Technical note

Sink before you settle: Settlement behaviour of Eastern oyster (*Crassostrea virginica*) larvae on artificial spat collectors and natural substrate

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ABSTRACT

Eastern oysters (*Crassostrea virginica*) are ecosystem engineers that are important to the ecological and economic sustainability of Atlantic Canada's estuarine resources. Recently, there has been an increased industry demand for oyster spat (i.e., newly-settled larvae), which is often collected using artificial spat collectors suspended in the water column. Little is known, however, about the interactions between artificial spat collectors and naturally occurring substrates, and whether or not such collectors can affect oyster recruitment to wild beds which are simultaneously fished. In the laboratory, we mimicked the typical set-up of artificial spat collectors used in Atlantic Canada to measure differences in spat recruitment between locally-used artificial collectors and natural shell substrate under real-life spat-collection scenarios. Larvae were allowed to settle on various substrates where they occur in a natural system and were subsequently counted. In both single substrate and choice experiments, results indicated that oyster larvae recruited in higher numbers to natural shell substrate located on the benthic surface compared to suspended shell; and there were no significant differences among suspended substrates. This experiment provides evidence supporting existing theories around recruitment behaviour by oyster larvae, mainly that settling oysters tend to sink before they settle, suggesting that spat collectors in the wild may have little effect on wild recruitment. With increasing densities of artificial collectors in estuarine systems to meet system demands, however, further research is needed to investigate the potential draw of oyster larvae away from benthic oyster beds under more realistic natural conditions before broad conclusions regarding spat collector effects on wild oyster populations can be achieved.

1. Introduction

The eastern oyster (*Crassostrea virginica*) has long supported a vibrant industry in Atlantic Canada, while also delivering essential ecosystem services (Poirier et al., 2017). As ecosystem engineers, oysters provide food and a structured habitat for a diverse group of estuarine organisms, as well as being able to improve water quality (Dumbauld et al., 2009; Grabowski et al., 2012; Hosack et al., 2006; Kennedy, 1996). In Atlantic Canada, wild oysters are found mostly in shallow estuarine bays and coastal areas with both soft and hard bottoms (Carriker and Gaffney, 1996). In 2016, the landed weight and value of oysters from both aquaculture and fisheries in Prince Edward Island (hereafter PEI) and New Brunswick was 4 751 715 kgs and $21 757 000

CAD (Statistics Canada, 2017).

Due to the economic importance of oysters in both commercial fisheries and aquaculture operations, there has been increased demand for oyster spat (i.e., newly-settled larvae) in order to maintain or expand production levels. Presently, there are 226 spat collection (fishing) licences issued on Prince Edward Island, and the demand for additional licences is progressively growing (Chris Mills, DFO Charlottetown, pers. comm.). For growing operations, oyster spat is collected by deploying artificial collectors in the water column after fertilization has occurred and free-floating veliger larvae develop in the water column for three to four weeks. During this process, oyster veliger larvae metamorphose into pediveliger larvae (afterward deemed "competent" for settlement; (Cranfield, 1973; Wallace et al., 2008) and
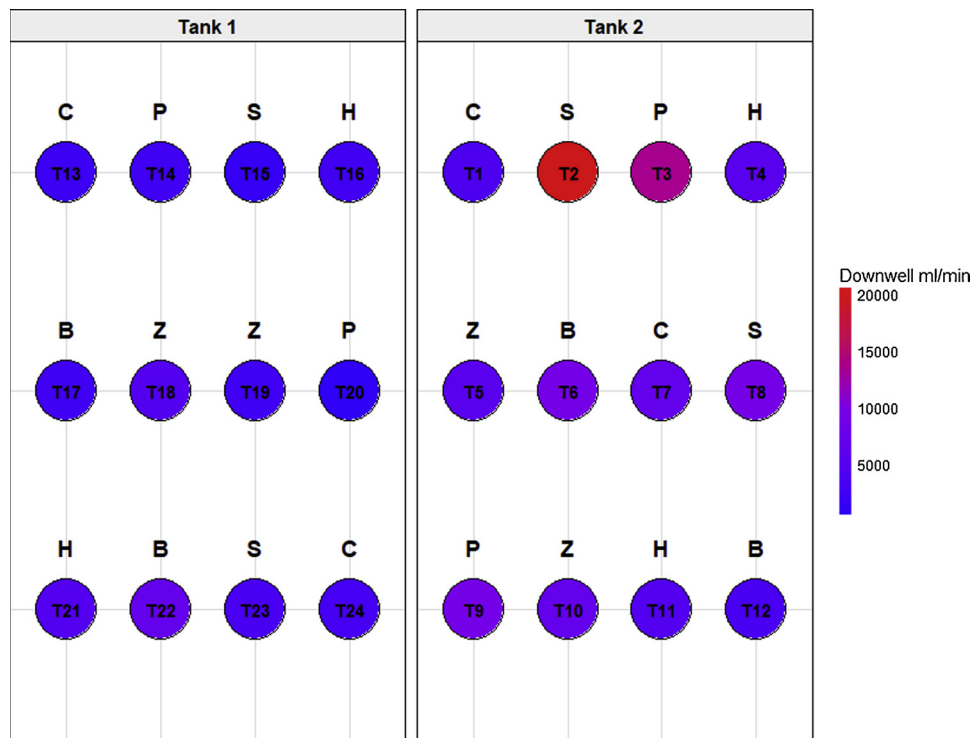

