Hard Clam Genetics and Breeding at Rutgers University

Ximing Guo, Paul Coyne, Zhenwei Wang, Samuel Ratcliff, Michael Acquafredda, Lisa Calvo, Michael De Luca

Haskin Shellfish Research Laboratory

Rutgers University

xguo@Rutgers.edu

New Jersey Aquaculture Growers Forum, May 20, 2024

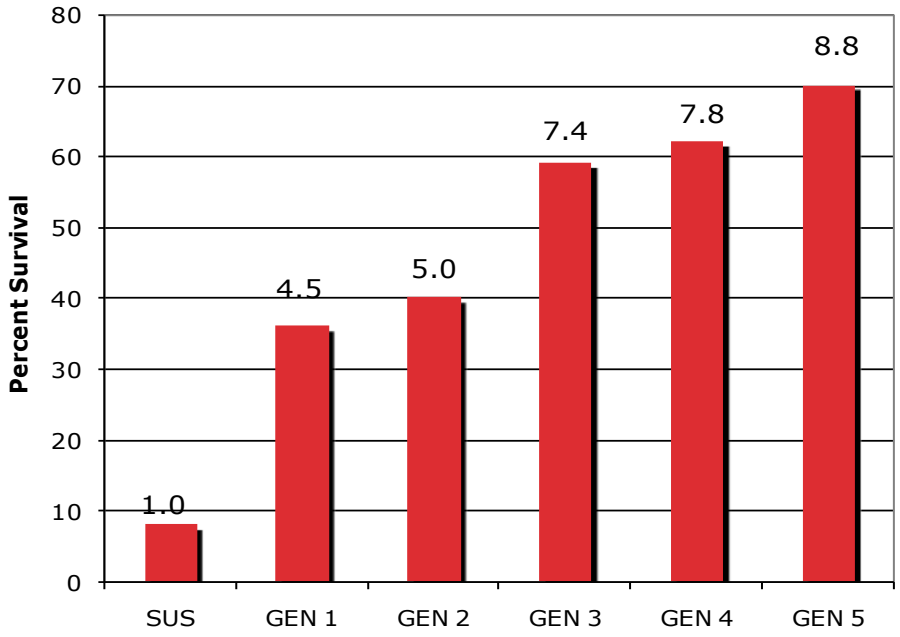
World Molluscan Aquaculture Production: 2020

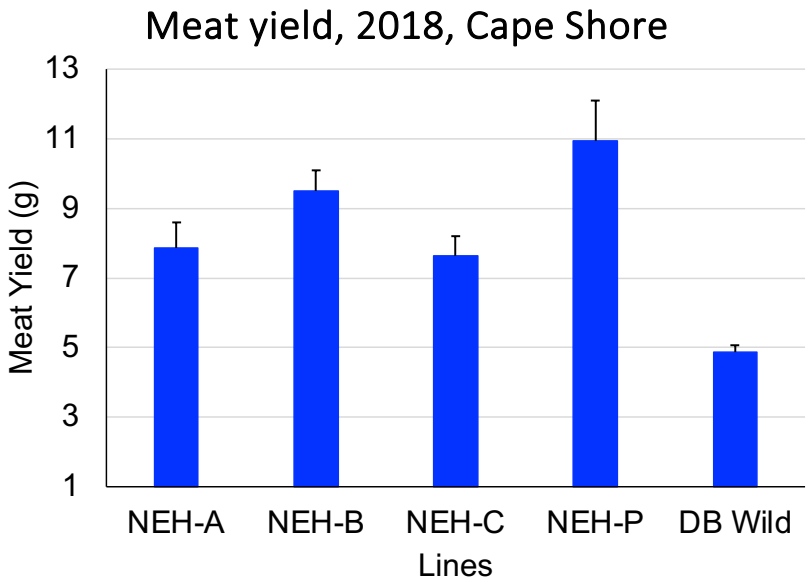
Species	Metric Tons
Oysters	6,260,194
Clams	5,742,807
Mussels	2,047,983
Scallops	1,984,209
Other marine molluscs	1,023,365
Abalones, winkles, conchs	489,277
Freshwater molluscs	192,689
TOTAL	17,740,526

