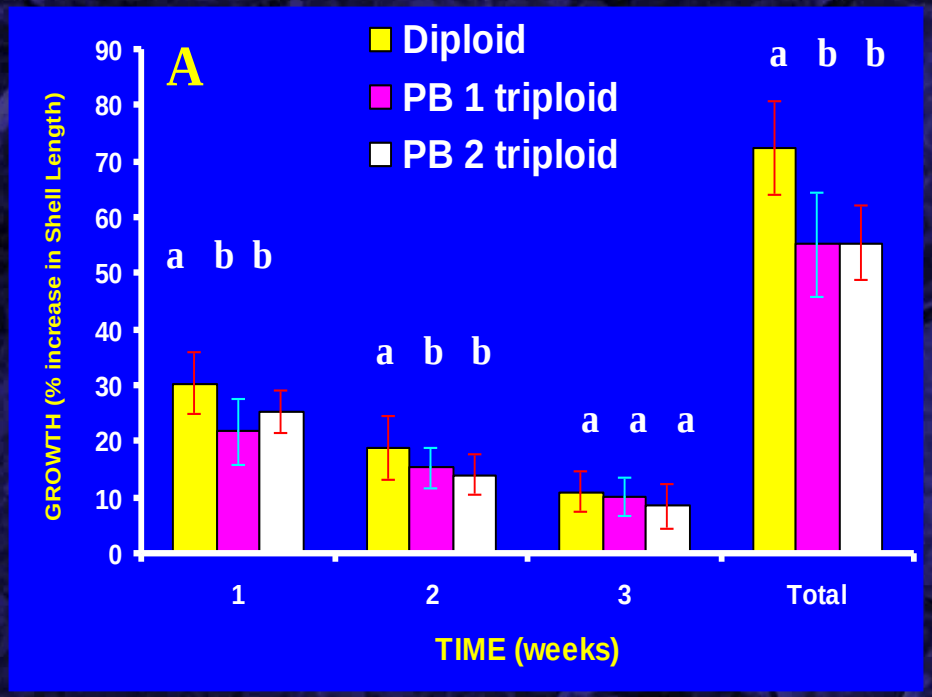


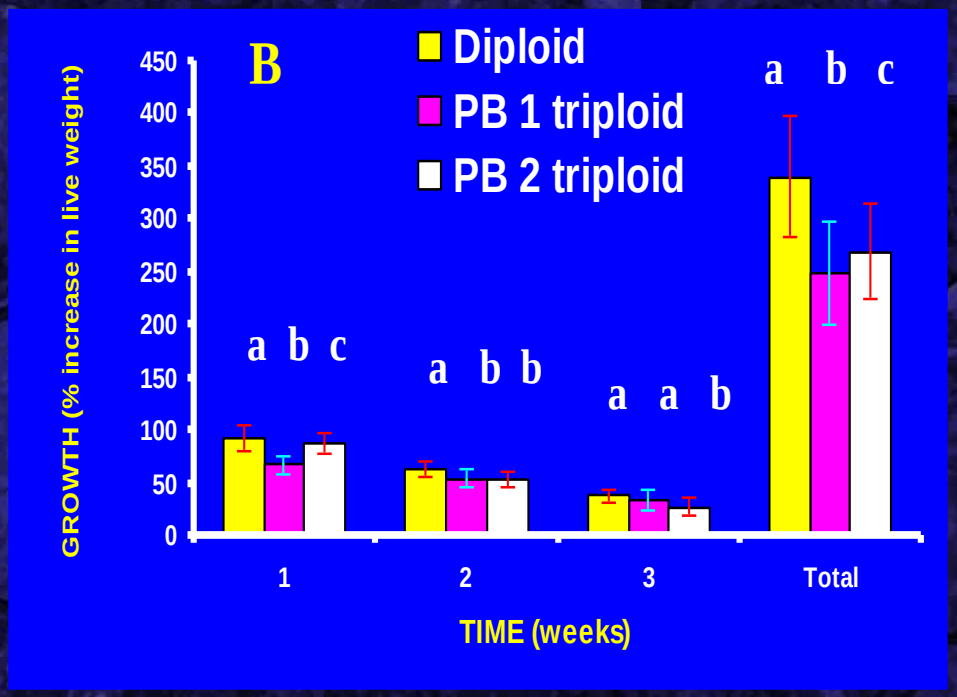
Cytological and Flow-Cytometric Data from Triploid Induction Experiments

