



PROPER DEVELOPMENT OF CLAM BROODSTOCK FOR SEED PRODUCTION

John Scarpa, Ph.D.
Elyse Steiner, M.S.
HBOI-FAU



HARBOR BRANCH

FLORIDA ATLANTIC UNIVERSITY



R/RL-A-46

Presented at the Clam Broodstock Development Workshop, 6 December 2011

So why are you here?



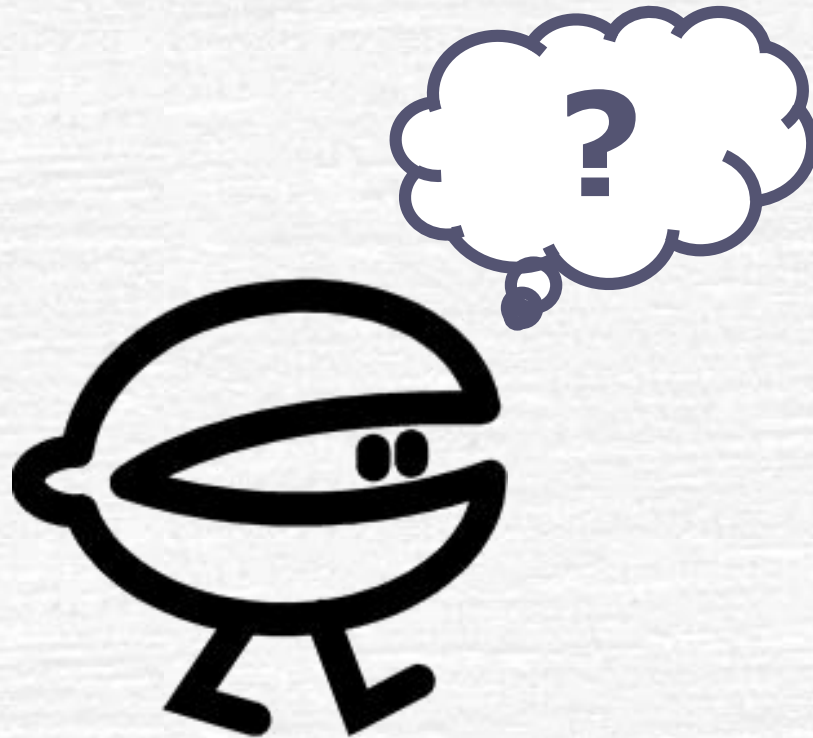
<http://forums.egullet.org>

So why are you here?

To ensure your success.

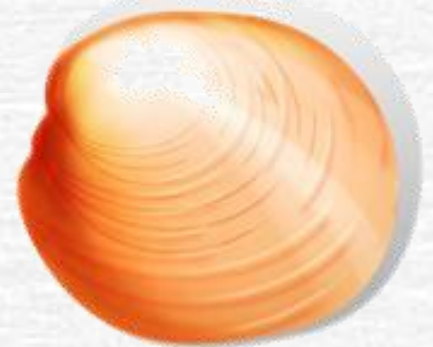


How are we going to do this?



By discussing:

1. Genetic variability and how it will help
2. Genetic Bottleneck and Founder Effect
3. Effective Population Number and Factorial Mating
4. Methods to maximize genetic variability in your broodstock
5. FDACS Best Management Practices



FDACS - Best Management Practices

http://www.floridaaquaculture.com/publications/P-01499-booklet-07_BMP_RULE.pdf

D. GENETIC PROTECTION

Best Management Practices:

- Aquaculturists who intend to sell or use hard clam seed stocks for further grow-out in the State of Florida must use broodstock which originated from Florida waters in their genetic selection program. Documentation of brood stock origin must be obtained by the hatcheries.

Best Management Practices (cont.)

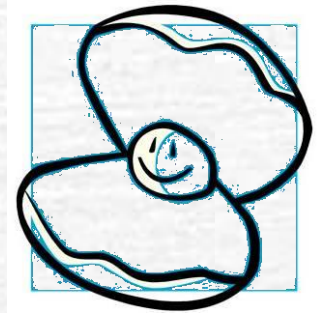
- Aquaculturists located on Atlantic coast waters, who intend to sell or use oyster seed stocks for further grow out in the State of Florida must use broodstock which originated from Florida Atlantic coast waters in their genetic selection program.
- Aquaculturists located on Gulf Coast waters, who intend to sell or use oyster seed stocks for further grow-out in the State of Florida, must use broodstock which originated from Florida waters of the Gulf of Mexico in their genetic selection program.

Best Management Practices (cont.)

- All shellfish must be transported or shipped in distinct containers identified by the producer's Aquaculture Certificate Number.
- If producers buy clam seed stocks from an out-of-state source, the hatchery must utilize Florida broodstock in their genetic selection program. Documentation of brood stock origin must be obtained from the hatchery.

Best Management Practices (cont.)

