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Measuring scallop fertilization success in the field: chamber design and tests

Skylar R. Bayer*, Richard A. Wahle, Peter A. Jumars, Damian C. Brady

Darling Marine Center, University of Maine, 193 Clark's Cove Road, Walpole, ME 04573, USA

ABSTRACT: We developed and tested a method to field test fertilization success, measured as the proportion of eggs fertilized, in the giant sea scallop *Placopecten magellanicus*, a commercially valuable and sedentary broadcast spawner in the northwest Atlantic. A laboratory sperm dilution series determined maximum fertilization success expected in the field and assessed gamete longevity. We also developed and flume-tested a fertilization chamber to assess ambient water-column sperm loads near field populations. We then conducted a series of dockside field trials using these chambers over the course of the late summer spawning season in coastal Maine, USA. Chambers were deployed among manipulated populations of scallops to assess effects of local spawner abundance and location on time-integrate fertilization success. In dilution-series experiments, maximum fertilization success occurred at sperm concentrations $>10^7$ sperm ml^{-1}. Egg longevity fell to zero between 8 and 24 h at ambient temperatures of 12°C, and sperm half-life shortened from 2 h to 9 min with a 10-fold dilution from a sperm concentration of 10^7 cells ml^{-1}. Flume trials demonstrated chamber artifacts: fertilization was lower inside the chamber and the effect was greater at higher flow rates, but chamber orientation to flow had no effect. Increasing the numbers of eggs tended to reduce fertilization efficiency. In dockside tests, a 30-fold difference in spawner numbers had a significant effect on fertilization success. Notwithstanding acknowledged chamber artifacts due to flow impedance, this study establishes the feasibility of time-integrated fertilization experiments with scallops and sets the stage for further investigations of fertilization dynamics in natural scallop populations.

KEY WORDS: Giant sea scallop · *Placopecten magellanicus* · Fertilization success · Fertilization ecology · Population density effect · Allee effects · Fisheries · Depensation

INTRODUCTION

Broadcast spawners, organisms that reproduce by releasing their gametes into the water column, are thought to pay a reproductive price in the form of sperm limitation when their populations are small or sparse (Petersen & Levitan 2001). Fertilization success and the potential for sperm limitation in broadcast spawners became a focus of study in the 1980s (reviewed in Levitan 1995, Levitan & Sewell 1998). The question remains, however, whether sperm limitation is common in wild populations of broadcast spawners and ultimately influences larval production and recruitment to adult populations, especially those that are commercially exploited and depleted (Coma & Lasker 1997). The degree to which these so-called depensatory or Allee effects (Allee 1931) operate in sparse natural populations is not well understood and remains a subject of debate for broadcast spawners in general (Yund 2000, Petersen & Levitan 2001, Gascoigne & Lipcius 2004) and, particularly, in managing commercially exploited sedentary invertebrates, including sea urchins (Quinn et al. 1993, Lundquist & Botsford 2004, 2010), scallops (Smith & Rago 2004), abalone (Shepherd & Partington 1995, Seamone & Boulding 2011), and oysters (Hawes et al. 2011). Sperm dilution in broadcast spawners is one type of depensatory effect (reviewed in Myers et al.

*Corresponding author: skylar.bayer@maine.edu

1995, Liermann & Hilborn 1997). In theory, as population sizes or densities decline, per capita fertilization is predicted to decline with increasing distance between adults. Experimental population manipulations further suggest that nearest-neighbor distances become more important to fertilization success as the overall population size gets smaller (Levitan & Young 1995).

Sperm dilution and its limitation in wild populations of broadcast spawners has been studied: (1) through observations of low fertilization success (fraction of eggs fertilized) during natural spawning events across a range of free-spawning taxa (bluehead wrasse *Thalassoma bifasciatum*, Petersen et al. 1992, Warner et al. 1995; hard corals, *Montipora digitata*, *Favites pentagona*, *Platygyra sinensis*, Oliver & Babcock 1992; *Acanthaster planci*, Babcock & Mundy 1992; soft corals, *Briareum asbestinum*, Brazeau & Lasker 1992; holothurians, *Bohadschia argus, Eupta godeffroyi*, *Stichopus variegatus*, *Holothuria coluber*, *Actinopyga lecanora, Bohadschia graffei*, Babcock et al. 1992; sea urchins, *Strongylocentrotus droebachiensis,* Wahle & Peckham 1999, Gaudette et al. 2006); (2) in laboratory and field experimental manipulations (Pennington 1985, Desrosiers et al. 1996, Babcock & Keesing 1999, Wahle & Peckham 1999, Metaxas et al. 2002); and, (3) using hydrodynamic simulation models of gamete dilution (Denny 1988, Denny & Shibata 1989, Claereboudt 1999). These examples indicate that we should expect a wide range of fertilization success under varying conditions in nature.

Field experiments with experimentally tractable species have contributed considerably to understanding the importance of sperm limitation in the wild, but they also illustrate the challenges of measuring fertilization *in situ*. Some investigators have collected naturally spawned gametes in the wild using various pumps, filters, suction samplers and large bags (e.g. Levitan 1991, Petersen et al. 1992, Warner et al. 1995, Styan 1997, Petersen et al. 2001). These methods may bias estimates of fertilization by artificially exposing eggs to high concentrations of sperm for longer than they would normally experience (Levitan 1995). Fertilization kinetic models predict that most sperm and egg collisions occur within the first few seconds of exposure and experimental artifacts biasing the measure of fertilization success may come from artificially altering the duration of exposure (Vogel et al. 1982, Styan 1998b). Several field studies have deployed freshly spawned, unfertilized eggs in synthetic mesh (e.g. nylon) chambers that retain the eggs but are permeable to sperm (Levitan et al. 1992, Levitan & Young 1995, Wahle & Peckham 1999, Gaudette et al. 2006). Such chambers inevitably alter natural transport of gametes and can produce experimental artifacts by impeding the flow through containers while keeping eggs stationary, which increases the amount of water and therefore the amount of sperm encountered by eggs (Levitan 1991, Levitan et al. 1992). Nonetheless, these methods have been recommended for eggs that are barely visible and at low concentrations (Styan 1997). Although egg chambers may alter absolute fertilization success, most experimentalists accept that such interventions provide a reasonable relative measure of fertilization success among treatments (Levitan et al. 1992, Levitan & Young 1995, Wahle & Peckham 1999, Yund & Meidel 2003, Gaudette et al. 2006).