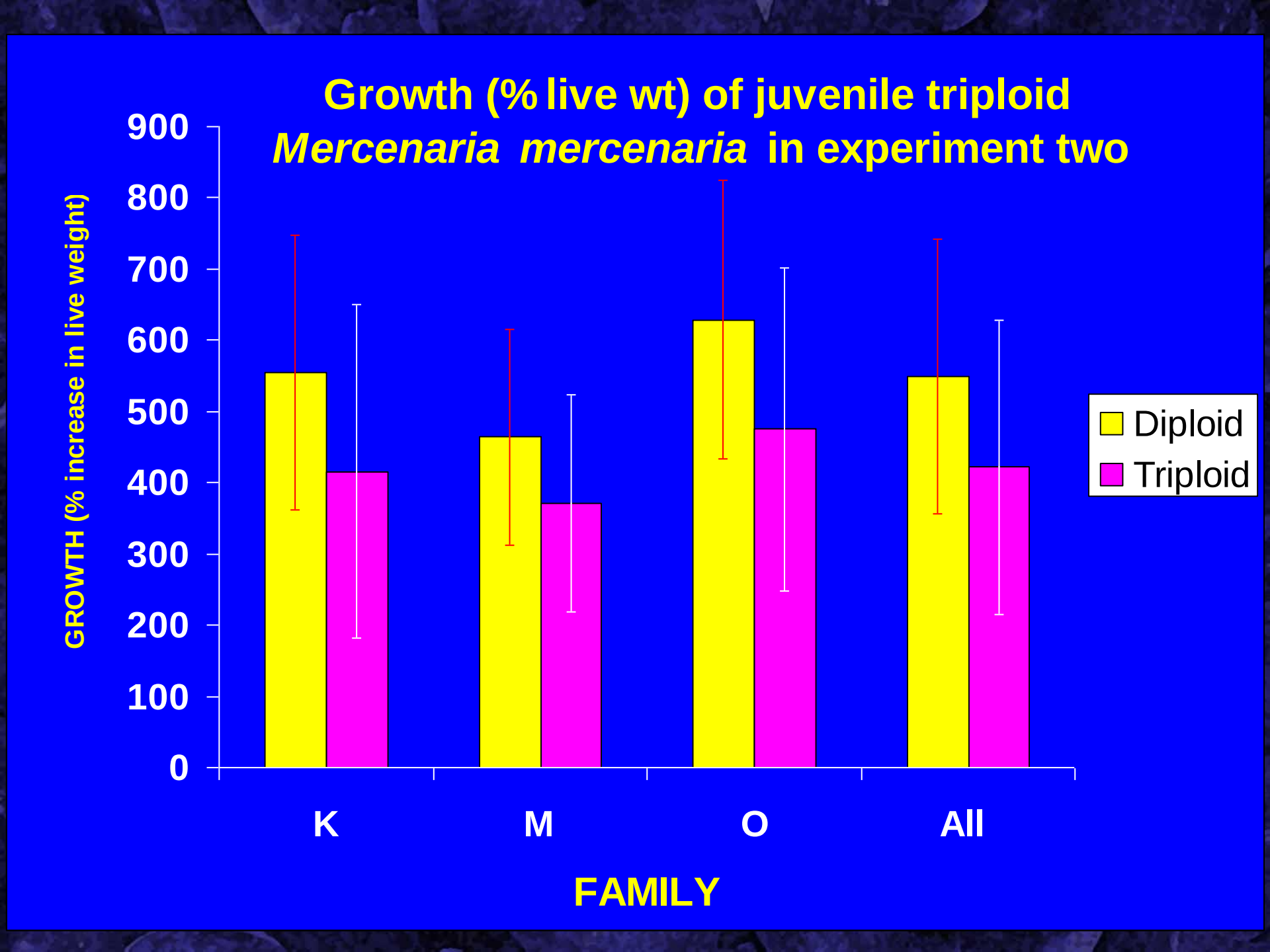
Trial #, treatment		Cytological Data (% of fertilized eggs)				Triploid (%)
		Pre-fertilized	Pre-PBI	Pre-PBII	Post-PBII	
1	PBI	55	50	7	43	39
	PBII		3	30	67	0
2	PBI	5	83	2	3	0
	PBII		23	77	0	0
3	PBI	1	100	0	0	0
	PBII		0	100	0	0
4	PBI	5	90	10	0	93
	PBII		0	44	56	33
5	PBI	0	85	0	0	33
	PBII		28	72	0	83
6	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*

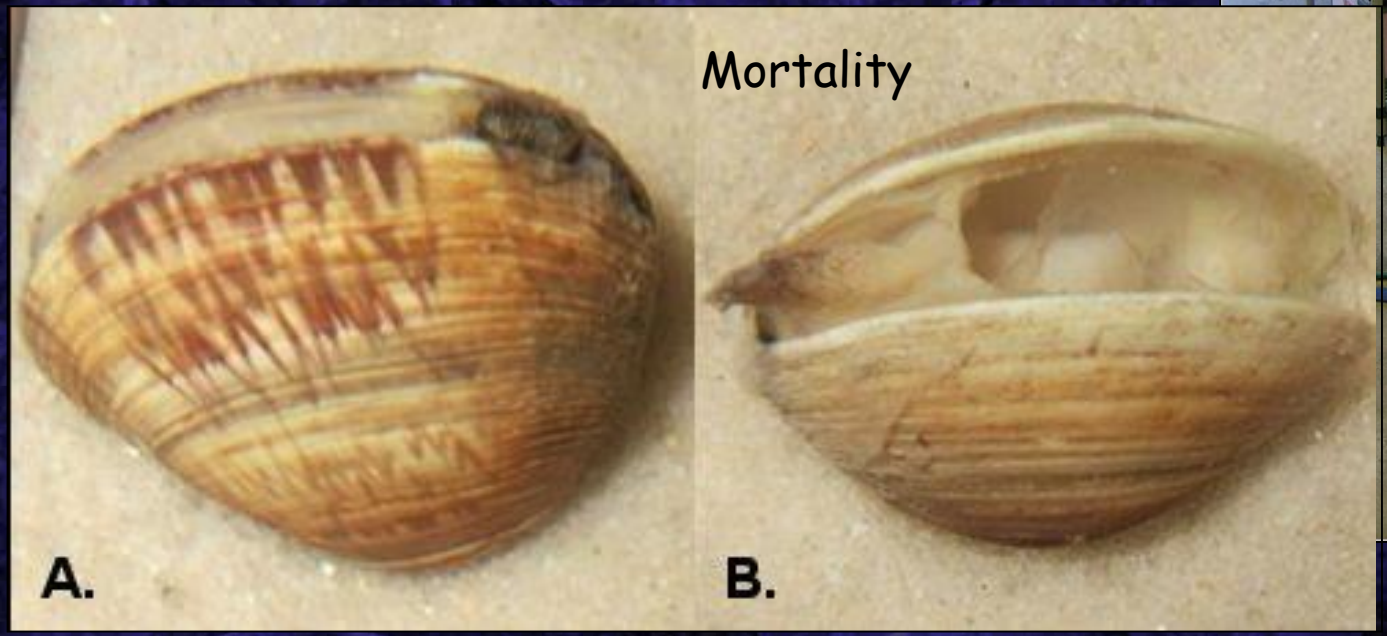





Growth (% live wt) of juvenile triploid
Mercenaria mercenaria in experiment two
GROWTH (% increase in live weight)
900
800
700
600
500
400
300
200
100
0
K
M
O
All
FAMILY
Diploid
Triploid

