A field based assessment of predation impacts on planktonic egg capsules across depth and flow gradients

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Abstract

The relative rates of mortality in benthic and pelagic environments have been proposed as a major factor structuring the evolution of complex benthic invertebrate life histories. Field methods, such as tethering, have provided indispensable measurements of mortality under natural conditions for the larvae of benthic invertebrates. However, few such field studies have examined mortality rates of benthic invertebrates' early life stages in size classes less than 1 mm. Here, we tethered egg capsules to quantify how rates of predation vary with environmental gradients, especially distance from the benthic substratum and flow velocity. We found that predation is consistently high on *L. scutulata* egg capsules and invariant at four positions in the water column and on the benthic substratum (average 34% loss across all treatments). Instead, the flow environment had the greatest effect on predation rate. We hypothesize that higher predation in the faster flow environment was due to higher encounter rates with advected predators.

Introduction

Predation is thought to be a primary driver of mortality in the early life history stages of benthic marine invertebrates. Indeed, predation and mortality for these free-living stages are often estimated to be up to 100% per day (Olson and McPherson 1987; Allen and McAlister 2007). Thus, a key to understanding how such complex, seemingly risky, life cycles can evolve and persist for millions of years lies in understanding how the environment structures mortality, and in particular, predation. Recent field studies have demonstrated variation across environments in larval mortality (Acosta and Butler

1999; Motro et al. 2005; Allen and McAlister 2007; Kerr et al. 2014). Most of these studies support the idea of higher predation risk near the benthic substratum and lower predation in the pelagic zone (e.g., Allen and McAlister 2007). This suggests that some complex life histories involving a planktonic phase may, in effect, allow early life stages to escape the higher predation on the benthic substratum (Vaughn and Allen 2010). However, the majority of these studies have been conducted on relatively large and mobile larvae, such as brine shrimp, megalope, and lobster postlarvae (Acosta and Butler 1999; Motro et al. 2005; Allen and McAlister 2007; Bullard and Whitlatch 2008; Kerr et al. 2014). Predation rates on smaller invertebrate larvae and eggs may be quite different because many predators are known to be strongly size selective (Allen 2008). Additionally, the effects of environmental variables other than proximity to benthic and surface boundaries in structuring the distribution of predation risk, such as the flow environment, have been little explored in a field setting.

Both field and laboratory methods have been used to estimate larval mortality (reviewed in Rumrill 1990; Vaughn and Allen 2010). Many existing estimates of larval mortality come from laboratory experiments, which provide useful information on prey defense (Kishida et al. 2010). However, controlled laboratory conditions may not provide accurate estimates of predation in the field. For example, predator-prey studies run under lab conditions typically lack natural prey communities (e.g., holoplankton, meroplankton). In the absence of natural prey assemblages, predators may be focusing on benthic invertebrate larvae purely because of a lack of alternative food sources, thereby artificially inflating predation rates on the focal species (Johnson and Shanks 1997).

Field based metrics for predation include tracking of larval cohorts through time (e.g., Tapia and Pineda 2007) and computing loss rates from adult populations with known fecundities (e.g., Rumrill 1990). Though these methods can produce robust estimates of mortality, these studies cannot determine the environmental structure of predation. Tethering based field approaches provide a means to quantify predation *in situ* conditions across environmental variations. Tethering methods have recently shown that predation on large (> 1 mm) prey varies with depth (Motro et al. 2005; Allen and McAlister 2007), lunar cycles (Acosta and Butler 1999), and diurnal cycles (Allen and McAlister 2007; Kerr et al. 2014).

To better understand spatial variation in predation risk for the very small (<1 mm) early life history stages of invertebrates, we compared survival rates of *Littorina scutulata* egg capsules (mean ± SD; 674 ± 38 μm) across three spatial gradients on a local scale. We deployed tethered *L. scutulata* egg capsules throughout the water column, stretching from benthic to pelagic habitats, and across two flow and tidal regimes. Based on previous measurements of predation rates (Motro et al. 2005; Allen and McAlister 2007), we hypothesized that depth would have the greatest effect on predation, with the highest predation occurring on the benthic substratum.