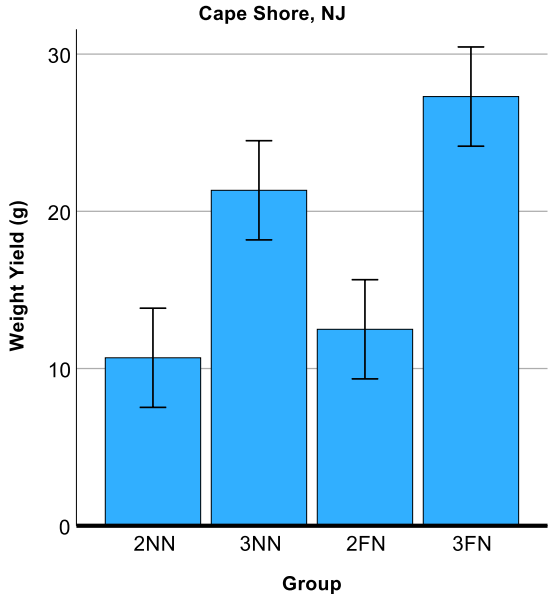
(FAO, 2022)

Oyster Breeding at Rutgers:

- 1960 Selection began after MSX outbreak
- 1990 New lines for Dermo resistance
- 1993 All-triploid Pacific oysters developed
- 1998 Growth, shell shape added to selection
- 2001 All-triploid Eastern oysters developed
- Disease-resistance lines released
  - **Haskin NEH®** – Derived from Long Island Sound, strong MSX resistance, Dermo resistance, fast growth.
  - **Haskin DBX** – Derived from Delaware Bay, strong resistance to MSX, resistance to Dermo.

