Gamete Ratio, Fertilization Success and Potential Sperm Limitation in Oysters

Mark W. Luckenbach[1], Elizabeth North[2],
Stephanie Bonniwell[1] and Roger Mann[1],

[1]Virginia Institute of Marine Science, College of William and Mary
[2]University of Maryland Center for Environmental Science

Funding was provided by the NOAA Chesapeake Bay Office and the Keith Campbell Foundation for the Environment.

Introduction

Fertilization success in free-spawning, sessile marine bivalves is dependent upon gametes making contact.

Many of the shellfish populations currently exist at low population density in some portions of their range, e.g.,

Argopecten irradians *Mercenaria mercenaria*
Crassostrea virginica *Arctica islandica*

Despite significant restoration efforts for oysters in Chesapeake Bay many areas continue to experience historically low recruitment rates.

The current paradigm assumes that this results from very low brood stock abundance, larval transport processes, poor habitat for settlement and/or early post-settlement mortality.

Introduction

High fecundity in these species lead to the expectation that a small population size is sufficient to effect a recovery.

For instance:
10^6 -10^7 eggs per ♀ at a density of 10^0 – 10^2 ♀'s m^{-2} yields 10^{10} – 10^{13} eggs $hectare^{-1}$

Assumptions about how many of these eggs are fertilized have implications for the outcome of our demographics models that vary over several orders of magnitude.

Objectives

1) Better understand the factors that affect fertilization success, including…

gamete concentrations
fertilization efficiency (avg. # sperm to fertilize an egg)
turbulent mixing

2) Ultimately, we want to use this, coupled with density, size and fecundity estimates from the field, to estimate not only egg production, but fertilization success in natural and restored bivalve populations.

Approach

1) Construct and parameterize a computational model which predicts fertilization success based upon contact between gametes in a turbulent medium.

2) Conduct laboratory experiments with *Crassostrea* gametes using field-relevant turbulent mixing conditions to test initial model predictions.

3) Refine the model predictions using experimental results.

4) Repeat 2 – 3 as necessary.

Fertilization Success Model

Predicts concentration of fertilized eggs over time when mixed with sperm of a given concentration:

$$F_{t+1} = F_t + fU_t E\delta t$$

$$U_t = U_{t-1} - F_t$$

where U = concentration of unfertilized eggs (number cm^{-3})

and

F = concentration of fertilized eggs (number cm^{-3})

f = fertilization constant (=1 if every contact results in fertilization)

dt = time interval (s)

E = contact rate of sperm with each egg (number s^{-1}).

Fertilization Success Model

The contract rate of egg and sperm (E) [After Rothschild & Osborn (1988), Evans (1989), Visser & MacKenzie (1998)]:

$$E = c_t \pi R^2 \left(u^2 + v^2 + 2w^2\right)^{1/2}$$

where c = concentration of 'prey' particles (e.g., sperm concentration, number cm^{-3}) and
R = reactive distance of 'predator' particles (e.g., effective egg radius, cm)
u = swimming velocity of 'prey' particles (e.g., sperm swimming speed, cm s^{-1})
v = randomly directed motion of 'predator' particles (e.g., $v = 0$ for eggs)
w = root-mean-square turbulent velocity, (cm s^{-1}):

$$w = a(\varepsilon d)^{1/3}$$

where a = constant [1.37 or 1.9 according to references in Visser and MacKenzie (1989)]
d = turbulence length scale [$d = R$ according to Visser and MacKenzie (1989)]
ε = turbulent dissipation rate (cm^2 s^{-3}).