Methods

Study organism: Littorina scutulata Gould 1849 is a prosobranch gastropod that lives in the high rocky intertidal of the northeastern Pacific. The genus *Littorina* has varied life history modes including planktonic egg capsules with planktotrophic larvae, benthic egg masses with crawl-away lecithotrophic larvae, and direct development (Reid et al.

1996). *Littorina scutulata* belongs to this first group, which is thought to be basal in the genus (Reid et al. 1996). *L. scutulata* egg capsules are flattened circular disks with a rim on one (Type A) or both (Type B) sides of the disk (Hohenlohe 2002). We used only Type B capsules in this study. Capsules can contain 1-11 eggs, but generally have 2-4 (Hohenlohe 2002). Since the outer diameter of egg capsules is significantly correlated with egg number (Hohenlohe 2002), to assure size uniformity of prey, we used only capsules with 3 to 4 eggs (mean ± SD: 674 ± 38 μm, n = 25). At 12-14°C, veligers of *L. scutulata* hatch after 9 days (Hohenlohe 2002), thus we used only egg capsules 1 to 4 days old to ensure that veligers did not hatch while the trials were underway. *Littorina plena* co-occurs with *L. scutulata* and has very similar adult morphology (Hohenlohe and Boulding 2001) and pelagic egg capsules. We distinguished *L. scutulata* from *L. plena* by egg capsule shape, size, and number of eggs (Hohenlohe 2002).

Field site: We deployed tethered *L. scutulata* egg capsules (Fig. 1) off the Friday Harbor Laboratories dock, San Juan Island, WA (N 48°54′, W 123°01′) from July 29 to August 2, 2014. Deployments were made off both sides of a floating dock; the high flow side faced the inlet of Friday Harbor and the low flow side was inside the U-shaped dock (Fig. 2). During our deployments we estimated surface flow using fluorescein dye. Estimated mean speed of surface flow for the high flow side was 29 mm/sec and 17 mm/sec for the low flow side of the dock. The benthic substratum off both sides of the dock was mud with patches of eel grass, cobbles, and boulders. Adult *L. scutulata* were collected in the high intertidal near the study site and kept in individual mesh containers at Friday Harbor Laboratories for 1 to 4 days while egg capsules were released.

Tethering egg capsules: Egg capsules were tethered to monofilaments, 75 µm in diameter, taken from 115 μm Nitex mesh. To remove water and dry the upper surface of egg capsules, capsules were first pipetted onto a 2 cm × 2 cm piece of 115 μm Nitex mesh and a Kimwipe® was wiped across the bottom of the mesh to draw out water surrounding the egg capsules. No more than 10 capsules were placed on the mesh at once to prevent excess drying before tethering could occur. Using a dissecting microscope, we attached the egg capsules to the Nitex filaments by dipping the tip of a filament into a small drop of cyanoacrylate glue (Gorilla Super Glue™: Gorilla Glue, Cincinnati, OH) and touching the glue covered tip to the rim of an egg capsule (Fig. 1). As soon as this attachment was made the capsule with its filament tether was submerged into water to harden the cyanoacrylate glue. Tethered capsules were stored individually in sea water filled 1.5 ml microcentrifuge tubes on a sea table. To avoid damage to capsules, they were kept in microcentrifuge tubes until immediately before deployment in the field.

Tethering units: Predation assays were conducted using tethering units designed to place *L. scutulata* egg capsules at five depths throughout the water column (Fig. 2). Each tethering unit consisted of a 4.5 m monofilament (6 lb test) attached at one end to a 650 g weight and at the other end to a float. Swivel ties were placed along the monofilament at the following five locations: 1) base, 2) 30 cm from the base, 3) 1 m from the base, 4) 3 m from the base, and 5) at the surface. Filaments with egg capsules had loops tied in the free end so that they could be attached to the swivel ties along the monofilament immediately before deployment. There was an additional float attached to the monofilament at 3 m. When tethering units were deployed in approximately 4 m of water the secondary float remained below the surface, keeping the lower portion of the line taut.

Tethering units were deployed at slack water (both high and low tides); even with slight tidal variations the four tethered larvae nearest to the benthic substratum remained at the same height above benthic substratum, while the surface egg capsule remained at or near the surface. Each tethering unit had eggs attached from the same *L. scutulata* mother; each mother (8 total) was used for 2 to 17 tethering units.

