

Evaluating the effectiveness of local materials for the wild oyster (*Crassostrea tulipa* Lamarck, 1819) spat collection in four coastal waterbodies in Ghana

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Abstract

The West African mangrove oyster, *Crassostrea tulipa* (Lamarck, 1819), has the potential to improve global shellfish food production and is being considered for commercial farming in many countries in West Africa. The current background information to support this venture is, however, inadequate especially with respect to identification of suitable materials for optimal collection of spat for large-scale production. We assessed the effectiveness of five locally available materials (coconut shell, oyster shell, nylon mesh, PVC, and ceramic tile) for harvesting *C. tulipa* spat from the Densu Delta, Narkwa Lagoon, Benya Lagoon and Whin Estuary, along the coast of Ghana from November 2017 to October 2018. Ceramic tile had the highest mean monthly spat settlement in the Narkwa Lagoon (3451 ± 206 spat m^{-2}), Benya Lagoon (1769 ± 145 spat m^{-2}) and Whin Estuary (373.1 ± 52.4 spat m^{-2}). This settlement was not significantly different from settlement on PVC slats ($P > 0.05$). Coconut shell consistently had the least *C. tulipa* spatfall in all four coastal water bodies ($P < 0.05$). The under-horizontal surfaces of collectors, [mean (S.E.); 2523.7 ± 66.9 spat m^{-2}] had significantly more *C. tulipa* spatfall than upper-horizontal surfaces [mean (S.E.); 775.2 ± 33.4 spat m^{-2}] in the main experiment ($P = 0.000$). In a separate experiment, a change of orientation from "Face down"/ 0° to "Face up"/ 180° did not change the observed profuse under-horizontal settlement of *C. tulipa* spat on the collectors, suggesting that under-horizontal surfaces were more attractive to *C. tulipa* spat. Larger-sized *C. tulipa* spat on under-horizontal surfaces, mean (S.E.) 9.88 ± 0.5 mm, compared to upper-horizontal surfaces, mean (S.E.) 5.99 ± 0.5 mm, of the collectors suggest earlier settlement on the undersides. Ceramic tiles and PVC slats were the most effective materials for *C. tulipa* spat collection, hence, their use recommended for large-scale *C. tulipa* farming.

1. Introduction

The mangrove oyster, *Crassostrea tulipa*, serves as a major source of animal protein for many coastal communities in West African (Adite et al., 2013; Asare et al., 2019). The shells are also used in the indigenous production of paints, traditional medicine and concrete for building (Yankson, 2004). The species is often found either on sandy-mud sediments or attached to mangrove roots and other hard objects in lagoons and estuaries along the coast of West Africa. It is naturally well adapted to the rigorous environmental conditions, surviving wide temperature ranges $20-30^\circ\text{C}$ (Ajana, 1980), $24 - 31.5^\circ\text{C}$ and $27- 36^\circ\text{C}$ (Obodai et al., 1991). Due to easy accessibility of its habitats, the fishery of the oyster is dominated by women (Theisen, 2010; FAO, 2014) who are estimated to earn as much as US \$ 150.00 per month mainly through the collection, processing and sale of the oyster (Cormier-Salem et al., 2010). In order to sustain and possibly enhance the earnings and nutritional benefits derived from *C. tulipa*, many coastal communities in West Africa are considering farming the oyster on a large scale (Ishengoma et al., 2011). Further, threats of mass mortalities due to diseases, in the tilapia aquaculture sector which is about 95 % of total aquaculture production in some West African counties e.g. from the Volta Lake in Ghana (FAO, 2018), raises deep concerns for the diversification of cultured species and geographical coverage in the development of aquaculture. Hence, the efforts to farm the native oysters.

The culturability of *C. tulipa* has been established within the last three decades (Kamara, 1982; Yankson, 1990). Recent studies have also farmed the oyster to marketable size within a reasonable period of time using cultches prepared from coconut shells (Asare et al., 2019). However, efforts towards large-scale culture of *C. tulipa* are impeded by myriad factors including inadequate scientific data on the local hydrodynamic conditions that promote the recruitment, larval export to new favourable areas, and suitable microhabitats for its growth. The biological, physiological and mechanical considerations in seed production and the methods for doing so are also not fully understood. In addition, research on appropriate hatchery techniques that can be used to mass-produce the spat of *C. tulipa* for onward transfer to suitable locations in coastal water bodies is limited. Yankson (1990) successfully produced gametes of the species from artificial fertilization through to settlement. This development was stalled by the unavailability of facilities for culturing marine microalgae to feed larvae and spat in the laboratory, which is expensive and not easily reproducible in poor areas (Dégremont et al., 2007; Mann, 1983; Tanyaros and Chuseingjaw, 2016).

Due to the foregoing limitations, and as done for other species of oysters in different parts of the world (Gosling, 2015; Quayle and Newkirk, 1989), spat for farming *C. tulipa* could be harvested from the wild using artificially introduced substrates or cultches. Elsewhere, these artificial collectors include concrete slabs (Pillay and Kutty, 2005), plastic mesh nettings in different forms and shapes (Friedman et al., 1998; Southgate and Lucas, 2008; Taylor et al., 1998a; Urban, 2000), plastic polyethylene sheet (Friedman et al., 1998; Nayar et al., 1980), polyvinyl chloride (PVC) materials (Helm and Bourne, 2004; Taylor et al., 1998a), empty oyster shells (Pillay and Kutty, 2005), tiles (Giese and Pearse, 1979; Miller and Hall, 1995) and empty coconut shells (Quayle and Newkirk, 1989). These records suggest that the spat of the oyster settle on a wide range of materials.

For the profitability of farming operations, the use of cheap, durable and locally available cultches are recommended (Vakily, 1989). It is also prudent to measure cost against yield to maximise profitability in any aquaculture business (Ahmed, 2007). Therefore, other strategic considerations on the suitability and effectiveness of collectors and their positional orientations would be worthwhile in the farming of *C. tulipa*. Previous studies suggest best yield of spat of other oyster species on collectors placed horizontally in water column (see for example Hopkins, 1937 [Olympia oyster, *Ostrea lurida*]; Schaefer, 1937 [Japanese oyster, *Ostrea gigas*]; Cole and Knight-Jones, 1939 [European flat oyster, *Ostrea edulis*]), with no record for *C. tulipa*. That aside, accounts of spat settlement and yield on the different surfaces of horizontally placed collectors are not consistent (Friedman et al., 1998; Soria et al., 2015; Taylor et al., 1998a), and needs to be investigated for different species of varying ecosystems.

As part of a broader scope of research on *C. tulipa* towards the large-scale farming of the species along the coast of West Africa, this study set out to assess the effectiveness of five locally available materials for the collection of *C. tulipa* spat for culture. The materials were coconut shells, empty shells of *C. tulipa*, nylon net, Polyvinylchloride (PVC) material and ceramic tiles. Their relative effectiveness and effects of their surface contour relative to horizontal orientation in the water column were evaluated in four coastal

water bodies along the coast of Ghana. We have discussed our results in the context of previous findings on other species of oysters from other ecosystems.

