Induction of Oyster Tetraploid Founders to Address the Triploid Seed Production for the Gulf Oyster Industry

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- Cedar Key Aquaculture Association

Industry collaborating farms

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- Clamtastic Seafood Inc., Cedar Key, FL
- Cedar Key Seafarms, Cedar Key, FL
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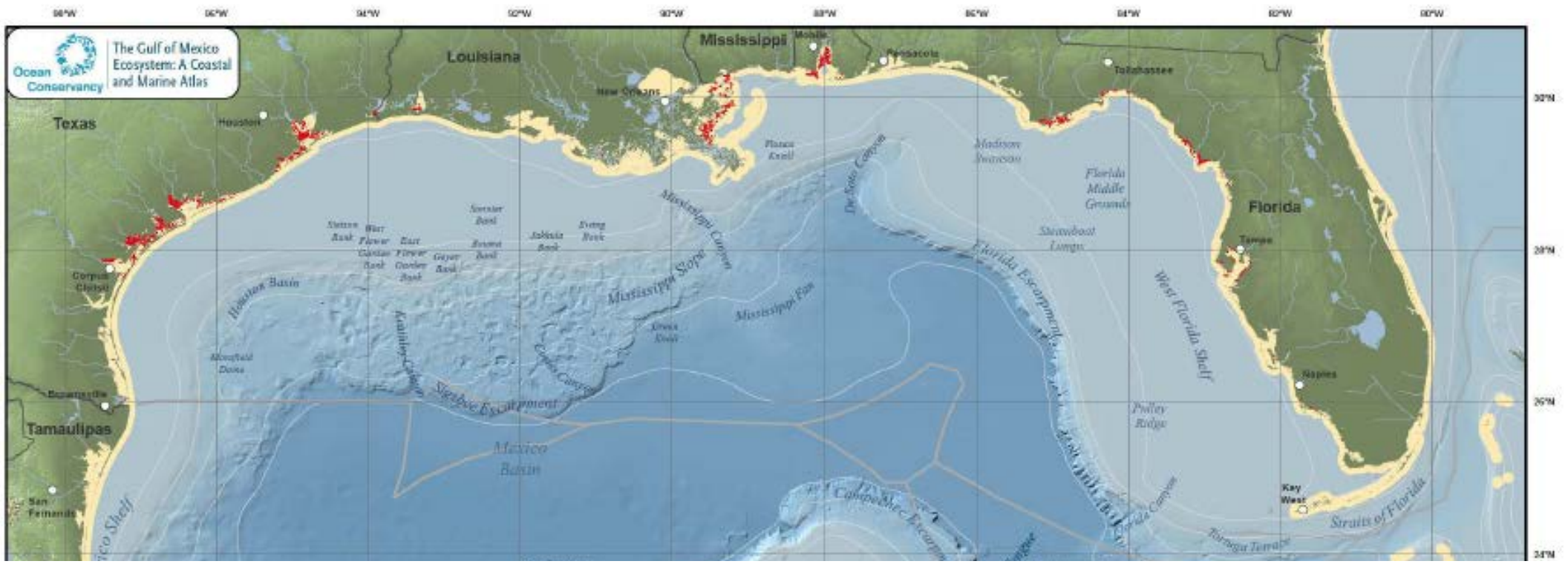


Fishery landing of eastern oysters in the Gulf region account for **85%** of national total in 2011 (NMFS 2012)

Eastern Oyster Reefs (*Crassostrea virginica*)