Fig. 1. Layout for the substrate preference experiment. Letters denote the substrate used. B – Bottom Shell, S – Suspended Shell, P – Corrugated PVC pipe, H – Oyster Hat, Z – Zapco© collector, C – Combination, or, all substrates included. Approximate downwelling amount of each cylinder used in the experiment (ml/min).

subsequently search for suitable hard substrates such as rocks, anthropogenic structures, or other oyster shells (Carroll et al., 2015; Fitt et al., 1989; Grant et al., 2013; Tamburri et al., 1992; Veitch and Hidu, 1971). Pediveligers then release a cement-like adhesive to become physically anchored to the hard substrate (Burkett et al., 2010; Metzler et al., 2016). In order to maximize spat collection, specially designed collectors made of various hard materials are deployed near particularly productive areas to collect settling spat. This spat is then used to provide additional oyster biomass for aquaculture operations and other wild oyster beds that do not recruit suitable numbers of oysters.

Some drivers of substrate selection during recruitment for oysters are known such as the presence of bacterial films and other organic compounds (Carroll et al., 2015; Grant et al., 2013). Additionally, oyster larvae are known to use chemical cues released by adult conspecifics that can act as an attractant and can induce settlement behaviour, which may imply that spat may settle most frequently on wild oyster reefs at the benthic surface (Bonar et al., 1990; Tamburri et al., 2007, 1992; Turner et al., 1994; Zimmer-Faust and Tamburri, 1994). However, there is a distinct need to explore this further in the context of Atlantic Canada's burgeoning *C. virginica* spat collection industry. Additionally, with an increase in the placement of artificial spat collectors in proximity to wild beds, the extent to which these collectors may draw from larvae production, and thus settlement on and recruitment to wild beds, is currently unknown. An opposite argument can be made that the increased presence of broodstock adult oysters from large culturing operations nearby on the surface would greatly increase the number of viable larvae in the water column, thus mitigating some of the effect on recruitment to wild beds. Unfortunately, the question of whether larval supply is limiting or saturated has not been explored. As such, interactions between wild beds and oyster farms in the context of larval recruitment remain unclear and contentious. These interactions are undoubtedly complex and may be driven by substrate preferences, uneven larval production and local hydrodynamics, among other variables.

It is apparent that understanding the interactions between wild oyster beds and aquaculture operations is imperative to ensure the

sustainability of shellfish aquaculture. As such, this study aimed to investigate C. *virginica* recruitment under varying scenarios of artificial spat collector presence under conditions of larval saturation in the laboratory. We mimicked the presence of three different types of artificial spat collectors, as well as suspended oyster shells (i.e., the presence of artificial oyster cages), in the water column above bottom oyster shell to determine whether or not artificial spat collectors reduced larval settlement to natural bottom substrate.