2. Materials And Methods

2.1 Preparation of collectors and construction of cultches

The spat collector materials investigated in this study were coconut shell, nylon net (mesh size = 2 mm), oyster shell, PVC and ceramic tiles (Figure 1). Coconut shells, with husk removed, were collected from fields around the study area, cleaned, washed in sea water and dried before using for the study. Fine mesh nylon nettings were cut out into sizes of $10 \times 10 \text{ cm}^2$. Oyster shells were obtained and treated the same as the coconut shells before use. PVC pipes (diameter $\approx 10 \text{ cm}$) were cut into smaller curved slats of height and surface area 10 cm and 106 cm^2 , respectively. Fifty-by-fifty cm^2 ceramic tiles were also diced into smaller sizes of $10 \times 10 \text{ cm}^2$.

Each collector type was strung together in threes on polypropylene ropes, using the approach described by (Chuku and Osei, 2017) in order to minimise wastage of cultch construction materials. The three collectors were fastened on each rope through holes (diameter $\approx 4 \text{ mm}$) drilled at the centre and kept equidistant from each other on the rope by knots to form a cultch. Collectors in a cultch were held horizontally on the ropes in order to ensure maximum spat harvest as observed in previous studies (Hopkins, 1937; Schaefer, 1937; Cole and Knight-Jones, 1939). The lengths of the ropes were varied, depending on average high water depth at each station within the water bodies selected for this study. The vertical location of collectors along the length of each rope was designated "top", "middle" and "bottom" at each experimental station in the selected water bodies.

2.2 Determination of surface area of irregular collectors

To standardize the estimate of total spatfall in this experiment, the surface area of the collectors was used ($Sf = Ns/Ac$; Where Sf = spatfall, Ns = number of spat on collector surface, and Ac = surface area of collector). For coconut and oyster shells, their surface areas were determined by first tracing the outline of their shapes on a square-grid paper (grid size = 2 cm subdivided into 0.2 cm minor grids) and the total grid area that fell within each outline taken as the area of one surface. Collectors made from nylon mesh, PVC, and ceramic tiles were cut into definite sizes (see section 2.1). Hence, their surface areas were determined by a multiplication of the length by breadth. The surface area values were then multiplied by two to obtain the total surface areas of the collectors.

2.3 Study sites, experimental design and data collection

Based on field surveys and previous reports on thriving populations of *C. tulipa* in Ghana (Asare et al., 2019; Janha et al., 2017; Obodai and Yankson, 2002; Yankson, 1990), four coastal waters bodies were selected for this study (Figure 2). These were the Densu Delta ($0^\circ 16' 43'' \text{ W}$, $5^\circ 34' 07'' \text{ N}$ and $0^\circ 20' 02'' \text{ W}$, $5^\circ 30' 21'' \text{ N}$), Narkwa Lagoon ($0^\circ 56' 22'' \text{ W}$, $5^\circ 12' 17'' \text{ N}$ and $0^\circ 54' 41'' \text{ W}$, $5^\circ 12' 32'' \text{ N}$), Benya Lagoon ($1^\circ 20' 50''$

W, 5°04'59" N and 1°21'26" W, 5°05'18" N) and Whin Estuary (1°46'47" W, 4°52'52" N and 1°46'04" W, 4°52'30" N). Three experimental stations (ST) were established in each water body to cover the head, middle and mouth regions, except for Densu Delta where ST3-DD was eliminated due persistent destruction of racks by local fishers (Figure 2). At each experimental station, three cultches each of the five different collector materials were deployed on a fixed bamboo rack; each cultch was made of three units of same collector material. A total of 45 collectors were, therefore, deployed per station and 540 for all the water bodies combined. Sampling was done monthly for one year from November 2017 to October 2018 by harvesting all cultches and replacing with a new set each month. Cultches were examined in the laboratory for settled spat on the collectors. The total number of spat on each collector was counted under a laboratory lamp; which provided a higher localised luminescence relative to laboratory ambience. A hand lens was used in instances of very small spat of shell height <1 mm. Mean spatfall per m² was then calculated using the equation:

$$\bar{X} Sf (m^{-2}) = [n^{-1} \cdot \sum_{i=1}^n (Sf_i) (cm^{-2})] \times 10000$$

Where $\bar{X} Sf$ = mean spatfall, Sf_i = spatfall on individual collectors, $i = 1, 2, 3 \dots n^{th}$ replicate collector, and n = number of replicate collectors/surfaces.

2.4 *Crassostrea tulipa* spat settlement on upper- and under-horizontal surfaces of collectors

The number of settled *C. tulipa* spat was counted separately for the upper- and under-horizontal surfaces of each individual cultch deployed. Another spatfall experiment was set up in March 2018, in the Densu Delta and Narkwa Lagoon, alongside the main experiment, to verify the general observation of relatively more spat setting on the underside of collectors than the upper side during the first three months of sampling. Since the two sides of each collector material were characteristically different either in texture, shape or contour, it was found necessary to test the influence of the surface in the horizontal position. In this confirmatory experiment for *C. tulipa*, collectors were strung in the opposite direction to the regular spatfall experiment as shown in Figure 3, on separate racks. The nylon mesh was excluded in this experiment. Spat were counted, shell heights (SH) were measured and a 0° (face down) versus 180° (face up) comparison of the surfaces was done statistically.

2.5 Data analysis

The comparative effectiveness of the different collectors within individual water bodies was done using a three-way Analysis of Variance (ANOVA). The sources of variance in this analysis were the different stations within each water body, sampling month and type of collectors used. A mixed model multi-way ANOVA was used to compare the effectiveness of the collectors between the different water bodies. Here, type of collector, water body, and sampling month were the fixed factors, sampling station was a random factor, whilst the response was spatfall on the collectors. The analysis was done using General Linear

Model function in Minitab®, version 18.1. The effect of surface contour on *C. tulipa* spatfall on the upper- and under-horizontal surfaces of the collectors within water body was determined using a three-way ANOVA (collector surface × collector material × collector orientation). The critical p-value for all the ANOVA was taken to be 0.05. Tukey's HSD ($\alpha = 0.05$) was used as the post-hoc test to determine which pairs of means were significantly different. Homogeneity of the variance in the data was verified using Levene's Test and data transformed to $\log_{10}(x + 1)$, where necessary, prior to ANOVA. Further, significant differences between the most effective collector material (highest mean spatfall) for harvesting *C. tulipa* spat and the other collectors, were identified using the Hsu's Multiple Comparison with Best (MCB) method (Hsu, 1992) using a 5 % family error rate. The difference of means was used to rank collectors relative to the best. Each spatfall value was obtained over a duration of one month.