Preliminary model run examples

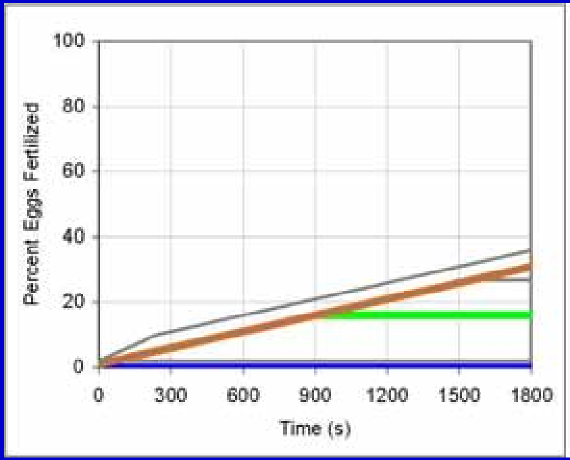
Turbulent energy dissipation rates

$\varepsilon = 0.007\ cm^2\ s^{-3}$

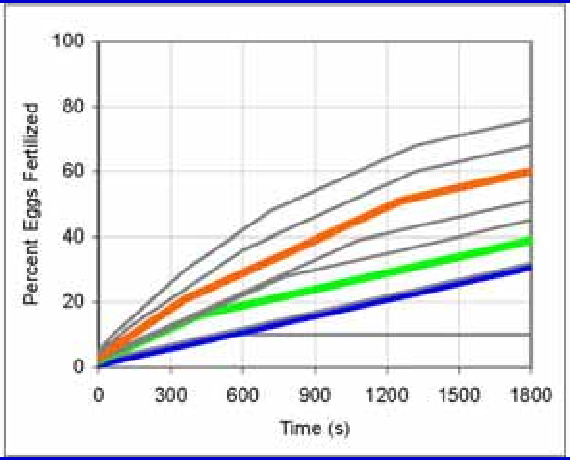
$\varepsilon = 0.034\ cm^2\ s^{-3}$

$\varepsilon = 0.180\ cm^2\ s^{-3}$

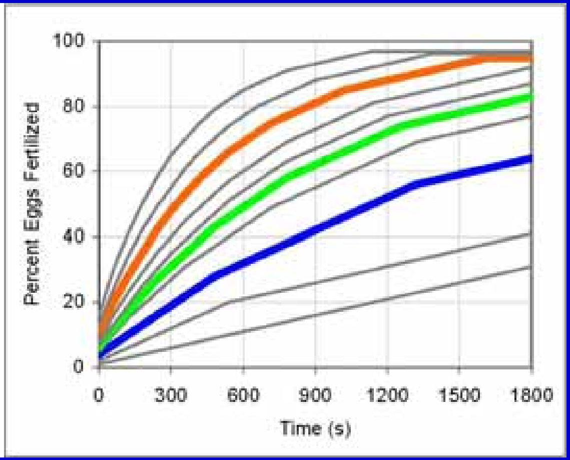
Unfertilized egg conc. = 100 cm^{-3}
Sperm concentration = 1000 cm^{-3}
Fertilization factor = 0.01



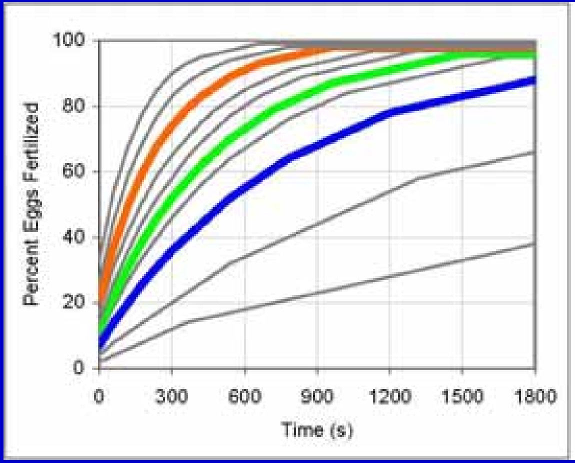
Unfertilized egg conc. = 100 cm^{-3}
Sperm concentration = 1000 cm^{-3}
Fertilization factor = 0.03



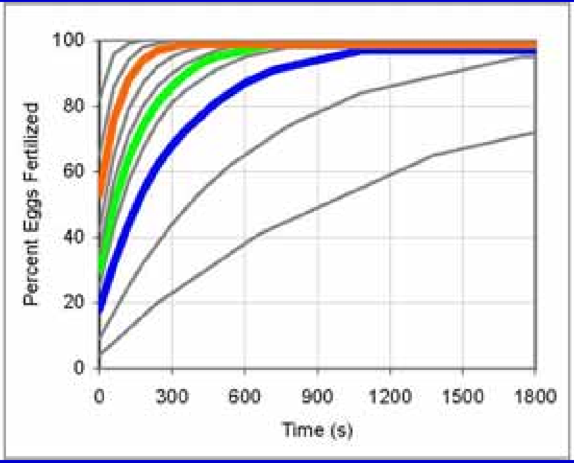
Unfertilized egg conc. = 100 cm^{-3}
Sperm concentration = 1000 cm^{-3}
Fertilization factor = 0.1