2. Methods

### 2.1. Oyster spawning and spat collection

Cultured adult oysters were collected in November 2017 from an oyster grow-out operation in Grand River, PEI (located in the Malpeque Estuary), and transferred to the Bideford Marine Centre in Ellerslie, PEI, which is operated by the Lennox Island First Nation. All adult oysters were then placed in 10 °C water which was gradually increased by 1 °C day^{-1} until 18 °C, at which the oysters were conditioned until spawning. On March 1st, the adult oysters were cleaned and rinsed with a 10% bleach solution and were then set in an 18 °C water bath for one hour after which the temperature was increased to 27.5 °C to stimulate spawning. Stripped gonads of several additional oysters were placed in the tanks to aid with spawning stimulation (Wallace et al., 2008). Once they spawned, the oysters were immediately removed from the water bath and placed in smaller, individual seawater containers at the same temperature (27.5 °C). Sperm and eggs were collected and mixed, with sperm being added at a low rate. The mixture was examined for fertilization and cell division periodically. After the majority of cells demonstrated division, approximately 44,000 larvae were transferred to each of the experimental cylinders (described below; larval density ~ 1 larvae mL^{-1}).

2.2. Experimental design and setup

The settlement experiments were conducted in two 400 L flow-
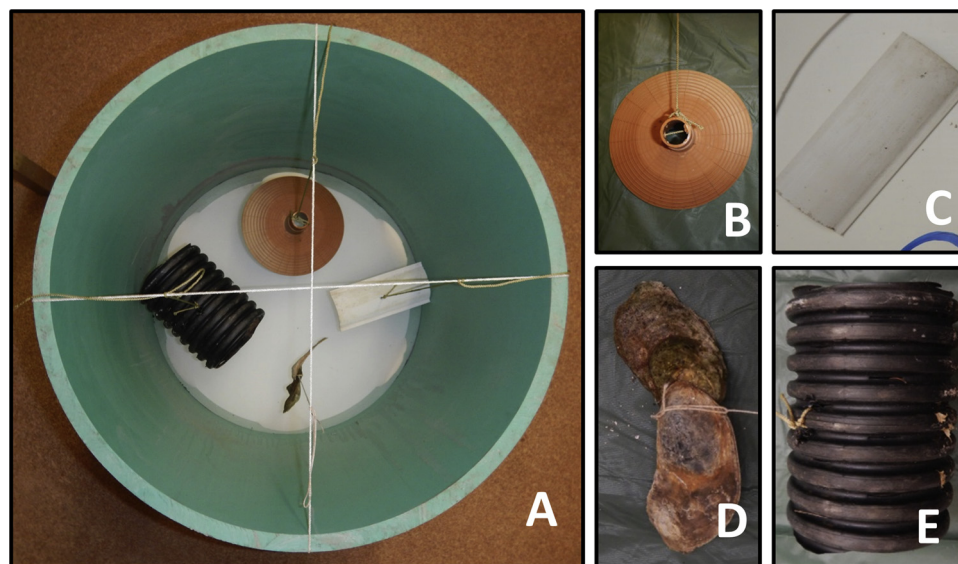
Fig. 2. Experimental substrates used in the experiment: A) combination of all four substrate types (bottom shell missing from photo), B) Oyster hat collector, C) Zapco© collector D) oyster shell, and E) PVC pipe collector.
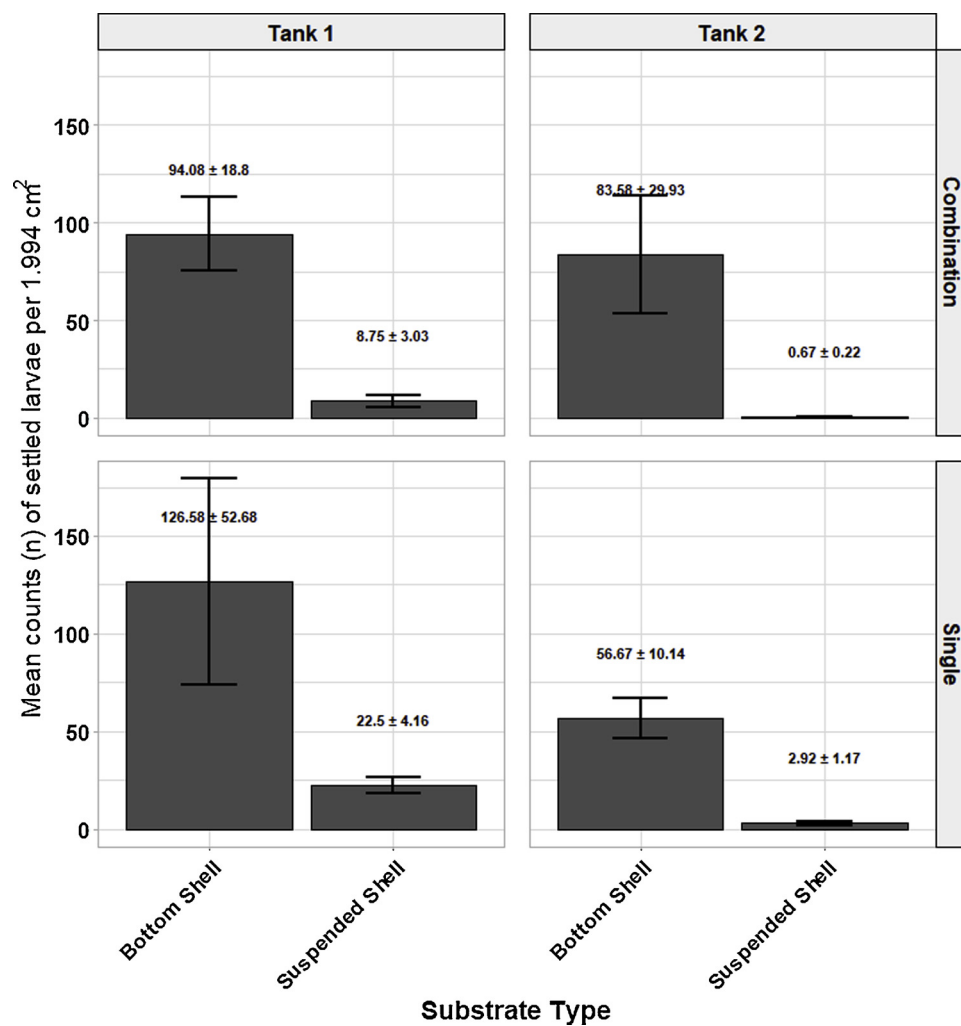
Fig. 3. Mean counts of settled larvae (spat) per 1.994 cm² on Bottom Shell and Suspended Shell. Facets indicate the mean counts across single and combination cylinders, as well as across tanks.
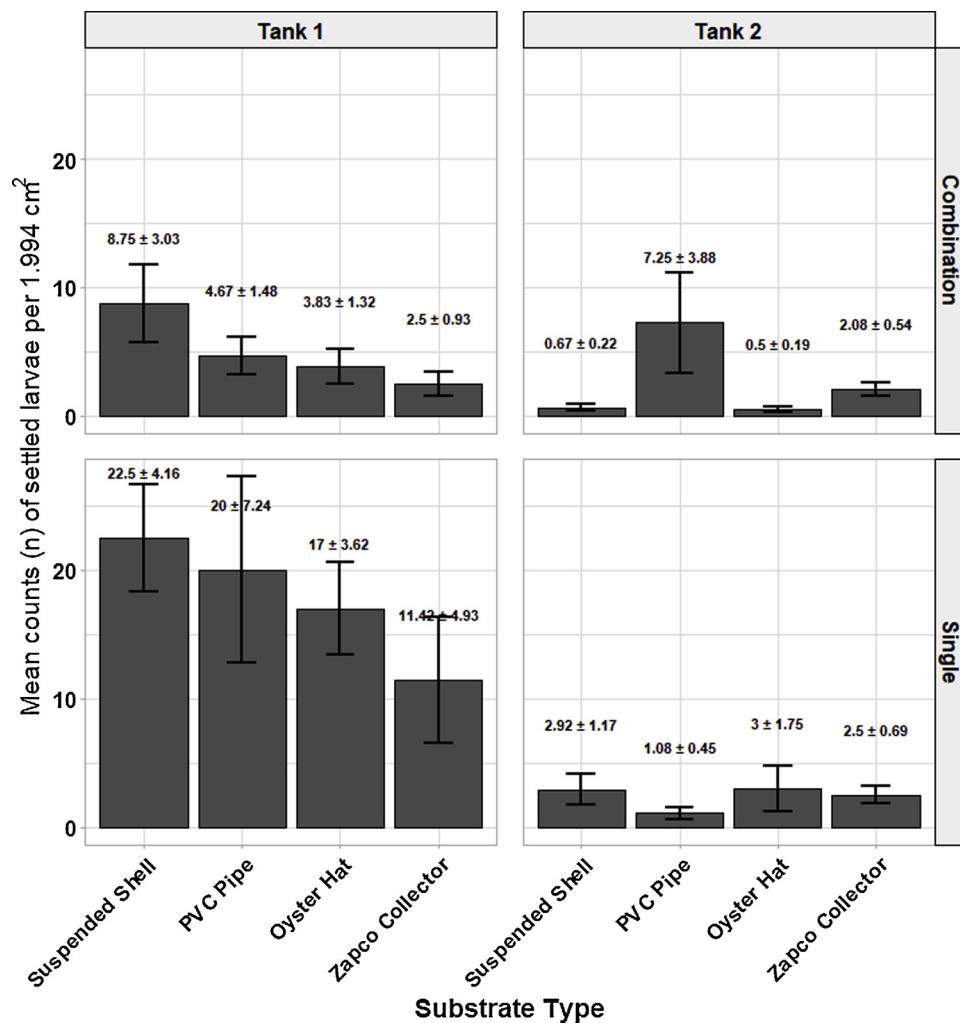
Fig. 4. Mean counts of settled larvae (spat) per 1.994 cm² on Suspended Shell, Corrugated PVC pipe, Oyster Hat. Facets indicate the mean counts across single and combination cylinders, as well as across tanks.