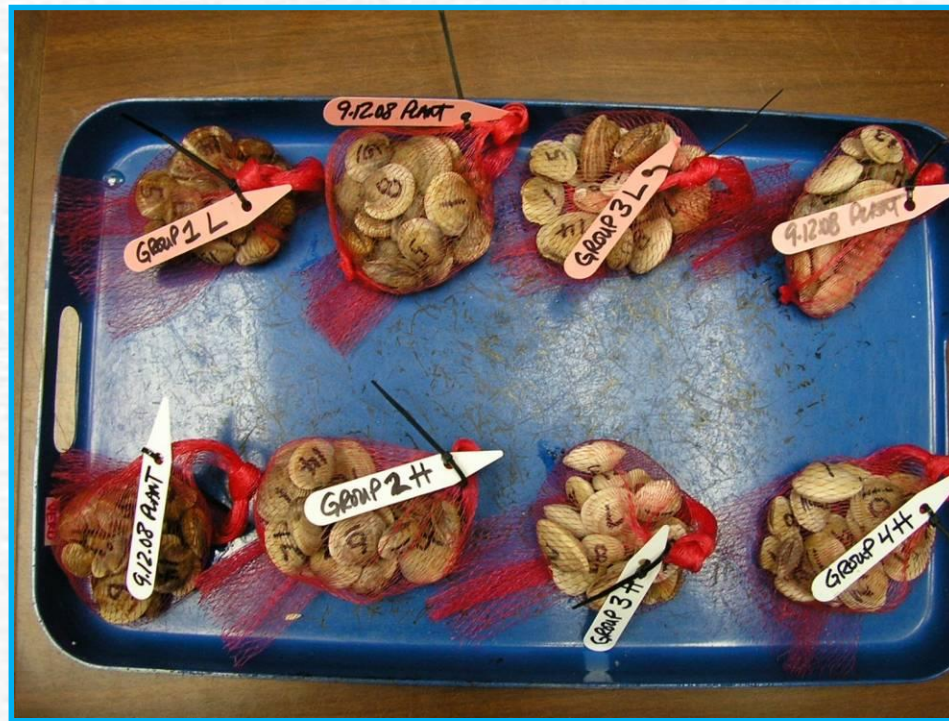
- Only the cultivation of indigenous, or hybrids of indigenous shellfish, should be placed on submerged lands. Each certificate holder shall notify the Division of the species of shellfish being cultured in Florida waters.



Why is this important to clam farmers?



To develop broodstock lines that provide greater genetic variation.




- By having large genetic variation you are able to select for more desirable traits.
- Large genetic variation increases environmental adaptability in individuals and within populations.

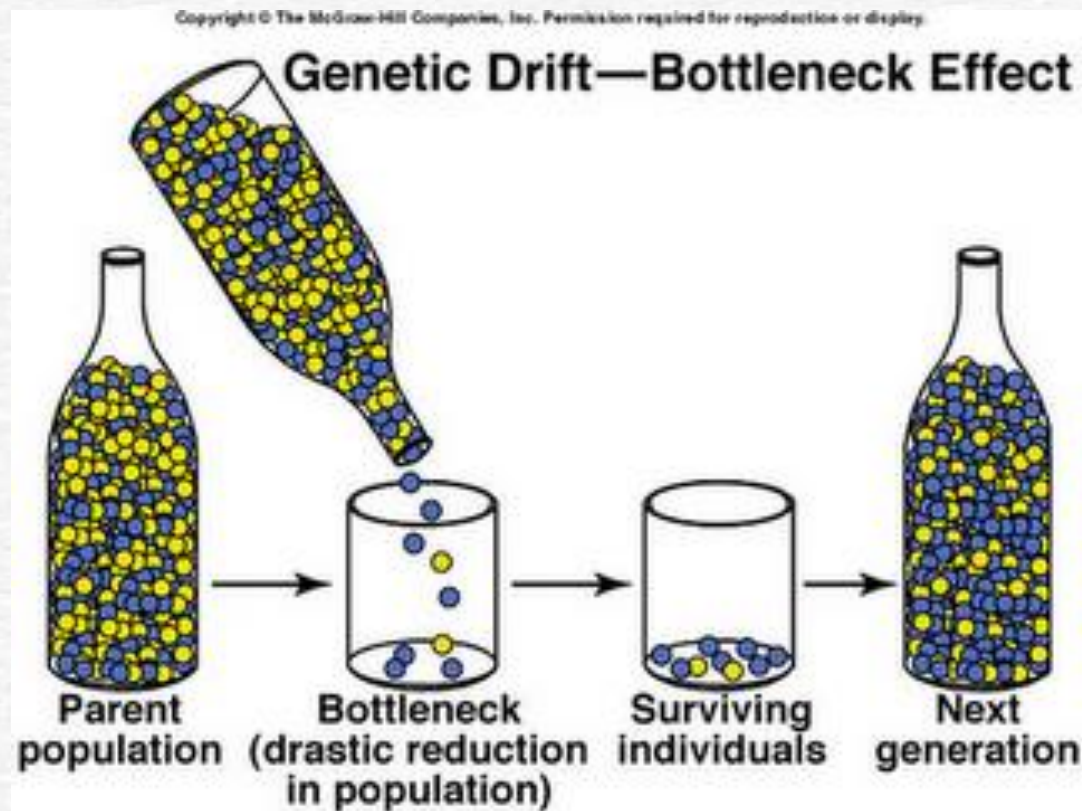




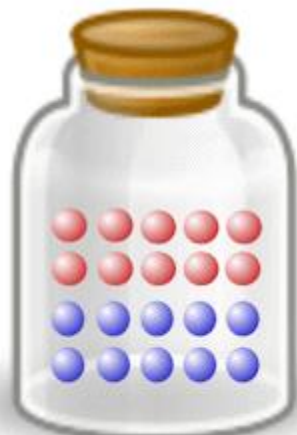
Four events can lead to reduced genetic variation in broodstock

1. Genetic Bottleneck (inbreeding)
 2. Founder Effect
 3. Adaptation to local conditions
 4. Barriers to movement
- 

What is a genetic bottleneck?



Random sampling and genetic drift



Original population



A genetic bottleneck can cause:

1. Inbreeding depression - the reduced fitness in a given population as a result of breeding of related individuals caused by:
 - Reduced fertility both in litter size and sperm viability
 - Increased genetic disorders
 - Lower survivorship
 - Slower growth rate
 - Smaller adult size
 - Loss of immune system function

(Frankham, 2010)

The background of the slide is a photograph of two Northern Elephant Seals in the ocean. The seals are dark brown with lighter, mottled patches on their heads and necks. They are both looking towards the camera, with their heads partially above the water. The water is choppy with white foam from the seals' movements.