Predation assays: On each tidal cycle during daylight hours, 6 to 8 tethering units (*n* = 52 units deployed over 7 cycles) were deployed approximately 30 cm from the edge of the dock in water depths of approximately 4 m. Twenty-six tethering units were deployed on both the high flow and low flow sides of the dock. Capsules that had been tethered in the laboratory were hooked onto the tethering unit via swivel ties and microcentrifuge tubes were removed just before each tethered capsule was lowered into the water. With a 10x hand lens, we checked for the presence of each egg capsule on the filament before submerging it into the water. Tethering units were deployed for 101 to 134 minutes (mean 118 min). When tethering units were brought to the surface we immediately checked for the presence or absence of egg capsules with a 10x hands lens.

Controls: In previous field predation studies, loss rates of tethered prey by factors other than predation appeared rare (Acosta and Butler 1999; Bullard and Hay 2002; Allen and McAlister 2007; Kerr et al. 2014). However, the size of the egg capsules deployed in our study were smaller than in most previous tethering studies and therefore the attachment site between capsule and filament was also smaller. We assessed the rate of potential loss due to egg masses being dislodged by factors other than predation.

To determine the rate of loss due to handling, deploying, and recovering tethering units (setting loss) we deployed tethering units identical to those in experimental trials (*n*

= 20), but retrieved them within 2 minutes of deployment and recorded capsule losses. We assumed that all egg capsules lost during these deployment loss controls were due to physical factors during deployment/ recovery and not predation.

To determine the rate of loss due to submergence time and currents (flow loss), tethered capsules were placed in a non-flowing sea table on moving ends of oscillating paddles that simulated current driven capsule movement. A rotating motor (8 rpm) pulled paddles, which were of two lengths to produce 'fast' (45 mm movement per cycle, 100 mm/s peak speed, n = 30) and 'slow' (25 mm movement per cycle, 57 mm/s peak speed, n =30) simulated flow speeds. Both simulated flows were faster than our fastest estimate of flow velocity in the field (29 mm/sec). Trials of loss due to flow were conducted for 120 minutes.

Data Analyses: Statistical analyses were conducted in R (version 3.03; http://www.R-project.org). Binomial data for egg capsules lost and recovered were analyzed using a generalized linear regression model (GLM) with a binomial error distribution and logit link function. Predictor variables included depth (benthic substratum, 30 cm, 1 m, 3 m, and surface), day deployed, location (high flow vs. low flow side of dock), tide (high vs. low), and mother (mother snail used). A GLM was also used to compare loss rates between control and treatment deployments. Daily instantaneous mortality (M) rates were calculated as $M = \ln\left({}^{N_t}/_{N_0}\right)/-t$ (Rumrill 1990), where N_0 is the number of tethered egg masses deployed and N_t is the number of egg masses recovered after a certain amount of time (t, in days). This equation was modified so that mortality rate from the treatments could be scaled by non-predation losses estimated from the setting and flow loss controls:

$$M = \frac{ln\left(\left(\frac{N_{ttreatment}}{N_{0treatment}}\right)+\left(\frac{N_{0settingcontrol}-N_{tsettingcontrol}}{N_{0settingcontrol}}\right)+\left(\frac{N_{0flowcontrol}-N_{tflowcontrol}}{N_{0flowcontrol}}\right)\right)}{-t_{days}}$$

Results

Controls: Out of 20 trials (n = 100 egg capsules), 9% of egg capsules were lost due to handling, setting, and/or pulling the tethering units out of the water. This setting loss rate was significantly less than predation treatment losses at all depths (Table 1, Fig. 3). No significant effect of depth or location was detected in our setting loss control trials (Table 1). No egg capsule losses occurred in our slow or fast flow loss control trials (n = 60 egg capsules).

Predation treatments: Across all treatment variables, our field predation treatments suffered 34% loss. We failed to detect significant effects of day, mother, tide, and depth (Table 2). Location was the only significant factor affecting egg loss, with the high flow dockside suffering 1.5 times as many losses as the low flow dockside (Table 2, Fig. 3). The mortality rate (M) was 5.32/day before adjustment for control losses; after control losses were accounted for the mortality rate was 3.70/day.