Known Eastern Oyster[1] Reefs (1969 - 2009) U.S. Gulf of Mexico Only

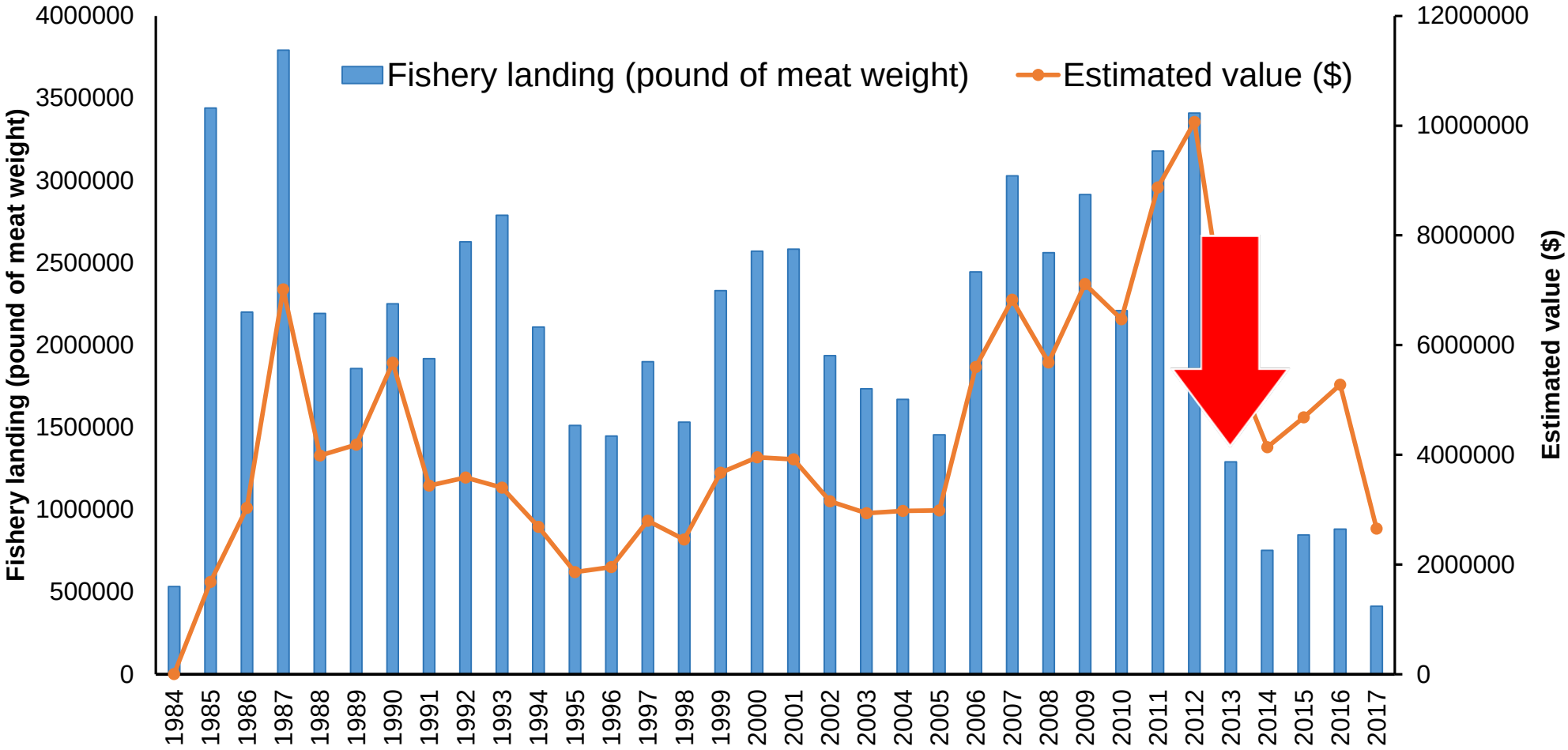
Species[2] Range

Exclusive Economic Zones

Contours (meters)

Sources: **(1)** Anson K., Arnold W., Banks P., Berrigan M., Pollack J., Randall B., and Reed D. (2011). *Gulf of Mexico, Eastern oyster* Stennis Space Center, MS: NOAA National Coastal Data Development Center. Retreived on July 21, 2011 from http://gulfatlas.noaa.gov/ **(2)** FAO Fisheries and Aquaculture Department. (2011, November 14). *FAO FishFinder*. Retrieved on July 22, 2011 from http://www.fao.org/fishery/fishfinder/en

Oyster fishery landing (catch of wild oysters) has been decreasing since 2012 in Florida (mtfwc.com). Therefore, oyster aquaculture is increasing to meet the seafood market needs



