Award Numbers: NA08NMF4540666 & NA10NMF4540483

Final Report

Developing Tools to Evaluate Spawning and Fertilization Dynamics of the Giant Sea Scallop, *Placopecten magellanicus*

October 12, 2011

Investigators:

Richard Wahle
University of Maine, School of Marine Sciences
Darling Marine Center
193 Clark's Cove Rd
Walpole, Maine 04573

Julien Gaudette
Department of Fisheries and Oceans, Canada
St. Andrews Biological Station

Michael Sieracki
Bigelow Laboratory for Ocean Sciences
180 McKown Point Road
West Boothbay Harbor, ME 04375

Kevin Stokesbury
University of Massachusetts Darmouth
200 Mill Road
Fairhaven, MA 02719-5252

Industry Participant:
Robert Maxwell
Owner: F/V Top Dog; F/V Snoopy II; F/V Sugar Foot
NEFMC Scallop Committee Member
190 North Street
West Creek, NJ 08092

Reporting Period: 04/01/2010 - 06/30/2011

EXECUTIVE SUMMARY

This project addressed NOAA research priorities on scallop biology, abundance and dispersion patterns, and the value of area closures as a fishery management strategy. This multi-institutional collaboration initiated new research into the long-standing question of whether aggregations of adult scallops increase fertilization success, and whether disturbing them may decrease reproductive success.

Our proposal had three objectives: (1) Conduct laboratory experiments to evaluate spawning rates and fertilization kinetics (i.e., dilution and temperature effects on fertilization rates) and to develop flow cytometry technology for gamete and embryo sorting and quantification; (2) Conduct field experiments in a natural flow field to evaluate the rate at which egg fertilization declines with distance from a spawning male; and (3) Examine existing camera survey images to characterize scallop aggregation and dispersion patterns inside and outside fishery closures in New England and Mid-Atlantic scallop beds. All objectives were addressed, however, objective 2 was revised during the project to be conducted in laboratory flume rather than in the field.

Ripe scallops were most responsive to induction cues during the late summer spawning season. During the spawning season scallops either spawned spontaneously or in response to artificial induction by temperature shock and flow. Maximum spawning rates occurred within 1-3 h of the onset of spawning, although low rates of spawning continued for several hours afterward. Maximum male spawning rates were on the order of 10^7 - 10^8 sperm s^{-1} with a cumulative total of approximately 10^{10} to 10^{11} sperm cells released during the spawning event. Maximum female spawning rates were on the order of 25,000 eggs per second, and maximum cumulative eggs spawned was on the order of 9 x 10^7 eggs spawned. These rates will be used to parameterize spawning models.

In sperm dilution series, fertilization rates followed the expected progression of increasing fertilization rates with increasing sperm concentration to a maximum above which the incidence of abnormally dividing cells increased. We observed maximum fertilization rates at sperm concentrations above 10^7 sperm cells ml^{-1}. In our study, the decline in fertilization rate and increased incidence of abnormally divided eggs above sperm concentrations of 10^8 cells ml^{-1} is likely due to polyspermy. We note that fertilization rates were generally lower, and the estimated fertilization maximum in the dilution series fell at a sperm concentration some 100-fold higher, than that observed in a previous study. This suggests the egg suspensions may have contained some non-viable eggs.

Age strongly affected gamete viability. By 8 h post-spawning egg fertilization rates had generally not yet fallen to half the maximum fertilization observed for newly spawned eggs, but by 24 h eggs were no longer viable. Dilution considerably reduces sperm half-life. Sperm at a concentration of 10^7 cells per ml had a half-life of 2h, but when diluted by a factor of ten, half-life fell to about 9 min at 12 °.

Flow cytometry was conducted by Bigelow Laboratory for Ocean Sciences. The FACScan flow cytometer enabled rapid counting and separation of scallop sperm cells from other co-occurring planktonic organisms of similar size. Quantification was possible over the range of sperm

concentrations where sperm limitation and polyspermy is likely to occur. Automated sperm counts by FACScan flow cytometry strongly correlated with manual counts by haemocytometer, although some systematic bias was detected in these counts that remains to be resolved. Nonetheless, the automated method holds great potential to save time in sperm counting. FlowCam Imaging flow cytometry image analysis algorithms were developed to distinguish fertilized from unfertilized eggs. While successful, sufficient uncertainty remains that manual techniques of distinguishing gametes and embryos is still more practical.

Flume experiments were conducted to evaluate flow rate and dosage effects on downstream fertilization rates over a distance of approximately 2 m. Higher sperm dosages and slower flow rates resulted in higher near fertilization rates near the sperm source, whereas flow tended to depress fertilization rates in the near-field while enhancing them downstream. Evidence of polyspermy was only found in the lowest flow and highest sperm dosages.

Assessment of scallop patterns of aggregation were conducted by K. Stokesbury (SMAST) from scallop surveys in mid-Atlantic and Georges Bank suggests that the degree of aggregation is positively related to population density and perhaps harvesting activity. Scallops tend to be aggregated in high density patches in areas where they are more abundant. We hypothesize that harvesters disrupt aggregations by fishing for maximum yield, through the targeting and depletion of high density patches.

The outcome of these studies will provide empirical data to stock assessment scientists in need of a better understanding of the role of depensatory effects in the population dynamics of free-spawners, such as scallops, at low population densities. The project also sets the stage for our second Scallop RSA project. Results will be disseminated to the NEFMC Scallop Committee, the Maine Scallop Advisory Committee and through professional journals.

PURPOSE

Objectives

This multi-investigator project had three main objectives:

Objective 1 - *Spawning and fertilization kinetics and quantification techniques:* Conduct laboratory experiments to **(a)** evaluate sea scallop spawning rate and fertilization response to changes in sperm concentration, temperature, and gamete longevity, **(b)** develop flow cytometry methodology to automate the determination of sperm concentrations, and in distinguishing fertilized from unfertilized eggs.

Objective 2 – *Flume Experiments:* Conduct fertilization experiments in a flume to determine flow velocity effects on downstream fertilization success. (This is a change from our originally proposed objective to conduct field trials.)

Objective 3 – *Patterns of Population Density & Aggregation Inside and Outside Closed Areas:* Examine available camera and dredge survey data to characterize density and degree of aggregation inside and outside closed areas on Georges Bank and Mid-Atlantic scallop grounds.

APPROACH

Objective 1a: Spawning and Fertilization in the Lab

Spawning Rate Experiments

In this experiment we measured the spawning rate of both male and female scallops. Spawning scallops ranged from 98 to 130 mm in shell height. All sea water used in these trials was subject to 5-µm-filtration. Scallops used for spawning trials were held individually in buckets with a measured amount of sea water that was enough to cover the shell. Several methods were employed to induce male and female scallops to spawn. Methods included temperature shock (warming from 12 to 18°C), mechanical stimulation by increase water flow, and serotonin injection, as described by Desrosiers and Dube (1993; 1996).Once spawning began, the time was noted, and scallops were transferred to a new bucket of filtered seawater. Transfers continued every 30 min thereafter until spawning appeared to stop. A 4.5 ml sample was taken from the bucket at the end of each interval. These samples were then fixed with buffered 37% formalin for counting. Sperm was counted by hemocytometer and FACscan flow cytometer and eggs were counted under a dissecting microscope. Spawning rate was calculated as the number of gametes released in the total volume of seawater over the given amount of time.

Sperm Dilution Experiment

In this experiment we prepared ten-fold dilutions of sperm, combined them with eggs and measured the percent of eggs fertilization in each dilution. Seawater used in these experiments was filtered through a 5 um filter and aged in for at least 2 days. Fresh gametes were collected from scallops induced to spawn; eggs were used within 5 h and sperm within 30 min of spawning. A highly concentrated sperm suspension was use to start a series of ten dilutions, each

diluted by a factor of ten from the previous (1 ml of sperm suspension to 9 ml of seawater). Freshly spawned eggs were allowed to settle in a test tube and 0.4 ml of the concentrated eggs (~2000 -5000 eggs) was added to a 4-ml sample of each sperm dilution, as well as to a seawater control with no sperm to evaluate the incidence of false positives. Eggs incubated at 12° C for 4 h and were mixed every 15 minutes during this period. Finally, samples were fixed with 66 ul of buffered 37% formalin.

A sample was taken from the original dilution vessel to determine sperm concentrations by FACScan. A selective sampling was also taken for an independent assessment of sperm counts by haemocytometer for comparison purposes. Up to approximately 300 eggs were examined under a compound microscope as they were encountered along a cross-slide transect. Eggs were assigned to the following developmental stages: (1) normal unfertilized, (2) abnormal undivided (3) fertilized with one polar body, (4) fertilized with two polar bodies, (5) embryo 2-cell, (6) embryo 4-cell, (7) embryo > 4-cell, and (8) abnormal divided. The proportion of fertilized eggs is presented in two ways: (a) as the proportion at or beyond the first polar body stage, and (b) the proportion at or beyond the two cell stage. The small number of abnormal undivided cells was excluded from the calculation. It is possible that the polar bodies may have been hidden in a small fraction of cells as viewed on a microscope slide, but that proportion of should remain constant. This count is therefore taken as a conservative estimate of fertilization. This is why we included cells that had undergone the first embryonic divisions as our second, more certain indicator that fertilization had occurred. However, a fraction of cells that were fertilized may not have gone on to divide. In subsequent fertilization experiments we used the proportion of cells at or beyond the two-cell stage as our index of fertilization.

Gamete Viability Experiments

Egg Viability: To evaluate how egg viability changed over time after spawning, we introduced fresh sperm to eggs of increasing age. Eggs were introduced to freshly spawned sperm suspensions at 1, 2, 4, 8, 24 and 48 h after they were spawned. Eggs aged in seawater at 12° C. At each time interval, fresh sperm was obtained and sperm dilutions were prepared as above. As in the dilution series experiment, 400 ul of eggs were added to 4 ml of sperm dilutions. Sperm dilutions 2, 3 and 4 were used to avoid the extremes in the dilution series where fertilization is either minimal or abnormal. Eggs were incubated, fixed and scored as above.