Genetic Bottleneck in Northern Elephant Seals

- Suffered major decline in population due to hunting
- Resulted in 20-30 survivors
- Populations have increased to over 175,000 with a very limited genetic variability

Frankham, et al 2010



Founder Effect

- When a small number of individuals breaks away from the population and form their own colony.
 - Usually what occurs in aquaculture.
- 

Why is genetic diversity important?

- **Genetic diversity helps organisms cope with environmental variability**
- **Diversity within populations reduces potentially deleterious effects of breeding among close relatives**
- **Genetic diversity is the primary basis for adaptation to future environmental uncertainty**

Examples in aquaculture

- Oysters – reduced fertility in animals which were closely related. (Longwell, 1973; Gaffney, 1993).
- Fish – inbreeding in rainbow trout decreased survival and percent hatch (Kincaid, 1976).

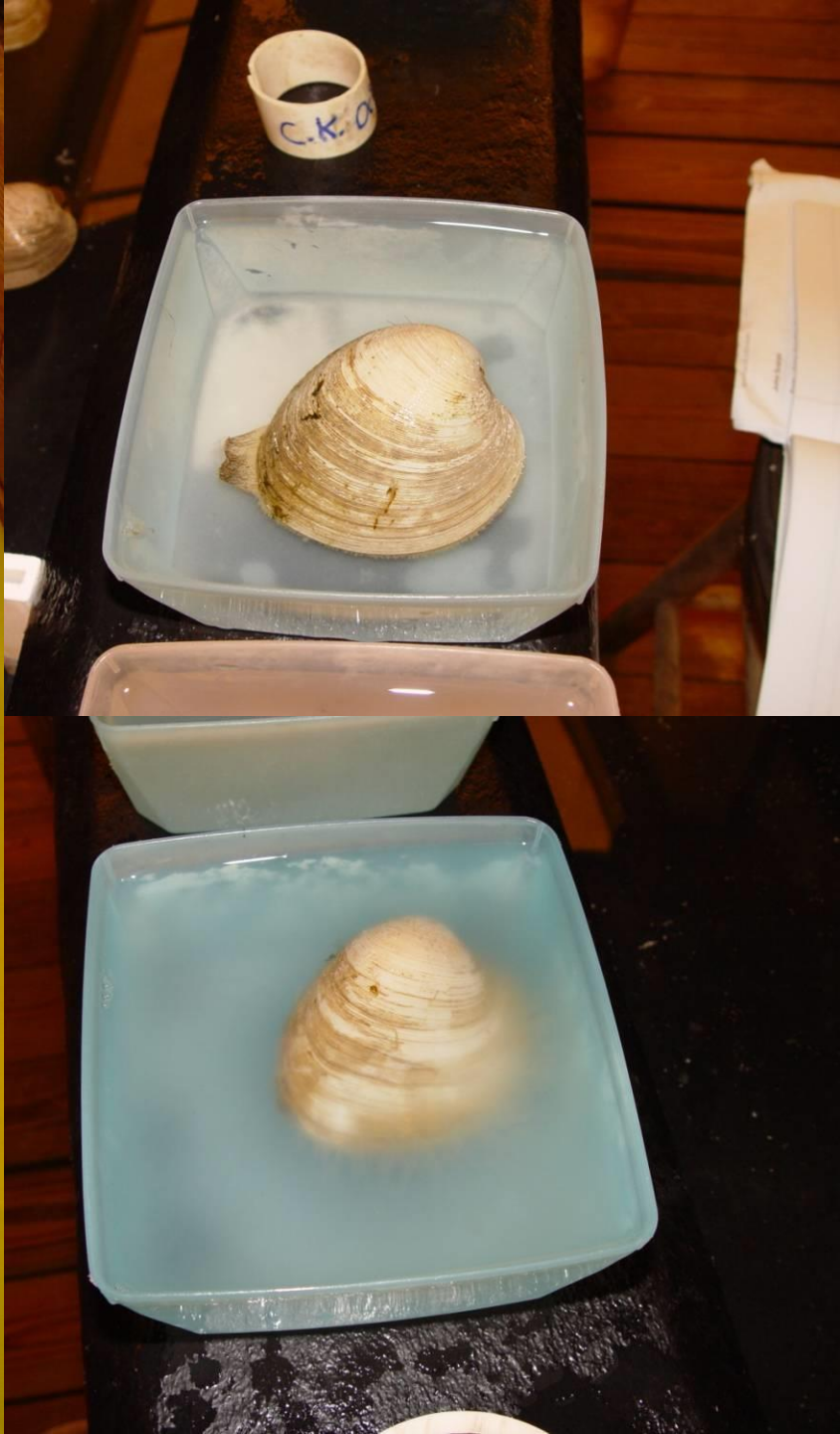
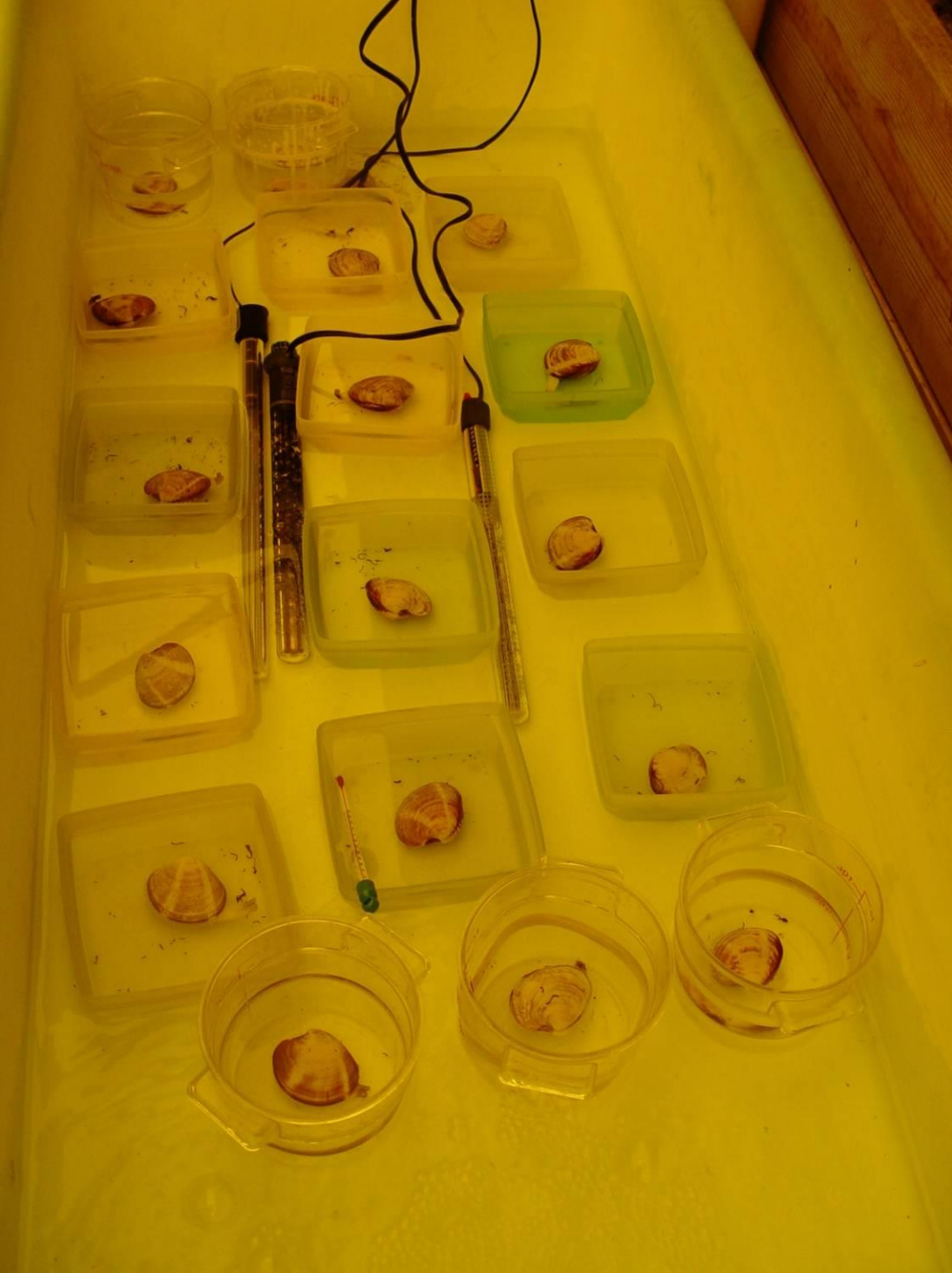
How to maximize genetic variability