Discussion

Recent field studies have shown that benthic predation rates on large planktonic larvae are significantly higher than predation rates in the plankton (Acosta and Butler 1999; Motro et al. 2005; Allen and McAlister 2007). Our study builds on previous work by measuring predation rates on marginally protected pelagic capsules on the benthic substratum and throughout the water column. We found no significant difference in

predation among any of our depth treatments: for small passive propagules (pelagic egg capsules) pelagic and benthic predation rates were indistinguishable in our study. This result contrasts with previous tethering studies that found a substantial increase in predation rate on the benthic substratum (Acosta and Butler 1999; Motro et al. 2005; Allen and McAlister 2007).

The different outcomes may be due to one or both of two aspects in which our study differed from previous work. First, the small size of *L. scutulata* tethered egg capsules (averaging 674 μm) contrasted with the much larger megalope and adult brine shrimp (~3-6 mm) tethered in some other studies. To our knowledge only two previous studies have tethered invertebrate larvae or egg capsules in the < 1 mm size class (Bullard and Hay 2002; Kerr et al. 2014). Our results appear within the range reported for 620 μm brine shrimp (~20-50% loss per half hour; Kerr et al. 2014). However, neither of these examined how predation varied with depth. Second, our passive egg capsules contrast with the active swimming behavior of previous tethered organisms. Together, the small size and passiveness of *L. scutulata* egg capsules, may explain why our results contrast with previous field tethering studies.

The limitations of tethering studies for measuring predation rates in the field have been discussed in detail (Bullard and Hay 2002; Allen and McAlister 2007; Vaughn and Allen 2010; Kerr et al. 2014). A limitation in previous studies has been the restriction of larval behavior, such as vertical migration and escape responses. However this was not a limitation of our study with egg capsules. An important limitation of this study is the exclusion of benthic suspension and filter feeders. Because our egg masses were tethered via a monofilament, they were protected from most benthic infaunal and epifaunal

bivalves and anemones. Thus, a significant guild of benthic predators was potentially excluded in the present study (Mercier et al. 2013). In previous tethering studies the primary predators were pelagic and demersal fishes (Motro et al. 2005; Allen and McAlister 2007; Allen 2008; Ng and Parker 2014). Due to the size difference between our egg capsules and the previously studied crustacean larvae, large fishes were probably not the primary predators in our study.

Despite the differences in size and motility between our focal prey and previous tethering studies, the high instantaneous mortality rate (3.7/ day) we observed is consistent with previous tethering and direct field observation studies (Olson and McPherson 1987; Allen and McAlister 2007). These tethering based mortality rate estimates are typically higher than rates estimated from indirect field methods, such as tracking larval cohorts and comparisons between larval production and subsequent recruitment (reviewed by Rumrill 1990).

The observed high instantaneous mortality rate of *L. scutulata* egg capsules strengthens the traditional idea of gamete and larval wastage in the plankton (Thorson 1950). Larval wastage posits that in order to compensate for high losses in plankton, adults must release huge quantities of gametes and larvae (Thorson 1950). The average *L. scutulata* female releases approximately 1000 eggs, distributed across approximately 333 capsules, over a 3 month period (Strathmann 1987; Hohenlohe 2002). According to our mortality rate estimate, the vast majority of these capsules will be consumed by predators. Once in the larval form, *L. scutulata* veligers have evolved morphologically plastic defenses induced by the presence of predators (Vaughn 2007), but egg capsules

have no known defensive mechanism. High egg capsule predation may be balanced by the enhanced predation defenses in the veligers that survive to hatch.