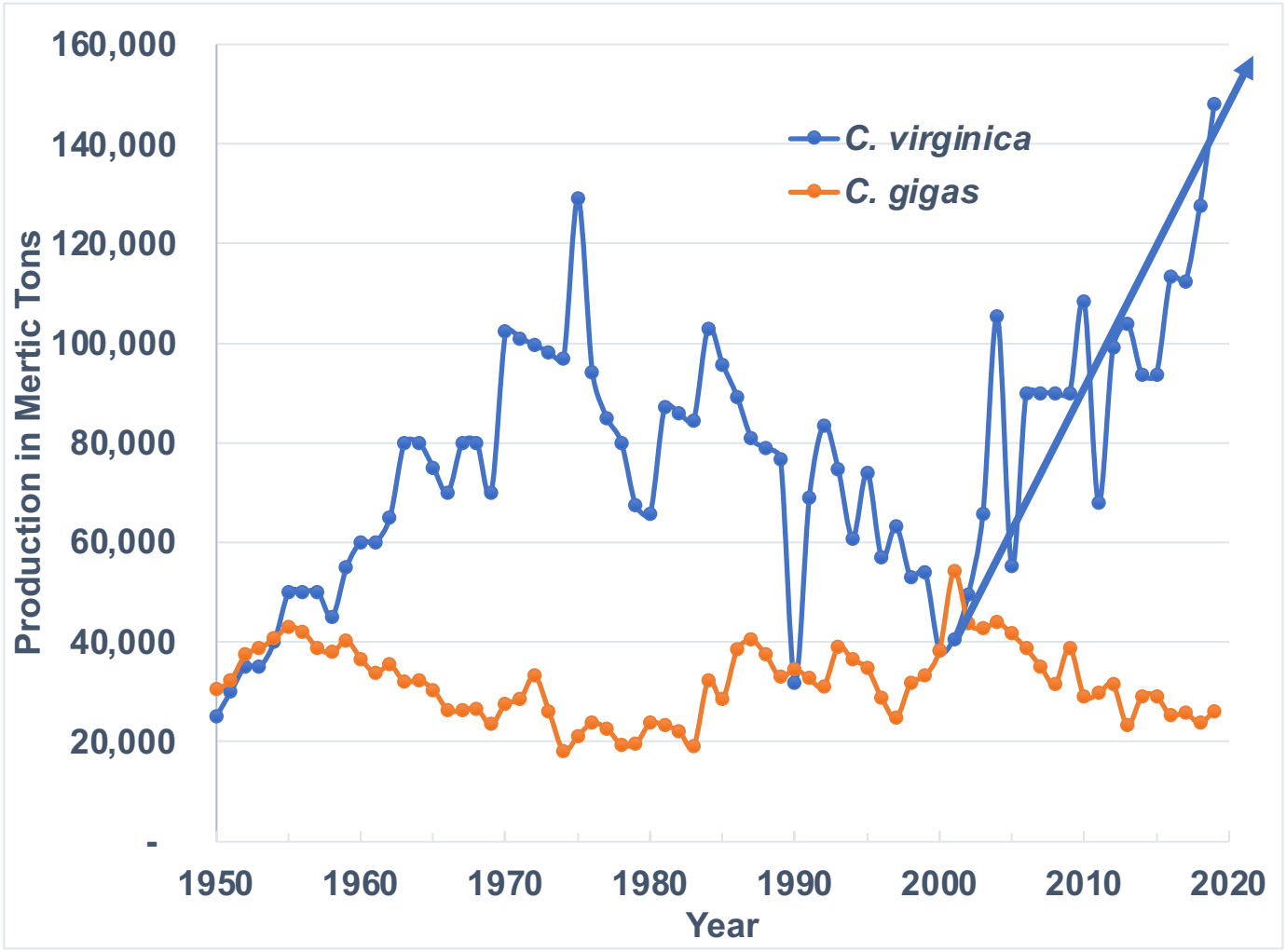






(S.E. Ford)

Oyster aquaculture in the US

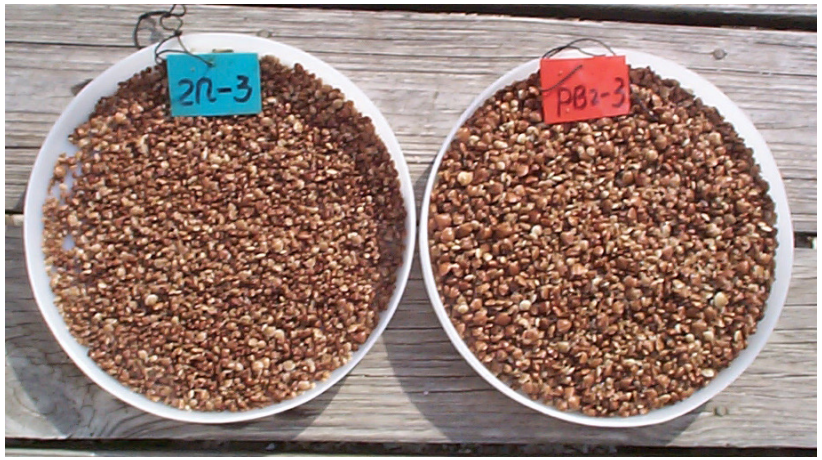


(FAO 2022)

2000 – 2005: Triploid-tetraploid Technology for Hard Clam Aquaculture

- Funded by NJCST and NJSGC
- Produced and evaluated triploid hard clam
- Tested tetraploid induction

- 2000: 3n = 2n (Yang & Guo, 2018)
- 2001: 3n >> 2n
- Tetraploids were induced but none survived to adult stage (Yang & Guo 2006)



Size of diploid and triploid clams at 15-month-old

Group	Parameter	Diploid (mean)	Triploid (mean)	3n over 2n (%)	t-test (p-value)
PB2-1	N	29	21		
	Length (mm)	20.3	22.4	10.3	0.003
	Whole Weight (g)	2.52	3.36	33.3	0.001
	Meat Weight (g)	0.40	0.56	40.0	0.001
PB2-2	N	11	37		
	Length (mm)	11.1	13.1	18.0	0.004
	Whole Weight (g)	0.44	0.85	93.2	0.008
	Meat Weight (g)	0.04	0.13	225.0	0.010
PB2-3	N	6	42		
	Length (mm)	11.9	16.0	34.5	0.005
	Whole Weight (g)	0.54	1.46	170.4	0.000
	Meat Weight (g)	0.07	0.25	257.1	0.001
PB2-5	N	9	38		
	Length (mm)	13.9	18.9	36.0	0.000
	Whole Weight (g)	0.85	2.19	157.6	0.000
	Meat Weight (g)	0.12	0.42	250.0	0.000

(Guo et al., unpublished)

2013-2018: Identification of QPX-resistance Markers in the Hard Clam

Ximing Guo[1], Guodong Wang[1], Carmelo del Castillo[2], Emmanuelle Pales Espinosa[2], Arnaud Tanguy[3], John Kraeuter[1] and Bassem Allam[2]

[1]Haskin Shellfish Research Laboratory, Institute of Marine and Coastal Sciences, Rutgers University, Port Norris, NJ 08345, USA.

[2]Marine Animal Disease Laboratory, School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY, USA.

[3]University of Paris 6, Station Biologique de Roscoff, Place Georges Teissier BP74, 29682 Roscoff, France.