Despite the fact that many species of bivalve mollusk are spawned in aquaculture and husbandry settings, to our knowledge fertilization experiments in the field have been conducted on only 1 of 2 congener species of scallop in southern Australia (*Chlamys bifrons*, Styan 1998a). The giant sea scallop, *Placopecten magellanicus* (Gmelin, 1971), of the northwest Atlantic has long provided one of the most profitable wild capture fisheries in the northeastern USA and Atlantic Canada. Although the fishery was widely depleted in the 1970s and 80s, subsequent management through effort controls and area closures resulted in an impressive resurgence of the population (Hart & Rago 2006). Historically, though, scallop landings have been notoriously variable, with peaks in abundance associated with the episodic recruitment of one or a few year classes (Hart & Rago 2006). Determinants of recruitment success are still not well understood, though predator abundance and presence of filamentous organisms are thought to be drivers (Stokesbury & Himmelman 1995). Although key components of recruitment, such as adult gamete production, larval transport and mortality have been subjects of intensive study (Langton et al. 1987, Tremblay et al. 1994, Wong & Barbeau 2003), recent literature exposes a critical gap in empirical data on spawning and fertilization (Stokesbury 1999, Smith & Rago 2004, Orensanz et al. 2006). Current larval production and recruitment models for scallops, indeed most free spawners, must make untested assumptions about the relationship between spawner abundance, degree of aggregation, and reproductive success (McGarvey et al. 1993, Smith & Rago 2004).

Although the commercial importance of *P. magellanicus* provides a compelling reason to study its fertilization dynamics, bivalve mollusks as a group do

not lend themselves well to such study. Unlike model taxa such as sea urchins, which are relatively easy to induce to spawn with simple potassium chloride (KCl) injections during their reproductive season (e.g. Pennington 1985, Levitan 1991, Levitan et al. 1992, Wahle & Peckham 1999), in our experience and for others (see Styan 1998a) scallops and are more difficult to induce predictably, sperm is more difficult to collect in concentrated form, egg longevity is shorter, and embryonic stages are more difficult to distinguish than for the echinoderm models used in previous studies.

In this study, we developed a reliable method to measure fertilization success in *P. magellanicus* which possibly can be applied to other species of scallops. First, we developed a fertilization chamber to assess ambient sperm loads in field populations of scallops. Next, we evaluated potential bias and artifacts of the fertilization chamber by conducting flume trials comparing fertilization success of eggs within the chamber versus outside, on the flume floor. Finally, we conducted a series of dockside, time-integrated fertilization field trials using these chambers to assess population size and location effects on fertilization success over the course of the late summer spawning season in coastal Maine, USA.

MATERIALS AND METHODS

Broodstock collection and spawning induction

Divers collected giant sea scallop *Placopecten magellanicus* broodstock from Muscongus Bay, Somes Sound, and the Damariscotta River, Maine. Scallops were held in lantern nets hung from a floating raft and in flowing seawater trays at the University of Maine's Darling Marine Center on the Damariscotta River. As seawater temperature approached 10–12°C in late June, we began rotating females from the rafts to flowing seawater trays. To prevent potential sperm contamination, all water used in the aquaculture facility was filtered to 5 µm, run through an ultraviolet treatment and aged for at least 24 h before female scallops were introduced. Scallops were fed twice daily with a phytoplankton mixture (50–80% *Tetraselmis chuii*, strains PLY and Plat P, with the remainder split between T and C strains of *Isochrysis galbana*, and *Chaetoceros mulleri* and *C. calcitrans*) from the center's algal culture laboratory. Males were held apart from females.

We used thermal shock and circulation to induce spawning in females. Females were kept at mid-summer ambient temperatures of about 15–18°C and were immersed in 10–12°C water as a thermal shock. We used air stones and submersible pumps to create water motion as an added spawning stimulus (Desrosiers & Dube 1993). To collect eggs from those females that spawned, we turned off the circulation, removed the scallops, separated them from the water and finer particles with a 45 µm sieve, and then transferred eggs to a beaker or test tube with fresh seawater to settle. To collect sperm, we either induced spawning with the same temperature shock method or dissected the gonad. With the induction method, to maximize the starting concentration of sperm, males were covered with only as much seawater as would immerse the shell. Sperm concentrations were verified by manual counts using a hemocytometer under a compound microscope. To obtain sperm from a single male gonad dissection, a small section of ripe male gonad was excised and dipped in approximately 50 ml of seawater to create a highly concentrated sperm source for the dilution series (strip-spawning). Further dilution was then usually needed to approximate the starting sperm concentrations used, as described in the methods below.

Sperm dilution series and gamete longevity experiments

To determine the relationship between sperm concentration and fertilization success, we measured fertilization success over a series of ten 10-fold sperm dilutions in test tubes. Seawater used in these experiments was filtered through a 5 µm filter and aged in 10 l carboys for at least 2 d. 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 used to start the dilution series. Freshly spawned eggs were allowed to settle in a test tube, and 400 µl of the concentrated eggs (~2000–5000 by number) were added to a 4 ml sample of each sperm dilution, as well as to a seawater control with no sperm to evaluate the probability of false positives. Eggs were incubated at 12°C for 4 h and shaken by hand every 15 min during this period. Finally, samples were fixed with 66 µl of buffered 37% formalin to be scored by developmental stage.

We conducted gamete longevity experiments because to our knowledge no such studies had been published for this species and we needed to quantify it for subsequent experiments and, specifically, to set the duration of field deployments of unfertilized

eggs. To evaluate egg longevity after spawning, we introduced fresh sperm to eggs of increasing age. Eggs were introduced to sperm suspensions at 1, 2, 4, 6, 8, and 24 h post-spawning. Eggs were aged in seawater at 12°C. At each time interval, fresh sperm were obtained and sperm dilutions were prepared as above. As in the dilution series, 400 µl of eggs were added to 4 ml of sperm dilutions. Saturating sperm concentrations of approx. 10^8 spermatozoa ml^{-1} were used to avoid sperm limitation, but not so high as to produce a decrease in fertilization presumably from polyspermy, as determined from the sperm dilution series. Eggs were incubated, fixed and scored as above. It is important to note that these egg viability experiments determined the duration of field trials.