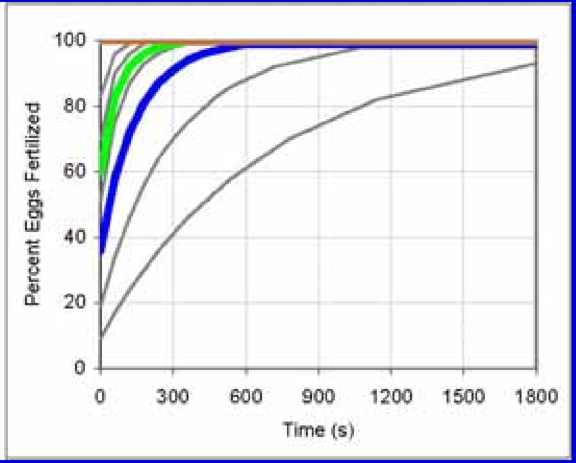
Unfertilized egg conc. = 100 cm^{-3}
Sperm concentration = 1000 cm^{-3}
Fertilization factor = 0.2



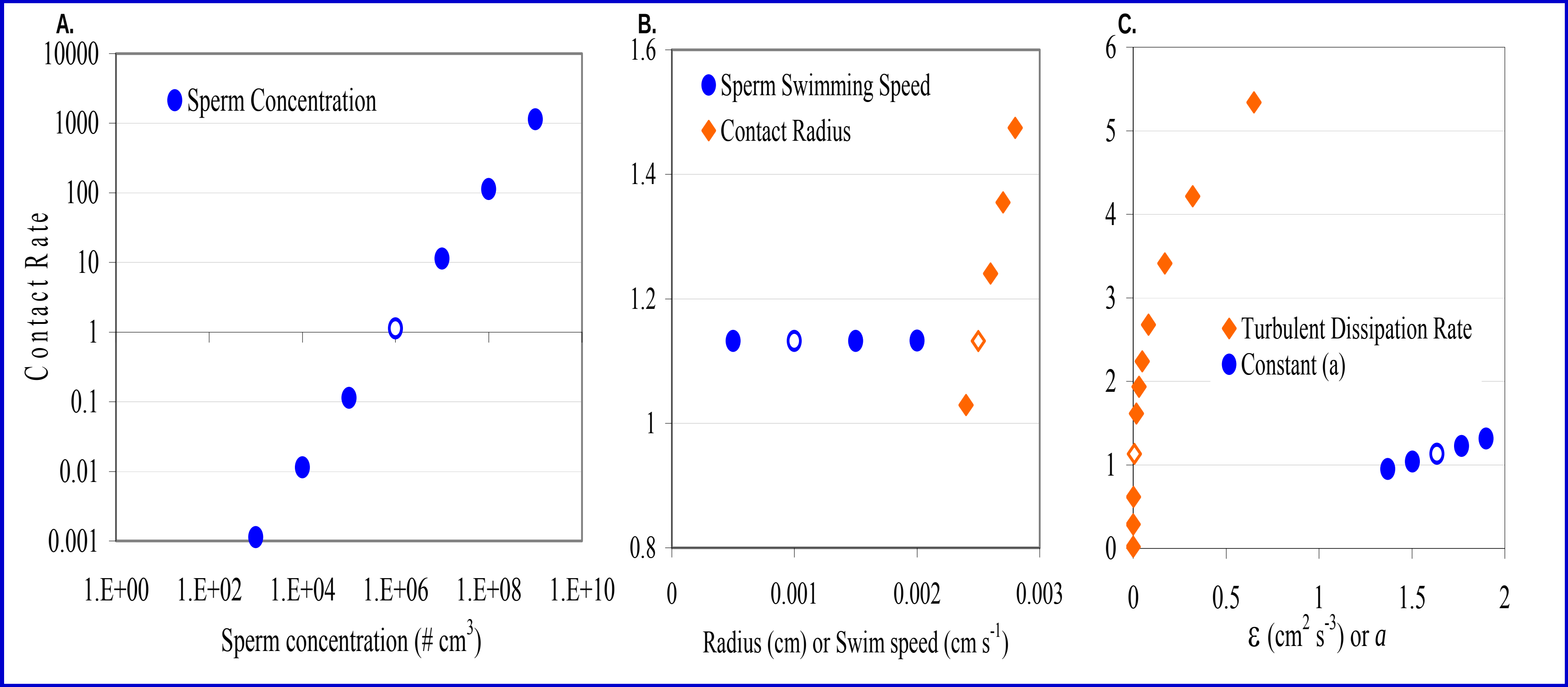
Unfertilized egg conc. = 100 cm^{-3}
Sperm concentration = 1000 cm^{-3}
Fertilization factor = 0.5