Sperm Viability: In this experiment the viability of sperm was determined by fertilizing fresh eggs with sperm aged 1, 15, 30, 60, and 120 minutes. Sperm dilutions were prepared and a blank control and allowed to age at 12° C. Three intermediate sperm dilutions (2, 3 and 4) were used from the standard ten-fold dilution series to evaluate further evaluate the interaction between dilution and age. At each time 400 ul of freshly spawned eggs possible were added to 4 ml of the aged sperm dilution.

Objective 1b: Developing Flow Cytometry Methodology

Sperm counts

Sperm were counted by FACScan located at the J.J. MacIsaac Facility for Aquatic Cytometry at Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME. Samples were run in

fluorescence trigger mode using FL1, green fluorescence based on DNA staining using PicoGREEN (Invitrogen/Molecular Probes, Inc.). Highly concentrated samples were diluted as necessary for accurate counting and the dilution factor recorded. The FACScan displays cytogram plots of particle size and light backscatter. The technician selects a region on the plot corresponding to the particles of interest – in this case sperm cells – in order exclude to count them to the exclusion of other particles.

Egg counts and distinguishing unfertilized from fertilized eggs

The imaging flow cytometer (FlowCAM, Fluid Imaging Technologies) was used to determine egg concentrations, egg size distributions, and discriminate fertilized from unfertilized eggs. The imaging software algorithm to distinguish fertilized from unfertilized eggs was developed during the spring/summer of 2010.

Objective 2: Flume Experiments

In 2010, 28 fertilization assays were conducted in the flume at the Darling Marine Center to evaluate flow and sperm dose effects on eggs positioned varying distances up and downstream from the sperm source. In this experiment fertilization rates at varied distances from a sperm source along a flume were determined under different flow regimes and with different volumes of sperm. A 2 meter long flume was used with different flow rates (no flow, low flow (~3 cm/sec), and high flow (4 cm/sec)). A glass pipette was used to spread approximately 10-15 ml unfertilized eggs over screen-covered platforms placed on the bottom of the flume at varying distances from the sperm source. Eggs settled in and were retained by the mesh, but were otherwise exposed to the overlying flow, hence simulating the roughness of fine sand seabed. Sperm was released 53 cm downstream from the flow inlet, and egg platforms were placed at six distances upstream and downstream from the sperm release point (-20, 0, 20, 50, 100, and 170 cm). Concentrated sperm was released from a syringe in one of two quantities (10 ml) or (60 ml) simulating two different spawning volumes over a period of 20-30 s. After 20 minutes eggs were collected from each platform, rinsed, and allowed to settle in filtered seawater at 12° C. Incubation, fixing and scoring protocols were as above.

Objective 3: Patterns of Population Density & Aggregation Inside and Outside Closed Areas

Existing SMAST (Stokesbury) scallop survey data were used to compare densities and aggregation indices of scallops inside and outside the Georges Bank and Mid-Atlantic closed area at 12 different locations.

We examined video survey data collected by the SMAST industry cooperative scallop survey on Georges Bank and in the Mid-Atlantic over fourteen research cruises; seven between 28 April and 30 June 2008 and seven between 27 April and 24 June 2009. In 2008 we surveyed 923 stations on Georges Bank and 932 stations in the Mid-Atlantic, covering an area of approximately 8,307 km^2 and 8,388 km^2, respectively (Figure 1). In 2009 we surveyed 899 stations on Georges Bank and 927 stations in the Mid-Atlantic, covering an area of approximately 8,091 km^2 and 8,343 km^2, respectively (Figure 2). Station locations in both areas and both years were separated by 5.6 km (3.0 nm).

Figure 1. Station locations in 2008, separated by 5.6 km.

Figure 2. Station locations in 2009, separated by 5.6 km.

Scallop population surveys were conducted using the SMAST Video Survey Pyramid deployed from a commercial scallop vessel (Figure 3). The pyramid supports three live-feed underwater video cameras (Stokesbury 2002, Stokesbury et al. 2004) and one high-resolution (10.1 mega pixels) digital still camera (DSC). The video cameras are configured such that two downward looking cameras view 3.24 m^2 and 0.78 m^2 (nested within the 3.24 m^2 view) quadrats, one downward looking still camera views 1.29 m^2 and one side-looking camera provides a view across the quadrat parallel to the sea bed (Figure 3).

Four quadrats were sampled at each station, increasing the sample area to 12.94 m^2. Video was recorded onto S-VHS and DVD and the time, depth, sediment type, number of scallops, latitude and longitude were recorded at each station. After each survey the video was reviewed in the laboratory and a still image of each quadrat was digitized. The field data were verified and the shell height (mm) of each scallop was measured in the still image using Image Pro Plus® software. Within each quadrat, scallops and associated macroinvertebrates and fish were counted and the substrate was verified (Stokesbury 2002).

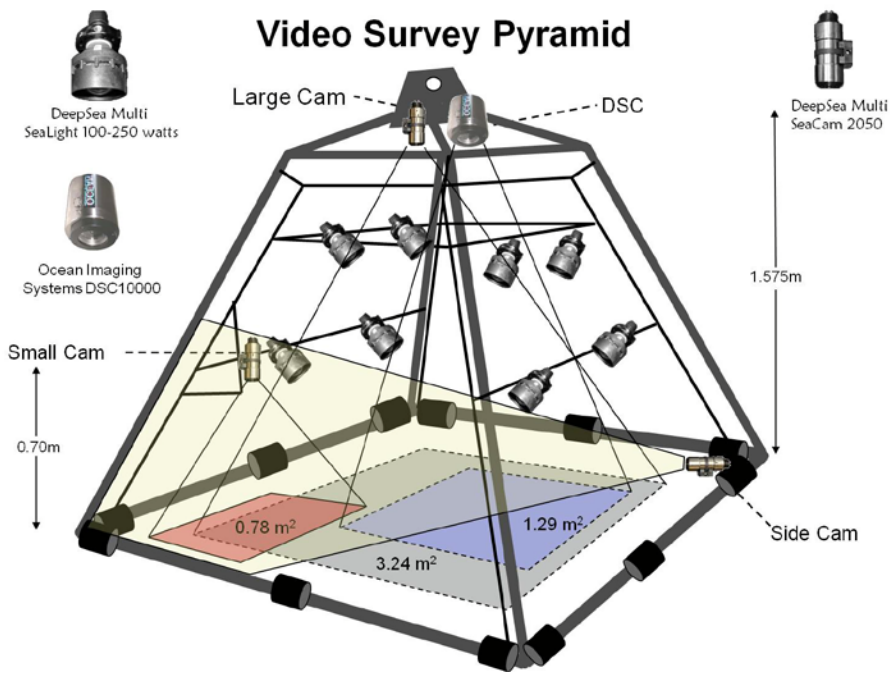

Figure 3. SMAST Video Survey Pyramid with large, small, and high resolution digital still camera quadrats shown in gray, red, and blue, respectively.

Stations were plotted by latitude and longitude and classified as open or closed to fishing. Mean scallop density and standard error was calculated in open and closed areas for each camera using equations for a two-stage sampling design (Cochran 1977):
The mean of the total sample is:

(1) $$\bar{\bar{x}} = \sum_{i=1}^{n} \left(\frac{\bar{x}_i}{n} \right)$$

where:
n = primary sample units (stations)
$\bar{x}_i$ = sample mean per element (quadrat) in primary unit i (stations)
$\bar{\bar{x}}$ = the mean over the two-stages

The standard error of this mean is:

(2) $$S.E.(\bar{x}) = \sqrt{\frac{1}{n}(s^2)}$$

where:
$s^2 = \sum^{n} (\bar{x}_i - \bar{\bar{x}})^2 / (n-1)$ = variance among primary unit (stations) means.

According to Cochran (1977, p. 279) and Krebs (1999, p. 297) this simplified version of the two-stage variance is possible when the sampling fraction n/N is small. This is the case for the SMAST survey, where hundreds of m^2 are sampled compared with millions of m^2 in the study area.

To be most relevant to sexually mature scallops, only scallop with shell height greater than 60 mm were analyzed. This shell height was selected based on gonad maturation; scallops smaller than 60 mm do not fully empty their gonadal follicles while those over 60 mm do (Leslie-Anne Davidson MSc. 1998) For each camera and year, scallop measurements were sorted by open and closed areas. The percentage of scallops greater than 60 mm was calculated for each area and applied to the respective total density and standard error, giving a density estimate of scallops greater than 60 mm. Since we were unable to assume equal variances, Welch's approximate t-test (Zar 1999) was used to compare density estimates of scallops between open and closed areas for the same camera and year.

To assess degree of aggregation, we compared the quadrat frequencies of mature scallops to expected Poisson and negative binomial distributions, in addition to calculating the variance-to-mean ratio and Morisita's index of dispersion. Georges Bank and Mid-Atlantic scallop counts and measurements were obtained for each camera and year, and classified as open or closed to fishing. Scallop measurements were used to create a table of quadrat counts of scallops greater than 60 mm.

These observed frequencies of mature scallops were compared to expected Poisson and negative binomial distributions using a chi-square analysis at the station level (Krebs 1999). Entire stations were removed from the analysis when one or more quadrat value was 'null.' Expected frequencies were grouped according to Cochran's rule so that < 20% had a value < 5. To correct for continuity when df = 1 we used the Yates correction (Zar 1999). Variance-to-mean ratio and Morisita's index of dispersion were also calculated using the above observed frequencies of scallops per station (Krebs 1999).

Project Management

Lead investigator, R. Wahle oversaw the entire project. Collaborating harvester, Robert Maxwell raised funds for this project through revenues from scallop harvesting. Laboratory fertilization kinetics and flume experiments were developed and executed by R. Wahle, J. Gaudette, Research Associate, Charlene Bergeron and graduate students, all at UMaine's Darling Marine Center. All flow cytometry work was done by M. Sieracki and N. Poulton at J.J. MacIsaac Facility for Aquatic Cytometry, Bigelow Laboratory for Ocean Sciences. Scallop survey data were collected by. K. Stokesbury (SMAST) and analyzed by graduate student, Jonathan Carey.