1. Maintain separate family groups based on where broodstock were obtained

- This will allow for better line development by allowing you to select for certain traits

2. Controlled spawning

- Controlled spawning is a better method to use than mass spawning because it allows one to know the parentage of each spawn group



3. Use factorial pair-wise matings to capture maximum genetic diversity



<http://forums.egullet.org>

		Males							
		1	2	3	4	5	6	7	8
Females	1								
	2								
	3								
	4								
	5								
	6								
	7								
	8								

Single-Pair (1 x 1)

		Males							
		1	2	3	4	5	6	7	8
Females	1								
	2								
	3								
	4								
	5								
	6								
	7								
	8								

2 x 2 Partial Factorial

		Males							
		1	2	3	4	5	6	7	8
Females	1								
	2								
	3								
	4								
	5								
	6								
	7								
	8								

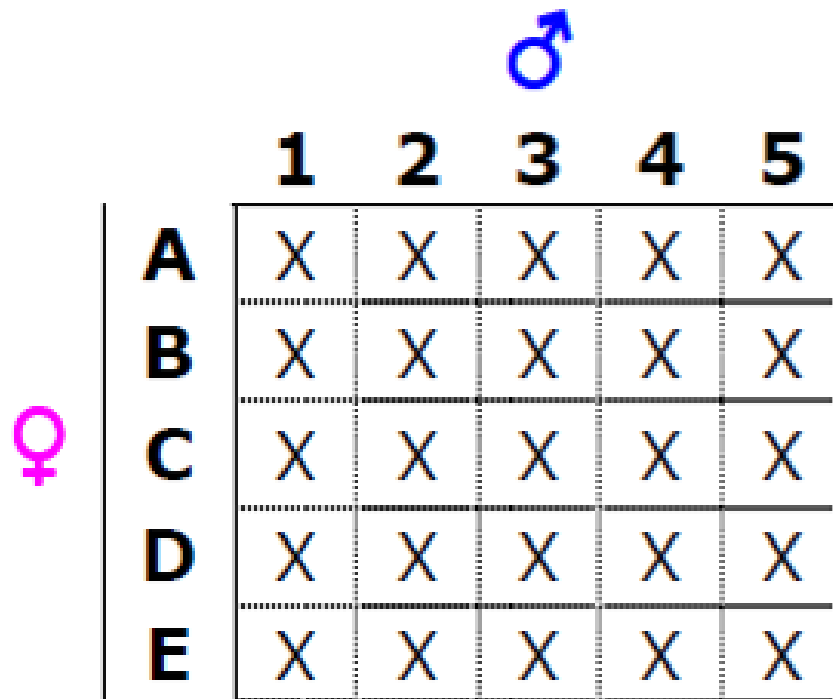
4 x 4 Partial Factorial

		Males							
		1	2	3	4	5	6	7	8
Females	1								
	2								
	3								
	4								
	5								
	6								
	7								
	8								

Full Factorial (8 x 8)

Factorial Mating

- Each male is mated with each female





A diagram illustrating factorial mating. A pink female symbol (♀) is positioned to the left of a 5x5 grid. A blue male symbol (♂) is positioned above the grid. The grid's columns are labeled 1 through 5, and its rows are labeled A through E. Every cell in the grid contains an 'X', indicating that every male is mated with every female.