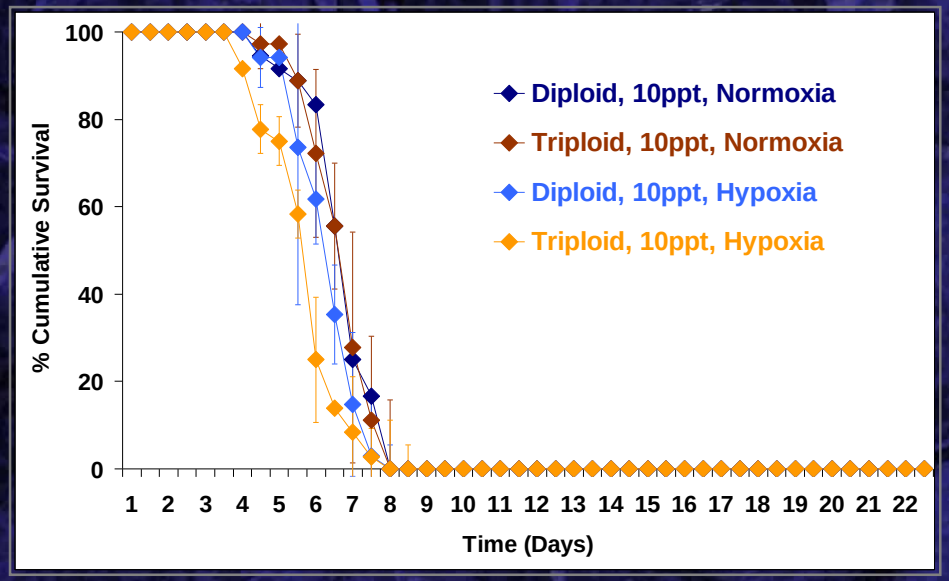
Survival under stress

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

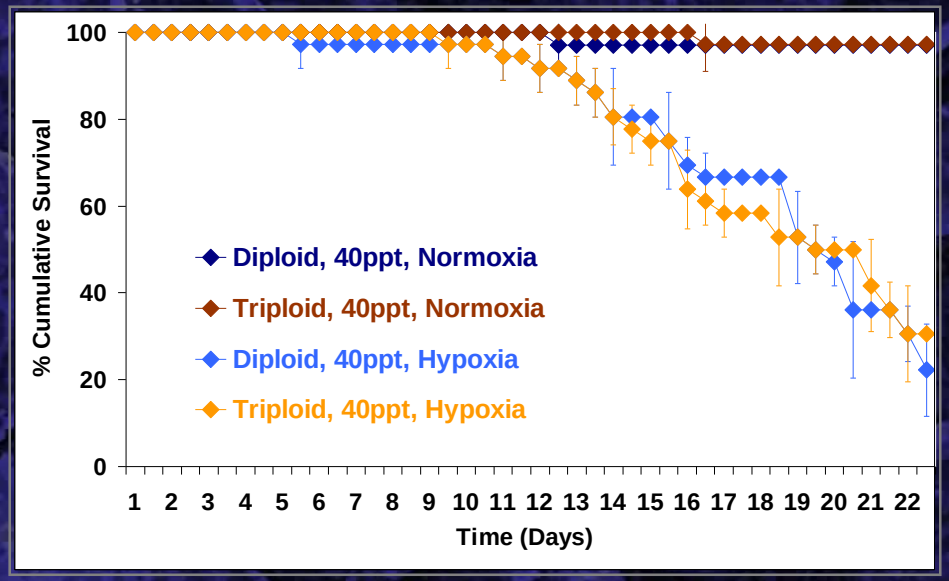


Survival - 10 and 40 ppt

10 ppt

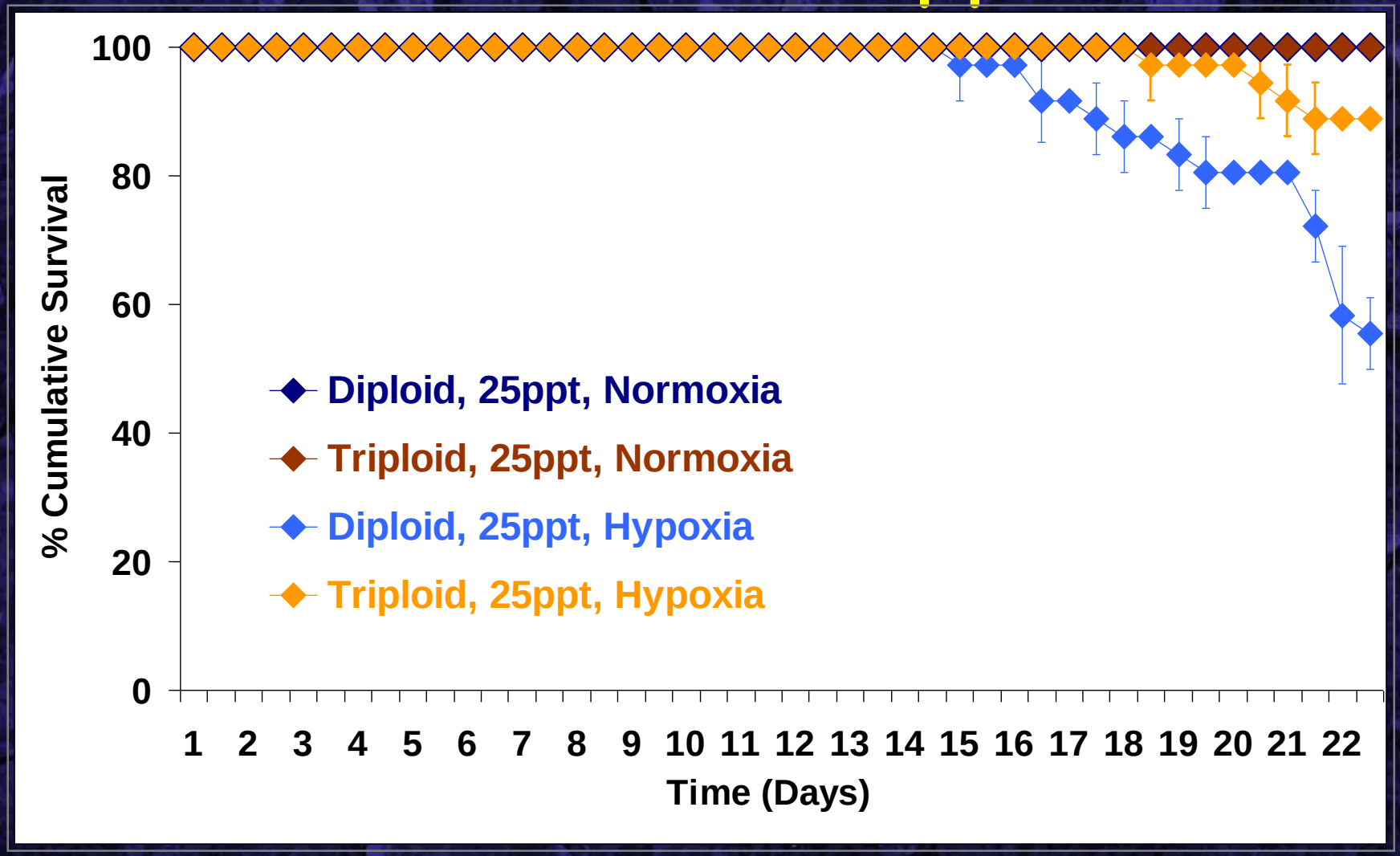


40 ppt