RUTGERS
NEW BRUNSWICK

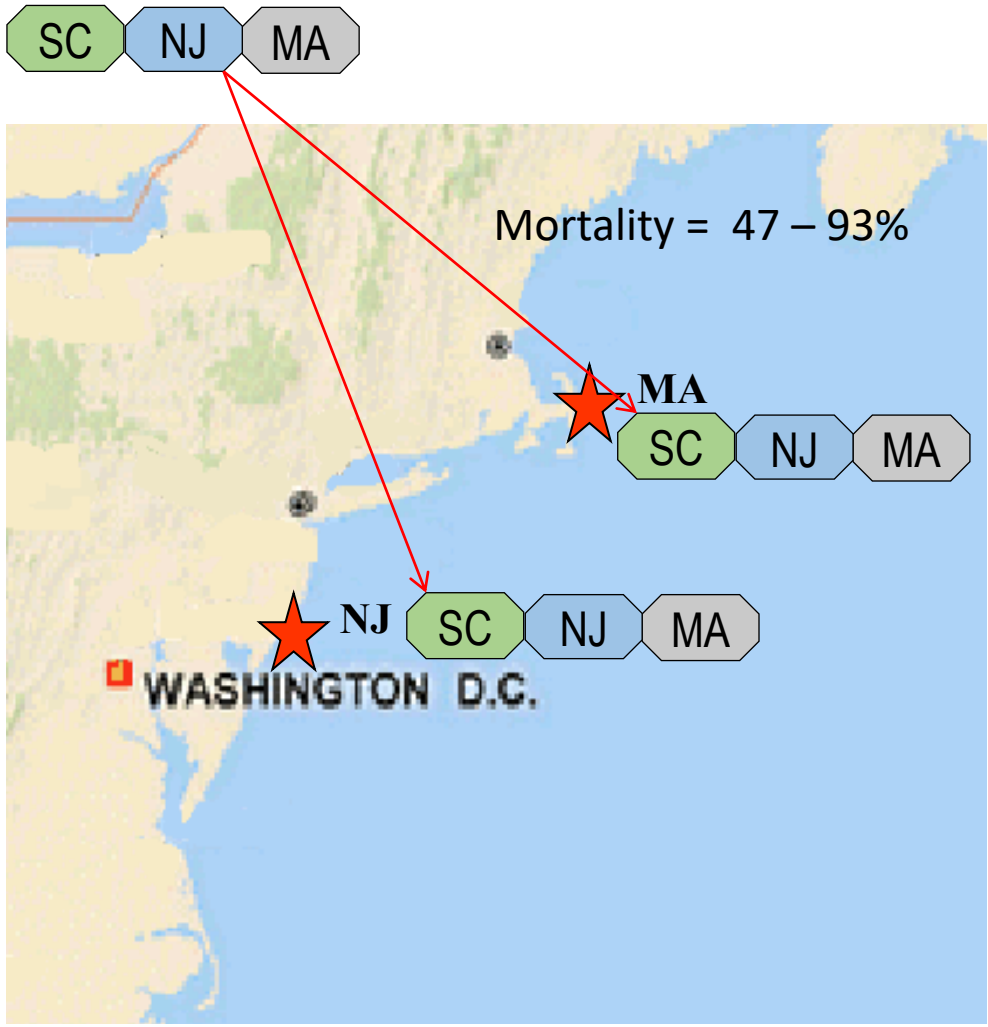
Stony Brook University

QPX Disease of the Hard Clam

- Quahog Parasite Unknown (QPX), phylum Labyrinthulomycetes
- A fatal parasite that can lead to 100% clam mortality
- Began in NS Canada in 1956, now endemic down to Virginia.

Study Design

- We deployed 3 stocks (SC, NJ & MA) in NJ and MA;
- Collected before and after-mortality samples at two locations (n = 56 – 64);
- Clam DNAs from each group were pooled into 9 samples (3 before and 6 after) for AmpliSeq at 373 immune related genes.
- SNPs were identified and changes in allele frequency determined;
- SNPs with allele frequencies consistently shifted after mortalities were considered as associated with QPX resistance.