FINDINGS

Objective 1a: Spawning and Fertilization in the Lab

Spawning Rate Experiments

Spawning rate varied considerably among the eight males and six females for which samples have been processed to date (Figures 4, 5). Samples from additional spawning males are still being processed; data will be forthcoming. In general, once spawning began, in both males and females the rate of spawning tended to peak within the first 3 h and diminished gradually over the next several hours. Some individual completed most of the spawning within 1 h, whereas others continued to release small amounts of gametes for more than 10 h.

Attempts to induce males to spawn in early summer, before they were fully competent to spawn, were only successful with serotonin injection. These scallops (#6-11 in Fig. 4) produced meager amounts of sperm over several hours compared to those spawning later in the summer when gonads were riper (#22, 25,31,32 in Fig. 4). At that time scallops either spawned spontaneously or in response to temperature shock and flow. In these scallops maximum sperm release rates were on the order of 10^7 - 10^8 sperm s^{-1}. These rates were not sustained for more than an hour (e.g., scallop #31) and little additional sperm was released after the first hour, for a total of approximately 10^{10} to 10^{11} sperm cells released during the spawning event. In contrast, the one late season scallop (#22) that was induced with serotonin sustained high rates of gamete release for several hours.

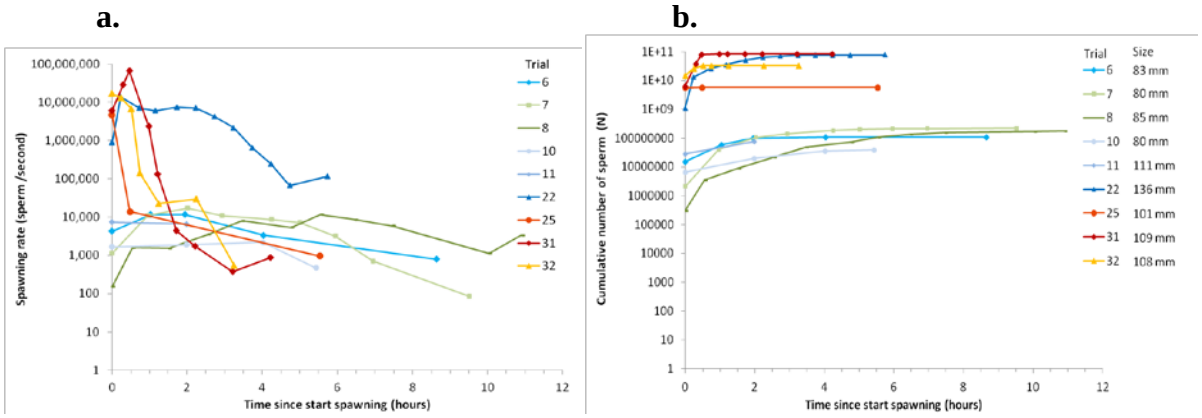

Figure 4. Male scallop spawning. Time course of (a) spawning rate, and (b) cumulative sperm cells spawned for eight male scallops (note log scale). Scallops #6-11 were induced in early summer by serotonin after attempts with temperature shock and flow were unsuccessful. Scallop 22 was induced with serotonin late in the summer; scallops 25, 31, 32 were late summer inductions by temperature shock and flow. Samples from additional spawnings are forthcoming. Scallop shell height (mm) given in panel (b).

Female spawning rate and total number of eggs spawned varied dramatically (Fig. 5). Spawning usually came in bursts and the rate typically peaked within the first 3 h, however some scallops continued to release gametes sporadically for over 10 h. Maximum spawning rates were on the order of 25,000 eggs per second (#29), but these spawning pulses lasted < 1 h, and little additional spawning occurred afterward. Maximum cumulative eggs spawned among those observed was on the order of 80-90 million eggs spawned (#28).

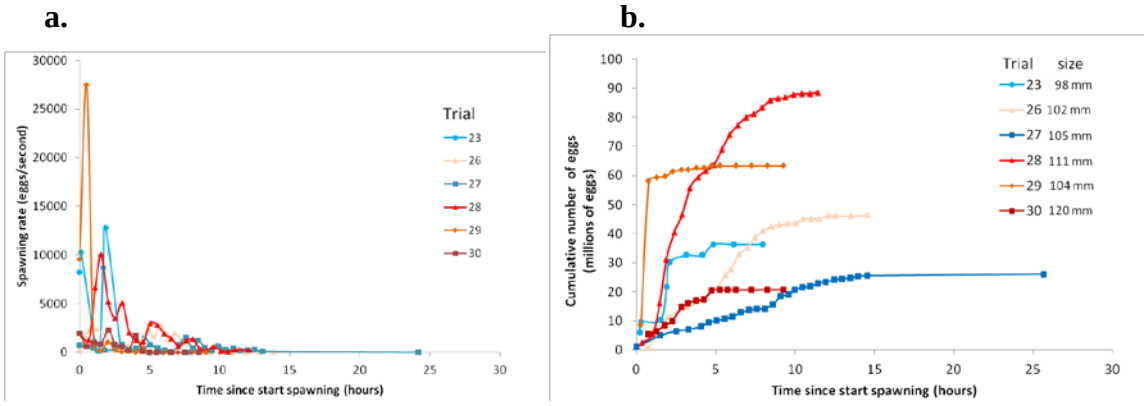

Figure 5. Female scallop spawning. Time course of (a) spawning rate, and (b) cumulative number of eggs spawned by six female scallops observed. Scallops #23 and 27 began spawning naturally without induction; the remainder were induced to spawn by temperature shock and flow. Scallop shell height (mm) given in panel (b).

Although we used spawning induction methods developed in the past, we did not systematically assess the most effective method to induce spawning. Spontaneous spawning occurred often during the spawning season. For example, between 24 July and 20 August 2009 at least 13 scallops spawned without induction, and on 12 August 2009 nine males spawned spontaneously in the laboratory. We found that serotonin induction worked more consistently in males than females with ripe gonads. Serotonin induced spawning in males ahead of the natural spawning season, but in limited quantities. Temperature shock alone produced no spawning in males or females, but water flow coupled with thermal shock had better results (Desrosiers and Dube 1993). Spawning typically occurred within 6 h of induction. With flow alone, 8% of the males 18% of the females spawned. Using these two methods together, 60% of males and 50% of females spawned. Systematic assessment of the efficacy of different spawning methods would be worthwhile.

Sperm Dilution Experiment

Fertilization rates followed the expected progression of increasing fertilization rates with increasing sperm concentration to a maximum above which the incidence of abnormally dividing cells increased (Figures 6). We observed maximum fertilization rates at sperm concentrations above 10^7 sperm cells ml^{-1}. The increased incidence of abnormally divided eggs above sperm concentrations of 10^8 cells ml^{-1} is likely due to polyspermy.

We note that our fertilization maximum falls at a sperm concentration some 100-fold higher than that observed by Desrosier et al. (1996). This discrepancy may be because of the different criteria used to define fertilization. Desrosier et al. (1996) used a DNA stain to distinguish monospermic and polyspermic fertilizations, whereas we used the presence of polar bodies and cell divisions as our criteria. We will continue to examine this issue in our current RSA project.

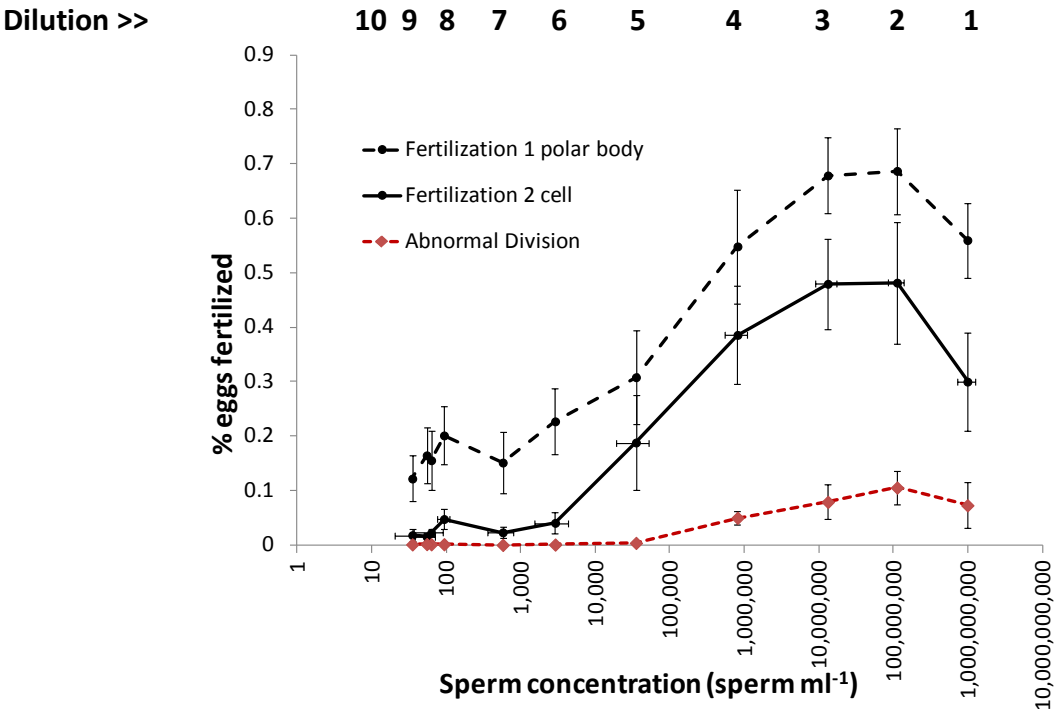

Figure 6. Indices of fertilization over a series of ten 10-fold sperm dilutions (N=7 trials). Dilution #1 is the highest sperm concentration measured. Dilution number at top of figure.

Gamete Viability Experiments

Egg Viability: Age strongly affected egg viability. In all sperm dilutions egg viability fell over the course of 24 h, but by 8 h fertilization rates had generally not yet fallen to half the maximum fertilization observed for newly spawned eggs (Fig. 7). However, by 24 h eggs were no longer viable.