We observed 1.5 times higher egg capsule mortality on the higher flow side of the dock than on the low flow side. The surface flow rate was 1.7 times higher on the high flow side of the dock. Our laboratory flow loss control showed that flow rates 3.4 times faster than those measured during our experiment did not dislodge egg capsules. Therefore, we interpret differences between sides of the dock as due to other factors. However, with a flow volume 1.7 times higher, the high flow side of the dock receives a greater amount of water flowing past the egg capsules. We hypothesize that this additional water flow increases contact rates with predators. In many encounter rate models, the encounter rate of predators with non-motile prey is proportional to predators' movement speeds (Gerristen and Strickler 1977). If we assume that predators of small, non-motile prey are themselves slow swimmers, the contribution of water flow to predator movement may be up to 1.7 times greater on the high flow side of the dock. The higher encounter rate implied by this higher relative movement may explain the 1.5 times greater predation rate on high flow dockside capsules. This higher encounter rate may be an artifact of tethering; in natural conditions prey would be flowing with predators, rather than remaining stationary with predators moving past them.

In a study examining predation rates on small larvae (veligers and plutei) using mesocosms, which also occurred off the Friday Harbor Laboratories dock, Johnson and Shanks found far lower mortality rates (0.012/ day) than observed in our study. These mesocosms maintained a constant number and composition of predators because the enclosures surrounding mesocosms prevented the flow of outside predators into the

experimental chamber. In contrast, our study design allowed a constant transport of new predators past tethered egg capsules, thereby considerably increasing encounter rates of predators with prey in our study compared to the mesocosm study.

Conclusion. Because tethering studies leave prey open to flow and the predator environment, they provide field based estimates for mortality rates of small marine plankton. Importantly, our results contrast with previous work that found strong benthic predation rates; instead, we found uniformly high mortality across all depths for *L. scutulata* egg capsules. These high mortality rates on fertilized egg capsules may place an added stress on later life history stages, perhaps promoting the evolution of phenotypically plastic predator responses in the veligers (Vaughn 2007). However, predation rates are not constant throughout the marine environment. Our study suggests that even within a few meters the patchiness of predation is apparent. Differences in flow rates, due to leaky physical barriers- like a dock (as in the present study), or small scale eddies- may lead to significant differences in predator encounter rates.

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Table 1. Analysis of deviance table for generalized linear regression model of field predation treatments and field controls. The controls suffered significantly lower losses than the predation treatments.

Model term	df	Deviance	Residual df	Residual deviance	p
NULL		3.17	346	411.21	
Treatment v. Control	1	27.45	341	380.59	**<0.0001**
Depth	4	28.08	341	401.25	0.53

Table 2. Analysis of deviance table for generalized linear regression model of field predation treatments. Location (high flow v. low flow) is statistically significant, with the high flow location suffering significantly greater losses than the low flow location across all depths.

Model term	df	Deviance	Residual df	Residual deviance	p
NULL			246	319.28	
Depth	4	0.29	242	318.98	0.99
Location	1	6.07	240	311.92	**<0.05**
Tide	1	0.38	239	311.54	0.53
Day	1	0.99	241	317.98	0.32
Mother	1	0.40	238	311.14	0.52

Figure Captions

Figure 1. *Littorina scutulata* egg capsule tethered to a 115 µm filament (250 µm scale).

Figure 2. Tethering unit for *Littorina scutulata* egg capsules. Inset: field deployment sites at Friday Harbor laboratories, San Juan Island, Washington. White = high flow deployment sites; black = low flow deployment sites; ovals = deployment sites at low tide; rectangles = deployment sites at high tide.

Figure 3. Mean percent egg capsule survival (± SD) across five depths in field predation treatments for low and high flow conditions and deployment controls. Deployment controls lost significantly fewer egg capsules than field predation treatments. There was a significant difference between flow regimes, but not among depths.