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

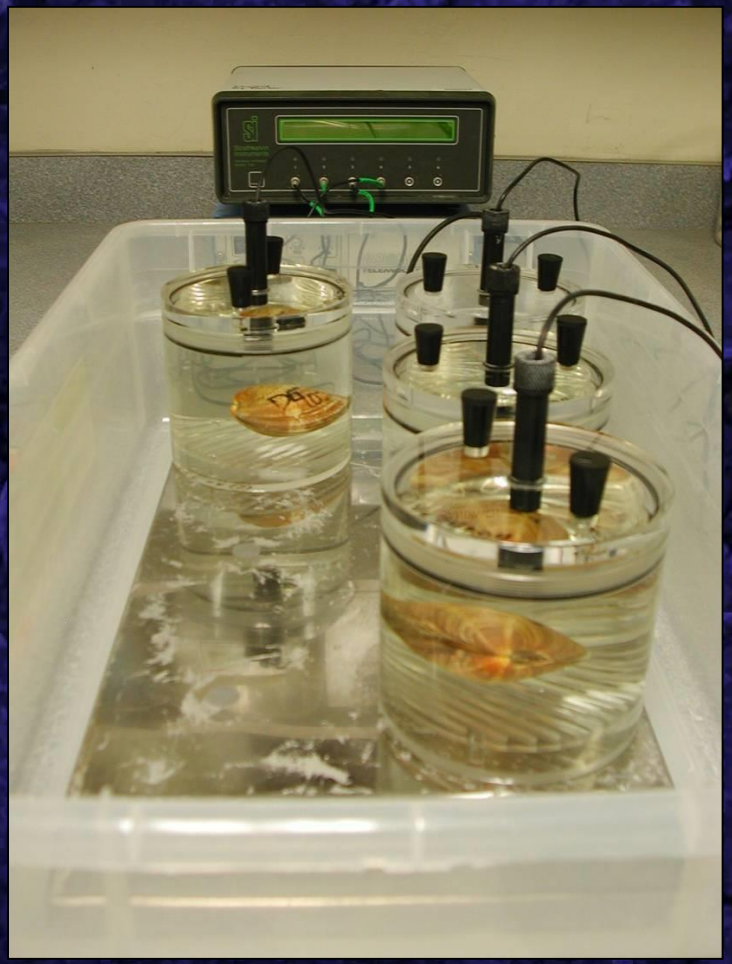
Survival - 25 ppt

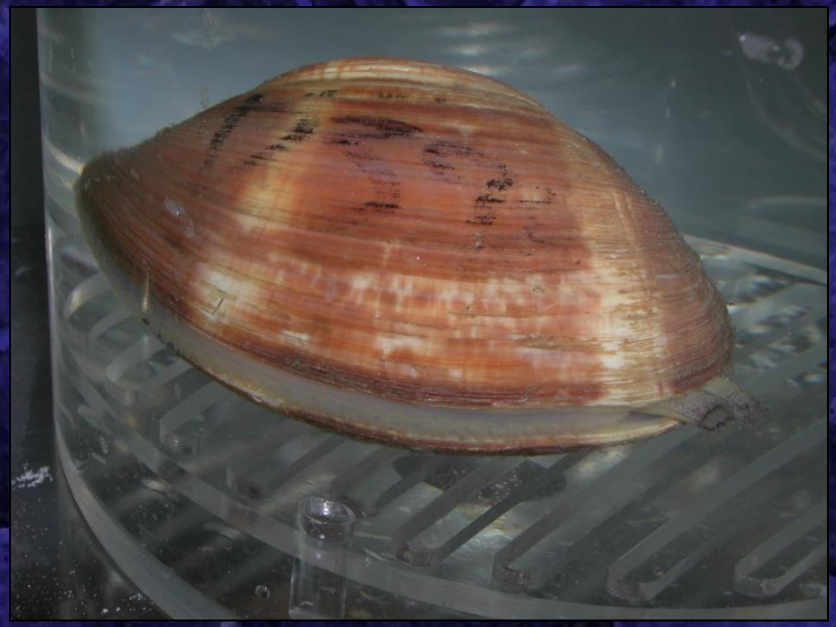


Triploidy may increase survival in hypoxia

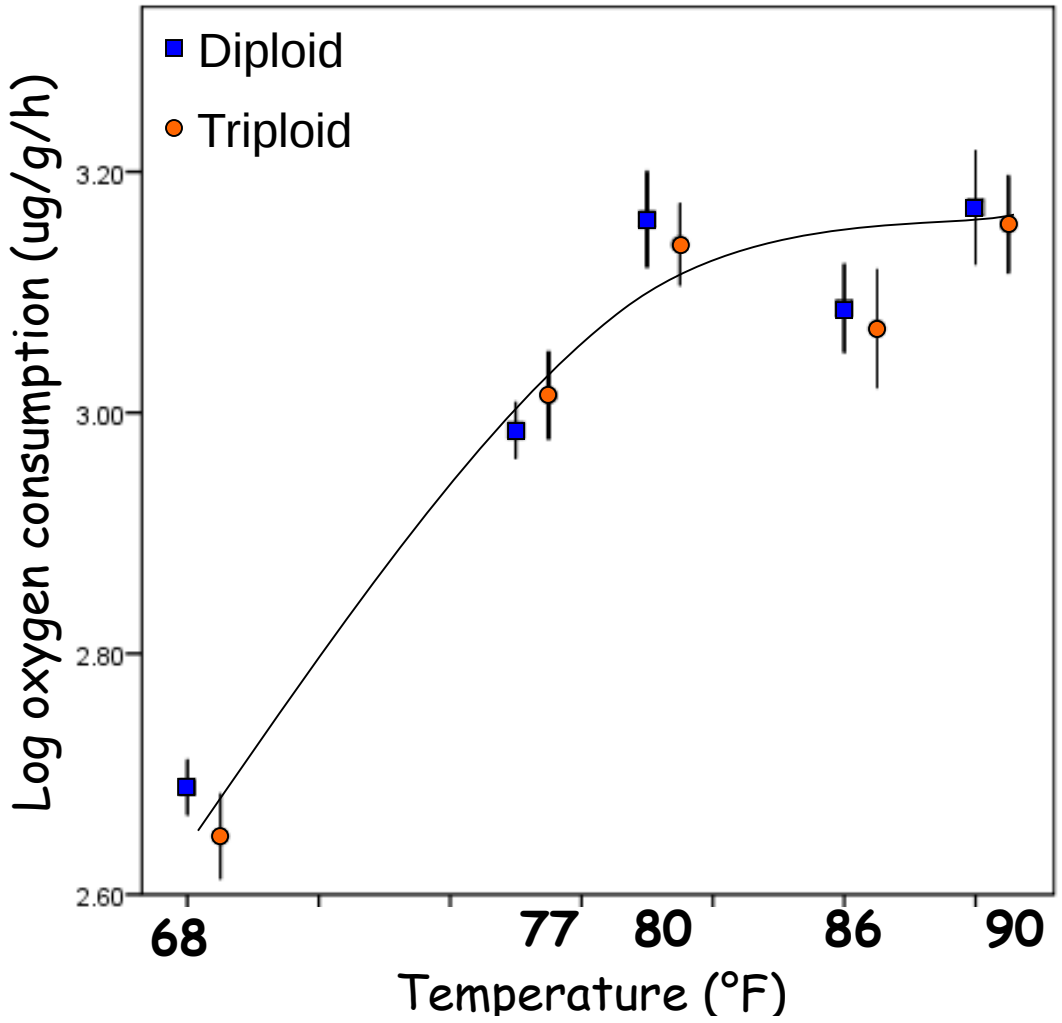
Physiological response to stress