	1	2	3	4	5
A	X	X	X	X	X
B	X	X	X	X	X
C	X	X	X	X	X
D	X	X	X	X	X
E	X	X	X	X	X



Factorial matings in
conjunction with mating equal
numbers of males and females
helps to maximize the
Effective Population Number

- 
- The effective population number can be defined as: the number of individuals in an ideal, randomly breeding population with 1:1 sex ratio which would have the same rate of heterozygosity as the real population under consideration (Wilson, 1975).
- 

Effective Population Number

$$N_e = \frac{(4N_m N_f)}{(N_m + N_f)}$$

Where:

N_e = Effective Population Number (=20?)

N_m = Number of Contributing Males

N_f = Number of Contributing Females

Effective "Parental" Number (N_e)



♀	♂	Total Spawners	N_e
10	10	20	20
9	11	20	19.8
8	12	20	19.2
7	13	20	18.2
6	14	20	16.8
5	15	20	15
1	19	20	3.8
7	18	25	20.2
6	30	36	20
5	195	200	19.5



What does this mean?

The most efficient way to maximize the parental number is to have equal numbers of males and females contributing to the gene pool.

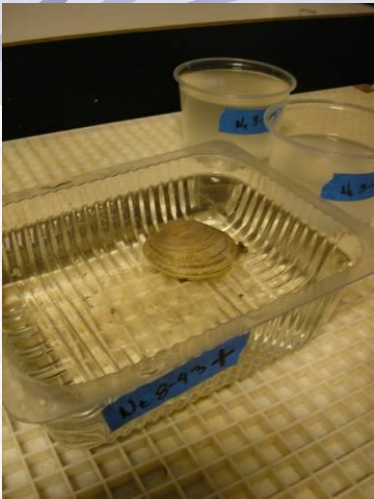


<http://forums.egullet.org>

Breeding Contribution

Equal Gametic (nuclear/mt)

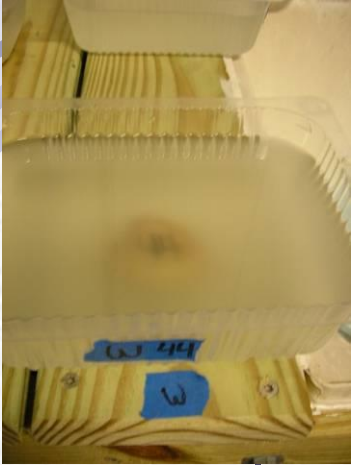
	♂a	♂b	♂c	♂d	♂e
♀A	Aa	Ab	Ac	Ad	Ae
♀B	Ba	Bb	Bc	Bd	Be
♀C	Ca	Cb	Cc	Cd	Ce
♀D	Da	Db	Dc	Dd	De
♀E	Ea	Eb	Ec	Ed	Ee



Breeding Contribution

Un-Equal Gametic

	♂a	♂b	♂c	♂d	♂e
♀A	Aa	Ab	Ac	Ad	Ae
♀B	Ba	Bb	Bc	Bd	Be
♀C	Ca	Cb	Cc	Cd	Ce
♀D	Da	Db	Dc	Dd	De
♀E	Ea	Eb	Ec	Ed	Ee



Breeding Contribution

Un-Equal Gametic

	♂ a	♂ b	♂ c	♂ d	♂ e
♀ A	Aa	Ab	Ac	Ad	Ae
♀ B	Ba	Bb	Bc	Bd	Be
♀ C	Ca	Cb	Cc	Cd	Ce
♀ D	Da	Db	Dc	Dd	De
♀ E	Ea	Eb	Ec	Ed	Ee

Breeding Contribution

Un-Equal Gametic/Larval Survival

	♂ a	♂ b	♂ c	♂ d	♂ e
♀ A		Ab		Ad	
♀ B					
♀ C			Cc	Cd	
♀ D					De
♀ E		Eb	Ec		

Breeding Contribution

Unintended Selection (nuclear/mt)