Oyster Aquaculture in the Gulf region - Florida Industry

Florida has established infrastructure for shellfish farming

- Hatchery: 9 list “hatchery” + 2 new this year
- Nursery: 31
- Growout: ~300

Based on the FDACS data in 2018

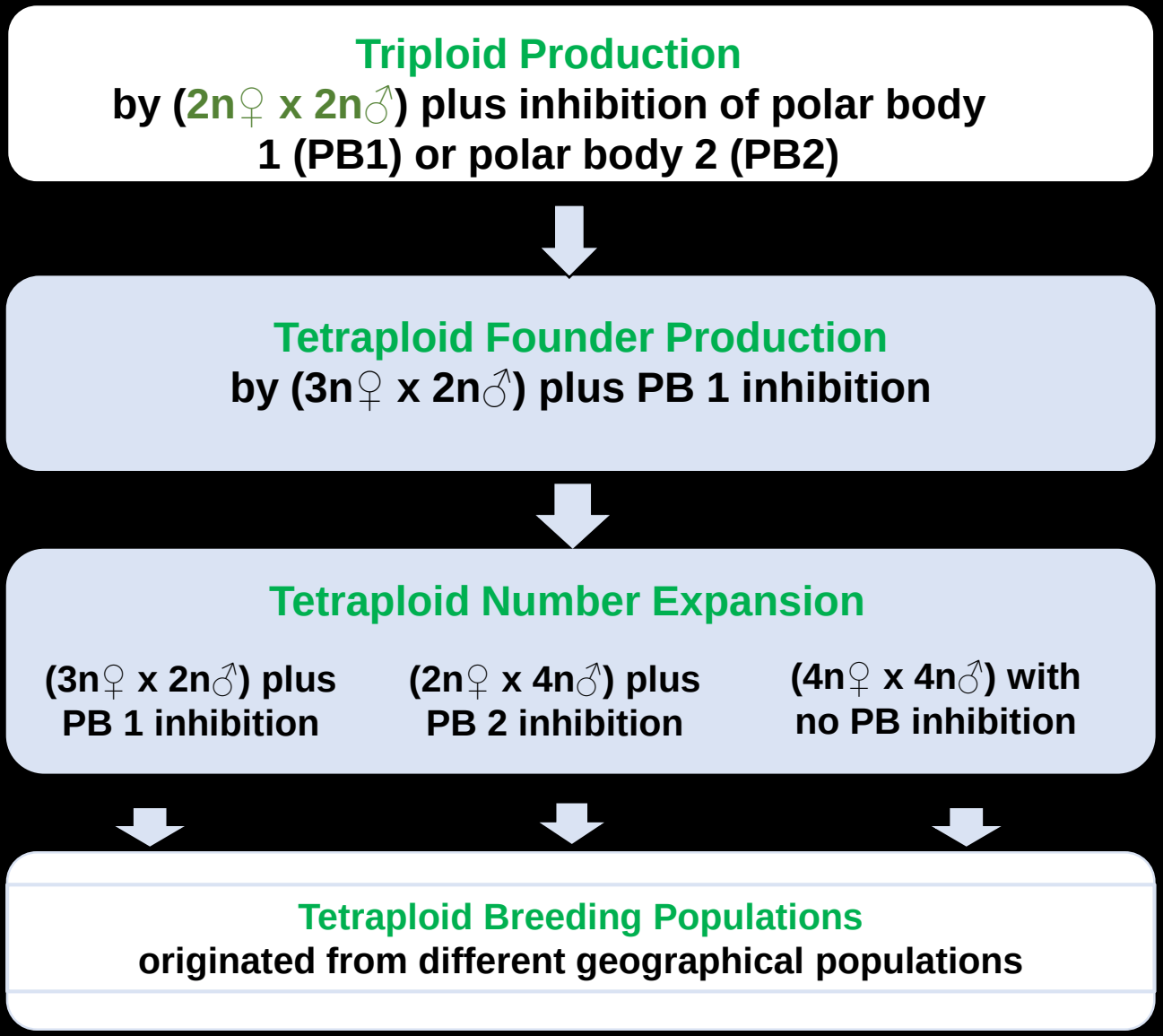
- Oyster: 92.03 Acres
- Oyster and clam: 380.49 Acres
- Hard clam: 847.2 Acres