Mortality and QPX Infection Intensity

Site	Stock	Mortality	Infection
MA	MA	73.9	1.3
	NJ	65.1	8.0
	SC	93.4	32.0
NJ	MA	58.2	0.0
	NJ	46.6	2.0
	SC	63.6	8.3

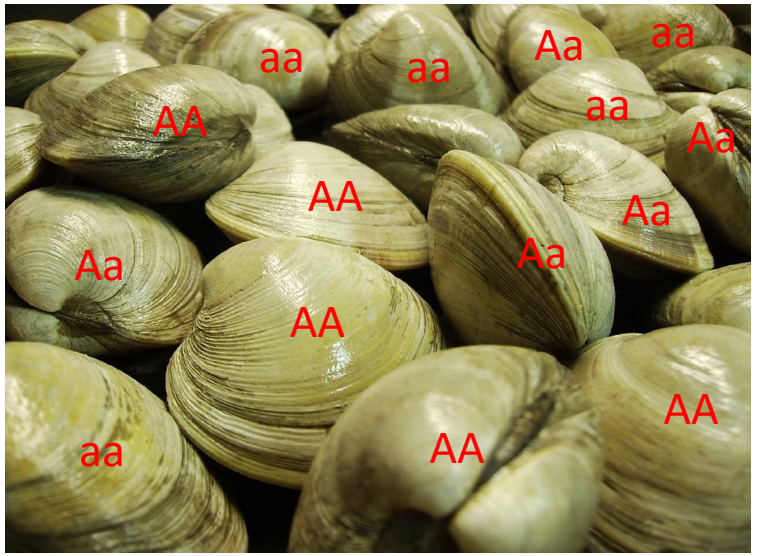
(Kraeuter et al. 2009)

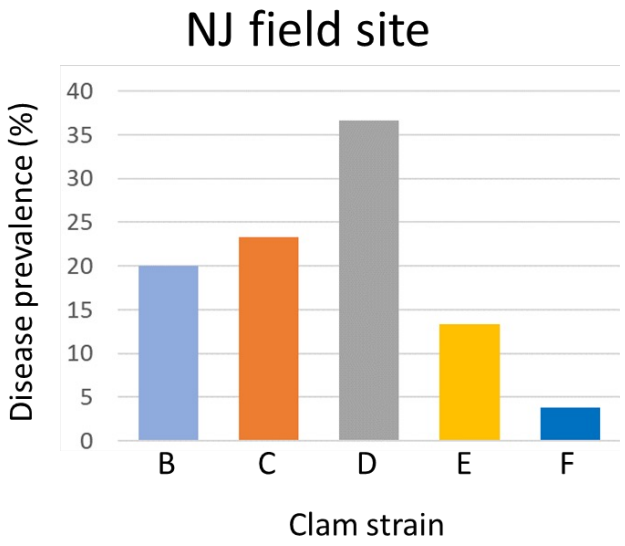
Markers associated with QPX-resistance:
9 markers in 7 genes changed allele frequencies after mortality

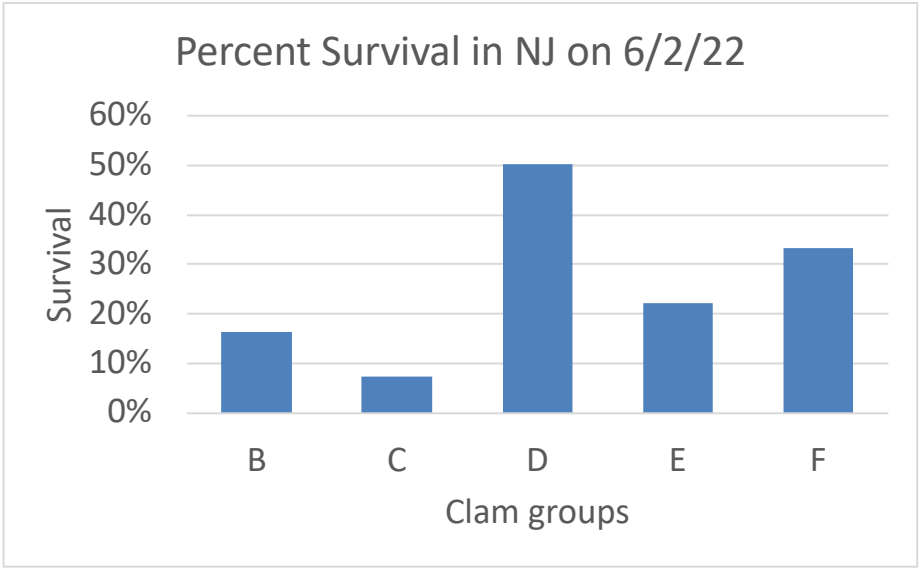
				After/before frequency ratio, stock-state						
Gene	**Position**	**Ref**	**Variant**	**M-MA**	**M-NJ**	**N-MA**	**N-NJ**	**S-MA**	**S-NJ**	**P-value**
GBP2	28	C	T	0.67	0.84	0.88	0.86	0.85	0.74	0.0029
CYB245	117	G	A	0.80	0.78	0.90	0.67	0.88	0.84	0.0023
MP10	83	C	T	0.68	0.78	0.83	0.68	0.82	0.74	0.0004
	127	G	A	0.72	0.90	0.74	0.65	0.85	0.92	0.0073
	244	A	G	0.77	0.84	0.75	0.76	0.79	0.85	0.0002
IAP1	116	G	A	0.77	0.85	0.94	0.65	0.63	0.97	0.0074
SMOC1	171	-	A	0.96	0.88	0.96	0.91	0.94	0.77	0.0094
HAAF	72	C	T	1.07	1.12	1.03	1.01	1.03	1.08	0.0084
PCDP7	486	A	T	1.48	1.99	1.25	1.04	1.29	1.27	0.0088

