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Effectiveness of different substrates for collecting wild spat of the oyster *Crassostrea tulipa* along the coast of 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. We assessed the effectiveness of five substrates (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 ($P > 0.05$) with the highest settlement in Densu Delta (2880 ± 294 spat m^{-2}). Coconut shell consistently had the least *C. tulipa* spatfall ($P < 0.01$). Spat collection with 2 mm nylon mesh was not successful. 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 ($P < 0.01$). 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. The effectiveness of collectors correlated positively with dissolved oxygen. Ceramic tiles and PVC slats were the most effective materials for *C. tulipa* spat collection, hence, their use is 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 Africa (Asare et al., 2019). 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 Gulf of Guinea coast. It is naturally well adapted to the rigorous environmental conditions, surviving wide temperature ranges of 20−36 °C (Ajana, 1980; Obodai et al., 1991). Due to easy accessibility of its habitats, the fishery of the oyster is dominated by women (Theisen, 2010) 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). The shells are also used in the indigenous production of paints, traditional medicine and concrete for building (Yankson, 2004).

At present, production of the oyster is mainly capture based. Regional data on capture production volumes is scarce. Available data for culture production of oysters, by live weight, on the coast of West Africa indicates some 415 tonnes in 2018 from Senegal and The Gambia (FAO, 2014). To sustain and possibly enhance the earnings and nutritional benefits derived from *C. tulipa*, many coastal communities are considering farming the oyster on a large scale (Ishengoma et al., 2011). Recent threats of mass mortalities due to diseases in the tilapia aquaculture sector further raise deep concerns for the diversification of cultured species and geographical coverage in the development of aquaculture. Nile tilapia (*Oreochromis niloticus*) constitutes up to 95 % of

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total aquaculture production in some parts of the Region [e.g. from the Volta Lake in Ghana (FAO, 2014)], hence, the efforts to farm the native oysters are to boost resilience of the sector.

The potential for farming *C. tulipa* was explored within the last three decades (Kamara, 1982; Yankson, 1990). Recent studies have also examined the farming of 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 a myriad of 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 *C. tulipa* 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 costal water bodies is limited. *C. tulipa* seed was successfully produced in the laboratory from artificial fertilization through to settlement of the larvae in a laboratory in Swansea, UK (Yankson, 1990). This development was stalled by the unavailability of facilities for culturing marine microalgae to feed larvae and spat in the University of Cape Coast laboratory, as it is expensive and not easily reproducible in poor areas (Dégremont et al., 2007; Tanyaros and Chuseingjaw, 2016). Due to the foregoing limitations, and as done for other species of oysters in different parts of the world (see Gosling, 2015; Quayle and Newkirk, 1989), spat for farming *C. tulipa* could be harvested from the wild using artificial substrates.

Many kinds of materials have been used to collect spat of some commercial bivalves such as pearl oysters (Friedman et al., 1998; Taylor et al., 1998a; Urban, 2000), scallops (Harvey et al., 1997; Pearce and Bourget, 1996) and edible oysters (Asare et al., 2019; Buitrago and Alvarado, 2005; Chaparro et al., 2008; Diadhiou and Ndour, 2017; Metz et al., 2015). Depending on their settlement habit, bivalves may be selective with surface preference for attachment and growth. Larval settlement of hatchery-reared silver-lip pearl oyster, *Pinctada maxima*, was enhanced by fibrous ropes and rough surfaces of grooved PVC slats owing to the greater tactile stimuli offered to crawling pediveligers (Taylor et al., 1998a). The giant scallop, *Placopecten mugellunicus*, was found to perform an active selection of chitinous substratum for byssal attachment (Harvey et al., 1997). Settlement of the pediveliger in some edible oysters is by cementation and is triggered immediately on first contact with a hard, conducive, substrate as reported for *Ostrea chilensis* (Chaparro et al., 2008). All of these suggest a wide range of substrate preference for bivalves but more importantly indicates selectivity of attachment surfaces by the pediveliger as it detects to be suitable.

Nonetheless, the use of cheap, durable and readily available collectors would be more profitable in commercial farming of bivalves. These considerations may, however, not be applied arbitrarily. It is 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 are worth considering in the farming of *C. tulipa*. Varying spat yield is reported on different surfaces of horizontally placed collectors (Diadhiou and Ndour, 2017; Holliday, 1996; Soria et al., 2015; Taylor et al., 1998a), and needs to be investigated for different species of varying ecosystems. This could result from differences in pre-settlement larval behaviour in different species. Prior to settlement, the shelled foot-bearing veliger larvae of bivalves often exhibit an upward swimming movement, accompanied by a cylindrical helical motion in some species, propelled by the beating of velar cilia (Bayne, 2017). Such characteristics may be instructive regarding the placement of collectors for optimum spat collection. Unfortunately, the actual settlement behaviour for *C. tulipa* larvae has not been studied, thus, knowledge of its larval movements prior to settlement, which could be useful in spat collection from the wild, is not available.

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 different substrates for the collection of *C. tulipa* spat for aquaculture. The effect of surface contour of collectors relative to horizontal orientation in the water column as well as hydrographic influences on collector effectiveness were evaluated. The study was conducted from November 2017 to October 2018. The duration spanned both the dry season, with warmer temperatures and low rainfall, and the wet season with contrasting hydrographic and climatic conditions. The findings of the study fill a critical vacuum of scientific literature on *C. tulipa,* which is required for optimising wild spat collection for farming the species.

2. Materials and methods

2.1. Study sites

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 water bodies were selected for this study (Fig. 1). These were the Densu Delta (0°16′43″ W, 5°34′07″ N and 0°20′02″ W, 5°30′21″ N), Narkwa Lagoon (0°56′22″ W, 5°12′17″ N and 0°54′41″ W, 5°12′32″ N), Benya Lagoon (1°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). These are generally shallow systems (with most parts not deeper than one and half metres at low tide) from which fish and shellfish resources provide livelihood support for the natives of adjoining communities, who are predominantly fishers. Bottle fishing, hook-and-line, and shellfish (oysters and cockles) harvesting are common in these water bodies. Two cardinal livelihood activities associated with the Benya Lagoon are artisanal fishing trade (including boat-building) and piggery. In the communities of the selected study sites, harvesting of oysters is a primary livelihood for women, who were usually observed plying the trade with their children.