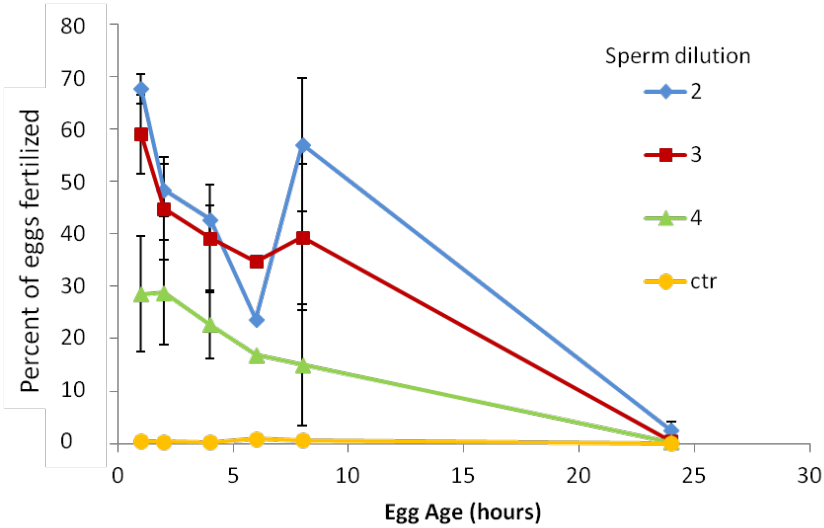

Figure 7. Age effects on egg viability in different sperm dilutions (N= 3 trials per age treatment, only one trial at 6 hours). See Fig. 6 for approximate sperm concentrations corresponding to the dilution number. Trials were conducted at 12°C.

Sperm Viability: The effect of age on sperm viability depended on the degree of dilution (Fig. 8). These results permit us to calculate the half-life of sperm at different dilutions. At the most dilute of the three treatments (#4 ~10^6 sperm ml^{-1}), sperm half life was on the order of 9 min. At the intermediate dilution (#3 ~10^7 sperm ml^{-1}) sperm half-life increased to 2 h, and in the most concentrated suspension (#2 ~10^8 sperm ml^{-1}) sperm half-life far exceeded the 2-h duration of the experiment. All trials contucted at 12°C.

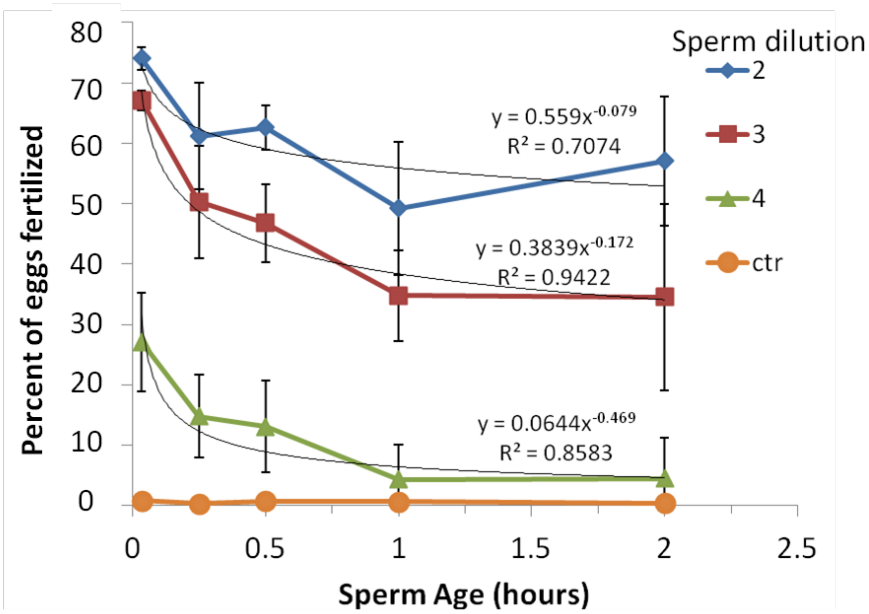

Figure 8. Age effects on sperm viability in three sperm dilutions with a ten-fold difference in sperm concentration (N=4 trials per age treatment). See Fig. 6 for approximate sperm concentrations corresponding to the dilution number. Trials conducted at 12°C. Fitted power functions permit calculation of sperm half-life stated in text.

Objective 1b: Developing Flow Cytometry Methodology

Sperm counts

The FACScan flow cytometer enabled rapid counting and separation of scallop sperm cells from other co-occurring planktonic organisms of similar size (Fig. 9). Quantification was possible over the range of sperm concentrations where sperm limitation and polyspermy is likely to occur (Figs. 10, 11, respectively). FACScan counts were comparable to the calculated sperm concentration throughout the dilution series. FACScan flow cytometer and hemocytometer counts were highly correlated (r^2=0.99), however manual counts from the hemocytometer were consistently higher than those from the FACScan by a factor of 0.6 (Fig. 11). It is possible we could be over-estimating sperm counts with the haemocytometer if non-sperm particles are being counted as sperm. On the other hand, it is possible the FACScan undercounts sperm if not all sperm are being scored; this could happen if some cells are not taking up the DNA stain, for example. Failure to stain could happen if the DNA in some sperm cells had disintegrated. The FACScan makes sperm counts considerably less labor-intensive and is capable of detecting sperm at very low concentrations. It therefore has great potential for continued use in sperm counts, and we will continue to work to reconcile these differences.

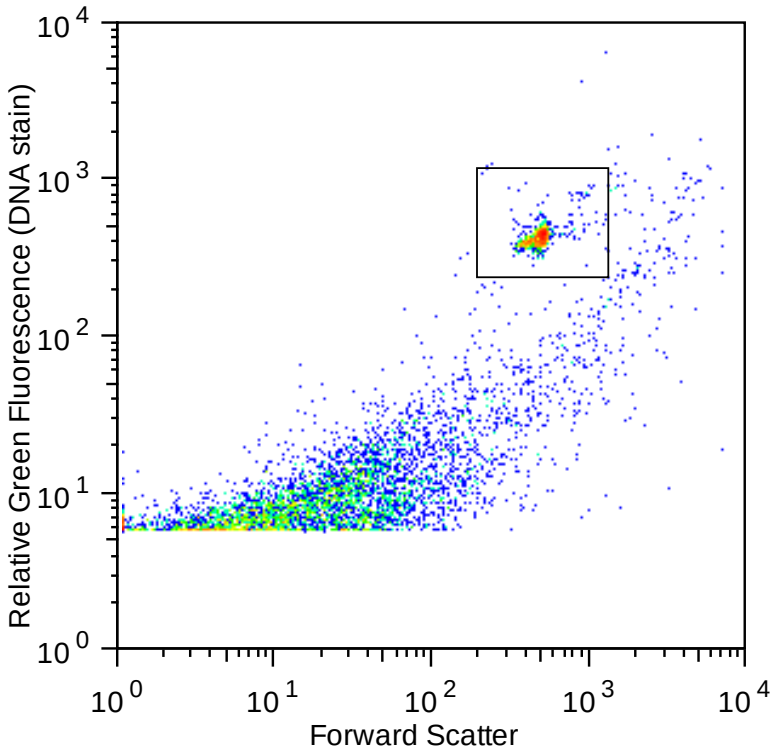

Figure 9. FACScan (BD BioSciences) flow cytometric dot plot of a DNA stained sperm sample/time-point. Relative Green Fluorescence indicates the DNA stain used to identify the sperm (PicoGreen, Invitrogen Inc.). Forward Scatter indicates cell size. The region in the box is the region of interest (ROI) used for enumerating the sperm within each sample. This dot plot represents sample 7.6 (time-point 2 hours) from the egg viability experiment and was stained without sample dilution.

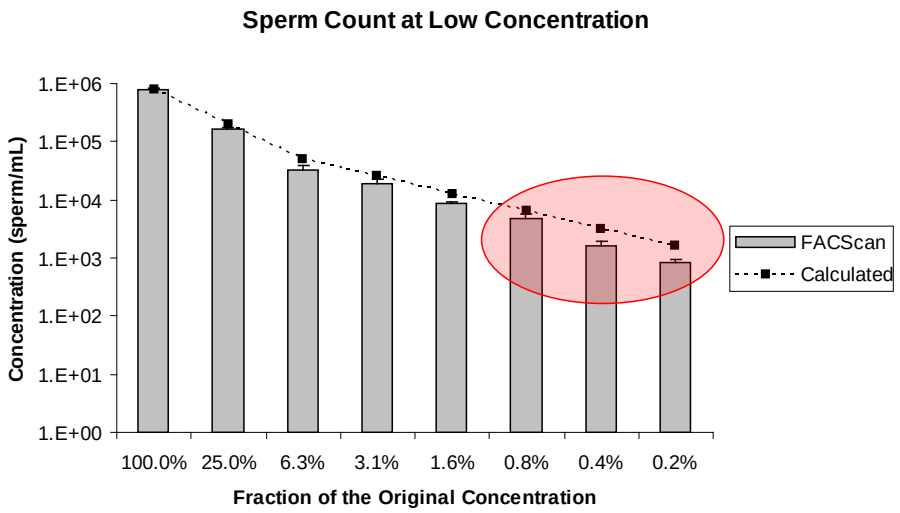

Figure 10. Sperm count determined by the FACScan at low concentration versus the calculated count from the dilution. Red area corresponded to concentration where sperm is anticipated to be seriously limiting fertilization (i.e. < 20% fertilization) based on Desrosiers et al (1996). The goal of this run was to determine whether the FACScan could count sperm at very low sperm concentrations. The calculated count for the dilutions was taken from the starting FACScan count at 100%.