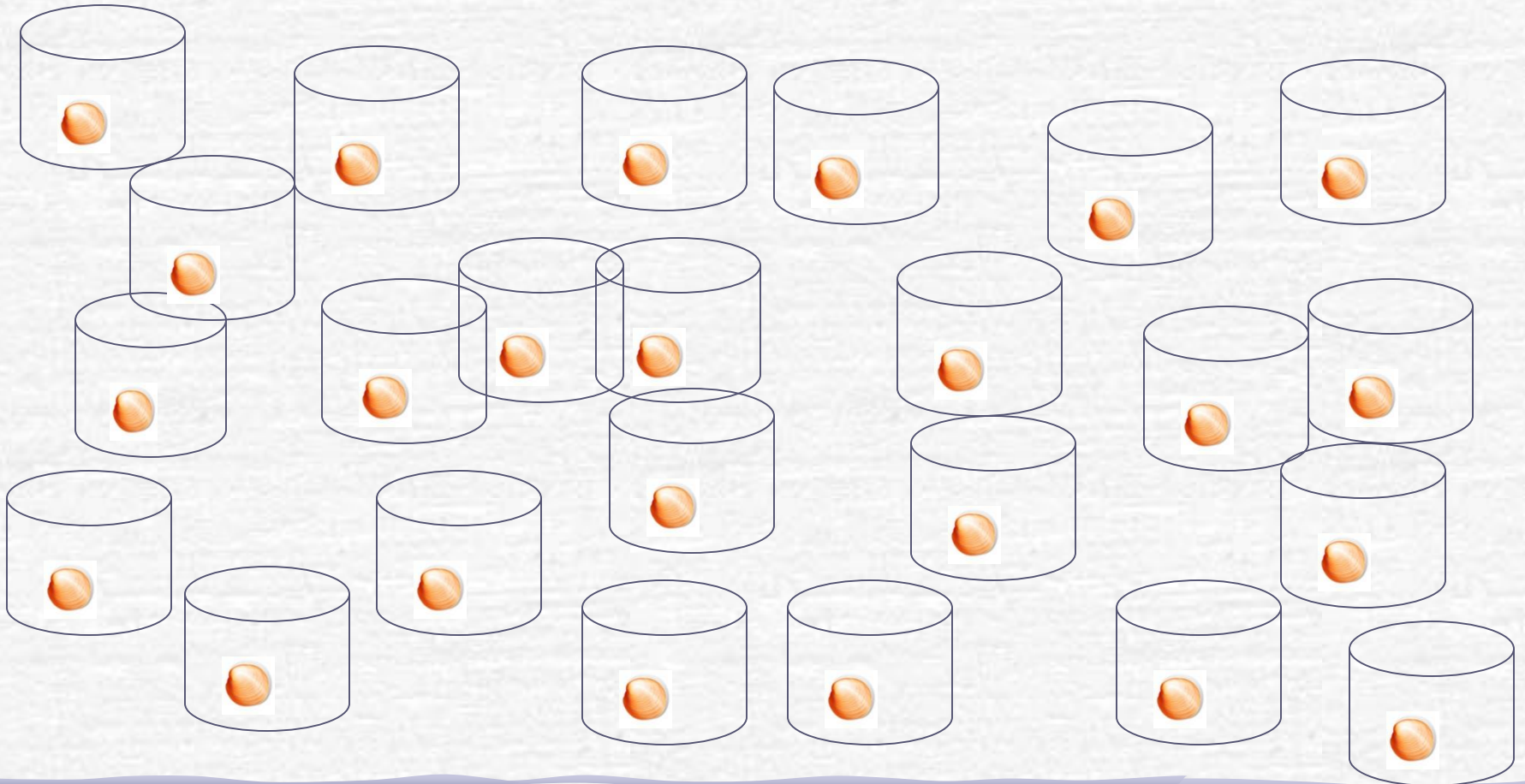
	♂a	♂b	♂c	♂d	♂e
♀A	Aa	Ab			
♀B	Ba	Bb			
♀C					
♀D					
♀E					



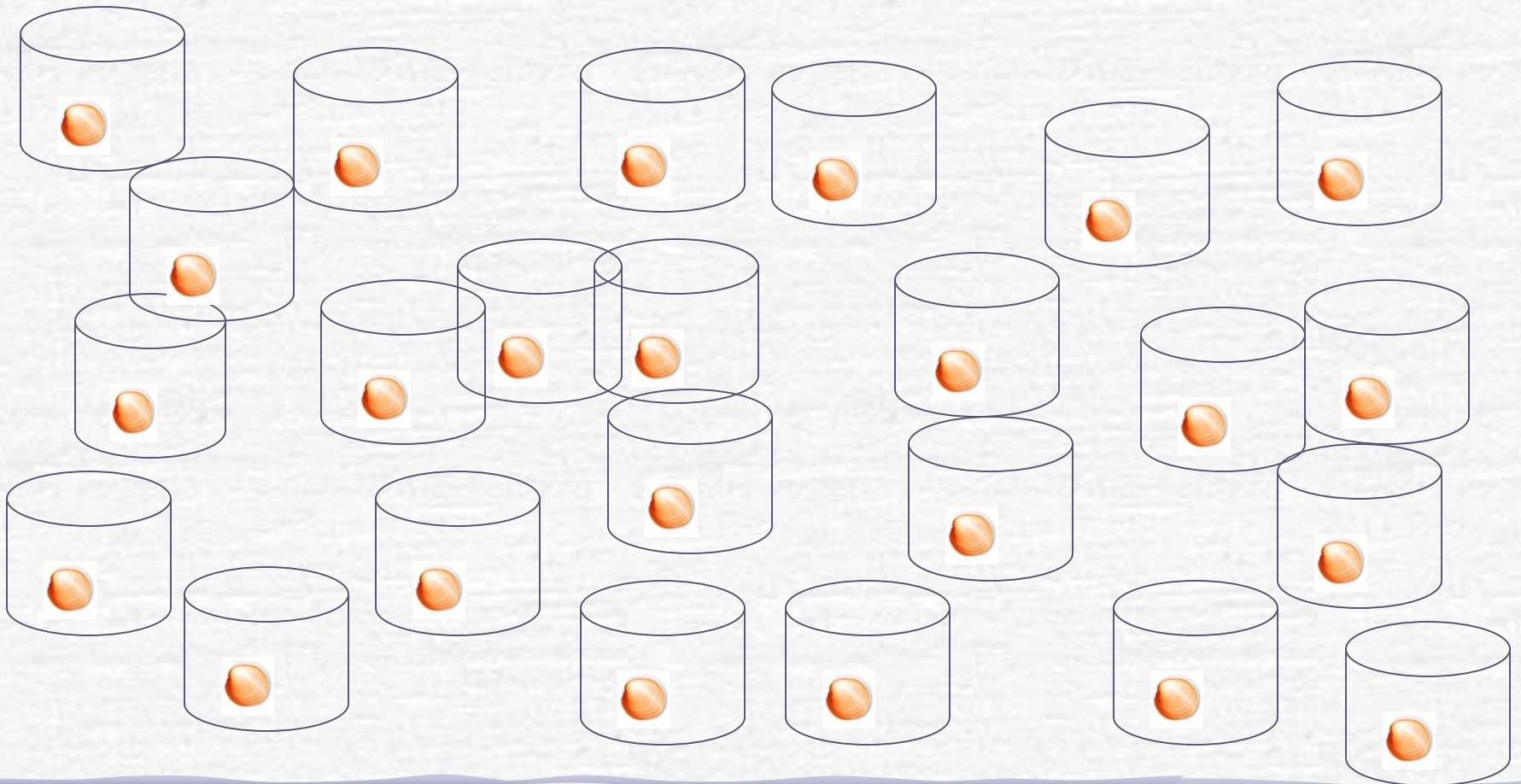
How can a hatchery do this?