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



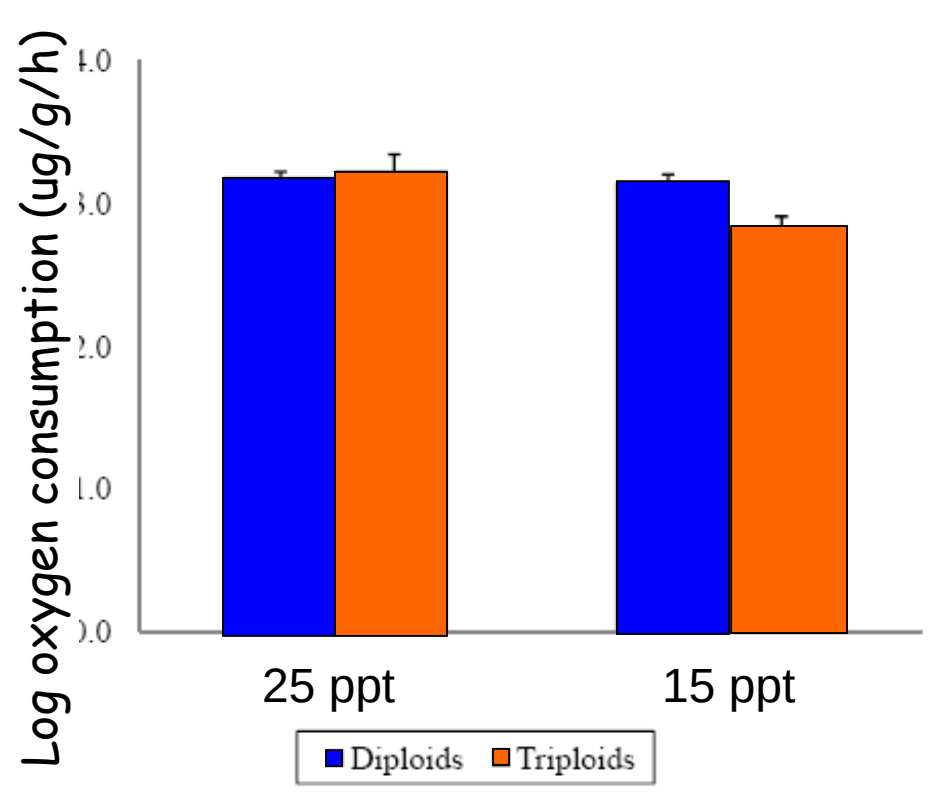


Oxygen uptake rate – temperature



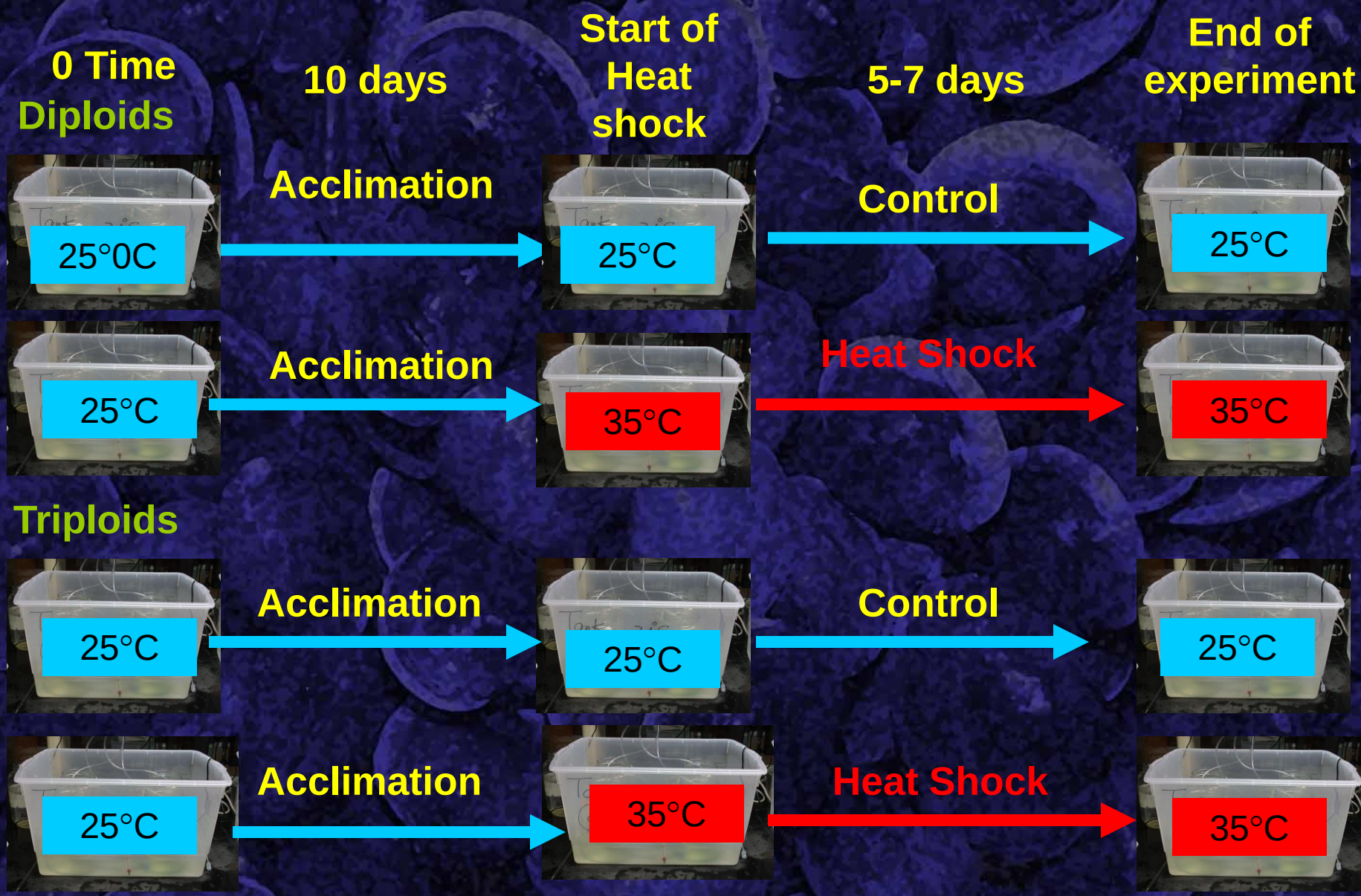
- Metabolic rate increases with temperature, but not above 80°F