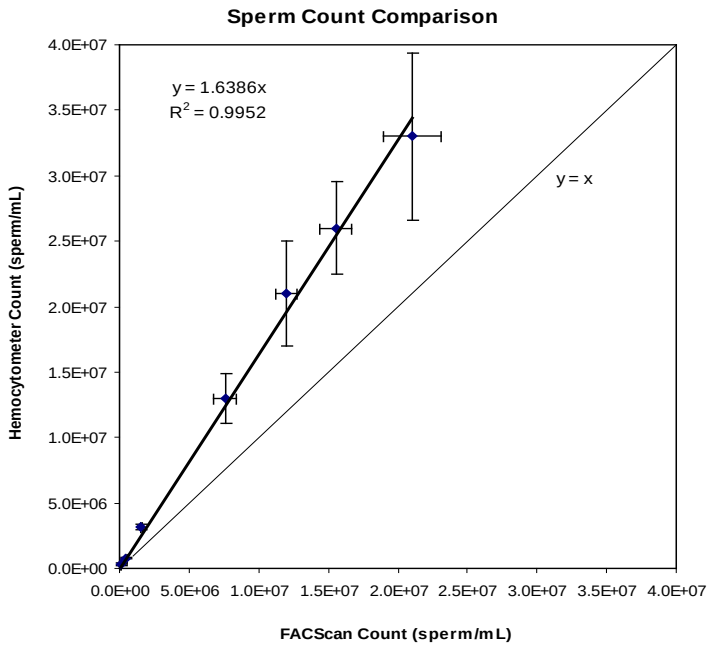

Figure 5. Comparison of sperm average concentration (± 1 SE) determine by haemocytometer (n=4) and FACScan (n=3).

Egg counts and distinguishing unfertilized from fertilized eggs

Unlike sperm, scallop eggs were too large to count by the FACScan flow cytometry. Nor did a Coulter Counter prove to be a viable alternative. Clumped and deformed eggs were not counted correctly in the Coulter Counter. Therefore, all female spawning rate experiment trials were counted manually using a 1 ml volume chambered counting slide.

The imaging flow cytometer (FlowCAM, Fluid Imaging Technologies) is able to determine egg concentrations, egg size distributions, and discriminate fertilized from unfertilized eggs. Egg size ranged from 60- 90 μm. An image analysis algorithm was developed to distinguish fertilized from unfertilized eggs. The program sorted cells into three categories: unfertilized eggs, those with one polar body, and embryos in the two cell stage (Fig. 12). Using the FlowCAM to count or categorize embryos, however, would likely not be cost effective at this stage because the trained human eye can still make these distinctions with greater certainty and more quickly.

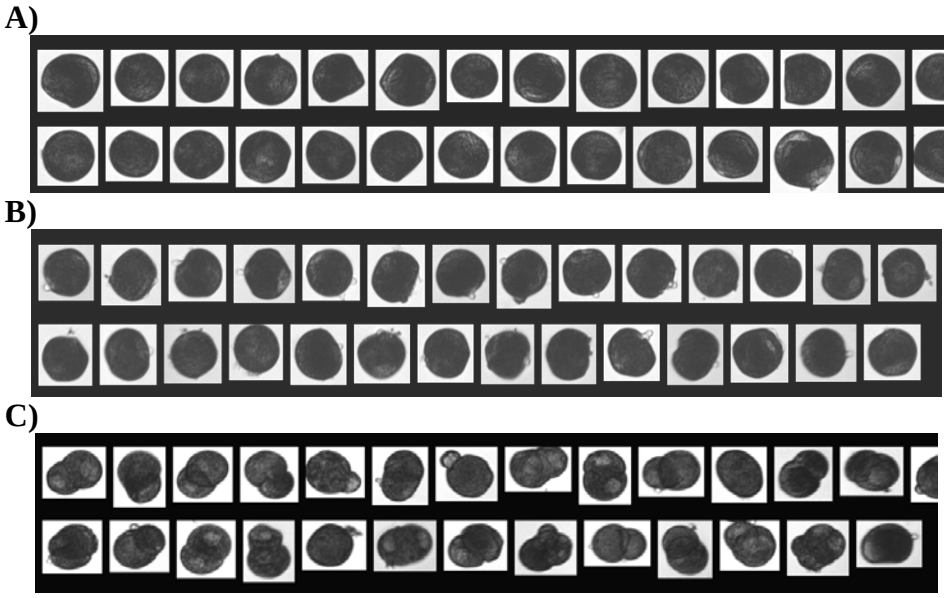

Figure 6. Scallop egg images captured by the FlowCAM and sorted into their development stages: (a) Unfertilized eggs, (b) Fertilized eggs with polar bodies, and (c) Two-cell eggs.

Other methods were also explored to distinguish fertilized from unfertilized eggs manually. A protocol using DNA stain with fluorescence microscopy was developed and refined. Without fluorescent staining, the criterion we use for a fertilized egg is the presence of two polar bodies. Fluorescent staining significantly improved our detection of fertilized eggs by approximately 6% (paired t-test, t-stat = -3.48, $p = 0.008$) (Figure 13). By regression analysis of the residuals, we determined that improvement in detection was constant regardless of the percent of eggs fertilized ($p=0.47$). Because this analysis still represents a relatively small sample, we will continue to refine the estimated correction, and will apply it to future fertilization estimates determined by light microscopy.

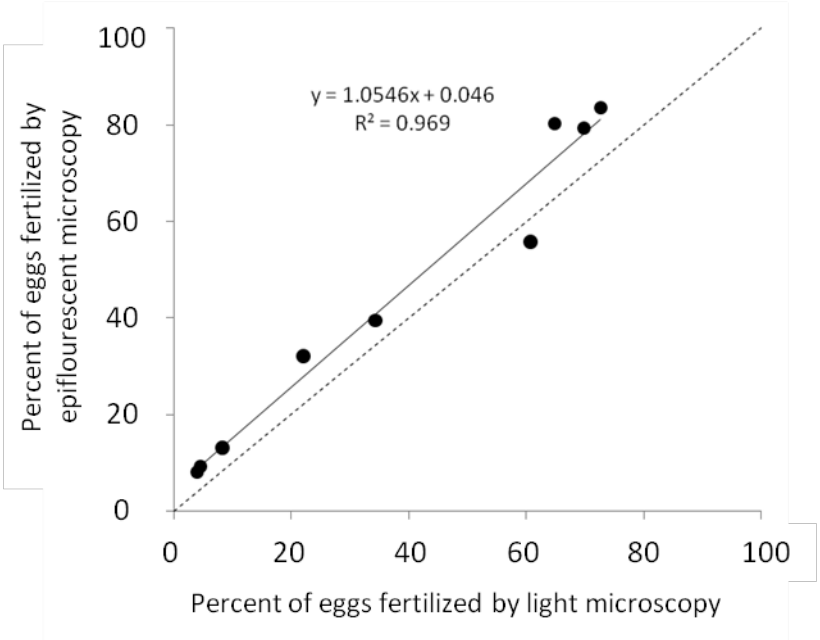

Figure 7. Relationship between estimates of fertilization measured by epiflourescent microscopy of DNA-stained scallop eggs and of unstained eggs by light microscopy. The one-to-one line (dashed line) is shown for reference.

Objective 2: Flume Experiments

Under all three flow rates, the higher dose of sperm resulted in higher fertilization rates, but most dramatically in the no-flow treatment (compare Fig. 14a to 14b). Distance effects were most evident in the no- and low-flow states. At the low sperm dosage and under no flow, fertilization was greatest within 20 cm of the sperm source and almost no fertilization occurred beyond 100 cm. Under flow upstream fertilization was depressed and elevated downstream. At the high sperm dose and no flow, fertilization rates were elevated dramatically over the entire area of the flume; under both levels of flow, although far fewer eggs were fertilized, fertilization rates remained most elevated near the sperm source.

Abnormally developing embryos only occurred under the high sperm dose treatment, and then only in the low and no-flow conditions. Under these conditions the percentage of abnormal embryos was greatest at locations nearest the sperm source. Taken together, the results suggest that polyspermic conditions can occur within 0.5 m of a spawning male under low or no flow conditions (Fig. 15).

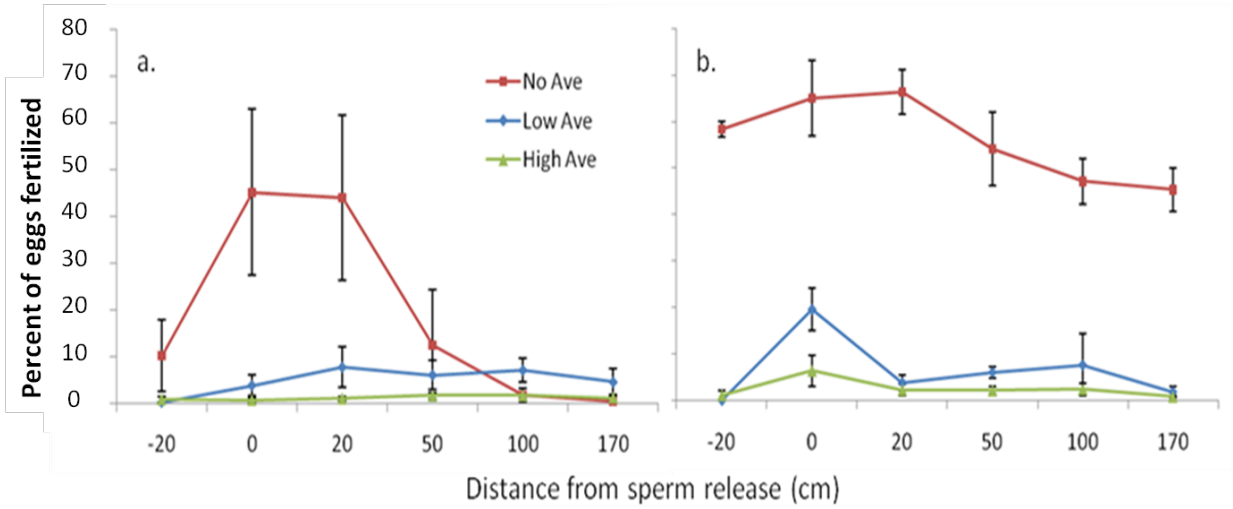

Figure 8. Mean fertilization (±1SE) at distance from sperm release point (Distance 0) under two doses of concentrated sperm (a) Low (10 ml) and (b) high (60 ml) and different flow regimes: No Flow, Low Flow (3 cm s^{-1}), and High Flow (4 cm s^{-1}).