Triploid Oyster are Preferred
by the Oyster Farms

Tetraploids are Needed

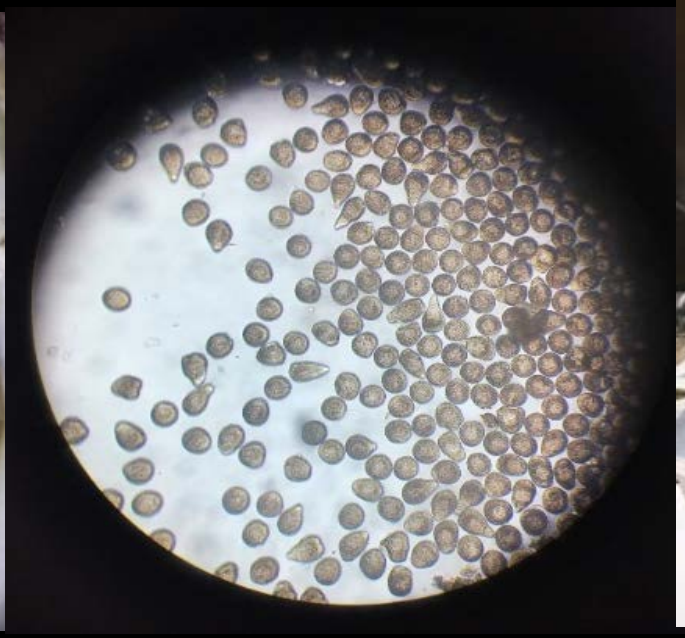
for Commercial 100% triploid seed production. 2n x 4n ➔ 100% 3n

Flow Chart for Oyster Tetraploid Production



Triploids were produced in 2017 and 2018

Broodstock were collected from different locations in Florida



Chemically Induced Triploid Seed harvested in three groups

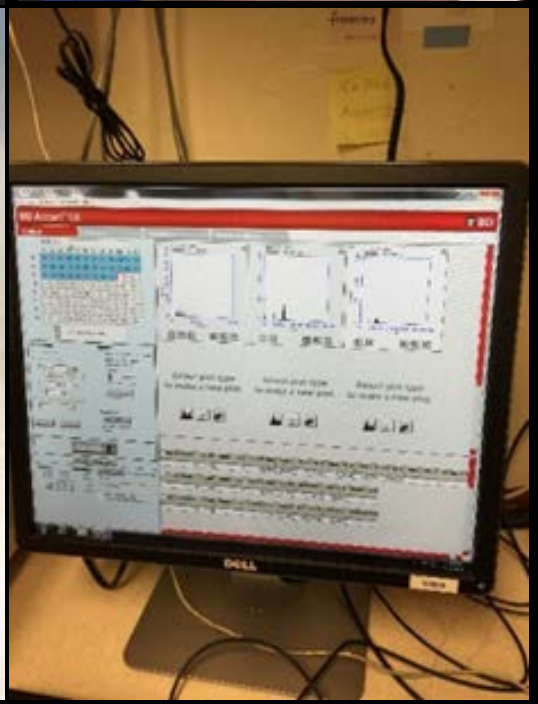
Labelling	Spawning Date	Broodstock source	Seed number (estimated)	Triploid (%) (Date to test)
2017CK1	April 5, 2017	Cedar Key	~30,000	38 (06/08/2017)
2017WA1	May 4, 2017	Wakulla	~20,000	57 (06/28/2017)
2017CK2	May 30, 2017	Cedar Key	15,525	53 (07/19/2017)