2.2. Preparation of collectors

Coconut shell, nylon net (mesh size = 2 mm), oyster shell, PVC and ceramic tiles were the spat collector materials investigated in this study (Fig. 2). The husks of coconut shells were removed and the hard shells washed in sea water and dried before using for the study. Fine mesh nylon nettings were cut out into sizes of 10 × 10 cm^2. Oyster shells were treated the same as the coconut shells before use. PVC pipes (diameter ≈ 10 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 × 10 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 in constructing stringed cultch masses. The three collectors were fastened on each rope through holes (diameter ≈ 4 mm) drilled at the centre and kept equidistant from each other on the rope by knots to form a cultch. Collectors were held horizontally in the water column in order to ensure maximum spat harvest as observed in previous studies (Diadhiou and Ndour, 2017; Holliday, 1996; Taylor et al., 1998a). The lengths of the ropes were varied, depending on average high-water depth at each experimental station. Water surface to bottom depths at the experimental stations in the water bodies ranged from 0.8 to 2.2 m.

2.3. 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. 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. This method is similar to the foil mold method

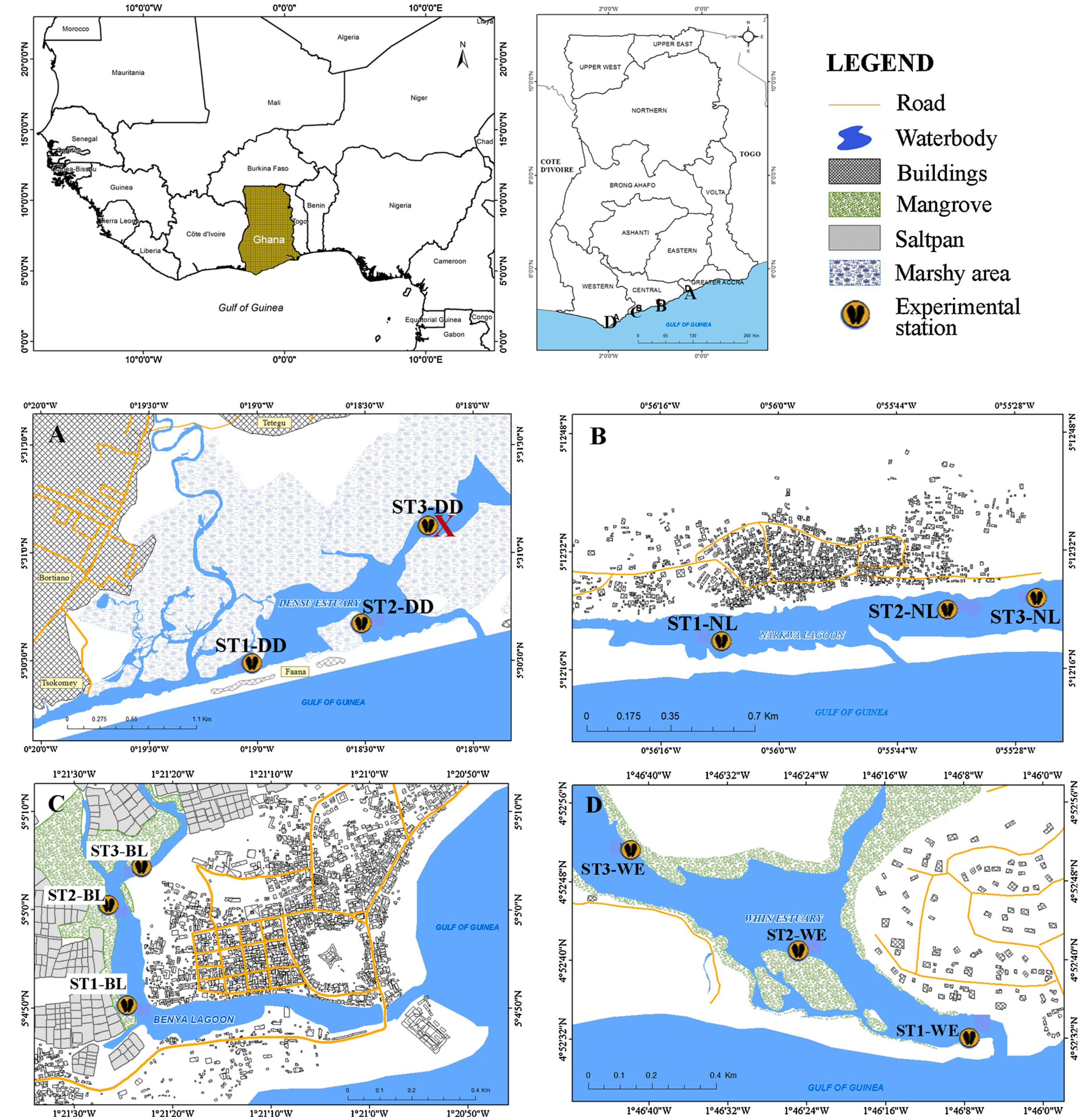

Fig. 1. Maps of study areas showing experimental stations (ST) in the (A) Densu Delta - DD, (B) Narkwa Lagoon - NL, (C) Benya Lagoon - BL, and (D) Whin Estuary - WE. (X = station eliminated due to recurrent destruction of rack).

(Morales-Alamo, 1993). Collectors made from nylon mesh, PVC, and ceramic tiles were cut into definite sizes (see Section 2.2). Their surface areas were determined by a multiplication of the length by breadth and multiplied by two to obtain the total surface areas of the collectors.

2.4. Experimental design

Five treatments of different oyster spat collector substrates (coconut shell, nylon net, oyster shell, PVC and ceramic tiles) were deployed to evaluate their effectiveness for harvesting *C. tulipa* spat (response is spatfall; number of spat m^{-2}). The experiment was replicated in four different water bodies (Densu Delta, Narkwa Lagoon, Benya Lagoon, and Whin Estuary) and repeated monthly for a year (12 months). Three experimental stations (ST) with bamboo racks mounted were established in each water body to cover the head, middle and mouth regions. For each collector type, 9 collectors were deployed on three strings (i.e. three collectors per string) affixed to a rack at each experimental station. Hence, a total of 45 collectors were deployed per station and 540 for all the water bodies combined (12 stations). In the Densu Delta, ST3-DD (see Fig. 1) was later eliminated due to persistent destruction of rack by fishers.