- Triploid clams have no advantage over diploids

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)



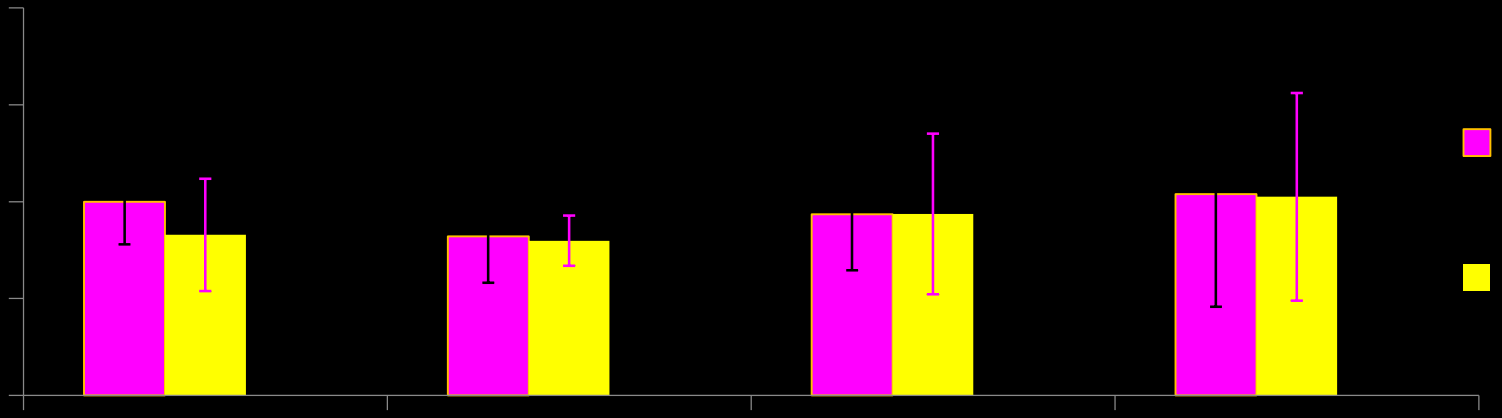
Experimental System

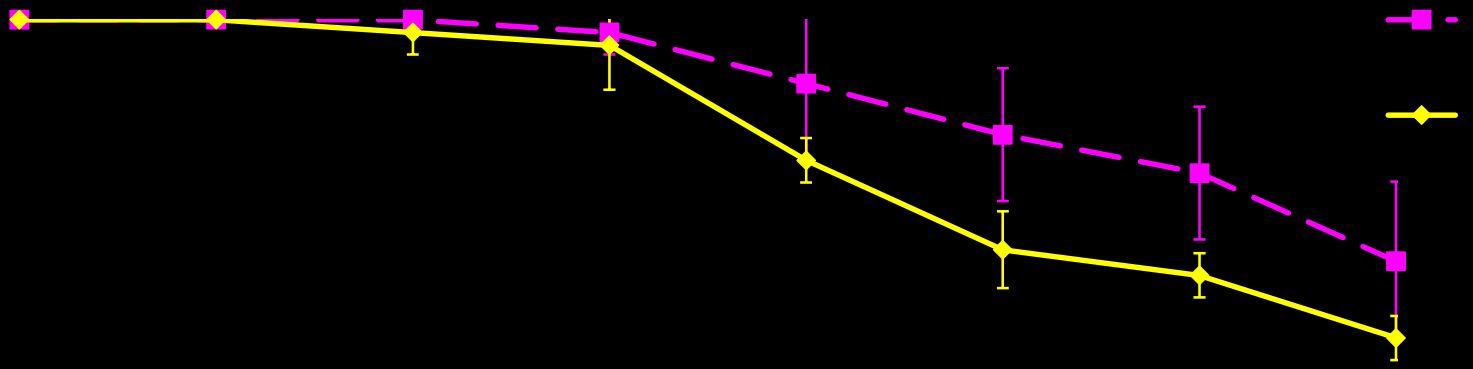
(1) Acclimation Unit : Sump connected to a chiller @ 25±1° C.