Unfertilized egg conc. = 100 cm^{-3}
Sperm concentration = 1000 cm^{-3}
Fertilization factor = 1.0



Effects of sperm concentration, sperm swimming speed, egg size and turbulence



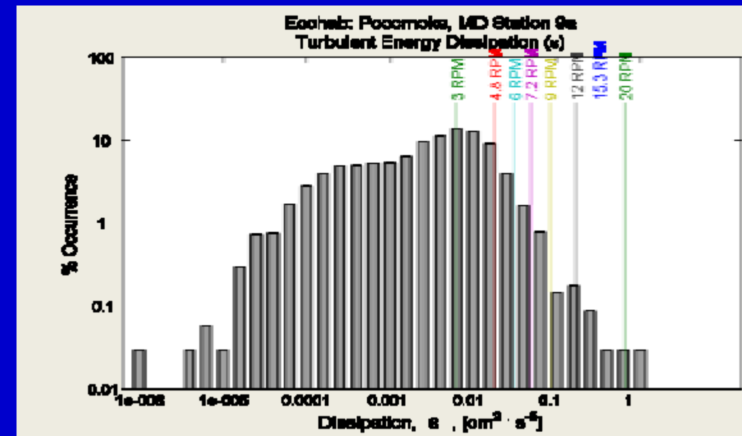
Solutions for contact rates when the following parameters were varied: A) sperm concentration (note the log scale), B) sperm swimming speed and contact (egg) radius, and C) turbulent dissipation rate (e) and the constant *a*. Open symbols indicate ‘base-case’ solutions.

Turbulent mixing

Grid-stirred chambers
Mixing controlled by motor speed.

Chambers constructed by S. Suttles, UNCES Horn Point Laboratory

Turbulent energy dissipation rates 1) measured in Chesapeake Bay and 2) occurring in the grid-stirred chambers with different motor speeds (colored lines).



Field data courtesy of Larry Sanford, UMCES Horn Point Laboratory

Examples of initial release of dye (left) and final distribution of dye (right) in grid chambers.

Turbulent energy dissipation rates (e, $cm^2\ s^{-3}$), average turbulent shear (g, s^{-1}), and Reynolds number at different motor speeds (RPM) predicted for the grid-stirred chambers.

RPM	average ε	average γ	Reynods number
1	0.00067	0.23	0.51
3	0.0070	0.80	1.53
4.8	0.020	1.39	2.45
6	0.034	1.82	3.06
7.2	0.053	2.28	3.67
9	0.090	3.00	4.59
12	0.18	4.29	6.12
15.3	0.34	5.83	7.81
20	0.69	8.20	10.21