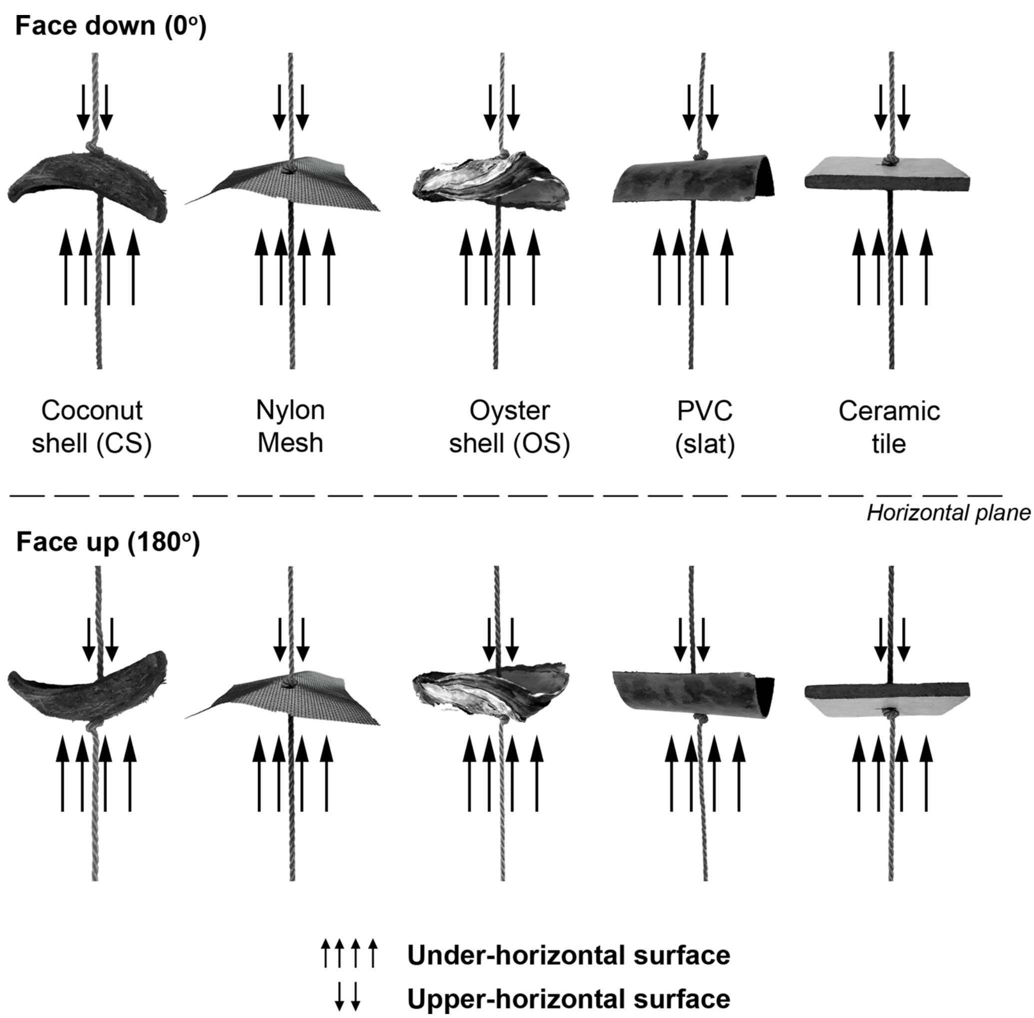

Fig. 2. An illustration of the different collectors/substrates depicting the "face down" (0°) and "face up" (180°) orientations and collector surfaces in the horizontal position.

2.5. Data collection and estimation of spatfall

Sampling was done monthly for one year from November 2017 to October 2018 by harvesting all collectors and replacing with a new set each month. Collectors were examined in the laboratory for settled spat on them. The total number of spat on each collector was counted. In instances of small transparent spat on collectors that were covered with dirt, a laboratory lamp was used to provide a higher localised luminescence relative to laboratory ambience. A hand lens was used in couple with the lamp to identify very small spat of shell height <1 mm. Spatfall on individual collector surfaces and mean spatfall per m^2 were calculated based on density, which is a common phenomenon. The application of density in this study follows absolute density as applied for distribution and abundance in ecological studies (see Krebs, 2013). The following equations were derived for spatfall.

$$\text{Sfi}\ (\text{cm}^{-2}) = \frac{\text{Nsi}}{\text{Aci}}$$

$$\overline{X}\ \text{Sf}\ (m^{-2}) = \left[n^{-1}.\ \sum_{i=1}^{n} (\text{Sfi})\ (\text{cm}^{-2})\right] \times 10000$$

Where Sfi = spatfall on individual collectors, Ns = Number of spat on collector surface, Ac = Surface area of collector material, $\overline{\overline{X}}$ Sf = mean spatfall, i = *1, 2, 3* … *n*th replicate collector, and n = number of replicate collectors/surfaces.

2.6. Settlement of Crassostrea tulipa spat 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 collector 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 (see Fig. 2) 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 Fig. 2, on separate racks. The nylon mesh was excluded in this experiment after three consecutive months of no spat settlement. Spat were counted, shell heights (SH) were measured and a 0° (face down) versus 180° (face up) comparison of the surfaces was done statistically.

2.7. Hydrographic and climatic parameters

The prevailing hydrographic conditions, namely water temperature, dissolved oxygen (DO), salinity, turbidity and pH in the water bodies were measured monthly during the period of the study. Temperature (°C), dissolved oxygen (mg/L), turbidity (NTU) and pH were measured with multi-parameter water quality instruments (YSI Environmental

EcoSence®) and salinity was measured using a hand-held refractometer. These measures were in triplicates at the various experimental stations. Data for monthly rainfall or precipitation (mm) in the vicinity of the study sites was extracted from the online global climate database (Tutiempo.net, 2019) for the nearest meteorological data stations. These were the Accra, Saltpond and Takoradi meteorological data stations for Densu Delta, Narkwa Lagoon/Benya Lagoon and Whin Estuary respectively.