3. Results

3.1 *C. tulipa* spatfall on artificial collectors

Figure 4 shows the effectiveness of different collectors, evaluated using the number of spat collected per unit area. No spat was observed on cultches made from nylon mesh net during the first three months of this study hence its use was discontinued. Of the remaining cultches, significant differences in the densities of *C. tulipa* spat were observed in all the water bodies combined (Mixed model multi-way ANOVA at $p < 0.05$; Table 1). On average, spatfall was highest on ceramic tile (2007.4 ± 85.5 spat m^{-2}) followed by PVC slat (spatfall = 1846.7 ± 88.2 m^{-2}), oyster shell (spatfall = 1697.2 ± 72.6 m^{-2}) and coconut shell (spatfall = 1046.5 ± 55.6 m^{-2}), in that order. This differential order was however not maintained in all water bodies throughout the study period as changes occurred in some months (see interaction between collector materials and the sampling month, $P = 0.000$, or interaction between collector materials, water body and sampling month considered in the statistical analysis, $P = 0.168$; Table 1). Hsu's MCB test following Tukey's HSD, showed spatfall on ceramic tile (highest mean spatfall) was significantly greater than those observed on both coconut and oyster shells ($P < 0.01$); it was not significantly different from the observation on PVC ($P = 0.156$). The performance of each collector, however, increased with increasing availability of spat from one water body to the other (Figure 4). The significant differences in effectiveness of spat collectors at harvesting *C. tulipa* spat in this study could not be related to third and fourth term interactions with water body, station, depth and/or month except for CM × WB × M (Table 1).

Table 1 - Results of multi-way ANOVA (mixed model) for the effects of type of collector material (fixed factor) on *C. tulipa* spatfall in three columns of water depth (fixed factor) at each of eleven stations (random factor - nested in water body) monthly (fixed factor) throughout the sampling year. Only interactions with type of collector material are shown.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Collector material (CM)	3	221.3	73.75	78.48	0.000
Water body (WB)	3	3459.8	1153.28	1227.28	0.000
Station(Water body) (ST)	7	295.8	42.26	44.97	0.000
Depth (D)	2	1479.9	739.96	787.44	0.000
Month (M)	11	5067.2	460.65	490.21	0.000
CM × WB	9	32.2	3.58	3.81	0.000
CM × ST	21	35.3	1.68	1.79	0.015
CM × D	6	46.6	7.77	8.27	0.000
CM × M	33	75.4	2.29	2.43	0.000
CM × WB × D	18	26.9	1.49	1.59	0.053
CM × WB × M	99	153.9	1.55	1.65	0.000
CM × D × M	66	50.6	0.77	0.82	0.857
CM × ST × D	42	27.1	0.65	0.69	0.938
CM × ST × M	231	177.2	0.77	0.82	0.980
CM × WB × D × M	198	137.1	0.69	0.74	0.998
CM × ST × D × M	462	221.2	0.48	0.51	1.000
Error	7920	7442.5	0.94		
Total	9503	24204.7			

There were also significant differences in spat settlement on collectors within each water body investigated in this study (Figure 4). In the Densu Delta system, spatfall (mean \pm S.E.) was highest on PVC ($2880 \pm 294 \text{ m}^{-2}$), followed by Ceramic tile ($2651 \pm 234 \text{ m}^{-2}$), oyster shell ($2344 \pm 200 \text{ m}^{-2}$) and coconut shells ($1238 \pm 128 \text{ spat m}^{-2}$) in decreasing order. A post hoc Tukey's Hsu MCB test showed no significant difference in spatfall on PVC slats and ceramic tiles ($P = 0.438$) as well as between the PVC and oyster shells ($P = 0.105$). In contrast, spatfall on PVC was significantly higher i.e. $\approx 233\%$ of spatfall on coconut shell (Hsu's MCB test; $P = 0.000$). However, spatfall on the coconut shells was $\approx 47\%$ of that on ceramic tiles (Tukey's Hsu MCB test; $P = 0.000$) and $\approx 53\%$ of the average spatfall on oyster shells (Tukey's Hsu MCB test; $P = 0.002$).

In the Narkwa Lagoon, *C. tulipa* spatfall (mean \pm S.E.) was $2168 \pm 157 \text{ m}^{-2}$, $3165 \pm 191 \text{ m}^{-2}$, $3112 \pm 203 \text{ m}^{-2}$, and $3451 \pm 206 \text{ m}^{-2}$ for coconut shells, oyster shells, PVC slats and ceramic tiles, respectively. The difference between the ceramic tiles and oyster shells, as well as the PVC slats was not statistically significant (Hsu's MCB test; $p > 0.05$). However, coconut shell harvested significantly lower numbers ($\approx 63 - 70\%$) of *C. tulipa* spat on the other collectors deployed in the Narkwa Lagoon (Tukey Hsu (MCB) Test: $P = 0.000 - 0.003$).

In the Benya Lagoon, spatfall (mean \pm S.E.) on coconut shells, oyster shells, PVC and Ceramic tiles were $710.2 \pm 75.6 \text{ m}^{-2}$, $1219.8 \pm 89.4 \text{ m}^{-2}$, $1393 \pm 122 \text{ m}^{-2}$, and $1769 \pm 145 \text{ m}^{-2}$ respectively. Again, average spatfall was highest on ceramic tiles; it was $\approx 249\%$ and 145% of the observations made on coconut and oyster shells respectively (Hsu's MCB Test; $P = 0.000 - 0.003$) but similar to that of PVC ($P = 0.080$). Spatfall on both the oyster shells ($P = 0.007$) and PVC ($P = 0.000$) was higher i.e. $\approx 172 - 196\%$ of spatfall on coconut shell. There was no significant difference between oyster shells and PVC ($P = 0.69$).

Similarly, in the Whin Estuary, a significant difference in *C. tulipa* spatfall on the different artificial collectors was observed (Figure 4; multi-way ANOVA; $P = 0.000$). Averaged over the entire study period, the fall on coconut shell was $134.0 \pm 23.0 \text{ spat m}^{-2}$; it was $276.3 \pm 36.2 \text{ spat m}^{-2}$, $346.3 \pm 51.6 \text{ spat m}^{-2}$, and $373.1 \pm 52.4 \text{ spat m}^{-2}$ on oyster shells, PVC and ceramic tiles, respectively. There was no significant difference between the observations made on ceramic tiles and PVC or oyster shells (Hsu's MCB Test; $P > 0.05$). There were, however, significant differences in *C. tulipa* spatfall on ceramic tiles and coconut shells ($P = 0.000$), as well as between the PVC slats and coconut shells ($P = 0.002$). Details of spatfall on artificial collectors at the different stations with respect to the collector with highest mean spatfall at each station compared simultaneously with the other collectors are shown in Table 2. Ceramic tile was the dominant *C. tulipa* spat collector, harvesting the most spat in 8 out of the 11 successful experimental stations.

Table 2 - Collector ranking at all eleven study stations (ST) in the Densu Delta (DD), Narkwa Lagoon (NL), Benya Lagoon (BL) and Whin Estuary (WE) using mean differences from the highest mean obtained from Hsu Simultaneous Tests for Level Mean (collector with highest mean *C. tulipa* spatfall is best; ranked 1st). For each station, spatfall on collectors at a lower step are significantly ($P < 0.05$) lower than the most effective (1st) collector.