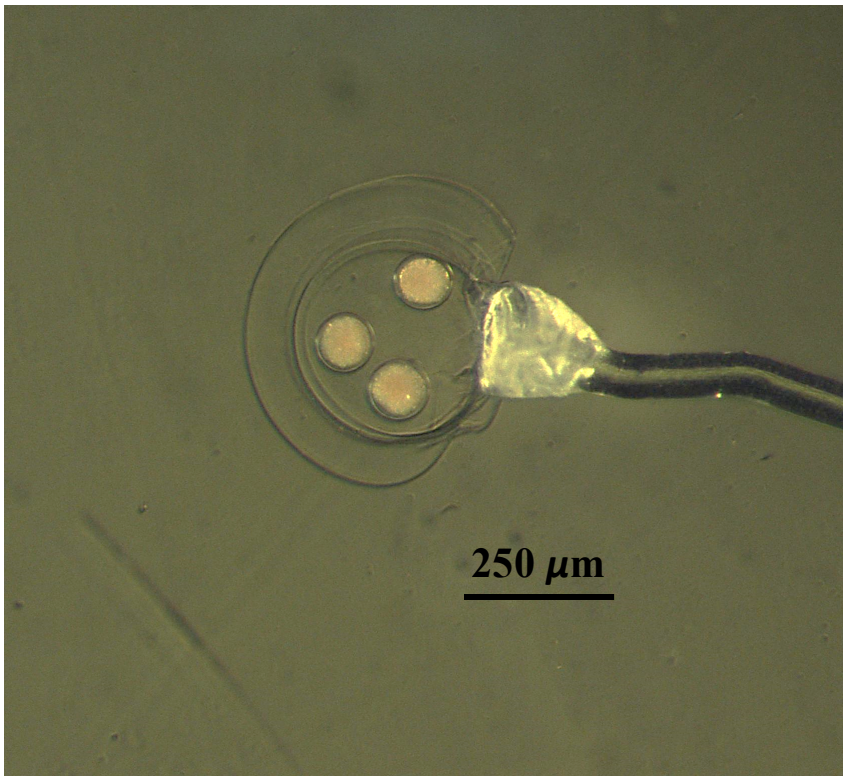

Figure 1

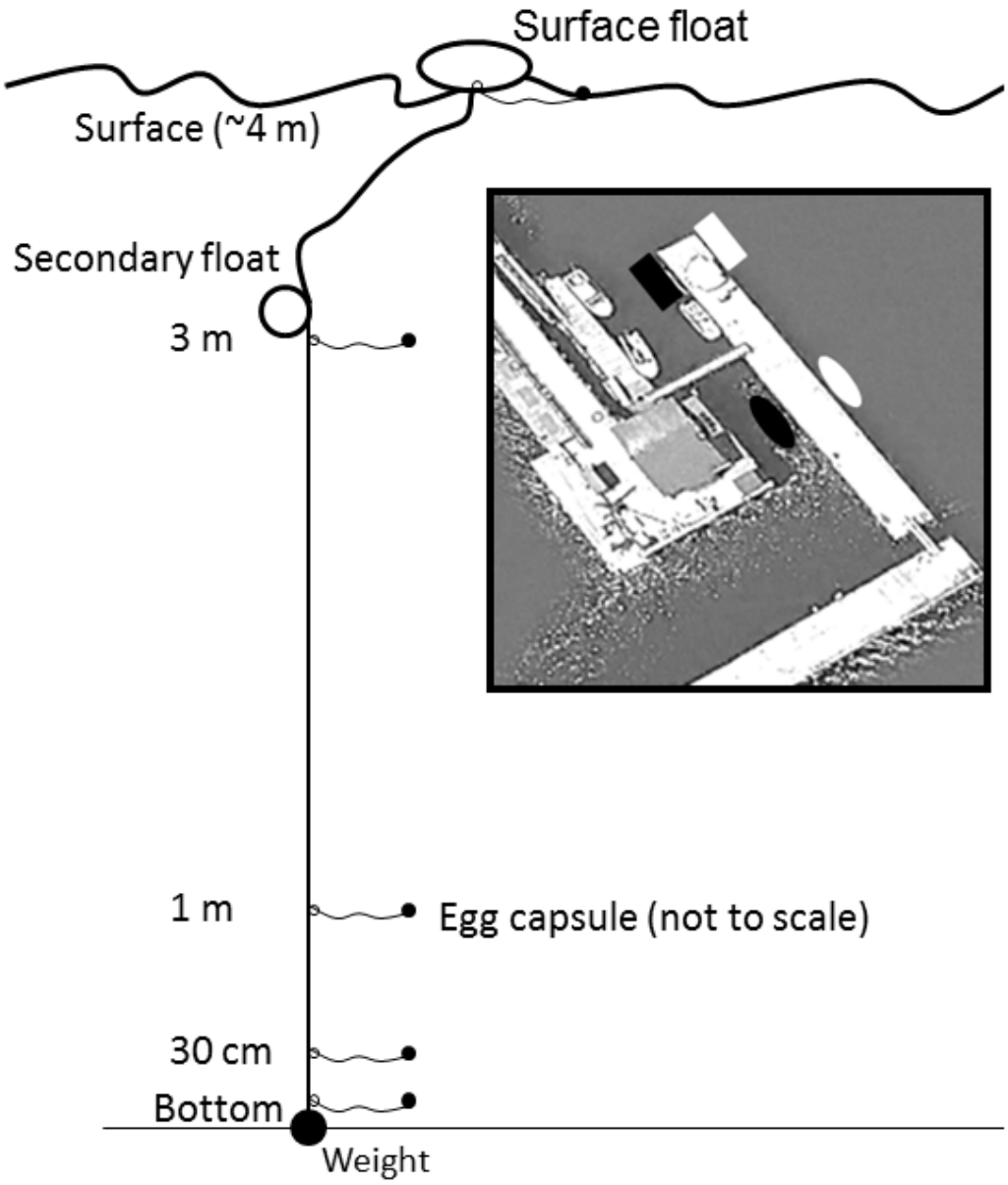
Surface float
Surface (~4 m)
Secondary float
3 m
1 m
Egg capsule (not to scale)
30 cm
Bottom
Weight