2018 spawning season – Screening triploids










Triploid Occurrence and Female Number

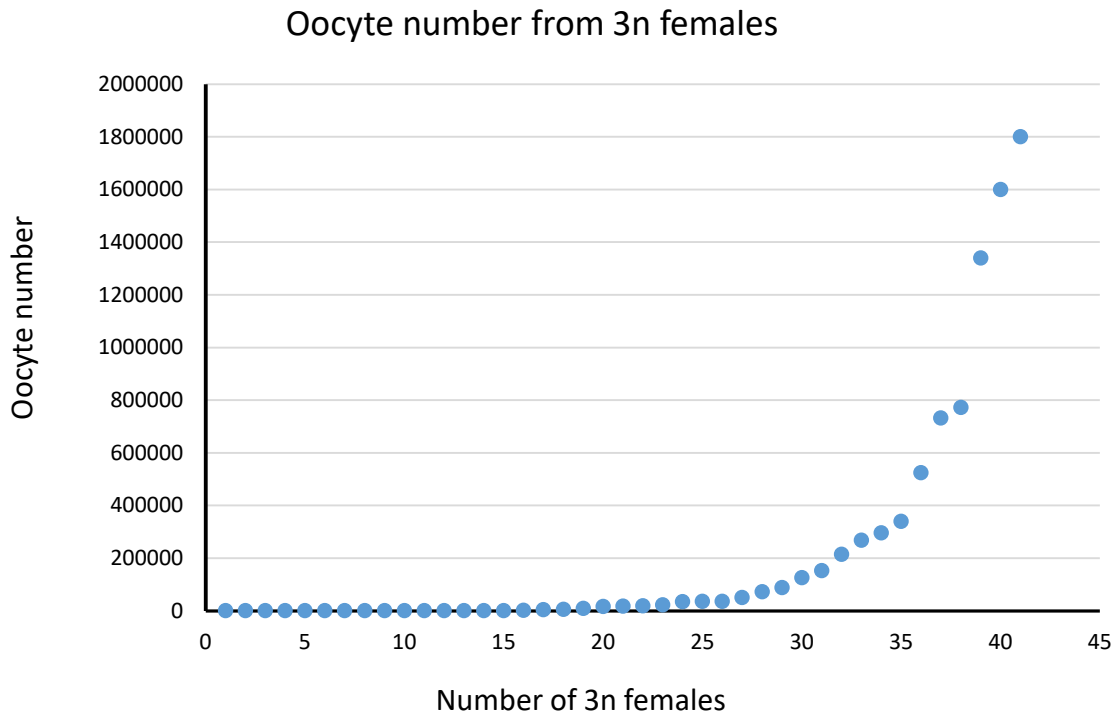
	Total Oyster	Confirmed 3N	% 3N	3N – no gonad	3n Female	2n-Female	2n-Male
	2017CK1 group: March 16, 2018 to May 25, 2019 (11 days)						
	1500	840	56%	835	5	365	209
	2017CK2 group: March 19, 2018 to May 25, 2018 (7 days)						
	992	630	63.5%	607	23	101	284
	2017WA group: March 28, 2018 to May 14, 2018 (13 days)						
	1842	1142	62%	1128	14	226	463
Total	**4334**	**2612**	**60%**		**41**	**692**	**956**

3n female % = 41/2612 = 1.57%

2n female % = 692/1648 = 42%

Oocyte production from 3n females (1-year-old)

15 Females:	100 - 1,000
4 Females:	1001 - 10,000
10 Females:	10,001 – 100,000
6 Females:	100,001 – 500,000 oocytes
3 Females:	500,001 – 1,000,000 oocytes
3 Females:	1-1.8 million