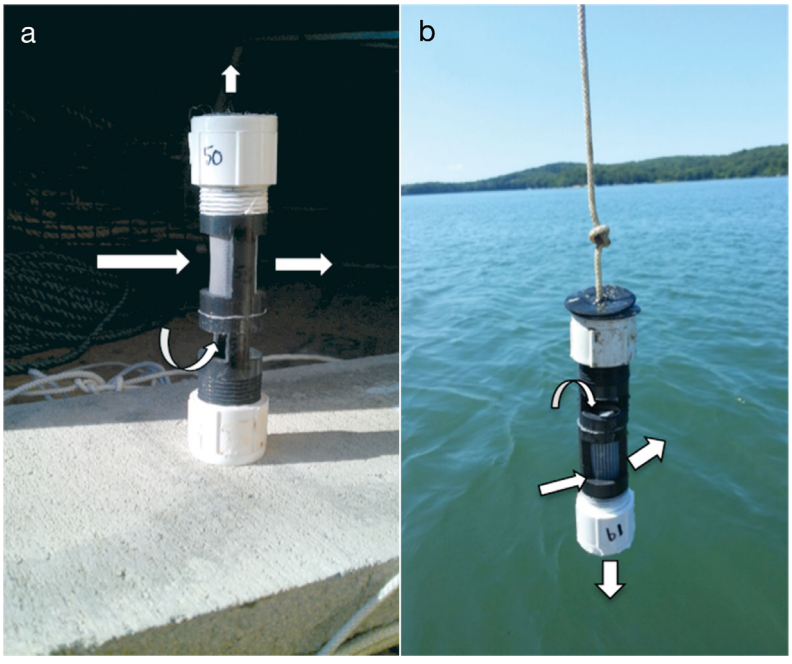

Fig. 1. Fertilization chamber (a) fixed to a cement block for bottom deployments (flume experiments) and (b) hanging on a weighted line for water-column deployments (field experiments). White arrows indicate flow-through areas with 40 µm nylon mesh. Chambers are 15 mm inner diameter × 70 mm high

Sperm longevity is well known to be strongly reduced by dilution in seawater because of the respiratory dilution effect (Chia & Bickell 1983). To quantify this effect in the sea scallop, we created 3 intermediate sperm dilutions based on the dilution series above. The viability of sperm was determined by fertilizing 400 µl fresh eggs (<1 h aged) with 4 ml sperm aged 1, 15, 30, 60, and 120 min at 12°C. The maximum time sperm was aged in this experiment was 2 h. Sperm half-life is the time it takes in hours for fertilization success to drop to half of its initial value with newly spawned sperm. This was estimated from a power function fitted to the observed data for the 3 sperm dilutions in Excel (v.14.5.4).

The flume

All flume experiments were conducted in a flow-through flume described by Yund & Meidel (2003). The flume measured 0.5 m wide and 8 m long from inlet to outlet. The working area of the flume, where we conducted our experiments, was limited to a 3 m segment 0.5 m wide. Large flow structures (vortices) were suppressed with a collimator as described by Thomas et al. (2013). The walls and floor were made of clear acrylic, 9 mm thick, and the floor was covered with Lego™ base plates (0.25 × 0.25 m) to approximately match the roughness Reynolds number observed in the field (Nowell & Jumars 1987). The flume was filled with seawater gravity fed from a head tank that received seawater pumped from the Damariscotta River. It was filled to a depth of 0.2 m above the Lego™ base plates. Flow rates were set by timing how long it took a bolus of dye released 2 cm above the bottom to travel the first 1 m of the working section of the flume. We adjusted a valve from the head tank until the desired flow velocity of 5 or 10 cm s^{-1} was achieved. We used the average of 10 dye tests to confirm the desired flow.

Fertilization chamber design

We designed a fertilization chamber constructed with PVC pipe (Fig. 1, 15 mm inner diameter × 70 mm height) that could be easily deployed for time-integrated fertilization trials in the field. To maintain flow, the chamber was open on the sides, bottom and top; the top consisted of a removable PVC screw cap. Openings, constituting a total area of 15.56 cm^2, were covered with 40 µm nylon mesh to retain scallop eggs, which average 70 µm in diameter. In flume experiments chambers were deployed fastened to a ceramic tile placed on the flume floor on a threaded

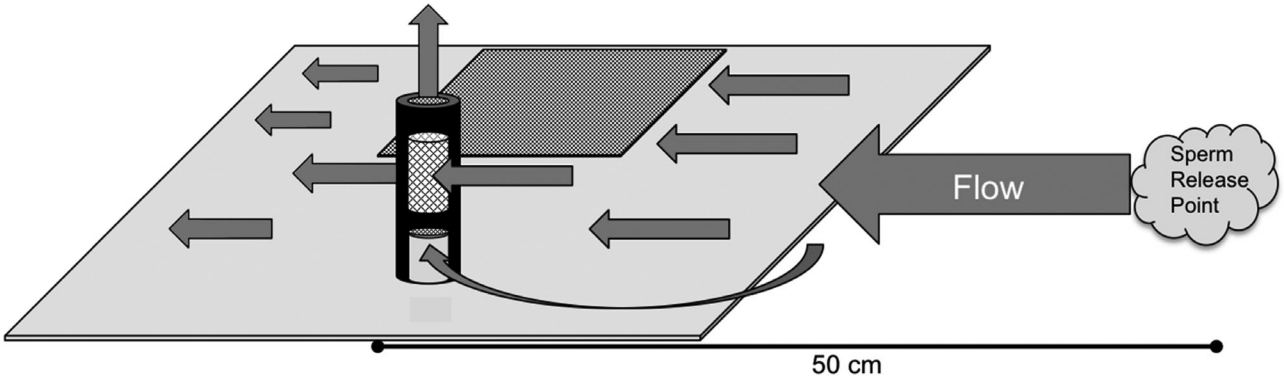

Fig. 2. Concept sketch of flume experimental layout of a textured tile and fertilization chamber. Textured tiles were used to prevent eggs rolling downstream

base (Fig. 1a). In the dockside field experiments they were suspended in the water column on a weighted line (Fig. 1b) because the experimental adult spawners were also suspended off the dock in lantern nets. In both configurations water was able to pass through both ends and on all sides of the chamber.