through tanks containing natural seawater from Bideford Bay that was filtered through a sand filter followed by sequential 25 μm, 10 μm, and 5 μm bags, followed by a 5 μm cartridge filter and finally through a 2 m UV filter. Each tank was partitioned into 12 experimental units that corresponded to an individual replicate from each experimental treatment ($N = 24$ units; $n = 4$ units treatment^{-1}; see next paragraph). The experimental units were comprised of 45 cm diameter PVC cylinders (dark grey colour; water volume ~44 L) equipped with a flow-through downweller and a 200 μm screen placed 2.5 cm from the bottom to prevent larval escape. We were unable to physically standardize downweller force across experimental units, but statistically controlled for random effects across experimental units (see *Data Collection and Analysis* section below). The relative displacement for each cylinder was calculated and is displayed in Fig. 1.

Each experimental treatment corresponded to the available substrate(s) provided to the oyster larvae. Substrates used in this experiment included typical artificial spat collectors used in Atlantic Canada, including corrugated PVC pipe, Oyster Hat, and Zapco© collectors, as well as natural adult oyster shells (Fig. 2); all artificial substrates were covered in a cement mixture to mimic industry practices. From these substrate types, we derived six treatments to quantify larval recruitment: suspended PVC only (P), suspended Oyster Hat only (H), suspended Zapco© only, suspended shell only (S), bottom shell only (B), and a combination treatment (C) where larvae were given a choice to settle on all five substrate types. All suspended substrates were placed at an approximate depth of 10–15 cm in the water. Because biofilms

occur naturally on substrates in the wild and can influence oyster settlement (Tamburri et al., 2008), natural biofilms were allowed to accumulate by exposing all substrates to unfiltered flow-through seawater for 72 h prior to experimentation. Experimental substrates were then randomly placed in experimental units according to the six treatments and larvae were subsequently added (as described in the previous section) and given approximately two weeks to settle. The number of larvae added to each cylinder was consistent across treatments as it was considered sufficient to saturate the column regardless of the amount of surface area present.