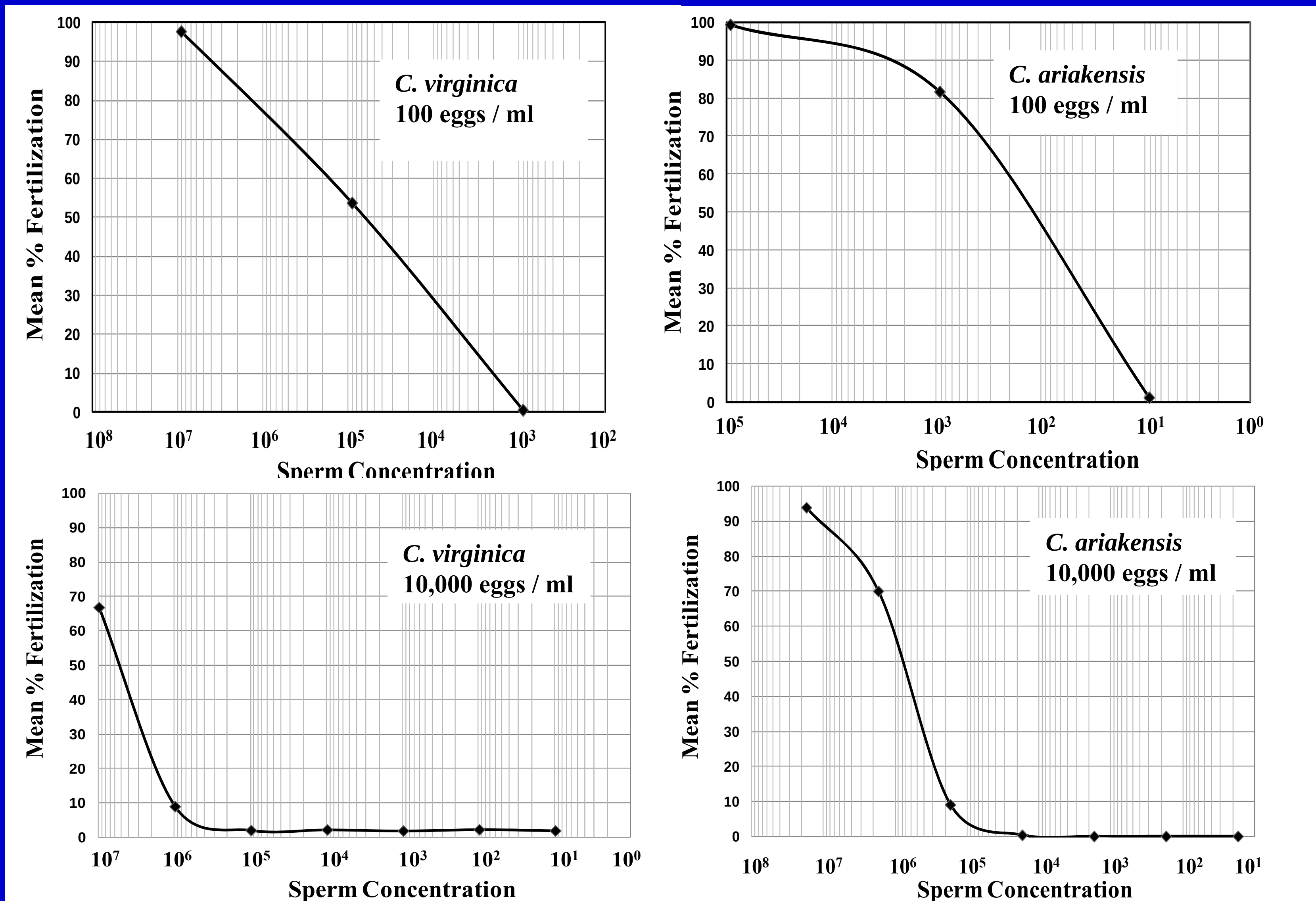
Summary of sperm dilution experiments with species, gamete concentrations, gamete ratios and mean % of eggs fertilized. Values for % fertilized are means of three treatment replicates.