Flume experiments to assess chamber artifacts

Laboratory flume experiments were conducted to evaluate artifacts associated with scallop egg chambers. Here we compared fertilization success of eggs enclosed in the fertilization chamber (container) to those exposed (no-container) on textured tiles under different flow conditions. If chambers inhibit or divert flow, we might predict that fertilization success from eggs in the chamber would be lower on average than those on the flume floor.

Chambers were fastened to a ceramic tile on the floor of the flume as in Fig. 1a. Textured tiles consisted of plastic tiles lying flat on the flume floor, textured to prevent eggs rolling downstream (Fig. 2). Fiberglass window screening (1.2 mm mesh) glued to the tiles provided the texture. The tiles represented a convenient simulation of a seabed that trapped sinking eggs, but otherwise did not constrain fluid exchange as chambers do. Except where otherwise noted, each trial used 0.25 ml of concentrated eggs. Concentrated sperm (60 ml) was collected from strip-spawned, ripe males. For each experiment, sperm was released at high concentration 0.5 m upstream of experimental eggs and 2 to 3 cm above the flume floor over a period of 5 s while the syringe was moved across stream from one side of the flume to the other.

We evaluated the effects of container vs. no-container over a range of velocities (5 vs. 10 cm s^{-1}) typically found within a few cm of the seabed in tidal estuaries where scallops are common. While flow speed and turbulence intensity are confounded with the time interval that the eggs are exposed to sperm, this comparison may be useful for a rudimentary understanding of flow effects and detection of gross container artifacts. In this experiment 5 trials were conducted at 10 cm s^{-1} and 9 at 5 cm s^{-1}. For this experiment the proportion of fertilized eggs (f) that developed to one polar body after a 4 h incubation were logit transformed (log (f/[1−f])) as recommended by Warton & Hui (2011) to improve normality. We evaluated effects with a 2-factor ANOVA on the transformed proportions of eggs fertilized. In 2 additional, separate, single-factor experiments, we tested the effect on fertilization success of the chamber's screen-face orientation to flow (0 vs. 90° in 6 trials) and the quantity of settled eggs in the chamber (0.25 vs. 1.5 ml in 10 trials). Results were logit transformed and analyzed with a single-factor ANOVA. All flume data were analyzed using the statistical software program JMP (v.5.1.2).

We ran separate control trials on each batch of eggs to confirm viability and to test eggs for sperm contamination or early cleavage without fertilization. Eggs were incubated for 4 h in the water they were released in and then fixed in buffered 4% seawater formalin. The criterion for successful fertilization was the appearance of one polar body (Desrosiers et al. 1996). If controls from a trial were contaminated or non-viable, those trials were not included the analysis.

Gonadosomatic indices and spawning season

The primary purpose of monitoring gonadosomatic indices (GSIs) was to be sure we were conducting our dockside fertilization experiments during the spawning period in the upper and lower river populations. To have an independent indication of onset and pro-

gression of the spawning season during our field experiment, we monitored changes in the GSI of separate groups of mature scallops (shell height: 78–153 mm) in the lower and upper Damariscotta River estuary. These populations were located ≥1 km from the dockside experiments. Starting in early July, at weekly intervals, we dissected a subsample of at least 20 individuals that had a minimum of 9 of each sex (male and female) randomly drawn from a starting stock of ~200 individuals. The GSI was measured as the wet mass of the gonad as a proportion of total soft tissue body mass without the shell (Barber et al. 1988, Langton et al. 1987, Parsons & Dadswell 1992). Tissue was blotted with paper towels before measurements. Although there are other methods to obtain more accurate measures of weight change during spawning (e.g, Bonardelli & Himmelman 1995), our method served the primary purpose of ensuring our fertilization experiments were concurrent with the spawning season of the local population. However, to ensure that shell height (as a measure of scallop maturity) did not significantly influence GSI we ran a simple ANCOVA using JMP (v.5.1.2) for the 2 dates (July 31 and August 7, 2013) when we saw the greatest decrease in GSI over the spawning season using shell height as a covariate for both population sites.

Dockside abundance manipulations

This experiment was a 2-factor design in which we manipulated scallop abundance (1, 4 or 30 male scallops paired with an equal number of females) at 2 locations (upper and lower segments of the Damariscotta River). Scallops were suspended in lantern nets (mesh size 21 mm, 3 m × 0.20 m^2 of net bottom) with the top of the nets 1–2 m below the surface off private docks available along the 2 locations of the river for each abundance treatment. The upper and lower locations of the river were separated by ~5 km, and docks within each location were situated at least ~1 km from each other in an effort to prevent sperm contamination among abundance treatments. There was a total of 6 abundance treatments for the 2 experimental locations in the river. On each dock we hung 3 fertilization chambers as in Fig. 1b within 1 m of the scallops in the net. Four 24 h trials were conducted during the spawning season (26, 30, 31 July and 3 August 2013).

To assess relative differences in flow rate at each site, we deployed clod cards (e.g. McClanahan et al. 2005). Clod cards are plaster of Paris cubes that erode with exposure to a flow field, thereby giving a relative index of flow. The percent change in weight of 2 clod cards at each site was normalized to controls that were submerged in calm seawater in a bucket for the same period. Clod cards were deployed for three 24 h periods in August, September, and October 2013 to estimate relative flow between sites. These results were logit transformed and used as a covariate in the statistical analysis of abundance and location effects on fertilization success.

As with the flume trials, we conducted parallel control assays to assure that eggs were viable and not contaminated with sperm. To assess egg viability in these longer-term, 24 h trials, we fertilized a sample of eggs with a saturating dose of sperm and set it aside to incubate for the duration of the trial at ambient water temperature. Unlike flume trials that incubated for only 4 h, the criterion for successful fertilization in this experiment included the blastula and all subsequent stages of embryonic development, if present. Although earlier cell division stages were present in these trials, we considered the blastula the most conservative measure of fertilization success given the occasional presence of earlier stages observed in our sperm-free controls. For all samples we subtracted the percentage of developed blastulae in controls from the experimental values observed in the field for that trial before conducting our statistical analysis of the field results.