2.3. Data collection and statistical analyses

After the two-week settlement period, substrates were carefully removed from the experimental units and larvae were counted using a random sampling method. To achieve random sampling, a one-inch wide ring (1.994 cm²) was randomly placed on both the top and the bottom of each substrate three times and a count of visible spat under a magnifying glass was taken (6 counts taken per substrate). The analyses of larvae counts per substrate type, for both single and combined choice experiments as described above were completed using R version 3.4.1 (R Core Development Team, 2017). Differences between counts on suspended versus benthic shell as well as differences between counts on all other suspended substrates were explored. The significance threshold was set at $\alpha = 0.05$. As we were analysing count data, negative Binomial Mixed effects analyses were conducted using the

Table 1

Results of negative binomial mixed effects models for counts of oyster spat. Factors were Substrate (Fixed), Downweller ID (Random) and Tank (Random). For the fixed effects, the reference group/substrate is bottom shell.

Single Cylinders
Fixed Effects

Response: Counts	Estimate	SE	z-value	p-value
Intercept	4.3459	0.7381	5.888	< 0.0001
Oyster Hat	−2.4833	0.4966	−5.000	< 0.0001
PVC Pipe	−2.8107	0.5083	−5.529	< 0.0001
Suspended Shell	−2.4090	0.4995	−4.823	< 0.0001
Zapco© Collector	−2.7861	0.4992	−5.582	< 0.0001

Random Effects

	Variance	Std. Dev.		
Downweller	0.3006	0.5483		
Tank	0.8488	0.9213		

Combination Cylinders
Fixed Effects

Response: Counts	Estimate	SE	z-value	p-value
Intercept	4.4480	0.3262	13.638	< 0.0001
Oyster Hat	−3.8387	0.3771	−10.178	< 0.0001
PVC Pipe	−2.7301	0.3540	−7.713	< 0.0001
Suspended Shell	−3.1090	0.3679	−8.452	< 0.0001
Zapco© Collector	−3.5523	0.3734	−9.513	< 0.0001

Random Effects

	Variance	Std. Dev.		
Downweller	0.1185	0.3443		
Tank	0.0383	0.1956		

Table 2

Marginal contrasts of fixed effects categories. Results are given on the log scale.

Contrast (single model)	Estimate	SE	p-value
Bottom Shell – Oyster Hat	2.486	0.497	< 0.0001
Bottom Shell – PVC Pipe	2.810	0.508	< 0.0001
Bottom Shell – Suspended Shell	2.409	0.500	< 0.0001
Bottom Shell – Zapco© Collector	2.786	0.499	< 0.0001
Oyster Hat – PVC Pipe	0.327	0.515	0.969
Oyster Hat – Suspended Shell	−0.074	0.507	0.999
Oyster Hat – Zapco© Collector	0.303	0.506	0.945
PVC Pipe – Suspended Shell	−0.401	0.513	0.936
PVC Pipe – Zapco© Collector	−0.025	0.518	1.000
Suspended Shell – Zapco© Collector	0.377	0.509	0.9470
Contrast (combination model)			
Bottom Shell – Oyster Hat	3.839	0.377	< 0.0001
Bottom Shell – PVC Pipe	2.730	0.354	< 0.0001
Bottom Shell – Suspended Shell	3.109	0.368	< 0.0001
Bottom Shell – Zapco© Collector	3.552	0.373	< 0.0001
Oyster Hat – PVC Pipe	−1.108	0.404	0.048
Oyster Hat – Suspended Shell	−0.730	0.377	0.298
Oyster Hat – Zapco© Collector	−0.286	0.404	0.954
PVC Pipe – Suspended Shell	0.379	0.396	0.875
PVC Pipe – Zapco© Collector	0.822	0.407	0.256
Suspended Shell – Zapco© Collector	0.443	0.394	0.793