Sampling station	Ranking of collector			
	1 st	2 nd	3 rd	4 th
ST1_DD	Ceramic tile	PVC	Oyster shell	Coconut shell
ST2_DD	PVC	Ceramic tile	Oyster shell	Coconut shell
ST1_NL	Ceramic tile	PVC	Oyster shell	Coconut shell
ST2_NL	Oyster shell	Ceramic tile	PVC	Coconut shell
ST3_NL	Ceramic tile	Oyster shell	PVC	Coconut shell
ST1_BL	Ceramic tile	Oyster shell	PVC	Coconut shell
ST2_BL	Ceramic tile	PVC	Oyster shell	Coconut shell
ST3_BL	Ceramic tile	PVC	Oyster shell	Coconut shell
ST1_WE	Ceramic tile	PVC	Oyster shell	Coconut shell
ST2_WE	Ceramic tile	PVC	Oyster shell	Coconut shell
ST3_WE	Ceramic tile	PVC	Oyster shell	Coconut shell

3.2 Settlement of C. tulipa spat on upper- and under-horizontal surfaces of collectors

There was a significant difference in *C. tulipa* spatfall on upper- [mean (S.E.) = 775.2 ± 33.4 spat m⁻²] and under-horizontal [mean (± S.E.) = 2523.7 ± 66.9 spat m⁻²] surfaces of collectors in the study conducted over 12 months (ANOVA $P < 0.05$). Pooled mean and range of distribution of spatfall on upper- vs. under-horizontal surfaces of collectors were (mean ± S.E.) 756.7 ± 58.4 (n = 864; Q1= 0.0; Median = 0.0; Q3 = 670.7) and 3800 ± 204 (n = 864; Q1= 0.0; Median = 1300.0; Q3 = 5283.0) spat m⁻² for Densu Delta, 2027 ± 106 (n = 1296; Q1= 0.0; Median = 179.0; Q3 = 2188.0) and 3920 ± 155 (n = 1296; Q1= 136.0; Median = 1448.0; Q3 = 5874.0) spat m⁻² for Narkwa Lagoon. Those for Benya Lagoon were 235.5 ± 18.4 (n = 1296; Q1= 0.0; Median = 0.0; Q3 = 188.7) and 2310 ± 103 (n = 1296; Q1= 253.0; Median = 900.0; Q3 = 2600.0) spat m⁻² whereas Whin Estuary had 75.09 ± 9.37 (n = 1296; Q1= 0.0; Median = 0.0; Q3 = 0.0) and 489.8 ± 40.9 (n = 1296; Q1= 0.0; Median = 0.0; Q3 = 236.7) spat m⁻². Thus, wider variations in spatfall were observed on under-horizontal surfaces of collectors than on the upper side (see also interquartile range boxes in Figure 5). In each water body, there was further evidence of this phenomenon on a month-by-month basis as indicated by the distribution of *C. tulipa* spatfall on the upper- and under-horizontal surfaces of the collectors deployed (Figure 5).

These profuse settlements on under-horizontal surfaces by *C. tulipa* spat on collectors, which were observed whilst collectors were placed in a “Face down”/0° orientation, were persistent in the experiments set up in March to test *C. tulipa* spat settlement on collectors oriented in the opposite “Face up”/180° direction. The interaction between orientation and collector surface was not significant (Table 3), thus, spatfall pattern on upper- and under- horizontal surfaces of collectors did not change with the change in orientation. Figure 6 shows details of higher spat settlement on the under-horizontal surface, recurring in both Densu Delta and Narkwa Lagoon in most instances for both orientations (T-test; $P_t < 0.05$). Nonetheless, irrespective of the lesser observations of *C. tulipa* spatfall on upper-horizontal surfaces of Oyster shells compared to their undersides, there were no significant differences (T-test; $P_t > 0.05$) between the two sides in a 0° orientation, in both water bodies (Figure 6). A typical example of *C. tulipa* spat settlement on upper- and under- horizontal surfaces of collectors deployed in this study is shown in Figure 7.

Table 3 – Results of three-way ANOVA for the effect of orientation on *C. tulipa* spatfall on collector surfaces (upper- and under-horizontal) of the different collector materials in the Densu Delta and Narkwa Lagoon, assessed in March 2018.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Collector Surface (CS)	1	37.361	37.3606	39.42	0.000
Collector material (CM)	3	17.057	5.6858	6.00	0.001
Orientation (O)	1	3.722	3.7219	3.93	0.049
CS × CM	3	5.514	1.8381	1.94	0.123
O × CM	3	6.616	2.2055	2.33	0.075
CS × O	1	2.451	2.4507	2.59	0.109
CS × O × CM	3	4.796	1.5986	1.69	0.170
Error	272	257.780	0.9477		
Total	287	335.297			

4. Discussion

Apart from the nylon mesh, which was eliminated after three months of not attracting spat, the other spat collectors proved to support the settlement of *C. tulipa* spat in natural ecosystems. The material structure, i.e. mesh size (2 mm diagonal) and thin nylon filaments of the mesh, coupled with instability in the water column may have contributed to unsuccessful *C. tulipa* spat settlement on the nylon mesh collector. Perhaps, a finer mesh-size material adapted to a more stable setup in the water column may produce positive results. Previous studies have demonstrated the use of wall tiles (Yankson, 1974), oyster shells (Yankson, 1974; Obodai, 1990) and coconut shells (Asare et al., 2019; Obodai, 1990), for collecting *C. tulipa* spat in lagoons and estuaries in Ghana. PVC was used to collect spat of other species of oysters; *Crassostrea gigas* in Wales (Laing and Earl, 1998), *Crassostrea virginica* in Georgia (Manley et al., 2008) and *Pinctada maxima* in Indonesia (Taylor et al., 1998a, 1998b, 1997). Ruwa and Polk (1994) successfully collected *Crassostrea cucullata* spat on coconut shells in Kenya.

In the present study, ceramic tile was the most effective *C. tulipa* spat collector, statistically similar to PVC but superior to both oyster shells (recycled) and coconut shells. Coconut shell collectors were the least attractive to spat in all the water bodies. The differences in efficiency among spat collectors used in this study could be due to the nature of their surfaces as Taylor et al. (1998a) identified surface contour to promote settlement of the pearl oyster *Pinctada maxima*. However, this phenomenon may not hold for *C. tulipa* in this study, as it would be expected that relatively contoured collectors, i.e. coconut and oyster shells, would have been most effective. Instead, the hard nature of materials such as ceramic tiles, PVC and oyster shells appeared to have provided a more stable substratum for the attachment of *C. tulipa* spat. On the other hand, the water absorption capacity of coconut shells (Rao et al., 2015) and the tendency to disintegrate in water may have rendered the relatively softer substrate, not as attractive as the other collectors for cementing by the pediveliger of *C. tulipa* during settlement.