2.8. Data analysis

The comparative effectiveness of the different collectors in general, within individual water bodies and among sampling months as well as the difference in spat settlement on under- and upper-horizontal surfaces were determined using Analysis of Variance (ANOVA). Homogeneity of the variance in the data was verified using Levene's test and data transformed to $\log_{10}(x + 1)$ prior to ANOVA. A two-way ANOVA was used to determine the influence of sampling month on the effectiveness of the collectors (collector material × month) for collecting *C. tulipa* spat. The effect of surface contour (based on the orientation of collector in water) on *C. tulipa* spatfall on the upper- and under-horizontal surfaces of the different collectors was determined using a three-way ANOVA (collector surface × collector material × collector orientation). Tukey HSD was used as the post-hoc test to determine which pairs of means were significantly different.

Significant differences between the most effective collector material (largest mean) for harvesting *C. tulipa* spat and the other collectors, were identified using the Multiple Comparison with Best (MCB) method (Hsu, 1992) with a 1 % family error rate. The Hsu MCB post-hoc performs a step-wise multiple comparison by controlling the best mean (it could be the largest or smallest mean, depending on what is deemed as best; in the case of this study, the largest mean is best) and conducting a pair-wise comparison with all other means. This differs slightly from the Tukey HSD, which is a simultaneous comparison of all means. As such, the Hsu MCB post-hoc test was only applied in ranking the collectors in the various water bodies. The difference of means was used to rank collectors relative to the best. All other post-hoc was done using Tukey HSD. Two sample T-test was applied to determine statistical differences between upper- and under- horizontal surfaces of collector types in the confirmatory experiment. Statistical significance was inferred at 99 % confidence interval and $\alpha = 0.01$. Linear regression analysis was used to determine relationships between the effectiveness of collectors (spat m^{-2}) and the observed hydrographic/climatic parameters at $\alpha = 0.05$. The effectiveness of collectors was deduced from spatfall values. Each spatfall value was obtained over a duration of one month. Analyses were done using the R-programme, and Hsu MCB post-hoc generated in Minitab®, version 19.

3. Results

3.1. Crassostrea tulipa spatfall on artificial collectors

There was no spat settlement on the nylon mesh net during the initial three months of this study hence its use was discontinued. Of the remaining collectors, significant differences in the densities of *C. tulipa* spat were observed throughout the study (Two-way ANOVA at $p < 0.05$; Table 1). Fig. 3 shows the effectiveness of different collectors from Nov 2017 to October 2018 evaluated using the mean number of spat per unit area, pooled for all the water bodies. Spatfall was highest on ceramic tile (2007 ± 86 spat m^{-2}) followed by PVC slat (1847 ± 88 m^{-2}), oyster shell (1697 ± 73 spat m^{-2}) and coconut shell (1047 ± 56 spat m^{-2}), in that order. This difference did not change statistically, from one month to the other [P (Collector material × Month) = 0.226; Table 1] considering all water bodies. Multiple comparison with the best mean, Hsu MCB, as post-hoc test showed spatfall on ceramic tile, which was the largest, was significantly greater than those observed on both coconut and oyster shells ($P < 0.01$). Spatfall on the ceramic tile was, however, not significantly different from the observation on PVC ($P > 0.01$). The independent effectiveness of each collector also differed significantly between water bodies ($P < 0.01$) and among sampling months ($P < 0.01$) within each water body.

Table 1
Results of two-way ANOVA for the effects of sampling month on the comparative effectiveness of collector material for *C. tulipa* spat collection from the wild.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Collector material	3	227	75.6	37.030	<2E-16	***
Month	11	4601	418.3	204.968	<2E-16	***
Collector material × Month	33	79	2.4	1.175	0.226	
Residuals	9456	19,298	2.0			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '' 1.

Significant differences in spat settlement on the collectors also occurred within each water body investigated in this study (Tukey HSD; $P < 0.01$). In the Densu Delta system, spatfall (mean ± S.E.) was highest on PVC (2880 ± 294 spat m^{-2}), followed by Ceramic tile (2651 ± 234 spat m^{-2}), oyster shell (2344 ± 200 spat m^{-2}) and coconut shells (1238 ± 128 spat m^{-2}) in decreasing order. Spatfall on PVC was ≈ 233 % of spatfall on coconut shell whereas coconut shells received ≈ 53 % less the spatfall on ceramic tiles (Tukey HSD; $P = 0.000$). Mean spatfall of *C. tulipa* in the Narkwa Lagoon was 2168 ± 157 spat m^{-2}, 3165 ± 191 spat m^{-2}, 3112 ± 203 spat m^{-2}, and 3451 ± 206 spat m^{-2} for coconut shells, oyster shells, PVC slats and ceramic tiles, respectively. Here also, coconut shell harvested significantly lower numbers (≈ 63–70 %) than the other collectors deployed (Tukey HSD; $P < 0.01$).

Average spatfall was highest on ceramic tiles in the Benya Lagoon; it was ≈ 249 % and 145 % of spat settlement on coconut and oyster shells respectively (Hsu MCB; $P < 0.01$) but similar to that of PVC ($P = 0.080$). Similarly, in the Whin Estuary, spatfall on coconut shell was the least and there was no significant difference between the observations made on ceramic tiles and either PVC or oyster shells (Hsu MCB; $P > 0.01$). The summarised graphical panel in Fig. 4 illustrates collector effectiveness by ranking in the four water bodies in this study. Since largest mean was set as the best, ranks were assigned using the mean difference between the best mean and all other means of the collectors, for each water body. Ceramic tile was the best *C. tulipa* spat collector, ranking first in three water bodies (Narkwa Lagoon, Benya Lagoon and Whin Esturay) and second in one (Densu Delta) (Fig. 4).

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

There was a significant difference in mean *C. tulipa* spatfall (S.E.) on upper- (775.2 ± 33.4 spat m^{-2}) and under-horizontal (2523.7 ± 66.9 spat m^{-2}) surfaces of collectors in the study conducted over 12 months (Tukey HSD; $P < 0.01$). The monthly distribution of spatfall on upper- vs. under-horizontal surfaces of the different collectors is represented visually in Fig. 5. Wider variations in spatfall were observed on under-horizontal surfaces of collectors than on the upper side (see also inter-quartile range boxes in Fig. 5). For each collector and in every water body, there was ample evidence of this phenomenon.