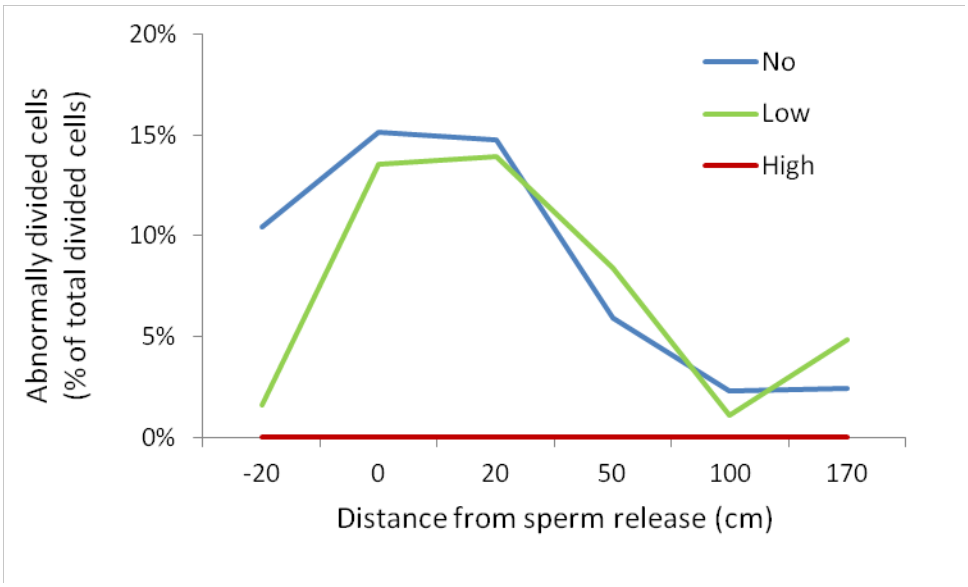

Figure 9. Percent of abnormally divided cells as a proportion of total divided cells by flow treatment and distance from sperm release point for high sperm concentration treatment (60 ml of sperm). No abnormal division was detected in the low sperm concentration (10 ml).

Objective 3: Patterns of Population Density & Aggregation Inside and Outside Closed Areas

For all cameras, years and areas the density of mature scallops appears to be consistently higher in areas closed to fishing than in those open to fishing (Table 1). Results of Welch's approximate *t*-test, however, indicate that these differences in density between open and closed areas are only significant in the Mid-Atlantic (Table 2).

Table 1. Density, standard error and CV% of scallops > 60 mm shell height by year, area, camera and presence of fishing. Areas are Georges Bank (GB) and Mid-Atlantic (MA).

Year	Area	Camera	Fishing	Stations	> 60 mm	Density	SE	CV
2008	GB	Large	Open	521	79%	0.068	0.008	11.9%
2008	GB	Large	Closed	411	83%	0.091	0.011	12.6%
2008	GB	Small	Open	497	80%	0.088	0.015	17.0%
2008	GB	Small	Closed	385	75%	0.142	0.025	17.4%
2008	GB	DSC	Open	437	51%	0.073	0.014	15.6%
2008	GB	DSC	Closed	300	57%	0.117	0.022	16.3%
2008	MA	Large	Open	516	69%	0.052	0.009	17.5%
2008	MA	Large	Closed	416	83%	0.262	0.030	11.5%
2008	MA	Small	Open	508	57%	0.058	0.008	14.2%
2008	MA	Small	Closed	405	80%	0.258	0.031	11.9%
2008	MA	DSC	Open	487	45%	0.043	0.007	15.4%
2008	MA	DSC	Closed	389	69%	0.234	0.027	11.7%
2009	GB	Large	Open	523	74%	0.091	0.017	18.2%
2009	GB	Large	Closed	376	87%	0.178	0.023	13.0%
2009	GB	Small	Open	529	74%	0.104	0.019	18.1%
2009	GB	Small	Closed	413	88%	0.135	0.020	14.9%
2009	GB	DSC	Open	504	57%	0.099	0.018	18.4%
2009	GB	DSC	Closed	366	82%	0.158	0.023	14.6%
2009	MA	Large	Open	501	96%	0.081	0.006	7.8%
2009	MA	Large	Closed	426	95%	0.183	0.015	8.3%
2009	MA	Small	Open	501	83%	0.072	0.007	10.3%
2009	MA	Small	Closed	427	90%	0.140	0.007	9.8%
2009	MA	DSC	Open	454	83%	0.081	0.007	8.7%
2009	MA	DSC	Closed	405	87%	0.157	0.015	9.6%

Table 2. Results of Welch's approximate *t*-test comparing density of spawning scallops between open and closed areas for each year, area and camera.

Year	Area	Camera	*t*'	df	ρ	Mean Diff
2008	GB	Large	0.51	771	0.306	0.023
2008	GB	Small	2.34	647	0.010	0.054
2008	GB	DSC	1.29	511	0.100	0.044
2008	MA	Large	2.06	492	0.020	0.210
2008	MA	Small	7.83	462	< 0.001	0.200
2008	MA	DSC	5.27	432	< 0.001	0.192
2009	GB	Large	0.94	720	0.173	0.087
2009	GB	Small	1.40	908	0.081	0.031
2009	GB	DSC	1.54	749	0.062	0.059
2009	MA	Large	1.91	571	0.028	0.102
2009	MA	Small	5.43	666	< 0.001	0.068
2009	MA	DSC	3.52	577	< 0.001	0.076

Observed frequencies were significantly different from expected frequencies of a Poisson distribution, indicating that the distribution of scallops was not random in any areas or years (Table 3). At the 5% level of significance, 18 of 24 tests revealed no statistical difference from a negative binomial distribution, suggesting that scallops are, in general, aggregated (Table 4). Three sets of data, highlighted in yellow, were not robust enough to meet the requirements of Cochran's rule as > 20% of expected frequencies had values < 5.

For each year, area and camera, the variance to mean ratio is consistently higher in closed areas than in open areas, indicating a higher level of aggregation of scallops > 60 mm in areas closed to fishing (Table 5). One exception to this is observed in samples from Georges Bank in 2009. This can be explained by a recruitment event observed in the open area of the Great South Channel in 2008. By the time of our survey in 2009 a large percentage of these scallops had grown to sizes greater than 60 mm. Morisita's Index of Dispersion shows a similar trend, where values are higher (more aggregated) in areas closed to fishing, with the exception of Georges Bank in 2009 (Table 6).

Taken together this analysis suggests that the degree of aggregation is positively related to population density and perhaps harvesting activity (Fig. 16). That is, scallops tend to be aggregated in high density patches in areas where they are more abundant. One hypothesis to examine more closely is whether harvesting activity not only depletes scallops, but disrupts aggregations either through harvesters targeting high density patches or by actively spreading them out. This analysis also did not take into consideration size structure and biomass. It would be valuable in the future to do compare at gametes production in closed and open areas by integrating fecundity potential.

Table 3. Results of a χ^2 analysis comparing observed frequencies of scallop counts per station to expected frequencies of a Poisson distribution.

Year	Area	Camera	Open/Closed	n	χ^2	df	ρ
2008	GB	Large	Open	521	203.88	2	0.000
2008	GB	Large	Closed	411	186.69	2	0.000
2008	GB	Small	Open	497	36.08	1	0.000
2008	GB	Small	Closed	385	47.10	1	0.000
2008	GB	DSC	Open	421	48.28	1	0.000
2008	GB	DSC	Closed	287	50.17	1	0.000
2008	MA	Large	Open	516	56.85	2	0.000
2008	MA	Large	Closed	416	625.00	6	0.000
2008	MA	Small	Open	508	4.42	1	0.036
2008	MA	Small	Closed	405	78.79	2	0.000
2008	MA	DSC	Open	487	23.71	1	0.000
2008	MA	DSC	Closed	389	128.66	3	0.000
2009	GB	Large	Open	523	641.09	4	0.000
2009	GB	Large	Closed	376	904.39	5	0.000
2009	GB	Small	Open	529	53.17	1	0.000
2009	GB	Small	Closed	413	53.44	1	0.000
2009	GB	DSC	Open	504	92.07	1	0.000
2009	GB	DSC	Closed	366	132.80	2	0.000
2009	MA	Large	Open	501	222.48	3	0.000
2009	MA	Large	Closed	426	360.09	6	0.000
2009	MA	Small	Open	501	3.63	1	0.057
2009	MA	Small	Closed	427	34.50	1	0.000
2009	MA	DSC	Open	454	23.06	1	0.000
2009	MA	DSC	Closed	405	46.52	2	0.000

Table 4. Results of a χ^2 analysis comparing observed frequencies of scallop counts per station to expected frequencies of a negative binomial distribution for each year, area and camera. Yellow highlighting indicates data did not conform to Cochran's rule.

Year	Area	Camera	Open/Closed	n	k	χ^2	df	ρ
2008	GB	Large	Open	521	0.181	4.84	5	0.435
2008	GB	Large	Closed	411	0.173	7.29	5	0.200
2008	GB	Small	Open	497	0.153	5.14	2	0.077
2008	GB	Small	Closed	385	0.185	0.40	3	0.941
2008	GB	DSC	Open	421	0.119	3.37	3	0.338
2008	GB	DSC	Closed	287	0.176	4.76	3	0.190
2008	MA	Large	Open	516	0.402	8.45	3	0.038
2008	MA	Large	Closed	416	0.385	16.36	11	0.128
2008	MA	Small	Open	508	0.656	***0.85**	1	0.357
2008	MA	Small	Closed	405	0.268	7.40	4	0.116
2008	MA	DSC	Open	487	0.300	***0.15**	1	**0.703**
2008	MA	DSC	Closed	389	0.264	8.94	6	0.177
2009	GB	Large	Open	523	0.131	21.42	8	0.006
2009	GB	Large	Closed	376	0.181	20.51	9	0.015
2009	GB	Small	Open	529	0.09958	6.39	3	0.094
2009	GB	Small	Closed	413	0.11869	0.44	2	0.802
2009	GB	DSC	Open	504	0.101945	8.95	4	0.062
2009	GB	DSC	Closed	366	0.131438	11.31	4	0.023
2009	MA	Large	Open	501	0.493	1.73	5	0.885
2009	MA	Large	Closed	426	0.491	19.76	9	0.019
2009	MA	Small	Open	501	1.047867	***0.18**	1	0.674
2009	MA	Small	Closed	427	0.315742	0.99	1	0.320
2009	MA	DSC	Open	454	0.549825	0.66	2	0.719
2009	MA	DSC	Closed	405	0.399743	13.36	3	0.004

Table 5. Variance to mean ratio comparing degree of aggregation between open and closed areas for each year, area and camera.