The proportion of fertilized eggs that were scored as blastula stage or more advanced were logit transformed before statistical analysis. Statistical analysis for the dockside experiment consisted of a 2-factor ANCOVA with site and abundance as fixed factors and using clod card weight change as a covariate (JMP v.5.1.2).

RESULTS

Sperm dilution series and gamete viability

We observed maximal fertilization success at concentrations $>10^7$ sperm cells ml^{-1}. As expected, fertilization success increased with increasing sperm concentration to a maximum. Above that, fertilization success decreased (Fig. 3a). Egg viability was strongly affected by egg age (Fig. 3b). In all sperm dilutions egg viability fell over the course of 24 h. By 8 h, however, fertilization success had generally not yet fallen to half the maximum fertilization observed for newly spawned eggs. By 24 h, eggs were no longer viable.

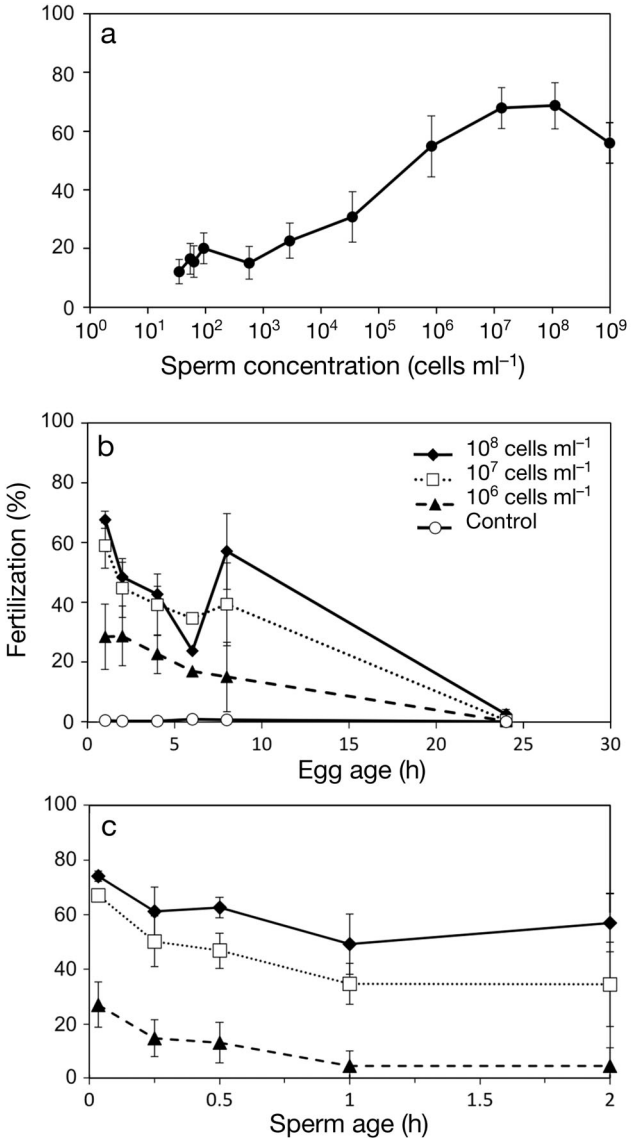

Fig. 3. Mean (±1 SE) fertilization success (a) over a series of ten 10-fold sperm dilutions (n = 7 trials), (b,c) under different sperm dilutions with (b) different egg ages (n = 3 trials per egg age treatment, only one trial at 6 h, control sperm dilution indicates no sperm in experiment [n = 1 trial]), and (c) sperm aged over 2 h (n = 4 trials per sperm age treatment)

The effect of age on sperm viability depended on the degree of dilution (Fig. 3c). At the most dilute of the 3 plotted treatments (~10^6 sperm ml^{-1}), sperm half-life was calculated to be ~9 min ($f = 0.06t^{-0.47}$, $R^2 = 0.86$). At the intermediate dilution (~10^7 sperm ml^{-1}) sperm half-life increased to ~2 h ($f = 0.38t^{-0.17}$, $R^2 = 0.94$), and in the most concentrated suspension (~10^8 sperm ml^{-1}) sperm half-life far exceeded the 2 h duration of the experiment.

Flume fertilization chamber artifacts

When we varied flow velocity, we again found a significant container effect, but not a significant overall effect of flow velocity (Fig. 4a, Table 1). Doubling flow velocity from 5 to 10 cm s^{-1} appeared to introduce enough variability in the outcome of the trials to render flow velocity and interactive effects non-significant at this level of replication. A post hoc test of container effects at the separate flow velocities revealed a significant negative effect of the chamber at the slower but not the faster flow velocity, although results at the higher flow velocity also trended in the same direction (5 cm s^{-1}, $t = -2.06$, df = 14.5, p = 0.03; 10 cm s^{-1}, $t = -1.43$, df = 7.7, p = 0.096.). Given the number of replicate trials for the flow speed experi-