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 2), thus, spatfall pattern on upper- and under- horizontal surfaces of collectors did not change with the change in orientation.

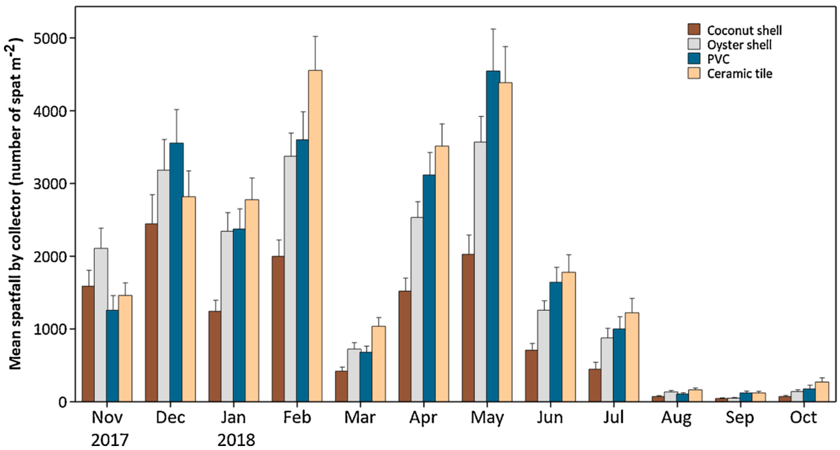

Fig. 3. *Crassostrea tulipa* spatfall (mean ± S.E.) on the collectors deployed in the selected coastal water bodies from November 2017 to October 2018. Means are pooled for the four water bodies.

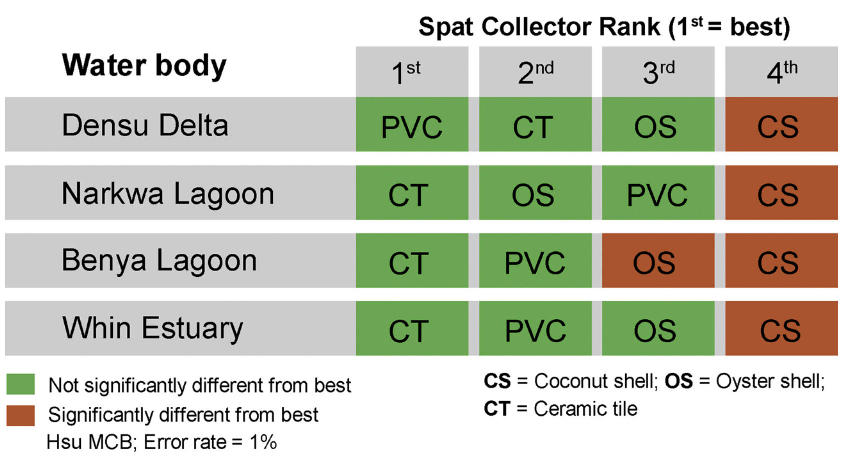

Fig. 4. Collector ranking in the water bodies using mean differences and Hsu Simultaneous Tests for Level Mean after ANOVA. Collector with largest mean *C. tulipa* spatfall is best; ranked 1st. Statistical differences among collectors within water body are shown in colour (α = = 0.01).

Fig. 6 shows higher spat settlement on the under-horizontal surface, recurring in most instances of the confirmatory experiment for both orientations (T-test; $P_t < 0.01$). 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; $P_t > 0.01$) between the two sides in a 0° orientation. A typical example of *C. tulipa* spat settlement on upper- and under- horizontal surfaces of collectors deployed in this study is shown in Fig. 7.

3.3. Relationship between prevailing hydrographic/climatic conditions and collector effectiveness

Mean water temperature in the systems studied was 28.68 ± 0.20 °C, ranging from 23.7 °C (Benya Lagoon - August) to 34.7 °C (Whin Estuary - February). Dissolved oxygen had a mean of 2.61 ± 0.22 mg/L with minimal levels recorded in September-October 2018 for all water bodies. Salinity averaged 21 ± 1 with a range of 0 (Whin Estuary - May, June; Densu Delta - July, September, October) to 41 (Benya Lagoon - August). Turbidity remained relatively high in May-June accompanied by high precipitation within the same period. The mean total monthly rainfall for the study period in the coastal belt (i.e. from the Accra, Saltpond and Takoradi meteorological stations for this study) was 50.50 ± 9.85 mm month^{-1} and ranged from 0.00 mm (January and March) to 218.95 mm (May). The water bodies were slightly alkaline averaging > 7 throughout the study period with pH ranging from 6.34 (Narkwa Lagoon) to 10.37 (Densu Delta). Monthly mean observations of these parameters are presented in Table 3.

A linear regression analysis of the effectiveness of spat collectors (number of spat per square metre) used in this study versus the hydrographic parameters/rainfall revealed, in most cases, a very low association between the data for the two variables except for dissolved oxygen. The regression coefficients (R^2) were 19.3 % ($P = 0.152$), 53.71 % ($P = 0.007$), 1.68 % ($P = 0.688$), 10.36 % ($P = 0.308$), 5.61 % ($P = 0.459$), and 0.34 % ($P = 0.858$) for temperature, dissolved oxygen, salinity, turbidity, pH and rainfall respectively (Sup. Table 1). Positive changes in dissolved oxygen levels in the water bodies appeared to induce significant commensurate levels of effectiveness of the collectors. This was true for the individual collectors inferring from Spearman's rank correlation analysis; r = 0.62 (coconut shell), 0.72 (oyster shell), 0.67 (PVC slat), 0.81 (ceramic tile) and $P < 0.05$ for all (Sup. Table 2). The inclusion of collector as a categorical predictor and subsequent introduction of an interaction term "DO × collector" in the linear regression model, however, produced statistically nonsignificant *P*-value of 0.345. The observation of increasing effectiveness of collectors with increasing DO, therefore, is not the resultant effect of significant interaction between dissolved oxygen and the collectors deployed within the water bodies.