"lme4" and "ggeffects" packages, and post-hoc tests were completed using the "emmeans" package (Bates et al., 2015; Lenth, 2018; Ludecke, 2018). Post-hoc test results were calculated on the log scale. Originally, Poisson distribution models were attempted; however, as the mean and variance were not equal, the Negative Binomial family of models was used. Model structure included Substrate type as a fixed effect, with Downweller cylinder ID (1–24) nested within Tank (1–2) as random effects. As AIC values were lower when random effects were included in the model structure, it was determined that their inclusion was warranted. Using this structure, cylinders that held only one substrate were compared, followed by comparing the substrates within the combination cylinders. Finally, we further tested whether or not the presence of artificial spat collectors in the water column could affect recruitment to

natural substrate (i.e., bottom shell) by comparing larval counts on bottom shell between the single and combined treatments using a two-tailed Student's *t*-test.

3. Results

In general, bottom shell substrate performed much better at recruiting spat than suspended shell. Mean counts per 1.994 cm^2 for the bottom shell substrate were several folds higher than suspended shell (Figs. 3 and 4). For example, in Tank 1, in the single treatment, the bottom shell substrate had an average spat count of 125.58 ± 52.68 (Mean ± SE) per 1.994 cm^2, while the suspended shell substrate had an average count of 22.5 ± 4.16. Counts between suspended substrates in the combination treatment ranged from 0.5 ± 0.19 (suspended shell) to 8.75 ± 3.03 (suspended shell), while in the single treatment, suspended substrate counts ranged from 1.08 ± 0.45 (PVC Pipe) to 22.5 ± 4.16 (suspended shell). Tank 1 counts on each substrate were all higher than Tank 2 counts except for PVC pipe in Tank 2 being higher than Tank 1 (Fig. 3). Downweller force was varied (Fig. 2), however, and overall trend of higher downwelling force can be seen in Tank 2.

In order to test for significant differences between substrate types, negative binomial mixed models were used. These models revealed significant differences between substrate types (Table 1). Contrasts revealed that the bottom shell substrate had better larvae settlement rates than suspended shell in both the single and combination groups of treatments (Table 2). In addition, there was no statistical difference in larval counts on bottom shell between the bottom shell only treatment (88.8 ± 84.8) and the combination treatment (91.6 ± 133.4) (two-tailed *t*-test: t$_{39}$ = 2.02, p = 0.932). With regards to the suspended substrates, only one slightly significant difference was noted; between the Oyster hat and PVC pipe treatment in the combination group of treatments (Table 2). Furthermore, when comparing the counts that were taken on the "top" versus the "bottom" of all the substrates, a paired *t*-test revealed that the bottom of the substrate performed significantly better than the top of the substrates (p = 0.006).

4. Discussion

Our results indicated that significantly more oyster larvae settled on oyster shell at the bottom of our experimental units than on suspended shell in the water column, both when substrate types were offered alone and when oysters were given a choice. Interestingly, an overall trend was noticed that spat counts did increase on artificial substrates when comparing multi-choice to single treatments, which may have occurred due to overall substrate availability, or optimal substrate selection. This result could be due to the inherent biology of oyster larvae. It is well documented that oyster larvae are not passive settlers, but actively seek out suitable substrate (Bonar et al., 1990; Cranfield, 1973; Hadfield and Paul, 2001; Turner et al., 1994; Zhao et al., 2003). Furthermore, the inherent biological drive to sink to the bottom when ready to settle (Tamburri et al., 1992), as well as their metamorphosis into the pediveliger larvae stage, likely contributed to our results. While it may be argued that oysters predominantly used chemical cues from other shells to settle or that shell offered the best substrate (Pawlik, 1992; Tamburri et al., 1996; Turner et al., 1994; Zimmer-Faust and Tamburri, 1994), oysters did not settle on suspended shell in comparable numbers to bottom shell, which indicates that location is likely more important than substrate when it comes to settling on natural shell. It should be noted, however, that we did not include tests of artificial substrates on the bottom because such scenarios are unrealistic given current techniques used in Atlantic Canada. As such, whether or not artificial spat collectors located on the benthos would affect larval recruitment to natural substrate remains unknown. Furthermore, as measured here and supported by other studies, spat were predominantly found on the underside of bottom shell where they would be most protected from