A comparative advantage of ceramic tiles could be their weight (each ceramic tile weighed 200 g; the other collectors weighed < 50 g each), probably making it relatively less perturbed by water currents and providing more stability for settlement of *C. tulipa* spat. However, it is extremely difficult to detach whole/live spat from ceramic tiles and oyster shells as experienced during monthly cleaning of collectors prior to re-deployment. In addition, tile collectors are brittle and break easily when they fall. This may increase losses and affect production cost if used in commercial spat collection. The utilisation of ceramic tiles as collectors on a *C. tulipa* farm will therefore require intensive labour for cleaning collectors and great care to prevent losses. In contrast, coconut shells require moderate effort whilst PVC requires minimal time and effort to detach almost all spat undamaged, corroborating the 95-100 % spat removal success reported by Wedler (1980) for PVC. This is probably the reason PVC is the most widely used in recent times for oyster culture (Gosling, 2015).

Further, preparation and construction of ceramic tiles and PVC collectors require skilled labour whilst coconut and oyster shells can be prepared by the culturist with little skill. The culturist, therefore, will have to decide on the type of collector based on availability, expertise and purpose. *C. tulipa* spat to be collected and detached for onward culturing in different grow-out facilities may be best done using PVC slats. Those meant for rearing on collectors would be ideal on ceramic tiles and oyster shells. The biodegradable coconut shells would be ideal for bottom culture (Quayle and Newkirk, 1989) and useful in oyster restoration programmes. In addition, growth and survival of spat may be critical in the choice of collectors by culturists beyond settlement.

Experimental racks in this study were fitted with vertical series of horizontally strung collectors. This was guided by conclusions on horizontally placed collectors yielding the greatest number of spat in other species of oysters by several studies in the past (Hopkins, 1937; Schaefer, 1937; Cole and Knight-Jones, 1939; Taylor et al., 1998a). However, reported observations by these authors on spat settlement on upper and under surfaces of horizontally placed collectors are inconclusive. In furthering information on the orientation effect of oyster spat collectors, the present study demonstrated the abundance of *C. tulipa* spat on under-horizontal surfaces of all the types of collectors in every month in each of the coastal water bodies, similar to the findings of Hopkins (1937) and Schaefer (1937) for *Ostrea lurida* and *C. gigas* spat respectively.

Earlier work by Cole and Knight-Jones (1939) however, showed a marked tendency for the larvae of *Ostrea edulis* to attach in daylight. In contrast, Shaw, Arnold and Stallworthy (1970) concluded that setting activity in mature larvae of *C. virginica* was encouraged by darkness and partially inhibited by light. Ajana (1979) also recorded best concentration of *C. gasar* (= *tulipa*) spat on shaded collectors. The observation of profuse settlement of *C. tulipa* spat on under-horizontal surfaces of artificial collectors in the present study demonstrates a possible escape from light or simply a quest for shaded areas (i.e. negative phototaxis) by the pediveliger. It could be assumed that undersides of the collectors used in this study received relatively lesser illumination and therefore attracted more spat than the upper surfaces.

The significantly larger sizes of *C. tulipa* spat settled on under-horizontal surfaces, mean (S.E.) 9.88 ± 0.5 mm, as opposed to the converse, 5.99 ± 0.5 mm, of the same collector (Figure 7), is most probably due earlier attachment. Thus, the descriptions of the morphological and anatomical structure of the larvae of *C. virginica* (Galtsoff, 1964) and *O. edulis* (Cole and Knight-Jones, 1939; Cranfield, 1974) would suggest that larval movement prior to setting could most likely account for the observation for *C. tulipa* in this study. The free-swimming veliger possesses a foot, for attachment, near the velum (which is an outgrowth of the prototroch of the previous trochophore larva) with cilia for swimming forward and upward with foot and velum uppermost (Galtsoff, 1964). Since larval formation is identical for *Ostrea* and *Crassostrea* (Galtsoff, 1964), the *C. tulipa* larvae, like other oyster larvae, swimming upside down with the foot uppermost, presents the best chance of attaching to undersides of horizontally suspended substrates even under turbulent natural conditions; upward swimming of competent larvae of *C. virginica* was found to persist in highly turbulent flow by Wheeler et al. (2013). In addition, Baker (1997) provides evidence of geotaxis, i.e. movement influenced by gravity, as a stronger settlement cue than phototaxis and rugotaxis for *C. virginica*, stating pediveligers of both *Crassostrea* and *Ostrea* possess statocysts, which are thought to be geosensory.

5. Conclusion

The 2 mm nylon mesh did not harvest any *C. tulipa* spat after three months of deployment in the coastal water bodies studied. On the other hand, coconut shell, oyster shell, PVC, and ceramic tiles demonstrated the capacity to harvest *C. tulipa* spat from the wild. Ceramic tile was the most effective *C. tulipa* spat collector among the four, although PVC material could be equally effective. *C. tulipa* farmers should consider other factors including cost of collector material, its amenability to culture technique and holding facilities, availability, and durability in making a suitable choice between ceramic tile and PVC. These collectors should be deployed suspended horizontally in the water column with their undersides exposed for maximum attachment of *C. tulipa* spat.

Declarations

Declaration of Competing Interest

We declare that we do not have any known financial interest or personal relationships that could be deemed a conflict of interest in relation with this work.