2018-2023: Marker-assisted Selection (MAS)

- Selection based on genotypes;
- Fast, can reach fixation in one generation;
- MAS is especially useful for breeding of disease-resistance when disease-exposure may be absent;
- MAS is effective when resistance is controlled by major effect genes







2019 – 2024: The East Coast Hard Clam Selective Breeding Collaborative (NOAA SG)

HardClamHub.org

Bassem Allam
Emmanuelle Pales Espinosa
Denis Grouzdev

Stony Brook University School of Marine and Atmospheric Sciences

MASL Marine Animal Disease Laboratory

Arnaud Tanguy

Sorbonne Université

Kimberly Reece

VIMS William & Mary Virginia Institute of Marine Science

Huiping Yang

UF University of Florida

Gregg Rivara

Cornell University Cooperative Extension

Joshua Reitsma

County of Barnstable Massachusetts

Antoinette Clemetson

Sea Grant New York

Ximing Guo

Rutgers

Objectives of the Hard Clam Breeding Collaborative

- Sequence, assemble and annotate the hard clam genome and develop a cost-effective genotyping platform (SNP array) for *M. mercenaria*
- Use this tool to enable genome-assisted selection for **QPX resistance** and **heat tolerance**
- Build a regional hard clam breeding program linking scientists, extension and the industry

Goals for Rutgers

- Establish a hard clam breeding program
- Apply genome-based selection
- Distribute improved clam seed to the industry

Tool Development

Hard clam genome (1.8 Gb) was sequenced:

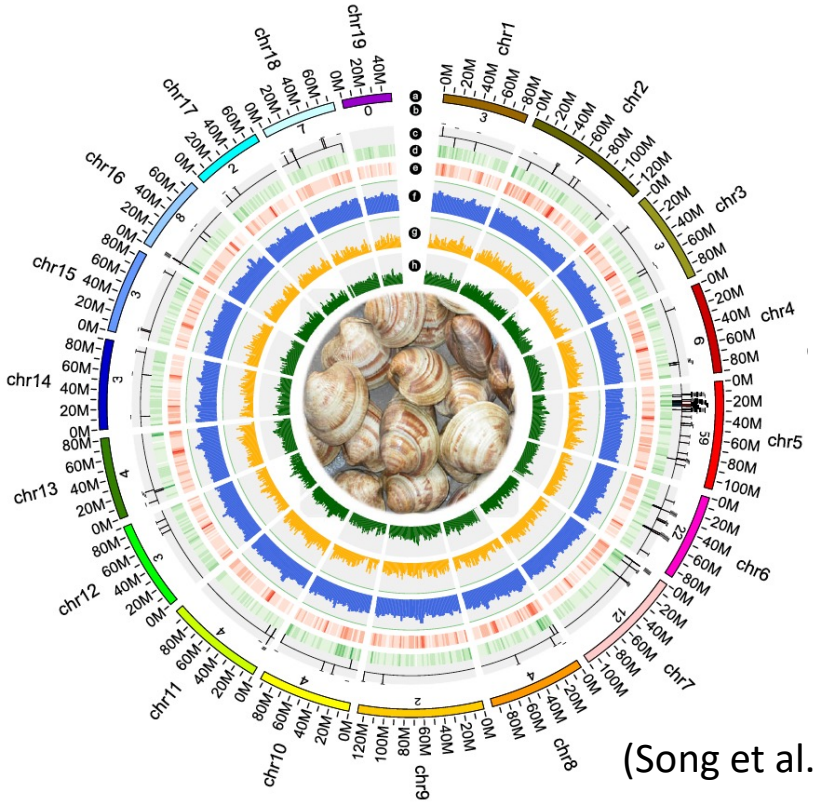
Song et al., 2021

Farhat et al., 2022

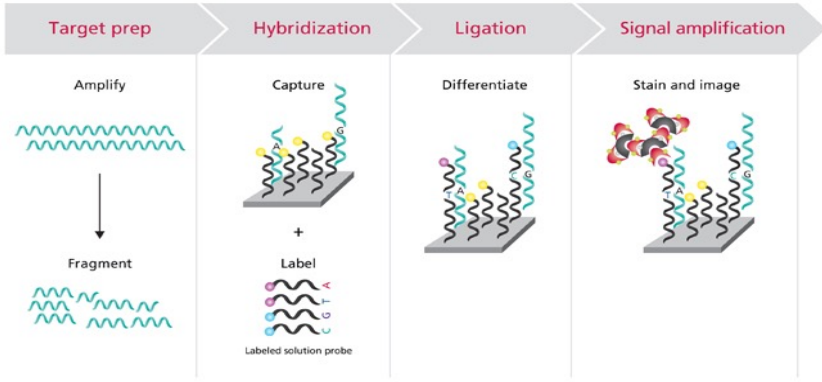
Hard clam has 34,283 genes

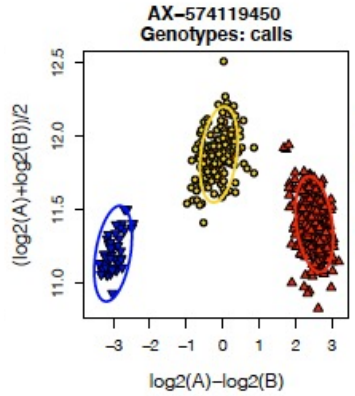
HSP, IAP and TNF greatly expanded

A 67K SNP array was developed.



(Song et al. 2021)





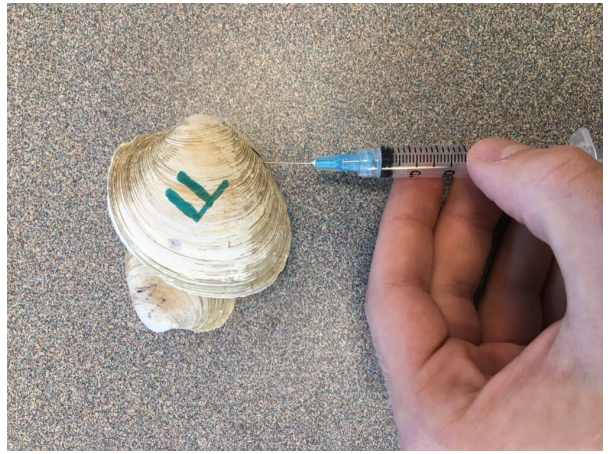
2023-2024: Establishment and Evaluation of Regionally Crossbred Hard Clam *Mercenaria mercenaria*

Paul Coyne, Samuel Ratcliff, Joseph J. Gabris III, Ximing Guo

RUTGERS
THE STATE UNIVERSITY
OF NEW JERSEY

Clam Broodstock

- NJ wild stock
  - Barnegat Bay
  - Great Bay
- NY/USDA selected stock
  - MAS selected for QPX resistance
- NJ Hatchery stock
  - Selected and used by farmers