Comparison of Diploid and Triploid Females

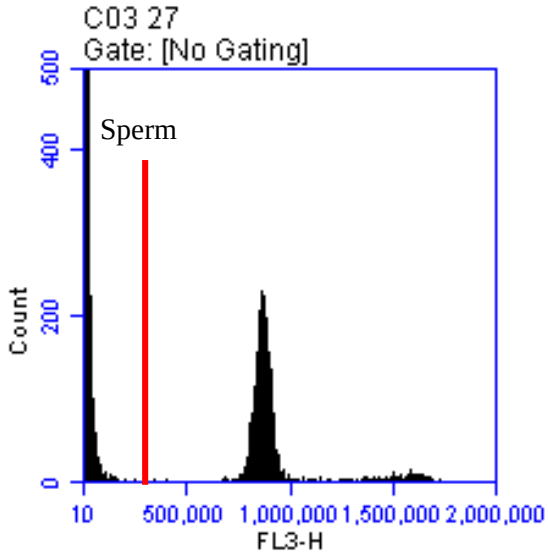
Triploid sizes vs. Diploids

	Group	Location	Female		Male		Measures	T-test (3n vs 2n)
			3N	2N	3N	2N		
3/27/2018 Friday	2017ck1	Cedar key	2	26	120	16	Number	Total = 164
			82.63 ± 0.85	87.32 ± 9.24	88.59 ± 8.12	87.70 ± 9.50	Height (mm)	0.2323
			54.41 ± 8.24	52.13 ± 5.81	55.56 ± 5.39	53.82 ± 4.92	Length (mm)	0.0034
			24.64 ± 1.24	23.19 ± 3.04	25.13 ± 3.37	23.35 ± 4.27	Width (mm)	0.0042
			50.6 ± 7.78	52.32 ± 12.23	62.51 ± 11.87	57.04 ± 17.79	Body weight (g)	0.0009

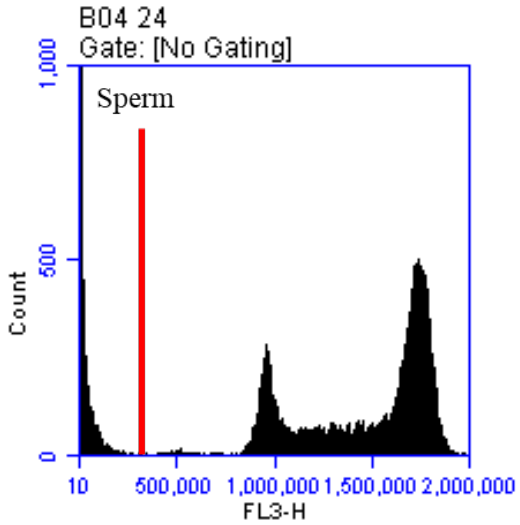
Date	Group	Location	Female		Male		Measures	T-test (3n vs 2n)
			3N	2N	3N	2N		
4/6/2018	2017WA (5/4/2017)	Cedar key	0	25	76	29	Number	TOTAL = 130
			NA	65.96 ± 8.93	71.74 ± 10.10	64.76 ± 9.05	Height (mm)	0.0002
			NA	46.72 ± 4.84	51.73 ± 7.37	47.77 ± 6.06	Length (mm)	0.0001
			NA	23.28 ± 3.28	24.84 ± 4.55	21.06 ± 3.31	Width (mm)	0.0001
			NA	46.63 ± 10.23	56.23 ± 17.31	38.27 ± 9.89	Body weight (g)	<0.0001

Date	Group	Location	Female		Male			T-test (3n vs 2n)
			2N	3N	2n	3n		P values (2n vs 3n)
			24	8	110	162	TOTAL = 304	
			62.83 ± 7.67	62.65 ± 5.02	64.94 ± 9.26	64.60 ± 1.32	Height (mm)	0.9618
5/8/2018	2017CK2	Wakulla	41.91 ± 4.77	42.96 ± 2.11	42.37 ± 4.24	42.32 ± 5.07	Length (mm)	0.9047
			21.94 ± 3.17	22.29 ± 1.98	21.71 ± 2.70	21.40 ± 2.57	Width (mm)	0.3305
			23.9 ± 7.23	24.4 ± 3.98	25.83 ± 8.29	25.58 ± 9.49	Weight (g)	0.9675

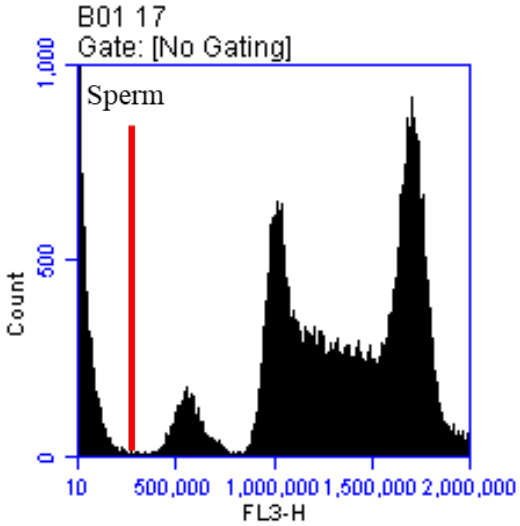
Analysis of 3n “male” gonadal sample n = 186