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Figures

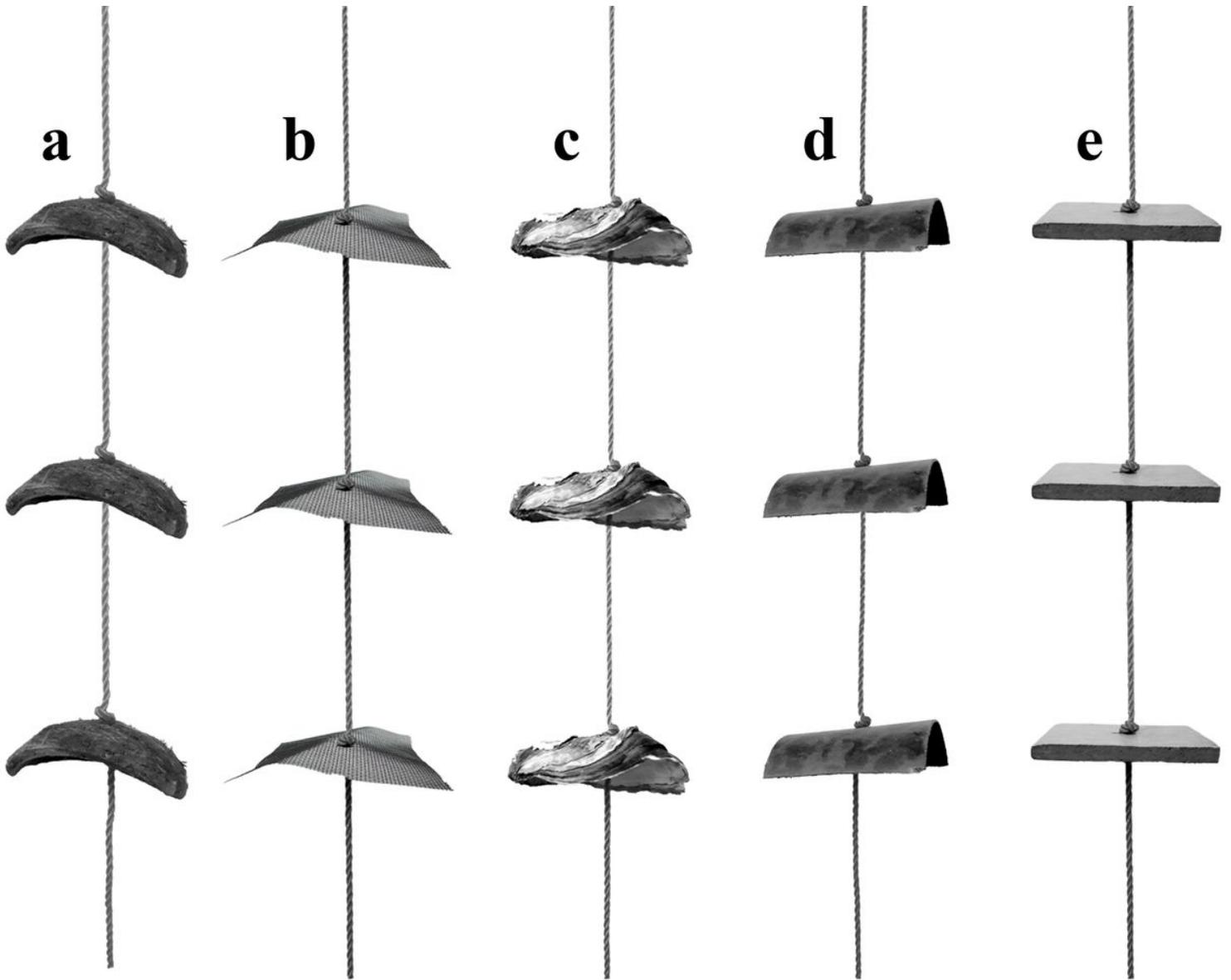


Figure 1

Collectors used for the study; (a) coconut shell, (b) nylon mesh, (c) oyster shell, (d) PVC slats and (e) ceramic tiles.

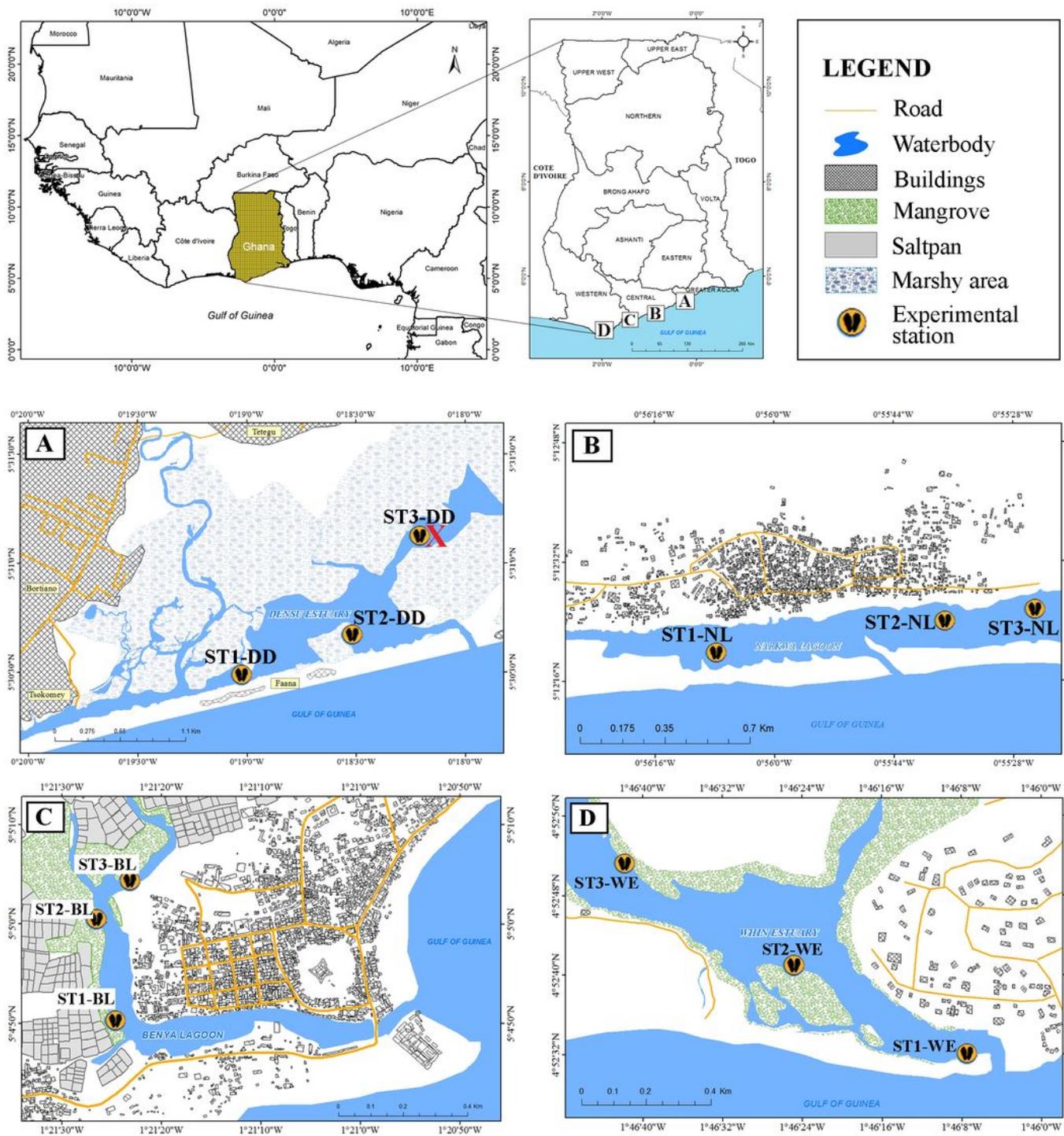
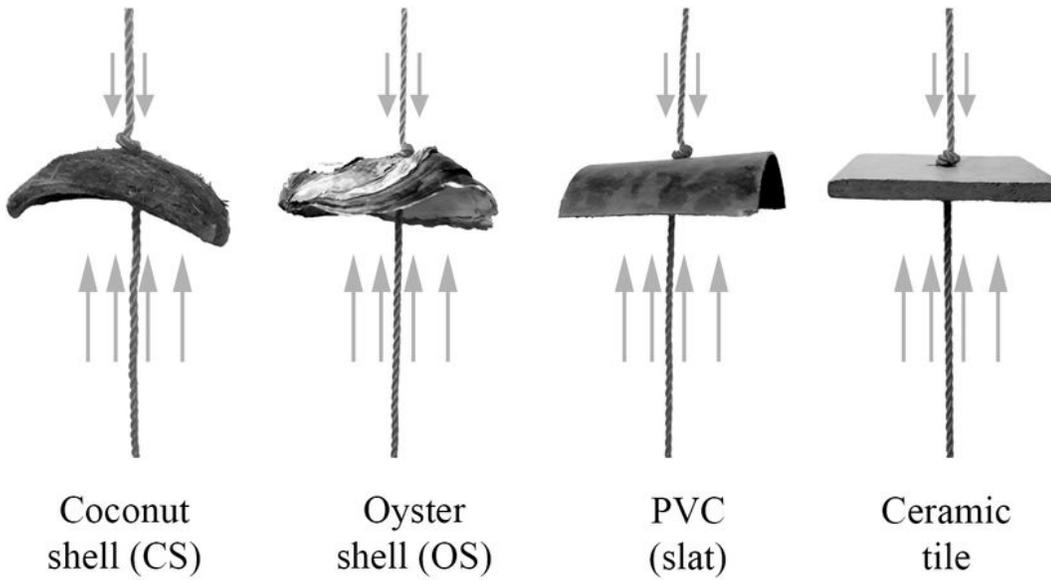