4. Discussion

The determination of suitable substrates is one of the precursors to upscaling oyster farming towards the development of coastal aquaculture along the West African coast. Coastal and marine food resources

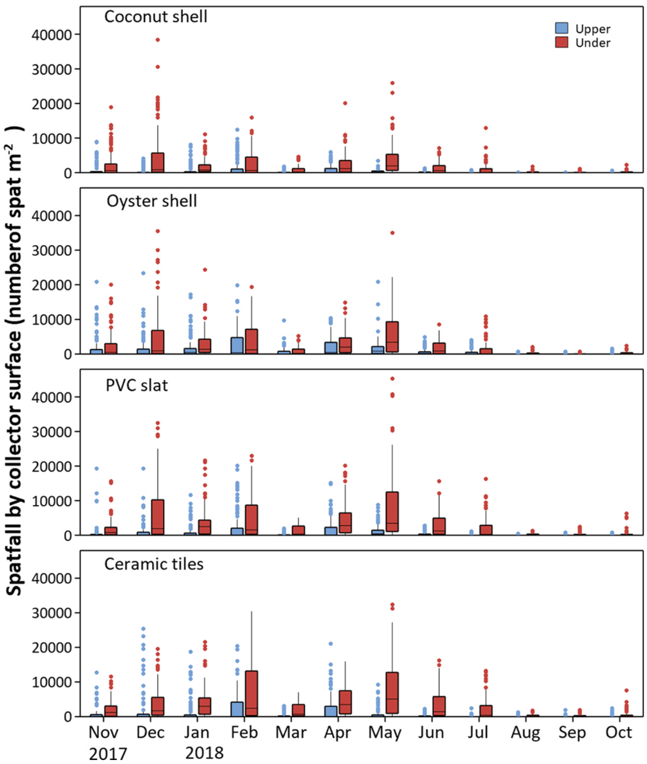

Fig. 5. Boxplot of the distribution of *C. tulipa* spatfall on upper- and under-horizontal surfaces of experimental collectors from November 2017 to October 2018.

Table 2
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.

Effect	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Collector material (CM)	3	17.06	5.69	5.999	0.000568	***
Collector surface (CS)	1	37.36	37.36	39.421	1.34E-09	***
Orientation (O)	1	3.72	3.72	3.927	0.048519	*
CM × CS	3	5.51	1.84	1.939	0.123478	
CM × O	3	6.62	2.21	2.327	0.074959	.
CS × O	1	2.45	2.45	2.586	0.108982	
CM × CS × O	3	4.80	1.60	1.912	0.108733	
Residuals	272	257.78	0.95			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

abound in this region yet aquaculture research related to their environments for food and conservation purposes is still limiting. The data on collector effectiveness in this study presents vital information for optimally harvesting seed of the native species, *Crassostrea tulipa* (Lamarck, 1819), from natural ecosystems as regards the most applicable collector. Aside 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 the natural ecosystems studied. Larval forms of the group *Crassostrea* measure less than 500 μm in diameter prior to settlement (Christo et al., 2010; Ginger et al., 2013; Tanyaros and Tarangkoon, 2016). The material structure, i.e. mesh size (2 mm diagonal) and thin nylon filaments of the mesh, could not provide adequate substrate for the settlement of such small competent larvae. Instability in the water column may have worsened the inability of the nylon mesh to collect *C. tulipa* spat. 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) and coconut shells (Asare et al., 2019), for collecting *C. tulipa* spat in lagoons and estuaries of West Africa. PVC was used to collect spat of other commercial 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). Spat of *Crassostrea cucullata* were successfully collected on coconut shells in Kenya (Ruwa and Polk, 1994). In the present study on *C. tulipa*, ceramic tile was found to attract the highest number of spat in the wild although statistically comparable to settlement on PVC slats. Spat settlement on ceramic tile was superior to oyster shell (recycled) and coconut shell, both of which were superior to coconut shell. Coconut shell collectors were the least attractive to *C. tulipa* spat in all the water bodies.

The differences in efficiency among spat collectors used in this study is probably due to the nature of their surfaces. Contoured surfaces could provide better tactile stimulus and enhance spat settlement as reported for the pearl oyster *Pinctada maxima* (Taylor et al., 1998a). This structural support may, however, not hold for *C. tulipa* in absolute terms as observed in this study, as it would be expected that relatively contoured collectors, i.e. coconut and oyster shells, would be most effective. Instead, between the collectors, 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. Edible oysters (Bivalvia: Ostridae) settle onto surfaces by cementing whereas the pearl oysters fasten to substrates with the byssus. Thus, rough and contoured surfaces may have influenced greater spat settlement in the pearl oysters (Taylor et al., 1998a), which is not clearly observed for *C. tulipa* in this study. Although colour was not selected as a factor for collector effectiveness in this study, the different colours of the collectors did not appear to play any role in their differential effectiveness, thus, did not warrant further investigation.

Further, a comparative advantage of the 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 a more stable substrate for settlement of *C. tulipa* spat, especially in the wet season with increased currents. Oyster culturists in the region may deploy collectors for *C. tulipa* spat collection in most times of the year from January to July, and November to December. The statistically nonsignificant interaction between collector type and months in this study shows that the relative effectiveness of collectors does not change throughout the year.

Nonetheless, depending on the season of the year, the abundance of *C. tulipa* spat harvested on collectors (absolute effectiveness) may increase or decrease. The diminishing effectiveness of spat collectors from June to an almost complete suppression from August to October coincided with increased precipitation within the vicinities of the water bodies during the period of study. Dissolved oxygen (DO) was minimal at this time. Decreasing DO seemingly lowered the spat collection potency of the collectors significantly and vice-versa. This occurrence is not a likely effect of a reaction between the collector materials and the oceanographic parameters in the water bodies as explained by the DO-collector interaction term in the linear regression model. Meanwhile, there was also not enough evidence of the direct isolated influences of temperature, salinity, turbidity, pH and rainfall on the effectiveness of the collectors deployed.