How can a hatchery do this?



How can a hatchery do this?



This is a lot of containers!!!

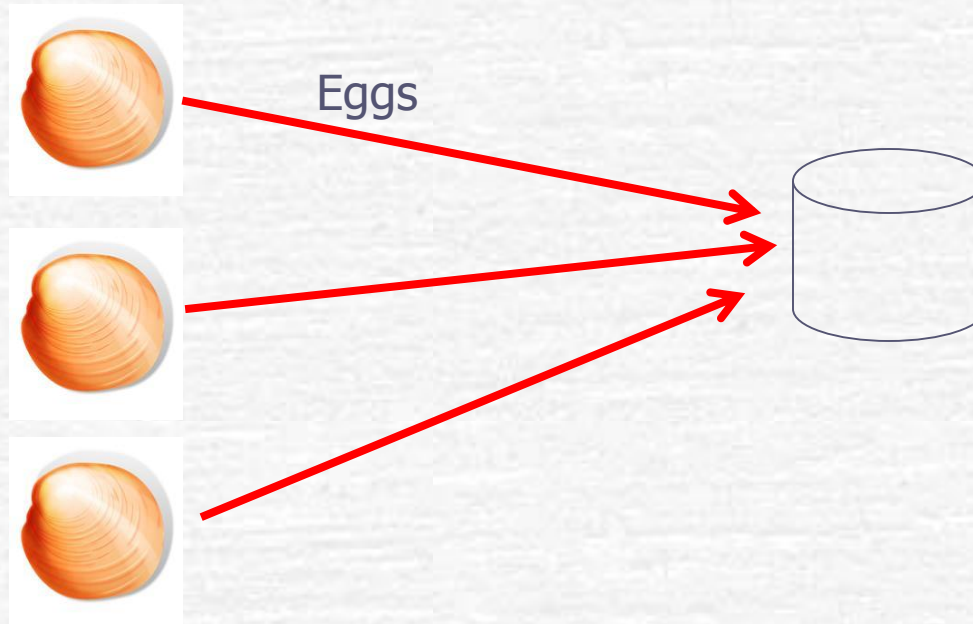


A better way

1. Let females spawn
2. Place equal amount of eggs together in containers
3. Inseminate each container of eggs with sperm