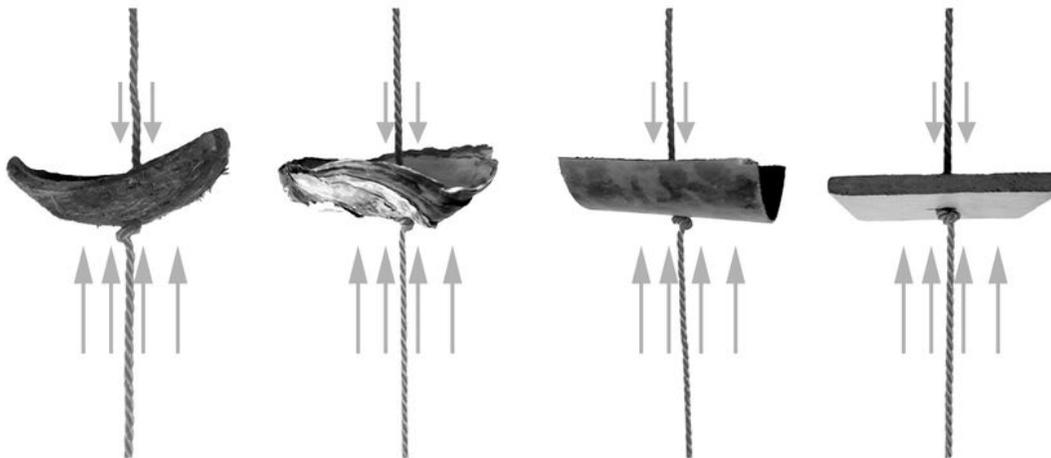
Figure 2

Maps of study areas showing experimental stations (ST) in the (A) Densu Delta - DD, (B) Narkwa Lagoon - NL, (C) Benya Lagoon - BL, and (D) Whin Estuary - WE. (X = station eliminated due to recurrent destruction of rack)

Face down (0°)



Face up (180°)



↑↑↑↑ **Under-horizontal surface**
↓↓ **Upper-horizontal surface**

Figure 3

An illustration of the different collectors/substrates depicting the “face down” (0°) and “face up” (180°) orientations and collector surfaces in the horizontal position

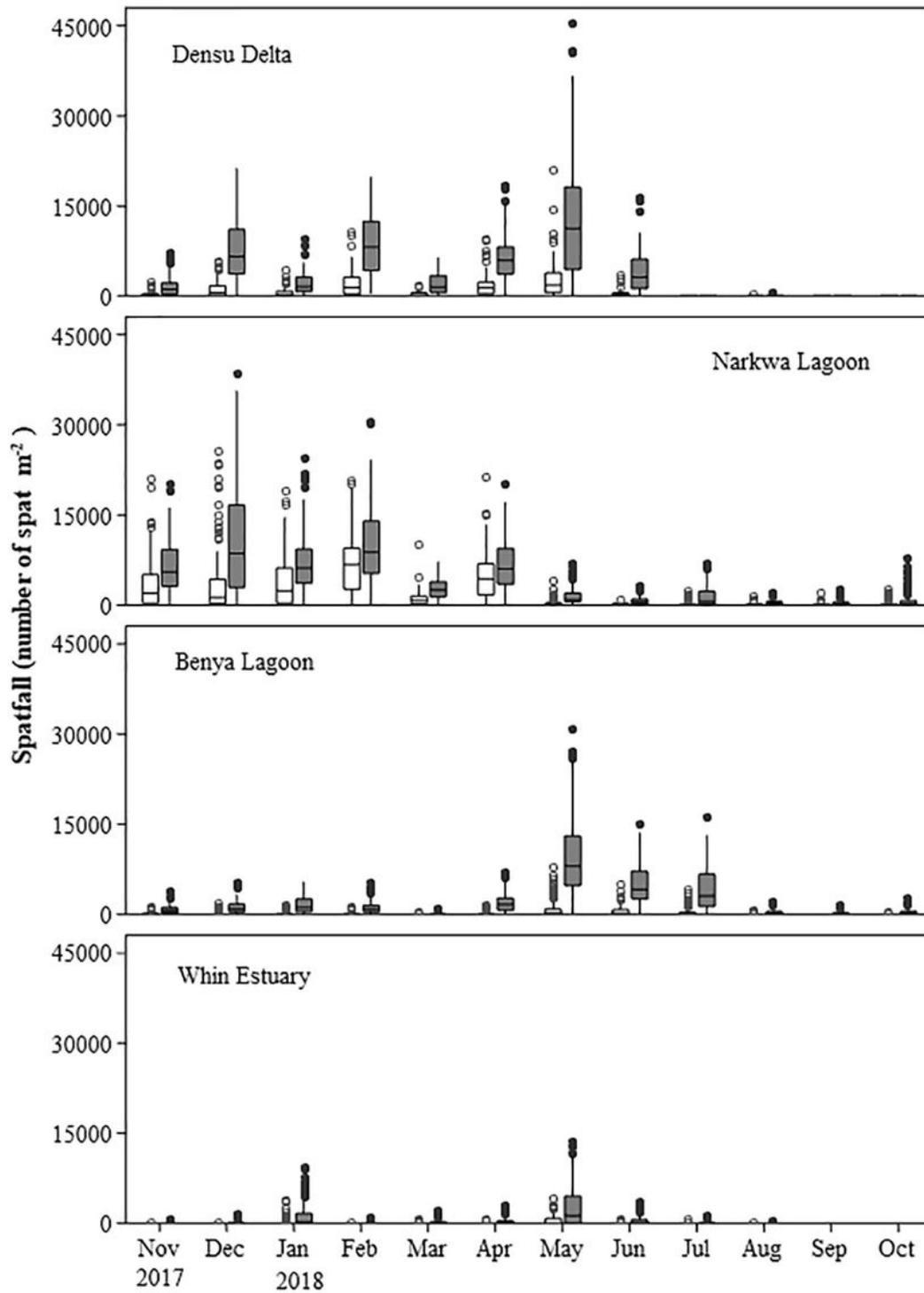


Figure 4

Crassostrea tulipa spatfall (mean \pm S.E.) on the collectors deployed in the selected coastal water bodies from November 2017 to October 2018. Means are pooled for the four water bodies.