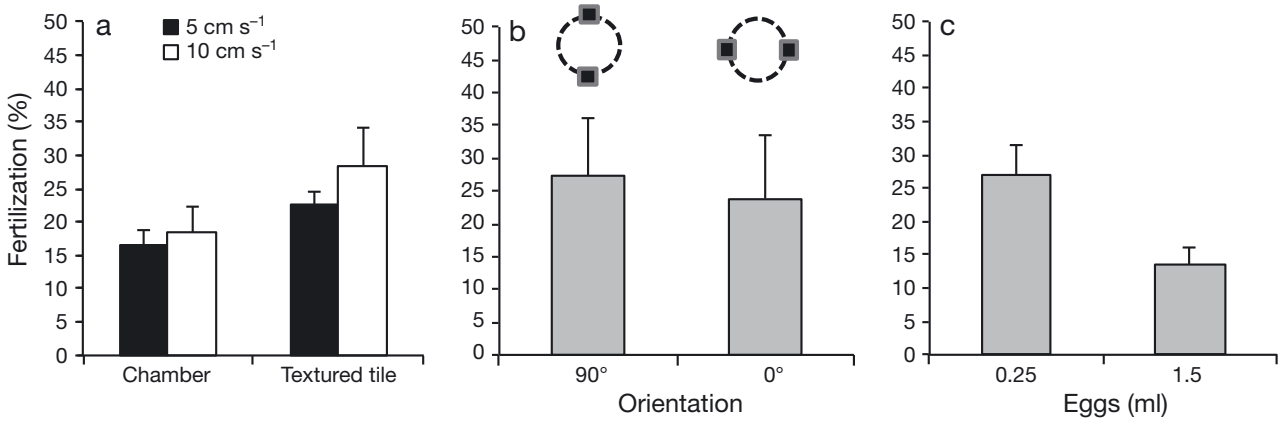

Fig. 4. Flume fertilization chamber artifact experiments. Comparison of mean (+1 SE) fertilization success in (a) nylon mesh chambers and on horizontal textured tiles at 2 different flow rates (n = 14 trials), as well as (b) the effects of chamber orientation to flow (n = 6 trials), and (c) quantity of eggs inside the chamber (n = 10 trials). Inset figures in (b) denote cross section of fertilization chamber with nylon screen windows (dashed line) and supporting frame (black squares)

Table 1. ANOVA for treatment effects on logit-transformed fertilization proportions given in Fig. 4: (A) container × flow, (B) chamber orientation, and (C) egg quantity. Significant p-values ($p < 0.05$) are in **bold**

Source	df	MS	*F*	p
(A) Container × Flow				
Container	1	0.2930	6.117	**0.021**
Flow	1	0.0460	0.959	0.337
Container × Flow	1	0.0050	0.112	0.741
Error	24	0.0420		
Total	27			
(B) Orientation				
Orientation	1	<0.0001	1.90×10^{-6}	0.999
Error	10	0.2550		
Total	11			
(C) Egg quantity				
Quantity	1	0.736	6.084	**0.024**
Error	18	0.121		
Total	19			

ment, sufficient statistical power ($\beta \geq 0.8$) resolved a difference of ~0.68 standard deviations or more.

Finally, in our single-factor experiments conducted at a flow velocity of 5 cm s^{-1}, orientation of the nylon mesh chamber to flow had no significant effect on fertilization (Fig. 4b, Table 1). However, a 6-fold increase in the quantity of eggs per container significantly reduced fertilization levels by approximately 50 % (Fig. 4c, Table 1).

GSI and spawning season

The seasonal peak of scallop GSIs occurred in mid July at both upper and lower river locations, and then dropped considerably over a 2 wk period (July 24 to August 7) signaling the spawning event (Fig. 5). In addition, we observed large standard deviations in GSI during this time; some scallops had spawned

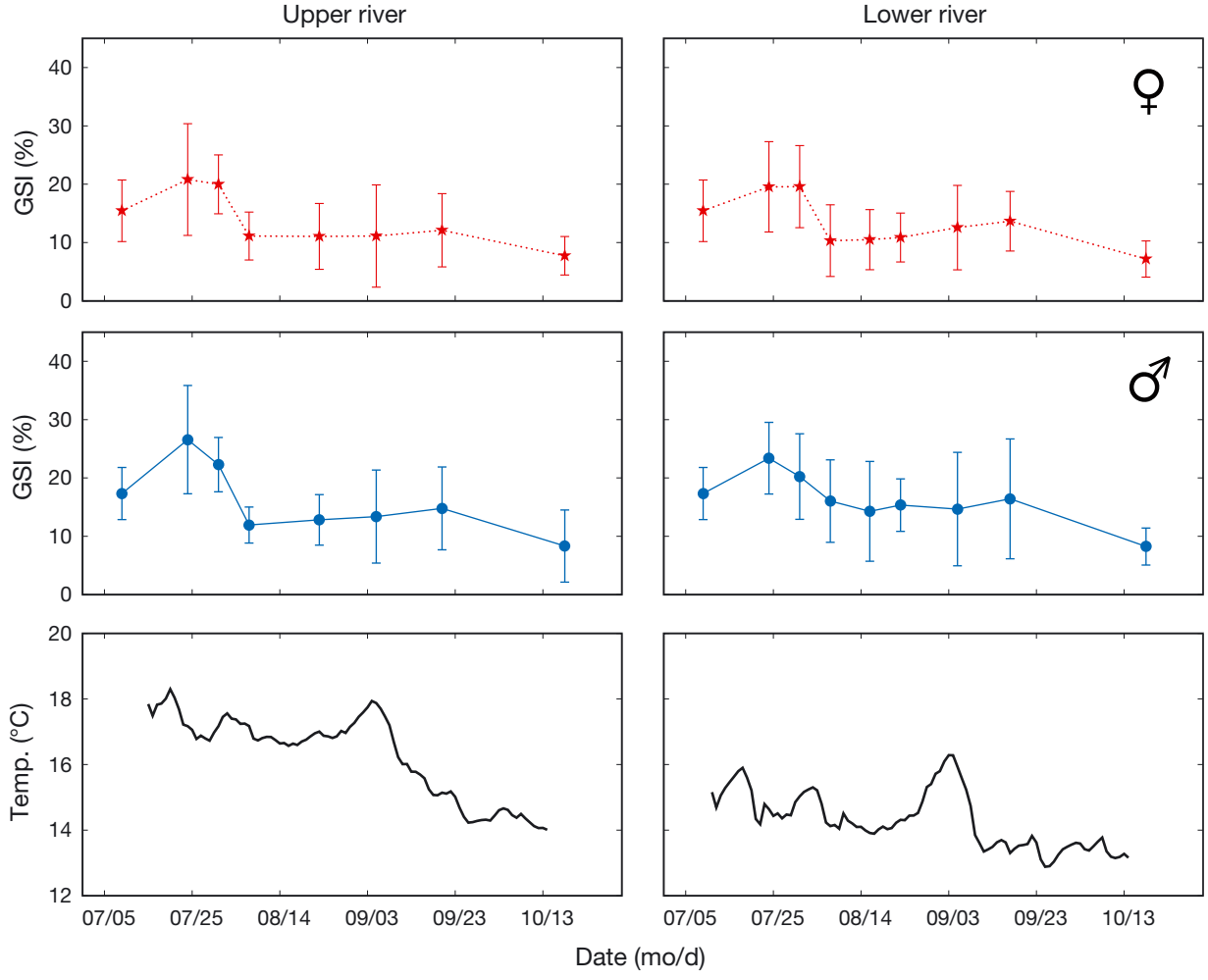

Fig. 5. Mean (±1 SD) gonadosomatic index (GSI) for female and male giant scallops and temperature time series from the upper and lower Damariscotta River in 2013. Minimum scallop sample size = 9 for both males and females