Year	Area	Camera	Open/Closed	mean	s^2	s^2/mean
2008	GB	Large	Open	0.620	2.751	4.438
2008	GB	Large	Closed	0.732	4.011	5.477
2008	GB	Small	Open	0.262	0.786	3.006
2008	GB	Small	Closed	0.369	1.244	3.372
2008	GB	DSC	Open	0.302	1.102	3.652
2008	GB	DSC	Closed	0.456	1.815	3.977
2008	MA	Large	Open	0.448	1.176	2.627
2008	MA	Large	Closed	2.337	36.441	15.596
2008	MA	Small	Open	0.157	0.196	1.245
2008	MA	Small	Closed	0.605	2.665	4.406
2008	MA	DSC	Open	0.177	0.273	1.548
2008	MA	DSC	Closed	0.902	7.439	8.244
2009	GB	Large	Open	1.063	14.170	13.329
2009	GB	Large	Closed	1.766	19.497	11.041
2009	GB	Small	Open	0.253	0.936	3.694
2009	GB	Small	Closed	0.269	0.799	2.973
2009	GB	DSC	Open	0.419	2.443	5.835
2009	GB	DSC	Closed	0.574	3.160	5.508
2009	MA	Large	Open	0.838	2.284	2.724
2009	MA	Large	Closed	1.991	12.212	6.135
2009	MA	Small	Open	0.160	0.182	1.143
2009	MA	Small	Closed	0.234	0.372	1.590
2009	MA	DSC	Open	0.348	0.563	1.618
2009	MA	DSC	Closed	0.635	1.836	2.894

Table 6. Morisita's Index of Dispersion comparing degree of aggregation between open and closed areas for each year, area and camera.

Year	Area	Camera	Open/Closed	n	Morisita's Index
2008	GB	Large	Open	521	6.55
2008	GB	Large	Closed	411	7.12
2008	GB	Small	Open	497	8.71
2008	GB	Small	Closed	385	7.46
2008	GB	DSC	Open	421	9.84
2008	GB	DSC	Closed	287	7.55
2008	MA	Large	Open	516	4.64
2008	MA	Large	Closed	416	7.24
2008	MA	Small	Open	508	2.57
2008	MA	Small	Closed	405	6.64
2008	MA	DSC	Open	487	4.13
2008	MA	DSC	Closed	389	9.03
2009	GB	Large	Open	523	12.60
2009	GB	Large	Closed	376	6.68
2009	GB	Small	Open	529	11.69
2009	GB	Small	Closed	413	8.39
2009	GB	DSC	Open	504	12.58
2009	GB	DSC	Closed	366	8.87
2009	MA	Large	Open	501	3.06
2009	MA	Large	Closed	426	3.58
2009	MA	Small	Open	501	1.90
2009	MA	Small	Closed	427	3.54
2009	MA	DSC	Open	454	2.78
2009	MA	DSC	Closed	405	3.99

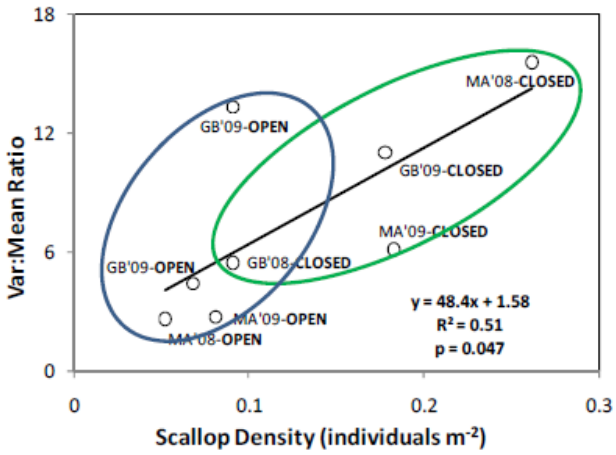

Figure 10. Relationship between population density of sexually mature scallops (>60 mm shell height) and an index of aggregation (variance:mean ratio). Data from mid-Atlantic (MA) and Georges Bank (GB) closed and open areas from surveys conducted in 2008 and 2009 by Stokesbury. Fitted regression statistics suggest the degree of aggregation is positively linked to population density and perhaps to harvesting activity. Ovals encompass areas closed (green) and open to fishing (blue).

Conclusions

These experiments build on previous laboratory experiments (Desrosiers and Dube 1993, Desrosiers et al. 1996) to provide a more complete picture of spawning rate and fertilization dynamics. Estimates of spawning rates, gamete longevity, dosage and flow effects are needed for subsequent calculation of gamete diffusion and modeling of fertilization dynamics at the population level (e.g., Denny and Shibata 1989). Further, automated techniques developed here to count and sort gametes with flow cytometry will dramatically speed sample processing in future studies.

Although dock-side *in situ* trials were not conducted, flume experiment results allowed us to evaluate flow, distance and sperm dosage effects efficiently, and provide the platform from which to launch our second NOAA Scallop RSA project. The flume will continue to be used to work out the methodology to evaluate ambient sperm loads on natural scallop beds in future projects, just as we have done for similar studies with the green sea urchin in the past (Wahle and Peckham 1999, Wahle and Gilbert 2000, Gaudette et al. 2006).

Problems Encountered

Early progress on the project was hampered by several unavoidable delays. First, although the project was awarded in June 2008, it was not possible to begin work that summer because harvesting needed to be conducted to generate funds for the proposed work. The project was under way by spring, 2009. Second, two of the investigators transferred to new positions during the peak of the summer scallop spawning season and into the autumn of 2009, forcing us to postpone some of the proposed work until the summer of 2010. Nonetheless, all objectives were eventually addressed.

Several methodological issues came up that we will continue to reconcile during our second RSA project. These issues are briefly summarized.

Spawning trials: Some spawning trial samples have not yet been completely processed, and will be completed within the next month. We learned that obtaining gametes dependably can be a problem at times, and additional research on the efficacy of different spawning induction techniques would be useful.

Dilution series experiment: Our fertilization maximum (Fig. 6) occurred at a sperm concentration some 100-fold higher than that observed by Desrosier et al. (1996). This discrepancy may be related to the use of different criteria to define a fertilized egg. It is also possible that not all eggs were viable within the suspention. We will continue to examine this issue because it is important to the development of fertilization kinetic models.

Flow cytometry methodology: We found a systematic difference between manual haemocytometer and automated FACScan sperm counts (Fig. 11). We will continue to address this discrepancy with Bigelow Laboratory's Flow Cytometry Laboratory.

Additional Work

In 2010 a new NOAA Scallop RSA project was funded, entitled "Developing Tools to Evaluate Spawning & Fertilization Dynamics of the Giant Sea Scallop – Phase II: Field trials in experimental populations." This collaboration addresses the long-standing question of whether methodologies can be developed to model and directly assess spawning and fertilization dynamics in wild scallop beds (e.g., Fig. 16). Lead investigator, R. Wahle, and industry collaborator, R. Maxwell, are joined in this project by Dr. Peter Jumars (University of Maine).

The investigators aim to advance beyond the current research by addressing two objectives: (1) Develop a two dimensional spatial model predicting the concentration of sperm and effective range of fertilization in a sperm plume at varying distances from a source population of spawning males at different population densities under scenarios of synchronous and asynchronous spawning; and (2) Conduct a sampling time series over experimental populations of scallops to quantify sperm loads and fertilization rates in order to (a) test model predictions from Objective 1 regarding the spatial pattern of sperm concentration and fertilization, (b) advance development of methodology to assess the reproductive performance of scallop populations, (c) determine the effect of a ten-fold difference population density on the spatial extent of the sperm plume; and (d) assess potential biases in fertilization estimates introduced by the fertilization assay technique. The outcome of these studies will provide empirical data to stock assessment scientists in need of a better understanding of the role of depensatory effects in the population dynamics of free-spawners, such as scallops, at low population densities. The project will also set the stage for future field studies of fertilization ecology to be conducted directly on Northwest Atlantic scallop beds. Results will be disseminated to the NEFMC Scallop Committee. Educational opportunities are made available in the project through a Graduate Research Assistantship and an Undergraduate Internship at the University of Maine.

Sperm advection-diffusion model:

At the core of the predictions we would like to make about the spatial pattern of sperm plumes from an aggregation is a simple two-dimensional advection-diffusion model (Denny 1988) that has been use in other fertilization studies to model sperm plumes (e.g., Levitan et al. 1992, Levitan and Young 1995, Claerboudt 1999, Metaxas et al. 2002). The model predicts the concentration (c) of particles at positions down- and cross-stream (x, y, respectively) from a sperm source:

$$c(x,y) = \left(\frac{Q\bar{u}}{2\pi\alpha_y \alpha_z u_*^{\,2} x^2} \right) * e^{-\left(\frac{y^2 \bar{u}^2}{2\alpha_y^{\,2} u_*^{\,2} x^2} \right)}$$

given knowledge of spawning rate (Q), average flow velocity ($\bar{u}$), coefficients of particle diffusion in seawater (α), and friction velocity (u_*), an indicator of shear stress on the sea bed. The sperm plume for an individual male, spawning at a rate of 10^8 sperm s^{-1} under a realistic range of current speeds is predicted to fall below effective fertilization levels (~10^4 sperm s^{-1}) within 1 m of the source (Fig. 16). We therefore are interested in the effect of surrounding males on the proportion of eggs fertilized.