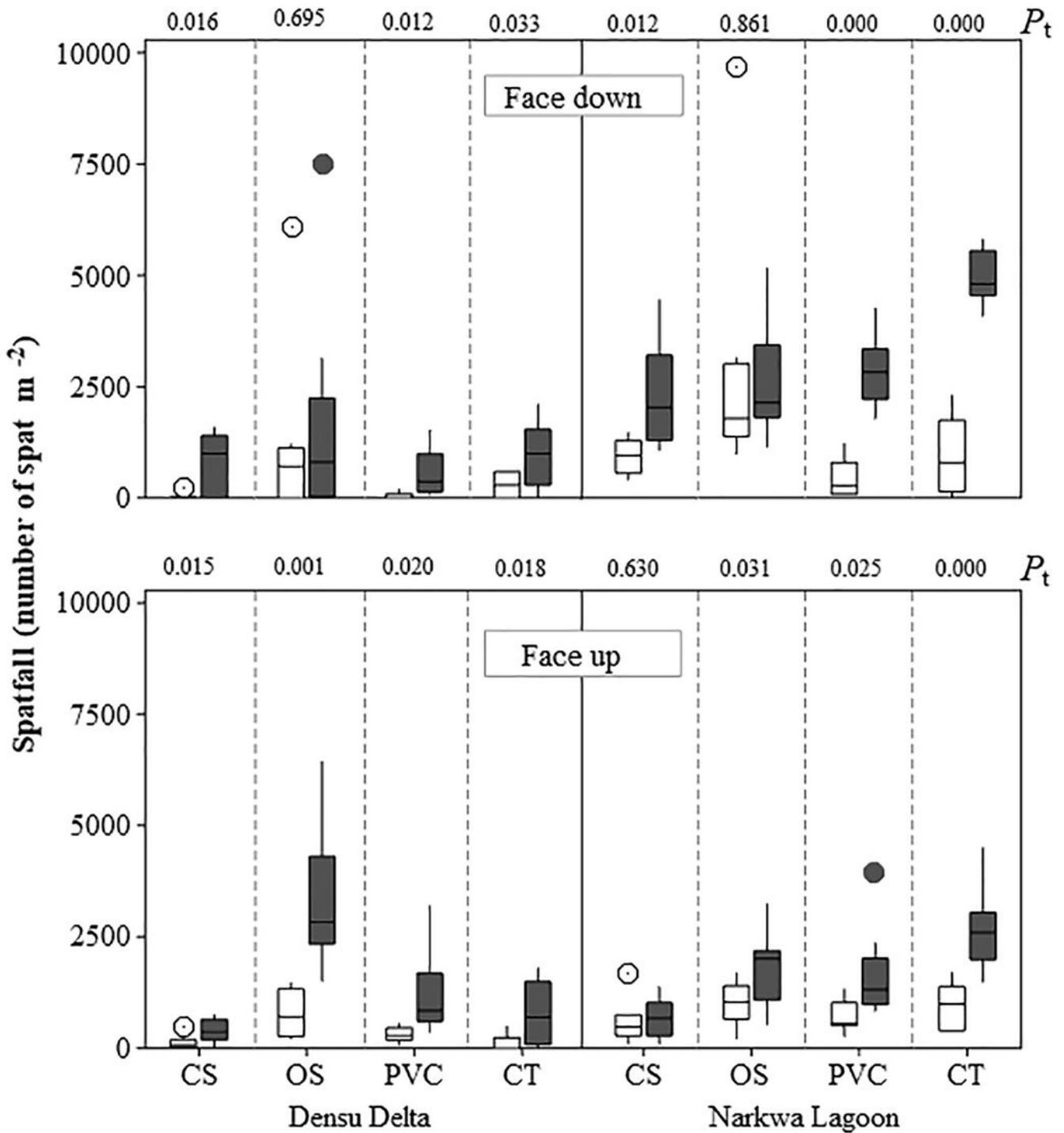


Figure 5

Statistical analysis of the effect of two positional orientations ("Face down" and "Face up") on *C. tulipa* spat settlement on upper- and under- horizontal surfaces of the artificial collectors (CS = Coconut shell; OS = Oyster shell; CT = Ceramic tile). P_t value is the statistical result for the t-test of the null hypothesis $H_0: \mu_{\text{upper}} - \mu_{\text{lower}} = 0$ between upper- and under- horizontal surfaces of collectors.

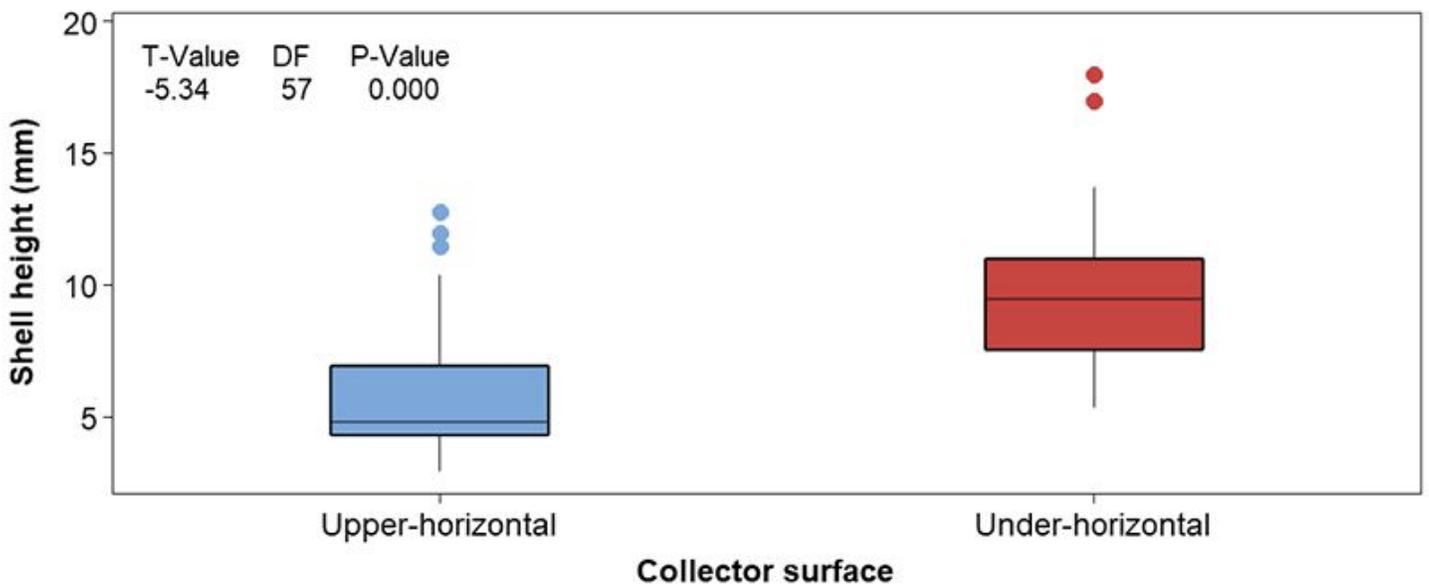


Figure 6

Typical occurrence of larger sizes and greater settlement of *Crassostrea tulipa* spat on the under-horizontal surface (right) than the upper-horizontal surface (left) of the same collector (In this illustration, 10 × 10 cm² ceramic tile).