Figure 2

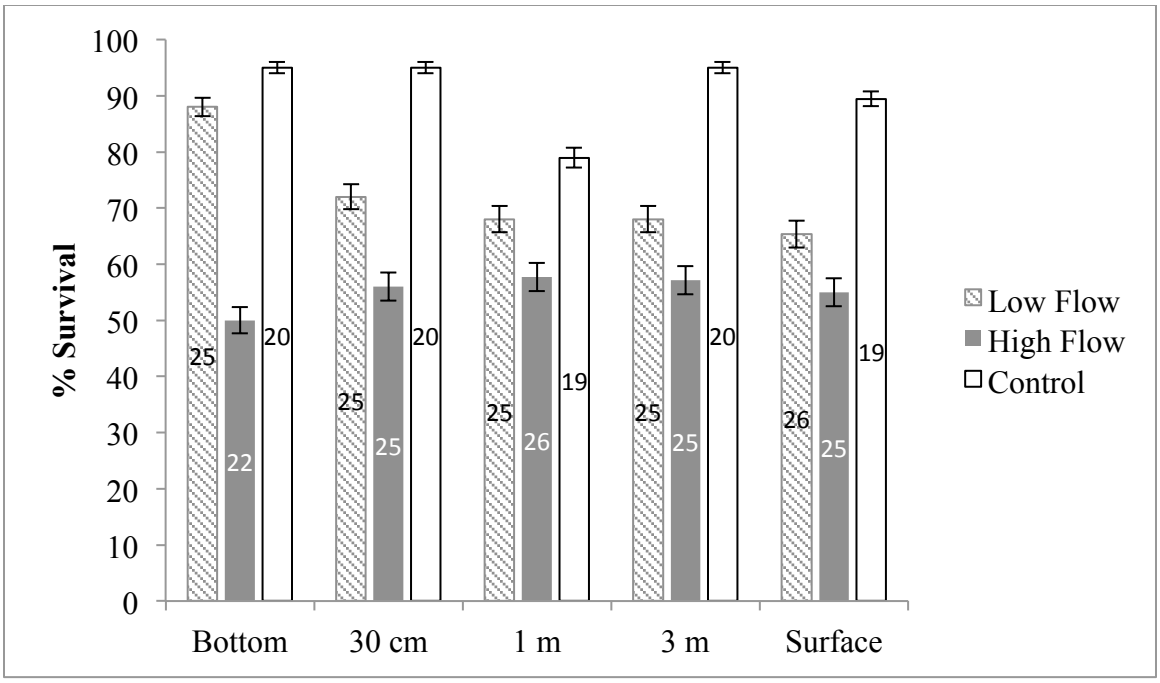
100
90
80
70
60
50
40
30
20
10
0
% Survival
25
22
20
25
25
20
25
26
19
25
25
20
26
25
19
Bottom
30 cm
1 m
3 m
Surface
Low Flow
High Flow
Control

Figure 3