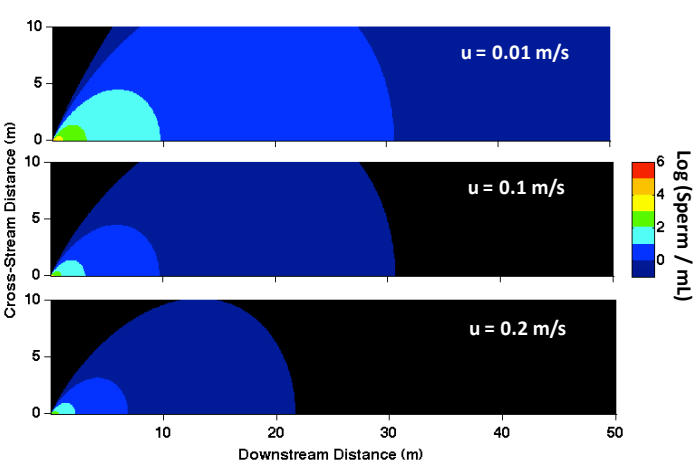

Figure 16. Numerical simulations of a downstream and cross-stream pattern of sperm concentrations in a sperm plume from an individual scallop spawning 10^8 sperm s^{-1} under three different flow speeds.

In our second phase of this work we propose to model sperm plumes from aggregations of males at different densities, and under different assumptions of flow velocity and spawning synchrony. Predictions will be made of sperm concentrations and fertilization rates achieved under these conditions at fixed locations within, and at a distance from, the aggregation using the approach described in Claereboudt (1999).

Applications

Outputs

The primary output is empirical data on spawning and fertilization dynamics that will be used to parameterize fertilization kinetic and population models.

Management Outcomes

The finding may have important implications for the management of scallop populations in closed and open areas. To date, however, no management outcomes have been proposed or implemented from this work.

EVALUATION

Attainment /Modification of Goals and Objectives

All objectives were generally achieved in this project. The main modification was to bring the proposed field work under objective 2 into a laboratory flume context. This worked to the advantage of the project because we could simulate flow conditions with more control and better set the stage for future field experiments in phase II of our research.

Dissemination of Results

Results of this study will be shared with NOAA and New England Fishery Management Council scallop fishery managers. The lead investigator is a candidate for Maine's Scallop Advisory Committee where he aims to assist in developing Maine's scallop fishery management plan. Findings will also be presented by the co-investigators and graduate students at fishery and academic meetings, such as the American Fisheries Society Meeting and the Benthic Ecology Meeting. Segments of the findings will be published in scientific journals.

FINANCIALS

Harvests of scallops generated the revenue to support this research. We requested $183,270 (27%) for the science and $494,448 (72%) in compensatory harvesting for the industry collaborator, for a total of $677,718. The scallop TAC requested was 88,015 lbs from Elephant Trunk Access Area (ETAA). Table 7 reports the harvests and revenues generated for this project.

Table 7. Scallop landings and revenues reported by vessel, trip, scallop size grade, and purchacer to generate funds for this project. Scallop sizes U/10 and 10/20 denote under 10 meats and 10-20 meats per pound, respectively.

Pounds	Size	Purchase	Price	Total	Boat
1398	10/20	V V	7.50	$ 10,485.00	Snoopy
3337	U/10	V V	7.80	$ 26,028.60	Snoopy
4735				$ 36,513.60	
1198	U/10	Agger	8.00	$ 9,584.00	Top Dog
549	10/20	V V	7.50	$ 4,117.50	Top Dog
3123	U/10	V V	7.80	$ 24,359.40	Top Dog
4870				$ 38,060.90	
150	10/20	BH	8.20	$ 1,230.00	Sugar Foot
439	U/10	Agger	8.00	$ 3,512.00	Sugar Foot
687	10/20	Agger	7.50	$ 5,152.50	Sugar Foot
2208	10/20	V V	7.50	$ 16,560.00	Sugar Foot
6399	U/10	V V	7.80	$ 49,912.20	Sugar Foot
9883				$ 76,366.70	
384	10/20	Agger	7.50	$ 2,880.00	Top Dog
2103	U/10	Agger	8.00	$ 16,824.00	
2517	U/10	V V	7.60	$ 19,129.20	
5004				$ 38,833.20	
1599	U10	Agger	8.0	$ 12,792.00	Snoopy
594	10/20	Agger	7.5	$ 4,455.00	
2522	10/20	V V	7.3	$ 18,410.60	
4715				$ 35,657.60	
200	10/20	BH	8.0	$ 1,600.00	Sugar Foot
2052	10/20	V V	7.3	$ 14,979.60	
5239	U/10	V V	7.6	$ 39,816.40	
7491				$ 56,396.00	
292	10/20	V V	7.30	$ 2,131.60	Top Dog
3208	U10	V V	7.60	$ 24,380.80	
3500				$ 26,512.40	
506	10/20	Agger	7.50	$ 3,795.00	Sugar Foot
1522	U10	Agger	8.00	$ 12,176.00	
1227	10/20	V V	7.30	$ 8,957.10	
4232	U10	V V	7.60	$ 32,163.20	
7487				$ 57,091.30	
1512	10/20	V V	7.30	$ 11,037.60	Snoopy
1489	10/20	V V	7.60	$ 11,316.40	
3001				$ 22,354.00	
1474	U12	Agger	8.25	$ 12,160.50	Sugar Foot
1202	10/20	Agger	7.50	$ 9,015.00	
4296	U12	VV	8.10	$ 34,797.60	
1569	10/20	VV	7.60	$ 11,924.40	
98	20/30	VV	7.30	$ 715.40	
8639				$ 68,612.90	
1049	10/20	Agger	7.5	$ 7,867.50	Sugar Foot
746	U10	Agger	9.35	$ 6,975.10	
509	20/30	VV	7.4	$ 3,766.60	
1834	10/20	VV	7.55	$ 13,846.70	
2143	U10	VV	9.3	$ 19,929.90	
6281				$ 52,385.80	
				$ 508,784.40	subtotal
550	U/10	Agger	9.25	$ 5,087.50	Sugar Foot
431	10/20	Agger	7.6	$ 3,275.60	
3949	U/10	VV	9.2	$ 36,330.80	
1600	10/20	VV	8.2	$ 13,120.00	
6530				$ 57,813.90	
627	U/10	Agger	9.00	$ 5,643.00	Sugar Foot
246	10/20	Agger	7.50	$ 1,845.00	
5057	U/10	VV	8.10	$ 40,961.70	
2809	10/20	VV	7.30	$ 20,505.70	
8739				$ 68,955.40	
4445	U/10	VV	9.40	$ 41,783.00	Sugar Foot
1457	10/20	VV	7.40	$ 10,781.80	
514	U/10	Agger	9.60	$ 4,934.40	
309	10/20	Agger	7.50	$ 2,317.50	
414	U/10	Agger	9.60	$ 3,974.40	
7139				$ 63,791.10	
Total Pounds				**Total $**	**Avg Price/lb**
88014				**$ 699,344.80**	**$ 7.95**

LITERATURE CITED

Claereboudt, M. 1999. Fertilization success in spatially distributed populations of benthic free-spawners: a simulation model. Ecological Modelling **121**:221-233.

Cochran, W.G. 1977. Sampling Techniques, 3rd Edition. John Wiley & Sons. New York. 330pp.

Davidson, L. A. 1998. Maturation gonadique du pétoncle géant *Placopecten magellanicus*, (Gmelin) du stade juvénile au stade adulte. Biologie. Moncton, Université de Moncton. M.Sc. Thesis, 112pp.

Denny, M. W. 1988. Biology and the mechanics of the wave-swept environment. Princeton University Press, Princeton, N.J.

Denny, M. W., and M. F. Shibata. 1989. Consequences of surf-zone turbulence for settlement and external fertilization. American Naturalist **134**:859-889.

Desrosiers, R. R. and F. Dube. 1993. Flowing seawater as an inducer of spawning in the sea scallop, *Placopecten magellanicus* (Gmelin, 1791). Journal of Shellfish Research **12**:263-265.

Desrosiers, R. R., J. Desilets, and F. Dube. 1996. Early developmental events following fertilization in the giant scallop *Placopecten magellanicus.* Canadian Journal of Fisheries and Aquatic Sciences **53**:1382-1392.

Gaudette, J., R. A. Wahle, and J. H. Himmelman. 2006. Spawning events in small and large populations of the green sea urchin *Strongylocentrotus droebachiensis* as recorded using fertilization assays. Limnology and Oceanography **51**:1485-1496.

Krebs, C.J. 1999. Ecological Methodology, 2nd Edition. Addison-Wesley Educational Publishers, Inc. Menlo Park, CA. 620pp.

Levitan, D. R., M. A. Sewell, and F.-S. Chia. 1992. How distribution and abundance influence fertilization success in the sea urchin *Strongylocentrotus franciscanus*. Ecology **73**:248-254.

Levitan, D. R., and C. M. Young. 1995. Reproductive success in large populations: Empirical measures and theoretical predictions of fertilization in the sea biscuit *Clypeaster rosaceus*. Journal of Experimental Marine Biology and Ecology **190**:221-241.

Metaxas, A., R. E. Scheibling, and C. M. Young. 2002. Estimating fertilization success in marine benthic invertebrates: a case study with the tropical sea star *Oreaster reticulates.* Marine Ecology Progress Series **226**:87–101.

Stokesbury, K. D. E. 2002. Estimation of sea scallop abundance in closed areas of Georges Bank, USA. Transactions of the American Fisheries Society **131**:1081-1092.

Stokesbury, K. D. E., B. P. Harris, M. C. Marino, and J. I. Nogueira. 2004. Estimation of sea scallop abundance using a video survey in off-shore US waters. Journal of Shellfish Research **23**:33-40.

Wahle, R. A., and A. E. Gilbert. 2002. Detecting and quantifying male sea urchin spawning with time-integrated fertilization assays. Marine Biology **140**:375-382.

Wahle, R. A., and S. H. Peckham. 1999. Density-related reproductive trade-offs in the green sea urchin, *Strongylocentrotus droebachiensis*. Marine Biology **134**:127-137.

Zar, J.H. 1999. Biostatistical Analysis, 4th Edition. Prentice Hall. Upper Saddle River, NJ. 663pp.