1. Let females spawn and place eggs together

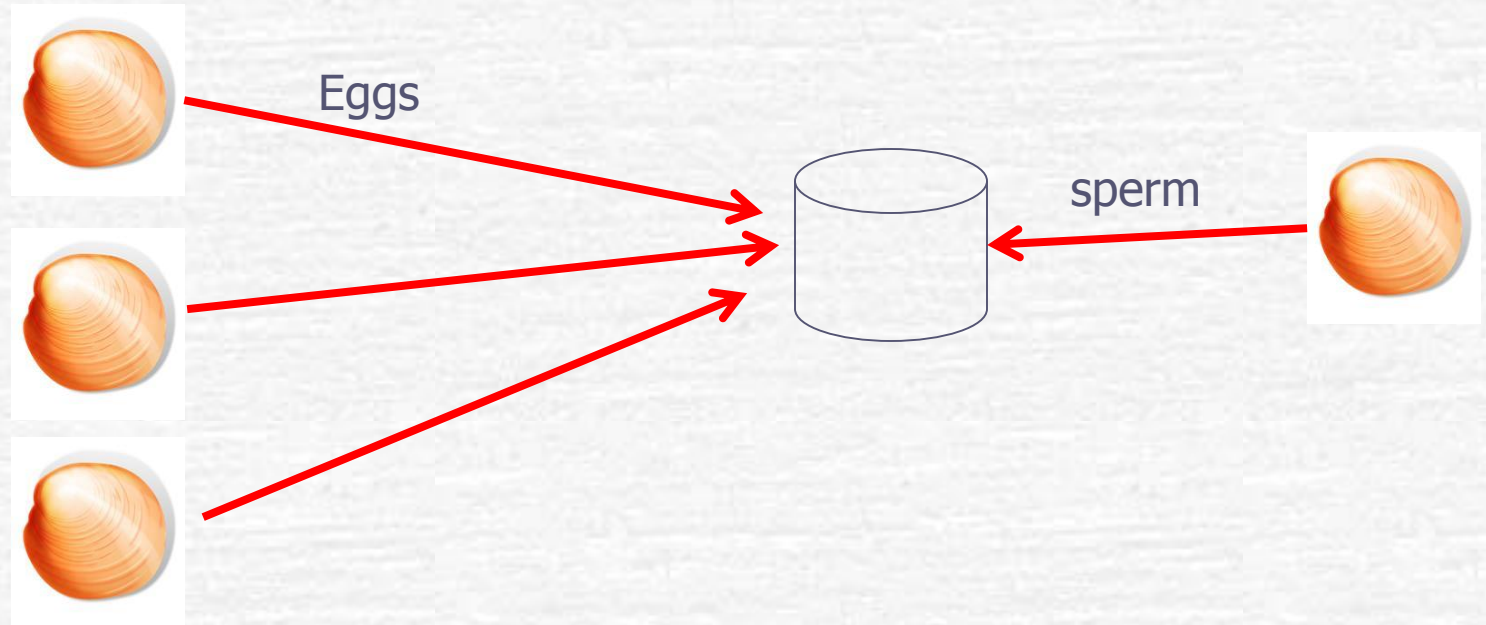
Females



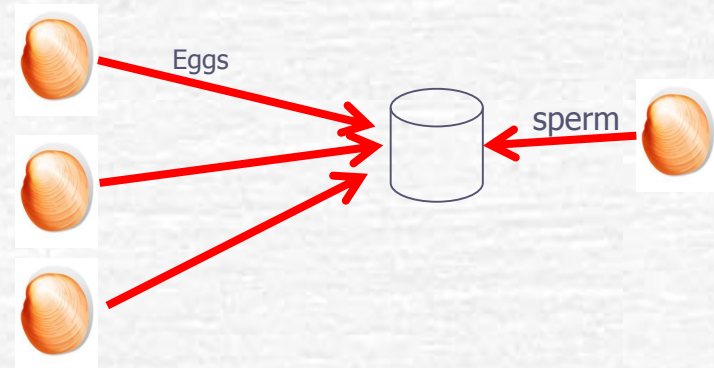
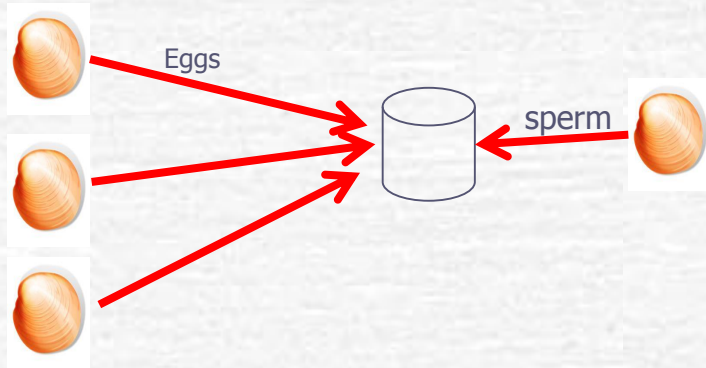
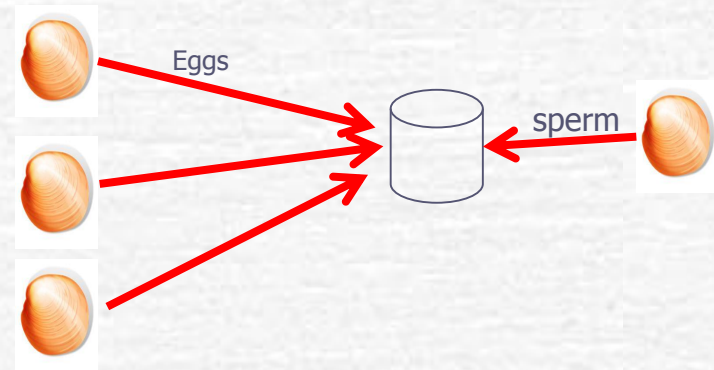
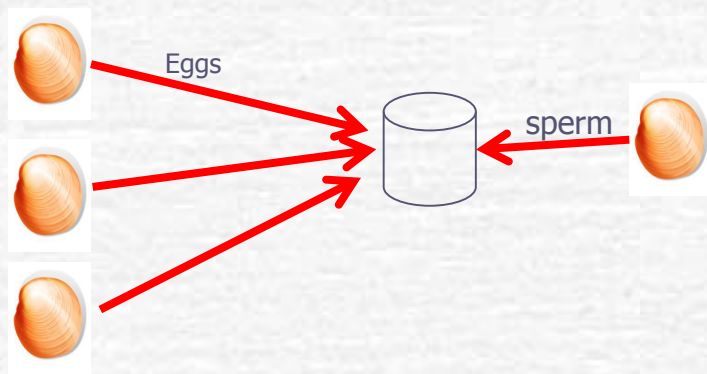
2. Inseminate each container with sperm

Females

male



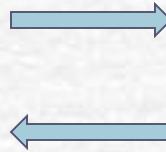
3. Repeat for all males





- By using factorial mating along with the concept of effective parental number, controlled spawning and good record keeping you will have a better brood stock line that will ensure your clams have greater genetic variation.

Questions?



References

- ✓ Aquaculture Best Management Practices . 2007. [http://www.floridaaquaculture.com/publications/P-01499-booklet-07 BMP RULE.pdf](http://www.floridaaquaculture.com/publications/P-01499-booklet-07_BMP_RULE.pdf)
- ✓ Busack, C., and Knudsen, C.M. 2007. Using factorial mating designs to increase the effective number of breeders in fish hatcheries. *Aquaculture*, 273(1): 24–32. doi:10.1016/j.aquaculture. 2007.09.010.
- ✓ Enger, E. D., F. C. Ross, et al. 2007. Concepts in biology. Boston, McGraw-Hill.
- ✓ Frankham, R., J. D. Ballou, et al. 2010. Introduction to conservation genetics. Cambridge, UK ; New York, Cambridge University Press.
http://books.google.com/books?id=vLZKnsCk89wC&printsec=frontcover&dq=Introduction+to+conservation+genetics&hl=en&src=bmrr&ei=Q2UoTvPhDeXj0QHH4-XbCg&sa=X&oi=book_result&ct=result&resnum=1&ved=0CCkQ6AEwAA#v=onepage&q&f=false
- ✓ Gaffney, P.M., C.M. Bernat and S.K. Allen Jr. 1993. Gametic incompatibility in wild and cultured populations of the eastern oyster, *Crassostrea virginica* (Gmelin). *Aquaculture* 115:273-285.
- ✓ Kincaid, H.L. 1976. Effects of inbreeding on rainbow trout populations. *Transactions of the American Fisheries Society*. 105: 273-280.
- ✓ Longwell, A.C. and Stiles, S.S. 1973. Gamete cross incompatibility and inbreeding in the Commercial American oyster, *Crassostrea virginica*. *Cytologia*, 38;521-533.
- ✓ Tave, Douglas; Food and Agriculture Organization of the United Nations. 1999. Inbreeding and brood stock management. Food & Agriculture Org. pp. 50. ISBN 9789251043400.
- ✓ Wilson, Edward. Sociobiology: The New Synthesis 1975, Harvard University Press, (Twenty-fifth Anniversary Edition, 2000 ISBN 0-674-00089-7)