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

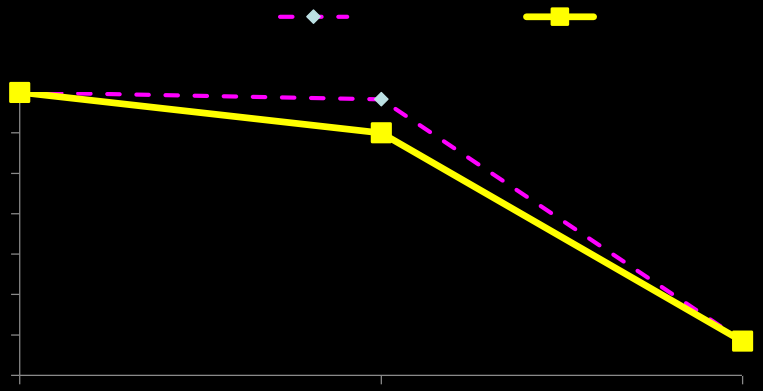
(3) Hot Treatment Unit: Sump with titanium Heater @ 35±1° C.

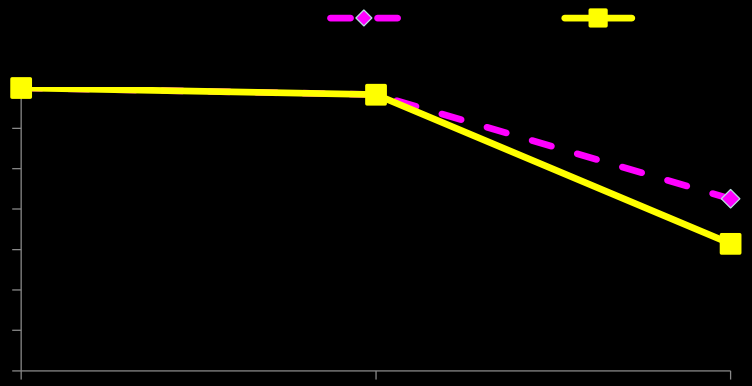
Hsp70 and Survival (± SD) in Triploid and Diploid Hard Clams (SL=31.6 ± 6.6) Following Severe Heat Shock (25-35°C).

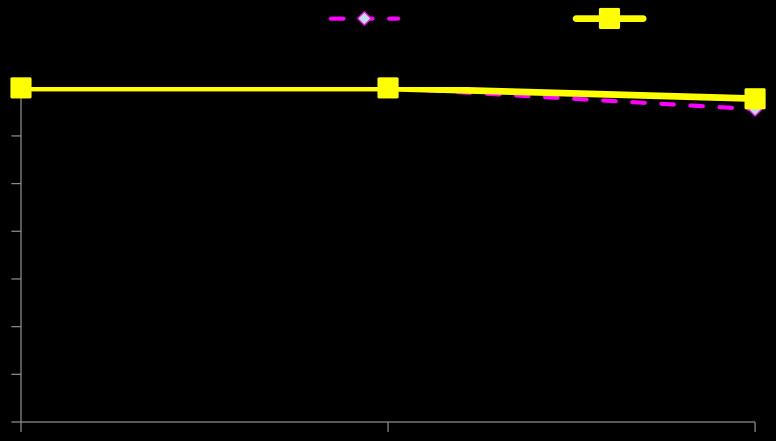


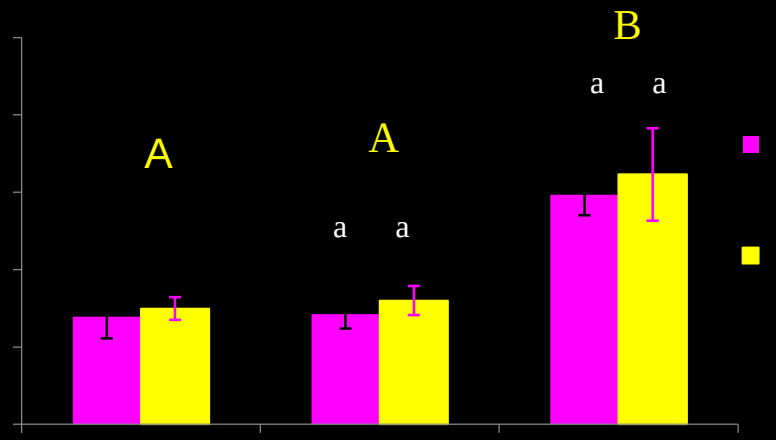


Survival (± SD) of hard clams (SL= 44.0± 3.3) exposed to severe heat shock (25-35°C) was influenced by genetic background represented by higher initial Hsp70 concentration (P= 0.042), than by ploidy (P= 0.184).