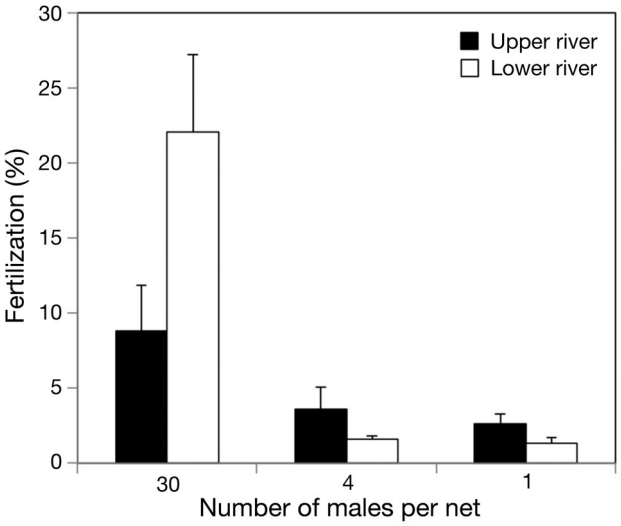

Fig. 6. Dockside scallop fertilization experiments showing mean (+1 SE) fertilization success at 3 scallop densities. Scallops housed in lantern nets spanned a 30-fold difference in abundance: high (30 males per net), medium (4 males per net) and low (1 male per net) at 2 locations in the Damariscotta River

almost completely while others had yet to spawn. The ANCOVA results demonstrated that shell height was not significantly influential as a covariate in our upper river ($F = 0.66$, df = 1, p = 0.42) and lower river ($F = 0.76$, df = 1, p = 0.39) populations. Our observations in how GSI changed during the summer indicates that our 4 fertilization trials were deployed during the peak spawning season of the Damariscotta River scallop populations. Decline in GSI coincided with the first recorded temperature drop (i.e. a 1 to 2°C decrease, Fig. 5) during summer at both sites, although temperatures tended to run about 2°C higher at the upper than the lower Damariscotta River study sites.

Dockside abundance manipulations

In our dockside experiment, higher male abundance had a strongly significant positive effect on fertilization success, whereas there was no significant location or interactive effect (Fig. 6, Table 2). Among-site differences in relative flow, our co-variate, as measured by clod cards, also did not explain a significant amount of the variability in fertilization success (Table 2). Fertilization success in experimental populations with 30 males per lantern net were between 2 and 10 times higher than those in the lower abundance treatments at 4 and 1 males per net.

Table 2. ANCOVA for the interactive effects of spawner abundance and location on logit-transformed dockside fertilization proportions given in Fig. 6. Significant p-values ($p < 0.05$) are in **bold**

.Source	df	MS	*F*	p
Abundance	2	3.368	4.956	**0.010**
Clod card	1	0.759	1.116	0.295
Location	1	0.331	0.487	0.488
Location × Abundance	2	1.408	2.071	0.134
Error	64	0.680		
Total	70			

DISCUSSION

This study represents the first assessment of giant sea scallop *Placopecten magellanicus* fertilization success in a field experiment. Our flume experiments show a statistically significant difference in fertilization success between chambers and unconfined eggs on the seabed at low flow velocities (~5 cm s^{-1}), but that a doubling of velocity of delivery of a short pulse of sperm does not result in a significant change in fertilization success. This result may not be surprising given that the shape of the sperm plume may become narrower and longer with increasing velocity and has a shorter contact period with the basket and over the screen at a higher velocity (Denny & Shibata 1989). Differences in duration of exposure and orientation of the chamber also had relatively little effect on observed fertilization success. These results allay concerns about the potential that the orientation to flow in field trials or the short duration of flume trials relative to field trials will significantly alter interpretation of field experiments that use chambers.

We did, however, observe an increase in fertilization success with lower egg concentration in the chambers. Our flume studies, therefore, indicate that (1) fertilization success recorded from chambers should be used as relative, not absolute, measures of fertilization success, and (2) the quantity of eggs should be standardized in field deployments to have reliable comparisons among treatments. The decrease in fertilization with higher numbers of eggs (Fig. 3c) suggests that eggs at artificially high densities in containers have the potential to compete for limited sperm, and thus the number of eggs in a container is an important variable to control for comparisons. Although perhaps not comprehensive, the above laboratory-based assessment provides valuable background regarding fertilization dynamics

under controlled conditions that set the stage for, and help interpret, field experiments.

Legitimate concern has been expressed in the literature about the potential for chamber artifacts in field fertilization studies (Levitan 1991, Levitan et al. 1992, Wahle & Peckham 1999, Yund 2000, Gaudette et al. 2006), but we are aware of no published experiments that quantify these effects. We therefore cannot compare our results to previously measured differences in fertilization success of eggs inside and outside chambers. Several authors have indirectly addressed this concern by measuring fertilization success of freely drifting eggs either in flume studies (sea urchins; Kregting et al. 2014) or *in situ* (sea stars; Metaxas et al. 2002). To our knowledge, fertilization studies of freely drifting eggs in the water column have only been attempted with one species of bivalve (*Chlamys bifrons,* Styan 1997).

Our dockside field experiment results indicate that less than a 10-fold difference in local scallop abundance (30 vs. 4 males per net in this case) can significantly affect fertilization success. The abundance treatments likely had a direct effect on fertilization success by influencing local sperm concentration at the fertilization chambers located within 1 m of the spawning scallops, as has been demonstrated in other broadcast spawners (Levitan et al. 1992, Babcock & Keesing 1999, Metaxas et al. 2002). It is also not possible to say whether female spawning in the dockside trials was synchronous with male spawning, although the GSIs indicated that both males and females were spawning during the weeks that the dockside fertilization experiment was conducted.