		Gamete Concentration			
Species	**Sperm Dilution**	**Sperm ml^{-1}**	**Eggs ml^{-1}**	**Sperm:Egg**	**Mean % Fertilized**
	10^1	**9.63 x 10^6**	**10^4**	9.63 x10^2	66.67
	10^2	9.63 x 10^5	10^4	9.63 x10^1	8.94
C. virginica	10^3	9.63 x 10^4	10^4	9.63 x10^0	2.06
	10^4	9.63 x 10^3	10^4	9.63 x10^{-1}	2.22
	10^5	9.63 x 10^2	10^4	9.63 x10^{-2}	1.89
	10^6	9.63 x 10^1	10^4	9.63 x10^{-3}	2.28
	10^7	9.63 x 10^0	10^4	9.63 x10^{-4}	1.94
	10^1	1.71 x 10^7	10^4	1.71 x 10^3	93.67
	10^2	1.71 x 10^6	10^4	1.71 x 10^2	69.83
C. ariakensis	10^3	1.71 x 10^5	10^4	1.71 x 10^1	9.00
	10^4	1.71 x 10^4	10^4	1.71 x 10^0	0.33
	10^5	1.71 x 10^3	10^4	1.71 x 10^{-1}	0.00
	10^6	1.71 x 10^2	10^4	1.71 x 10^{-2}	0.00
	10^7	**1.71 x 10^1**	10^4	**1.71 x 10^{-3}**	**0.00**
	10^0	1.46 x 10^6	10^3	**1.46 x 10^3**	92
C. ariakensis	10^2	1.46 x 10^4	10^3	**1.46 x 10^1**	12.5
	104	1.46 x 102	103	1.46 x 10-1	0
C. ariakensis	10^0	9.19 x 10^5	10^2	**9.19 x 10^3**	**99.25**
	10^2	9.19 x 10^3	10^2	**9.19 x 10^1**	81.63
	10^4	9.19 x 10^1	10^2	**9.19 x 10^{-1}**	1.25
C. virginica	10^1	9.16 x 10^5	10^2	**9.16 x 10^3**	97.5
	10^3	9.16 x 10^3	10^2	**9.16 x 10^1**	53.5
	10^5	9.16 x 10^1	10^2	9.16 **x 10^{-1}**	0.33
C. virginica	10^1	3.43 x 10^5	10^3	**3.43 x 10^2**	55.67
	10^3	3.43 x 10^3	10^3	**3.43 x 10^0**	13.50
	10^5	3.43 x 10^1	10^3	**3.43 x 10^{-2}**	0.50
C. virginica	10^1	1.42 x 10^6	10^1	**1.42 x10^5**	90
	10^3	1.42 x 10^4	10^1	1.42 x10^3	94
	10^5	1.42 x 10^2	10^1	1.42 x10^1	66.17