Economic and technical considerations are worthwhile in selecting one of the substrates in this study for harvesting wild seed of *C. tulipa*. 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

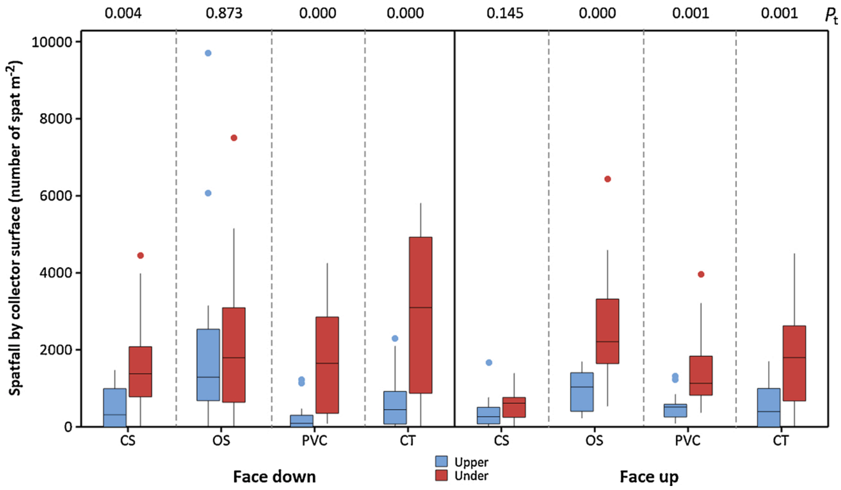

Fig. 6. Statistical analysis of the effect of two positional orientations ("Face down" and "Face up") on *C. tulipa* spat settlement on upper- and under- horizontal surfaces of the artificial collectors (CS = Coconut shell; OS = Oyster shell; CT = Ceramic tile). P_t value is the statistical result for the *t*-test of the null hypothesis H_0: $\mu_1 - \mu_2 = 0$ between upper- and under-horizontal surfaces of collectors.

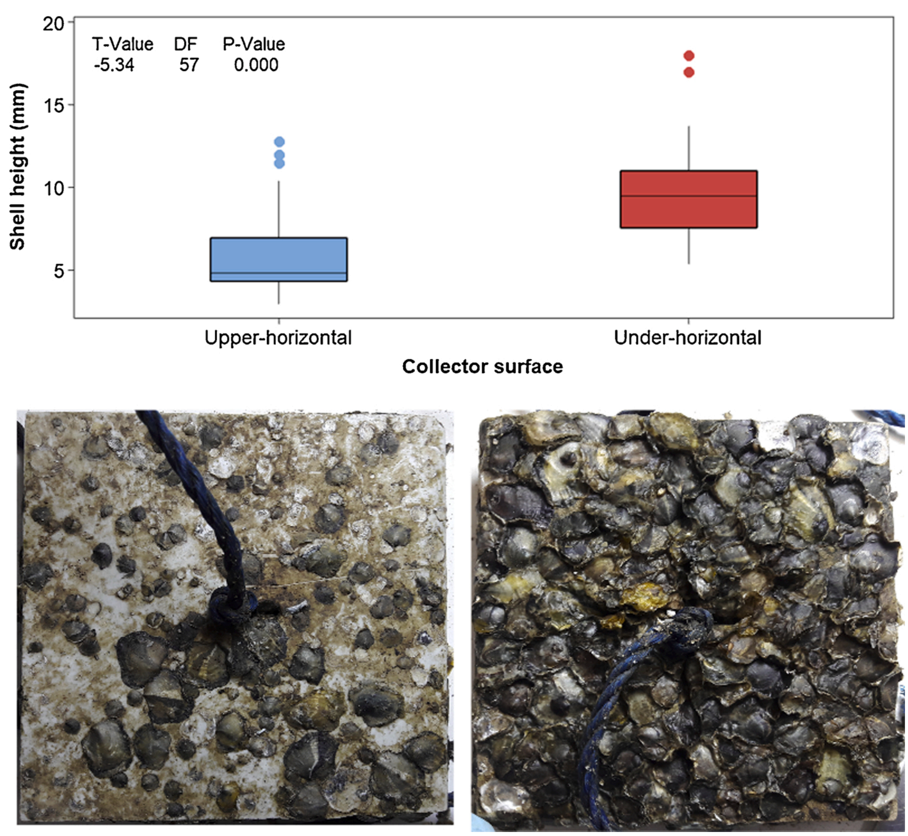

Fig. 7. Typical occurrence of larger sizes and greater settlement of *Crassostrea tulipa* spat on the under-horizontal surface (right) than the upper-horizontal surface (left) of the same collector (In this illustration, 10 × 10 cm^2 ceramic tile).

when they fall. This may increase losses and affect production cost 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 for a flexible plastic (Wedler, 1980). This is probably the reason PVC is the most widely used in recent times for oyster culture (Gosling, 2015; p 349). 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.

Oyster farmers 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 the collectors would be ideal on ceramic tiles and oyster shells. The biodegradable coconut shells would be ideal for bottom culture and useful in oyster restoration programmes. In addition, growth and survival of spat are important factors in the choice of collectors, beyond settlement. Arbitrary deployment of the collectors into open water systems for spat

Table 3
Summary means (± S.E.) of water temperature, dissolved oxygen, salinity, turbidity and pH in the coastal water bodies studied and mean total rainfall in their vicinities.