physical force of water movement (Bartol and Mann, 1997). Thus, it seems most likely that the settlement counts herein were driven by active behavioural selectivity exhibited by the oyster larvae, which is in agreement with previous studies (Tamburri et al., 2008, 1992).

The results of our experiment suggest that the presence of artificial spat collectors in the water column is unlikely to impact natural oyster recruitment. It is difficult, however, to extrapolate the results observed herein to what might be observed under natural conditions in the wild, particularly given that the larvae in our experiment were from cultured broodstock and not from wild populations, and that the degradation of natural benthic substrate due to increased siltation from erosion is widely reported (Comeau, 2014; Comeau et al., 2014; Mallet et al., 2006). Further, anecdotally, fishers report high levels of variability on spat collectors and there is a lack of information regarding natural larval densities of eastern oysters in nearshore PEI waters. We also did not account for larval viability and condition pre- and post-settlement, which could also differ from wild larvae and thus impact the transferability of our results to natural conditions. For instance, predation pressure may be much higher on benthic substrates, whereas suspended substrates may be more susceptible to fouling. Furthermore, results from our small-scale tank experiment should be confirmed in larger systems (i.e., bay-scale systems) where the density of artificial spat collectors is likely to be quite different from our experiment. Field experiments staining and tracking larvae in the wild during periods spat collection may help to better understand how these artificial collectors affect recruitment under natural conditions. Coupling such a study with detailed hydrodynamic modeling would provide a more robust understanding of how spat collectors might affect wild recruitment. Such information could be fed into large-scale numerical models to fully comprehend interactions between wild oyster populations and co-existing oyster farming.

Unfortunately, we weren't able to control downwelling force which could direct larvae to the bottom of the cylinders, rather than keep them suspended in the water column (Dekshenieks et al., 1996). This seems unlikely, however, given that one would expect downwelling forces to create rotational mixing within the experimental chambers rather than a linear cline of seawater. Furthermore, we observed some settlement on the suspended artificial substrates, which was predominantly evident on sheltered sides of the artificial substrates, indicating that the oyster larvae certainly had access to and could select for the suspended substrates (rather than larvae just passively collecting on the top of these substrates as the larvae sank). This, coupled with the observed selection for the underside of bottom shell, it seems likely that the oysters in our experiment actively selected for optimal substrate. Even more, if the strength of downwelling pushed larvae to the bottom and influenced settlement, one would expect higher settlement indices on bottom shell from Tank 2, which tended to have a stronger downwelling force (Fig. 1); however, we observed higher settlement indices in the less energetic waters of Tank 1 (Fig. 3).

Ultimately, the results of our experiment suggest that the presence of artificial spat collectors in the water column (based on current spat collection methods in Atlantic Canada) likely has little effect on oyster recruitment to bottom shell substrate when larval densities are saturated. Furthermore, when comparing shell substrate location (i.e., natural bottom shell vs. suspended shell mimicking suspended oyster farming), oyster larvae prefer to settle on the benthos rather than suspended shell in the water column. When comparing the various suspended substrates, there appear to be no clear differences between substrate types. While this suggests that spat collectors likely exert little influence on natural oyster populations when benthic shell substrate is available, field studies coupled with numerical modeling are needed to confirm or refute such a hypothesis. Only then will a detailed and robust understanding of artificial spat collector effects on wild recruitment be established. Nonetheless, our results provide a starting point for better understanding the facets of wild-farmed shellfish interactions and open the door to future studies.

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