Interaction of spawning synchrony and population density in the field has not been well studied, despite laboratory evidence indicating that synchrony could be influenced by localized conspecific spawning cues (Miller 1989, Hardege & Bentley 1997, Soong et al. 2005). In broadcast-spawning corals (Oliver & Babcock 1992, Babcock et al. 1994), abalone (Babcock & Keesing 1999), urchins (Gaudette et al. 2006) and scallops (Mendo et al. 2014), individuals in closer proximity to conspecifics and in larger aggregations are more likely to spawn synchronously. Styan & Butler (2003) found that larger, more fecund male scallops did not release more sperm per spawning or at a greater instantaneous rate than smaller scallops. Consequently, they speculated that larger scallops must spawn more frequently and thus increase chances of spawning simultaneously (Styan & Butler 2003). Synchrony can also be triggered by external environmental cues such as temperature, food supply, or lunar periodicity, and can be further enhanced by positive feedbacks from surrounding conspecifics. For example, Gaudette et al. (2006) observed that sea urchins in both large and small aggregations (10^4 vs. 10^2 ind., respectively) all exhibited spawning maxima during full and new moons, but only those in large aggregations produced a mass synchronous spawning. As for *P. magellanicus,* Bonardelli et al. (1996) observed that downwelling events that change bottom temperature often coincided with spawning events. Food supply or its interaction with conspecific signals may be a potential spawning cue in some invertebrates (Starr et al. 1990, Reuter & Levitan 2010), but no phytoplankton trigger has been found for giant sea scallop spawning (Bonardelli et al. 1996). In the present study, changes in temperature were similar between locations and coincided with the decline and greater variance in the gonad index for both males and females (Fig. 5). As local differences in temperature and planktonic food supply may have existed among the docks in this study, we cannot rule out the possibility that these factors may have contributed to variable spawning intensity despite the lack of a significant site effect on fertilization success.

Our field study did not separate effects of scallop density at the population level and local aggregation size on fertilization success. For example, in the sea biscuit *Clypeaster rosaceus,* Levitan & Young (1995) observed that nearest-neighbor distances became increasingly important to fertilization success as aggregations got smaller. In short, as the size of the overall gamete pool from neighboring spawners shrinks, proximity to individual spawners makes a bigger difference to per capita reproductive success. A similar effect has been observed with population manipulations of the green sea urchin, *S. droebachiensis* (Wahle & Peckham 1999, Gaudette et al. 2006). Giant sea scallops in the wild are often highly aggregated, and it has been assumed that aggregative behavior during the spawning season favors fertilization success (Stokesbury & Himmelman 1993), although this relationship has never been empirically tested. Mendo et al. (2014) argued that to rigorously test the hypothesis that aggregative behavior enhances fertilization success, spatial patterns and nearest-neighbor distances need to be measured before, during and after the spawning season of a motile broadcast spawner. The present study takes a first step toward that end by developing the tools to measure fertilization success *in situ,* and we have initiated studies to assess fertilization dynamics under more natural

conditions with manipulated scallop populations on the seabed (S. R. Bayer et al. unpubl.).

Determining how *P. magellanicus* aggregation patterns change through the spawning season may indicate how scallops affect their ability to reproduce on a small scale and whether it is relevant to fishery management. Mendo et al. (2014) determined with an empirically informed model that for the scallop *Pecten fumatus,* the probability of spawning increased with a decrease in nearest-neighbor distance for adults with larger gonads. In the green sea urchin *Strongylocentrotus droebachiensis*, Gaudette et al. (2006) found that natural aggregations 40 times larger in population size than smaller aggregations resulted in mass, synchronous spawning and fertilization success near 100%, unlike the smaller populations. This suggests that, at least in sea urchins, aggregation size, in addition to density, may be relevant to observed changes in gonad indices and measured fertilization success.

Local differences in average flow velocity or turbulence intensity that affect dissolution of clod cards did not explain local differences in fertilization in our dockside field study. Although relative flow, as measured by clod cards, did not prove to be a significant covariate, we cannot rule out turbulence intensity as a variable influencing fertilization success. Clod cards may give a relative measure of average flow rates during deployments, but they confound mean flow speed and turbulence intensity. They are not universal integrators of 'water motion' (Porter et al. 2000). Because these dockside experiments were located on variable dock types and scattered among various inlets and coves, turbulence at each location likely varied in frequency and intensity. Turbulence has been demonstrated both empirically and with modeling to affect fertilization success significantly (Denny 1988, Denny & Shibata 1989, Crimaldi & Browning 2004, Crimaldi & Zimmer 2014). These physical processes may bring eggs and sperm together (Lasker et al. 1996, Simon & Levitan 2011) or dilute them rapidly before fertilization can occur (Denny 1988). Future studies examining the effect of scallop population abundance or density on fertilization success using more natural flow conditions at the seabed should be conducted. Furthermore, because bottom boundary layer flow dynamics in a wild scallop bed likely differ considerably from flow conditions in lantern nets suspended in the water column, gamete dispersal is likely to differ. Scallop eggs are negatively buoyant (S. R. Bayer & R. A. Wahle unpubl.) and eventually settle to the seabed under typical flow conditions, and it would not be unreasonable to expect a downward bias in sperm motion (Falkenberg et al. 2016). Our results therefore only begin to depict the complexities of scallop fertilization dynamics in the wild.

Further studies using our fertilization chamber method have the potential improve estimates of fertilization success in wild populations and would be especially relevant to comparisons of scallop reproductive performance inside and outside fishing closures, a common management tool for scallops (e.g. *P. magellanicus,* Stokesbury 2002; *Pecten maximus,* Beukers-Stewart et al. 2003). In addition, fertilization success estimates from this method and experimentation can be incorporated into population models that estimate gamete production, subsequent larval production, export and ultimately connectivity within scallop stocks (i.e. *P. magellanicus*, Davies et al. 2015).

In conclusion, with this study we have (1) developed a tool to assess scallop fertilization success in the field, (2) used flume studies to quantify experimental artifacts that may influence interpretation of fertilization success measured in the field, and (3) detected a significant effect of scallop aggregation size on per capita fertilization success in a field experiment that approximated the range of densities near the high end of observed populations in nature. Together, these results set the stage for further studies to compare fertilization success in naturally occurring populations on the seabed to better evaluate whether depensatory or Allee effects may occur in wild scallop populations.

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