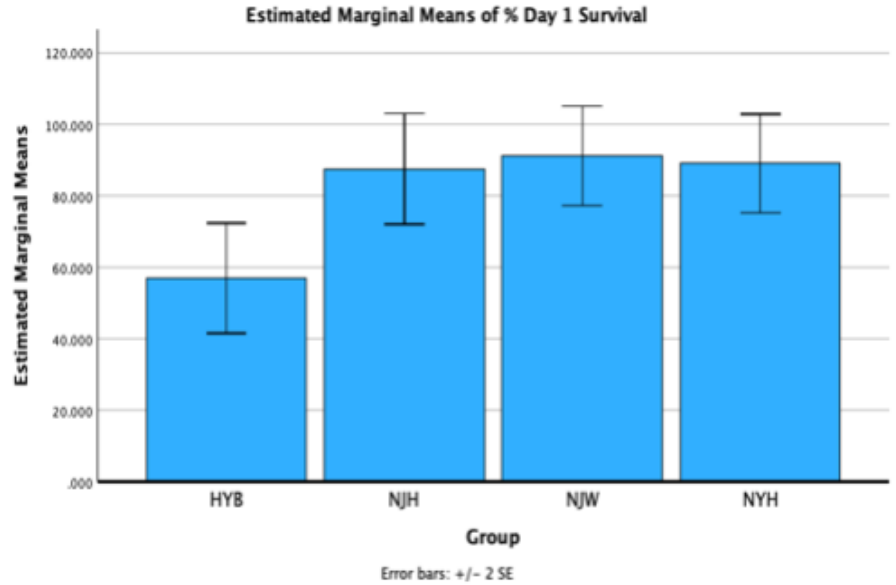
Results

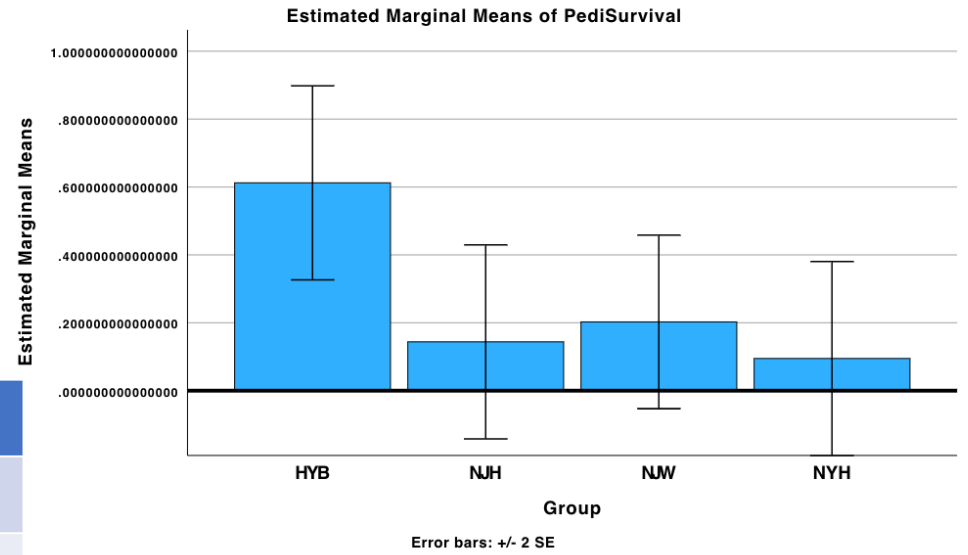
- Survival from fertilization to D-Stage
  - Significantly lower in the hybrid group ($p<0.05$)
  - ~56% in Hybrid; ~90% in others

- Survival from Day 1 tto Day 7 and 14
  - Significantly higher in the hybrid group ($p<0.05$)
  - ~90% in hybrid; ~46% in others (7)
  - ~52% in hybrid; ~14% in others (14)

- Juvenile Survival from Day 1
  - Higher in hybrid group ($p<0.1$)
  - 3.6% in Hybrid, 1% in NJW, 0.39% in NJH, 0.21% in NY

Group	Hybrid	NJW	NJH	NY
Attempted Spawns	1 (4)	3	2	5
Day 1	5,725,000	18,000,000	5,500,000	11,000,000
Day 14	3,313,000	4,000,000	725,000	3,900,000
Juveniles	51,780	24,500	2,450	1,788





Ongoing and Future Research

- Nursery and field evaluation of clam stocks
- Biopsy and genotyping of clams with the SNP array
- Conduct genomic selection
- Produce regionally cross-breed clams
- Triploid and tetraploid clams

Perspectives

- Triploid clams may grow fast and reduce production cycle. Its application depends on the development of tetraploids.
- Genomic selection is promising, and validation is needed.
- Hybrid clams may show hybrid vigor and offer benefits.

Challenges:

- How to deploy clams in replicates and get solid data?
- What are the most important traits for hard clam culture?
- Genetic health of clam stocks in NJ and beyond: free test

Acknowledgements

John Kraueter
Susan Ford
Paul Coyne
Josh Kiernan
Sean Towers
Sam Ratcliff
Zhenwei Wang
Jillian Jamieson
Siena Stucki
Emma Hutzinger
Noah Chriss
David Bushek
Dale Parsons
Michael De Luca
Lisa Calvo
Bassem Allam

Funding:
NJCST
NRAC
NJSGC
NOAA Sea Grant