Month	Water temperature (°C)	Dissolved Oxygen (mg/L)	Salinity	Turbidity (NTU)	pH	Total rainfall (mm)
November 2017	29.25 ± 0.47	2.25 ± 0.30	17 ± 4	18.08 ± 2.28	8.25 ± 0.26	17.78 ± 17.40
December	27.99 ± 0.33	2.04 ± 0.31	14 ± 3	33.63 ± 9.60	7.90 ± 0.09	42.17 ± 18.77
January 2018	28.98 ± 0.67	5.28 ± 0.79	23 ± 1	12.48 ± 1.48	8.37 ± 0.13	0.00 ± 0.00
February	30.45 ± 0.69	7.01 ± 1.21	28 ± 1	12.22 ± 2.17	7.95 ± 0.07	33.19 ± 19.08
March	31.17 ± 0.57	2.23 ± 0.28	27 ± 1	9.18 ± 1.14	7.86 ± 0.17	1.35 ± 1.35
April	30.77 ± 0.24	4.51 ± 0.43	27 ± 2	10.73 ± 1.01	7.93 ± 0.08	48.09 ± 21.60
May	29.03 ± 0.54	2.72 ± 0.62	16 ± 4	27.60 ± 7.83	7.49 ± 0.09	161.29 ± 46.01
June	29.15 ± 0.32	1.51 ± 0.24	16 ± 3	25.82 ± 6.71	7.62 ± 0.09	73.58 ± 12.90
July	25.04 ± 0.29	1.33 ± 0.12	19 ± 4	18.66 ± 2.39	7.15 ± 0.16	10.07 ± 7.37
August	26.52 ± 0.88	1.08 ± 0.13	29 ± 3	11.52 ± 1.96	7.93 ± 0.08	88.74 ± 54.80
September	27.74 ± 0.39	0.63 ± 0.06	16 ± 4	16.94 ± 3.44	7.66 ± 0.04	24.64 ± 15.38
October	28.10 ± 0.23	0.69 ± 0.07	15 ± 4	18.00 ± 1.39	7.47 ± 0.05	105.15 ± 17.57

collection may not yield optimum harvests as the effect of collector surface is established for *C. tulipa* spat in this study.

The fitting of experimental racks with horizontally strung collectors was guided by literature on horizontally placed collectors yielding the greatest number of spat in other species of edible and pearl oysters (Diadhiou and Ndour, 2017; Holliday, 1996; Taylor et al., 1998a). Their observations of spat settlement on upper and under surfaces of horizontally placed collectors are, however, 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. Setting activity in mature larvae of *C. virginica* was encouraged by darkness and partially inhibited by light (Shaw et al., 1970). Best concentration of *C. gasar (=tulipa)* spat were recorded on shaded collectors (Ajana, 1979). The observation of profuse settlement of *C. tulipa* spat on under-horizontal surfaces of artificial collectors in the present study suggests 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 lower abundance on upper sides of collectors could also be a result of predation (Newell et al., 2000). Under-horizontal surfaces of the collectors could provide shelters for settled spat against predation, whereas spat attached to the upper sides remained vulnerable to predation, resulting in lower abundance.

The significantly larger sizes of *C. tulipa* spat settled on under-horizontal surfaces as opposed to the converse of the same collector, is most probably due to earlier attachment. The descriptions of the morphological and anatomical structure of the larvae of bivalves (Bayne, 2017) suggest larval movement prior to setting could account for this observation for *C. tulipa*. The free-swimming veliger possesses a foot for attachment near the velum (which is an outgrowth of the prototroch of the previous trochophore larva) and cilia for swimming forward and upward with foot and velum uppermost (see Bayne, 2017). Since larval formation is identical for *Ostrea* and *Crassostrea*, 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 even in highly turbulent flow (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. The profuse settlement coupled with larger-sized *C. tulipa* spat on under-horizontal surfaces of collectors give cues on the settlement behaviour, affirming the need for further studies on the larval behaviour of *C. tulipa*. This adds a different dimension to the “sink before you settle” analogy propagated for larvae of the Eastern oyster *Crassostrea virginica* (Poirier et al., 2019). The indication here is that for *C. tulipa* in open water, settlement of the larvae may actually occur in the rise (upward swimming) (Galtsoff, 1964; Hopkins, 1937; Kim et al., 2010; Wheeler et al., 2013) before the fall (sinking). An understanding of the pre-settlement behaviour of *C. tulipa* would, therefore, throw more light on the differential upper- and under-horizontal settlement pattern observed in this study.

5. Conclusion

Coconut shell, oyster shell, PVC slat, and ceramic tile substrates introduced into coastal water bodies supported the settlement of large numbers of *C. tulipa* spat within a period of one month. The collection of *C. tulipa* spat from the wild using a 2 mm nylon mesh was not successful. Ceramic tile was the most effective *C. tulipa* spat collector among the substrates assessed, ranking first in three of four water bodies studied. PVC slats were also found to be as effective as the ceramic tile collector. Coconut shell was conspicuously the least effective spat collector. It was inferred that the solid and hard surfaces of ceramic tile, PVC and oyster shell collectors provided a stable substratum for the attachment of larvae superior to the soft and easily degradable substratum of coconut shells.

The absolute effectiveness of each collector was dependent on the month of deployment. All the successful collectors in this study harvested larger numbers of *C. tulipa* spat from November 2017 to May 2018 and reduced to very low harvests from August to October 2018, most likely due to temporal availability of competent *C. tulipa* larvae in the water bodies. A positive linear relation between the effectiveness of the collectors and concentration of dissolved oxygen was observed in the ecosystems studied yet no interaction between the two variables was statistically established for this occurrence. There was a preponderance of settlement of *C. tulipa* spat on the under horizontal surfaces of all successful collectors deployed in this study. Similarly, larger-sized *C. tulipa* spat were observed on the undersides of all collectors. These findings represent additional information required to successfully culture *C. tulipa*.

It is recommended that prospective *C. tulipa* farmers in the region of the study should consider other factors including cost and availability of collector material, its amenability to culture technique and holding facilities, and durability in making a suitable choice between ceramic tile and PVC for large-scale spat harvesting. The collectors should be deployed suspended horizontally in the water column so that their undersides will be exposed to enhance settlement. Information on the effectiveness of collectors assessed in water bodies of varying hydrodynamics, i.e. an open lagoon (Benya Lagoon), a complex, intermittent lagoon estuary (Narkwa Lagoon), a classical river estuary (Whin Estuary) and a delta (Densu Delta), in this study, is representative of the different brackish water environments available for the farming of *C. tulipa* along the coast of West Africa. Thus, it is expected that the findings of this study would be applicable in the emerging large-scale aquaculture of the species in many parts of the region.

CRediT authorship contribution statement

Ernest Obeng Chuku: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft. **Kobina Yankson:** Conceptualization, Methodology, Validation, Writing - review & editing, Supervision. **Edward Adzesiwor Obodai:** Methodology, Validation, Writing - review & editing, Supervision. **Emmanuel Acheampong:** Formal analysis, Writing - review & editing. **Eunice Efua Boahemaa-Kobil:** Methodology, Investigation.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aqrep.2020.100493.

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