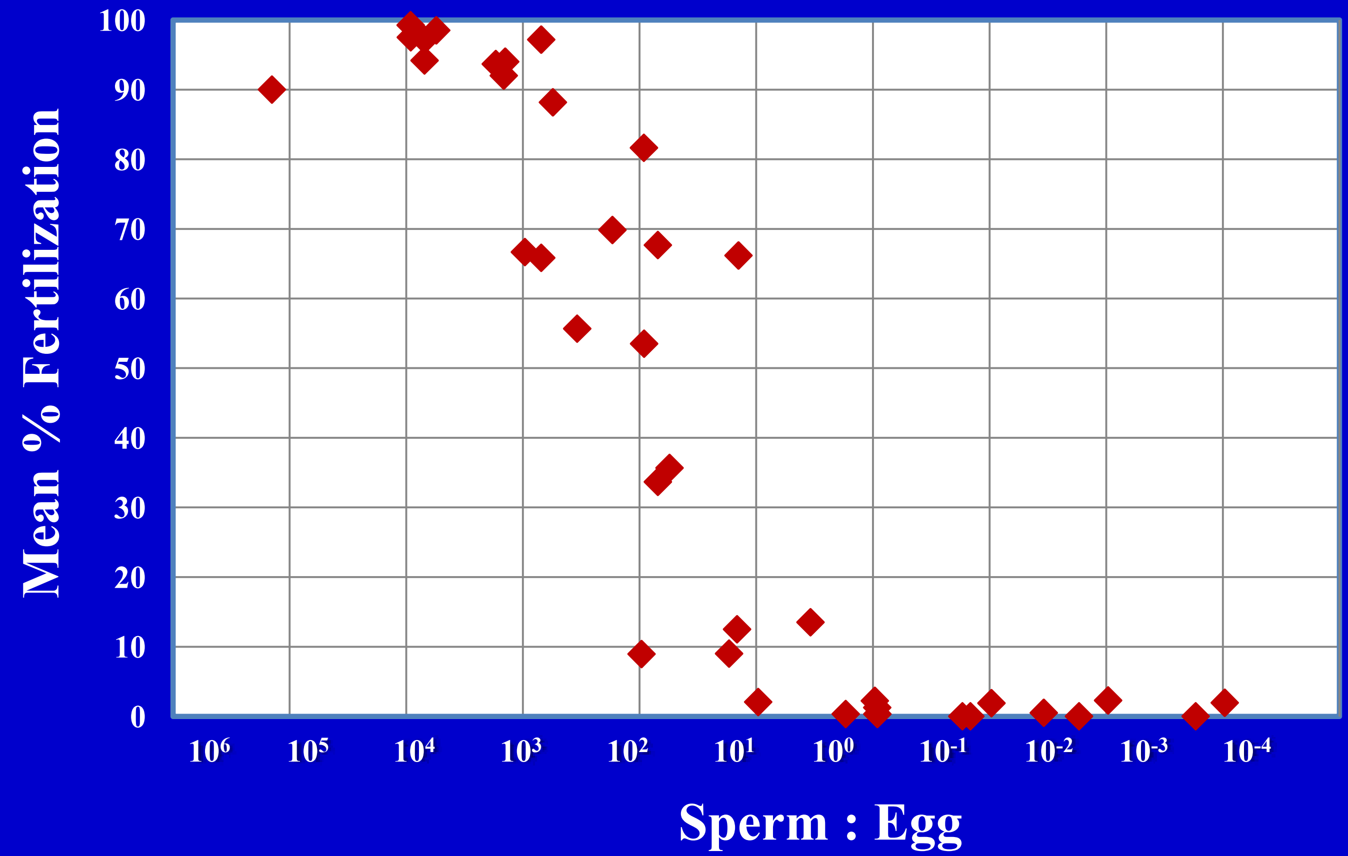
Summary of egg dilution experiments with species, gamete concentrations, gamete ratios and mean % of eggs fertilized. Values for % fertilized are means of three treatment replicate.

		Gamete Concentration			
Species	**Egg Dilution**	**Sperm ml^{-1}**	**Eggs ml^{-1}**	**Sperm:Egg**	**% Fertilized**
C. virginica	**10^3**	**5.54×10^4**	**10^1**	5.54×10^3	**98.50**
	10^2	5.54×10^4	10^2	5.54×10^2	88.17
	10^1	5.54×10^4	**10^3**	**5.54×10^1**	35.67
C. virginica	**10^3**	6.98×10^4	10^1	6.98×10^3	97.17
	10^2	6.98×10^4	10^2	6.98×10^2	97.17
	10^1	6.98×10^4	10^3	6.98×10^1	67.67
C. virginica	**10^3**	**6.98×10^4**	10^1	6.98×10^3	94.17
	10^2	6.98×10^4	10^2	6.98×10^2	65.83
	10^1	6.98×10^4	10^3	6.98×10^1	**33.67**

Examples of mean % eggs fertilized at varying sperm density



Percent fertilization as a function of sperm : egg ratios



Fertilization Efficiency $= f = 0.01e^{(4.3233+31.95/x+0.0286)}$

where x was the ratio of initial sperm and unfertilized egg conc.

Conclusion

Fertilization success declines rapidly at sperm:egg ratios $\leq 10^3$ and is very low at 10^2 . . .
. . . even at high gamete concentrations
. . . and even in well-mixed suspensions w/o dilution.

Implications

♂ produce ~ 10^{10} sperm; ♀ produce ~ 10^{7} eggs
To achieve a sperm:egg ratio of 10^{3} need ~ 1:1 sex ratio.

Natural or "restored" populations with few age classes may have sex ratios which differ substantially from 1:1.

Anthropogenic factors, such as exposure to ED's, that alter oyster sex ratios may have significant effects on the proportion of eggs that are fertilized, thereby dramatically affecting population dynamics.