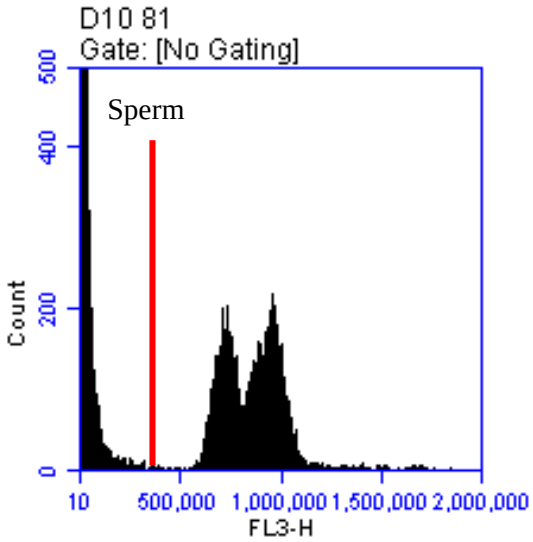
Type I: 3n peak (66%)



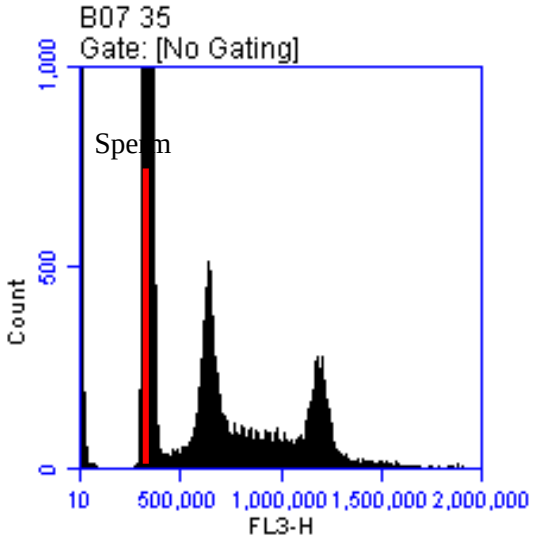
Type II. 3n and 6n peaks (17%)



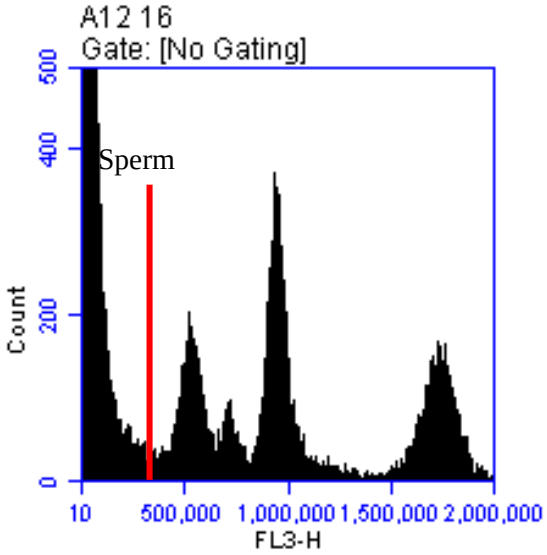
Type III 2n, 3n and 6n peaks (8%)



Type IV 2n and 3n peak (6%)



Type V. n, 2n, 4n peaks (3%)



Others (<1%)

Challenges and Solutions I:

limited availability of oocytes from triploid females

- Only very few individual triploids produce a few oocytes (Wang et al. 2002, Gong et al. 2004). For example: 1 out of 1,600 triploid eastern oysters had a few oocytes (Supan 2000).

- Our data: 1.57%; 100 – 1,800,000 (year-1 triploids)

Further Improvement:

- Use of older triploids

- Conditioning of triploids for gonad enhancement

Challenges and Solutions II:

Poor survival of induced tetraploid larvae

- For Pacific oysters, the survival of putative tetraploid larvae was reported as 0 in two replicates and 0.0739% in one replicate (Guo and Allen 1994).

- Our data: most larvae died out at Day 7-10

Further Solutions

- To increase the egg quality by conditioning the triploids in a temperature controlled system

- To take more care of larval culture

Thank you!

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