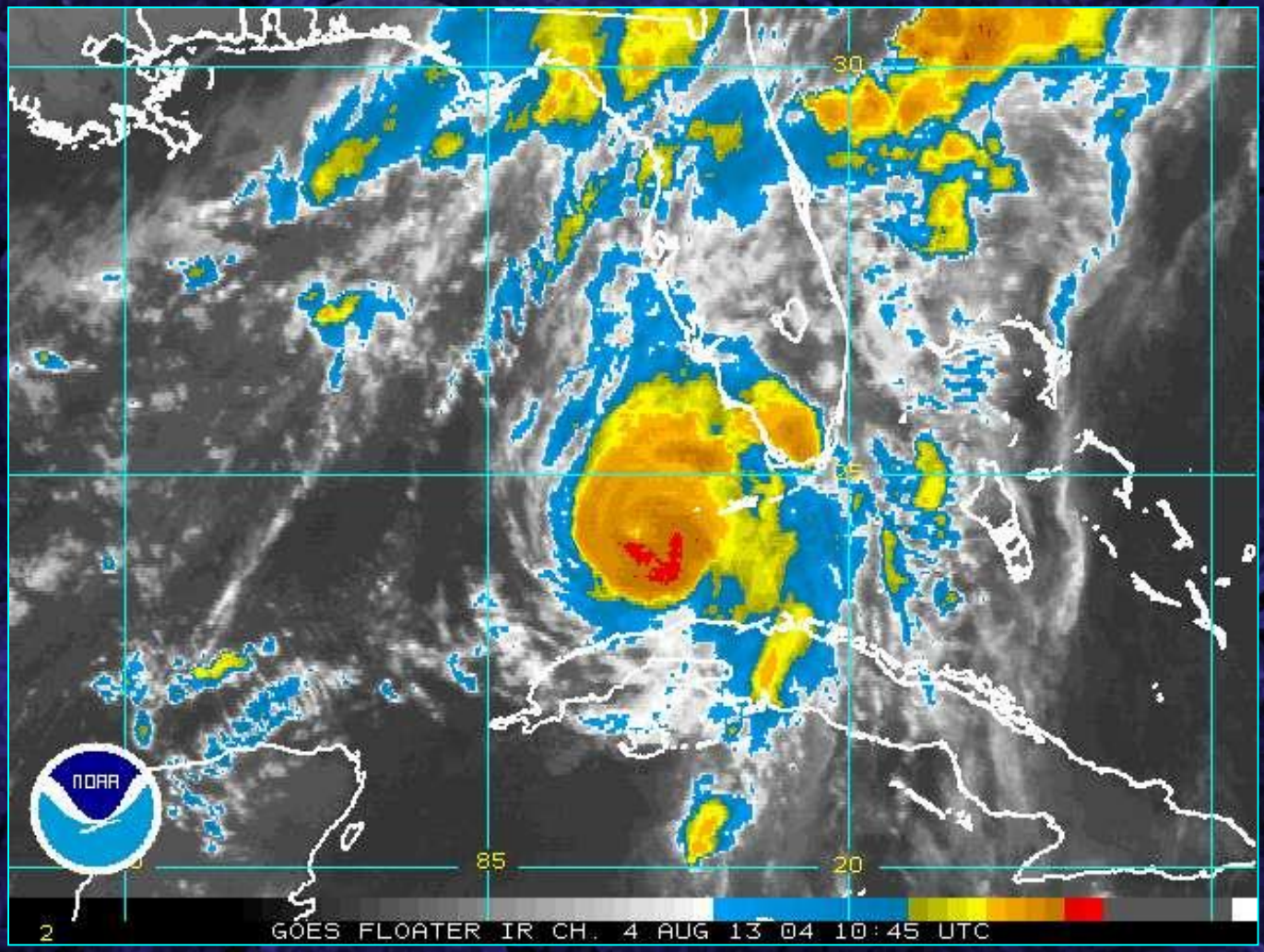




GROW-OUT

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

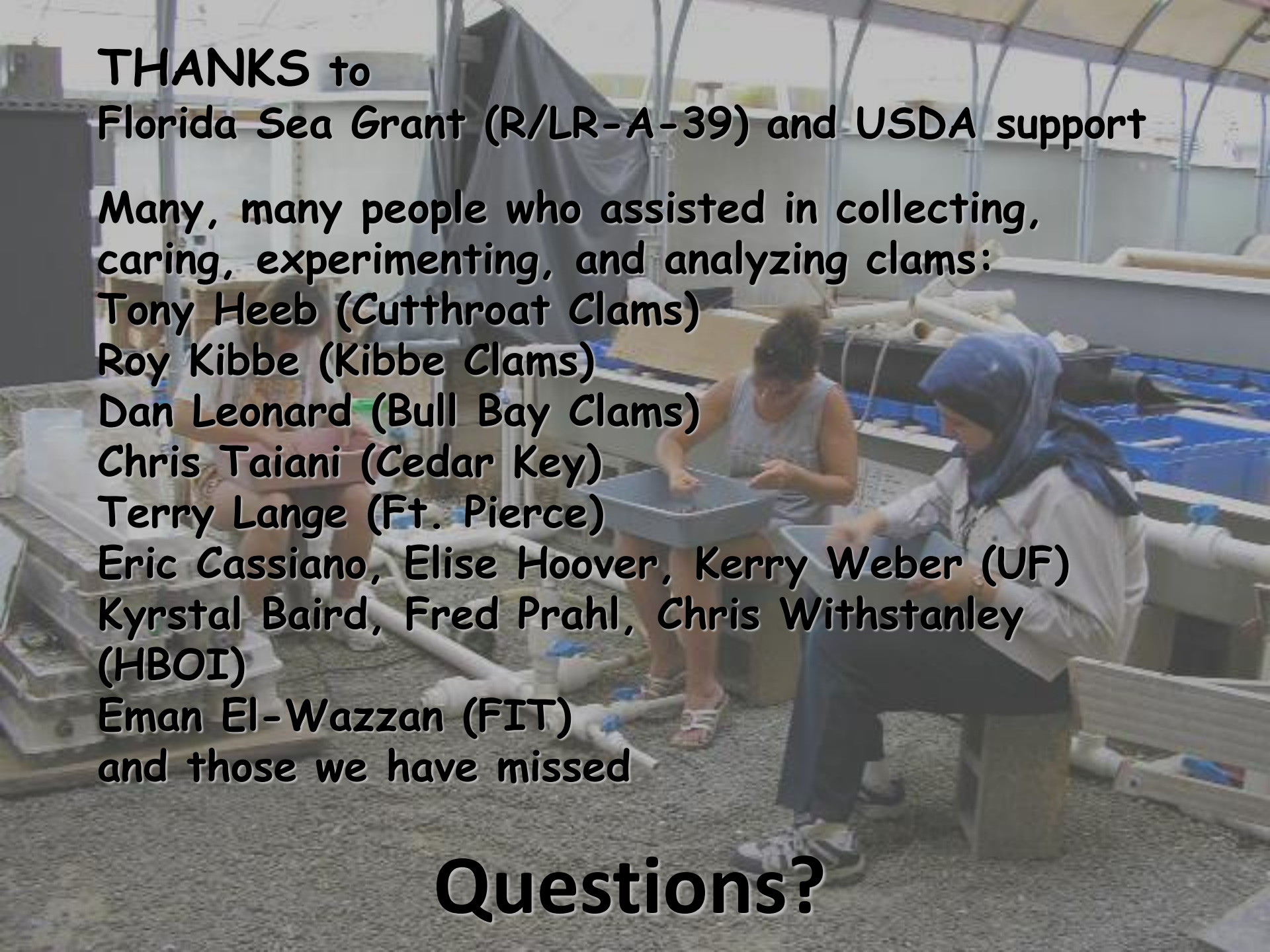
Ploidy	Length (mm)	Weight (g)	Cond. Index	Survival (%)
2N	50.8	**43.8**	4.65	**48.6**
3N	48.2	33.9	4.65	43.1

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)
Roy Kibbe (Kibbe Clams)
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Kyrstal Baird, Fred Prahl, Chris Withstanley (HBOI)
Eman El-Wazzan (FIT)
and those we have missed

Questions?