

ORIGINAL ARTICLE

Effects of Tidal Exposure on Bivalve Molluscs: First Stab at the Case of West African Mangrove Oyster (*Crassostrea tulipa*) in Benya Lagoon, Elmina, Ghana

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ABSTRACT

It is widely recognized that growth of sessile intertidal organisms is largely driven by tidal exposure. However, data supporting exact manifestation of this effect on *Crassostrea tulipa* is lacking, limiting management of the species to enhance nutrition and livelihood opportunities for coastal communities in West Africa. We investigated the growth of the species at 3 tidal heights [13 – 22 cm, 23 – 32 cm and 33 – 41 cm] above neap tide mean low water (MLW)] focusing on 3 parameters, i.e. shell height, condition index (CI) and gonadal development. Three hundred and thirty-three individual oysters from 10 colonies randomly selected from 2 stations, 500 and 600 m from the sea, around an open lagoon were investigated. CI was determined as the ratio between dry meat weight and inner shell volume; gonadal development was determined via histological analysis of gonad tissues. Shell height and CI of the oyster increased with tidal elevation irrespective of whether they were closer to or further from the sea. These findings agree with reports on other bivalves, suggesting that short tidal inundation promotes growth of *C. tulipa*. In terms of gonads, five stages of development were identified; all occurred among oysters located at lowest tidal levels (13 – 22 cm above MLW), possibly due to continuous breeding as a result of increased food supply by long inundation. The proportion of ripe gonads increased with tidal height; ≈67% of gonads found at the highest tidal levels were ripe suggesting discontinuous breeding in the higher-level oysters.

Keywords: Condition index, gonadal development, meat content, shell height, shell length, tidal height

Introduction

Tides are perhaps the most important environmental variable that affect the growth performance of sessile intertidal marine organisms. Depending on their intensity and direction, tides can (i) promote local recruitment [the supply of early juvenile stages to an established population/community] by retaining the larvae of intertidal organisms in the vicinity of their spawning communities (Connell, 1985), (ii) export larvae away to unfavourable areas, and thus lead to the loss of new recruits (Ellien et al., 2000), or (iii) limit the availability of favourable microhabitats by disturbing and redistributing bottom sediments (Cabaço et al., 2009; Nicastro et al., 2012). In addition to these effects, tides determine the extent of food delivery, desiccation/thermal stress, and predator access to intertidal areas (Mills & Berkenbusch, 2009).

These tidal effects lead to significant differences in the biological rates (e.g., growth) and morphometric features (e.g., shell size) of sessile organisms located

at different heights within intertidal environments (Littlewood, 1988; Hobday, 1995; McQuaid et al., 2000). This zonation has long been recognised and documented for many bivalve molluscs (Connell, 1961, and reviews by Underwood, 1979).

However, to the best of our knowledge, a quantitative assessment of tidal exposure effect on West African mangrove, *Crassostrea tulipa* (Lamarck, 1819) has not yet been done. The oyster grows on soft sediment substrates or attached to hard objects such as mangrove roots at different tidal heights (Angell, 1986). Its flesh is a cheap source of animal protein to many coastal communities; the shell is used as a building material, a poultry feed ingredient and in traditional medicine (Yankson, 1996). In support of efforts to manage the production of the species to benefit rural coastal communities (see, for example, Asare, 2017; Crow & Carney, 2013), we assessed the shell size, condition index, and gonadal development of the oyster using naturally occurring clusters of the animal located at

different tidal heights. We have discussed our results in the context of previous findings and presented possible adaption of *C. tulipa* to environmental factors (e.g., food availability, parasite infestation) that are directly linked to local tidal regimes.

Materials and Methods

The Study area

Crassostrea tulipa investigated in this study were sampled from two sites within a *Rhizophora* mangrove forest fringing an open lagoon called Benya in Elmina, Ghana (05° 03' N, 01° 02' W; Figure 1). The oysters grow hanging on the stilt roots of the mangrove trees. The lagoon is flushed by a semi-diurnal tidal regime with a

mean tidal range of ≈ 1.6 m during spring tides and ≈ 0.3 m during neap tides (Wellens-Mensah et al., 2002). The two sampling sites were set approximately 100 m apart (Figure 1). One site (Site 1) was placed farther away, ≈ 600 m away from the mean low water mark (MLW) of the sea during neap tide, in an upland area where oysters are inundated for a relatively short period of time. The other site (Site 2) was placed at about 500 m away from the MLW of the sea, in a downstream area inundated longer by tidal flows.

Sampling

The oysters were collected during low tide to ensure easy access to the animals. The sites were sampled in July 2015. Sampling was limited to colonies exposed at

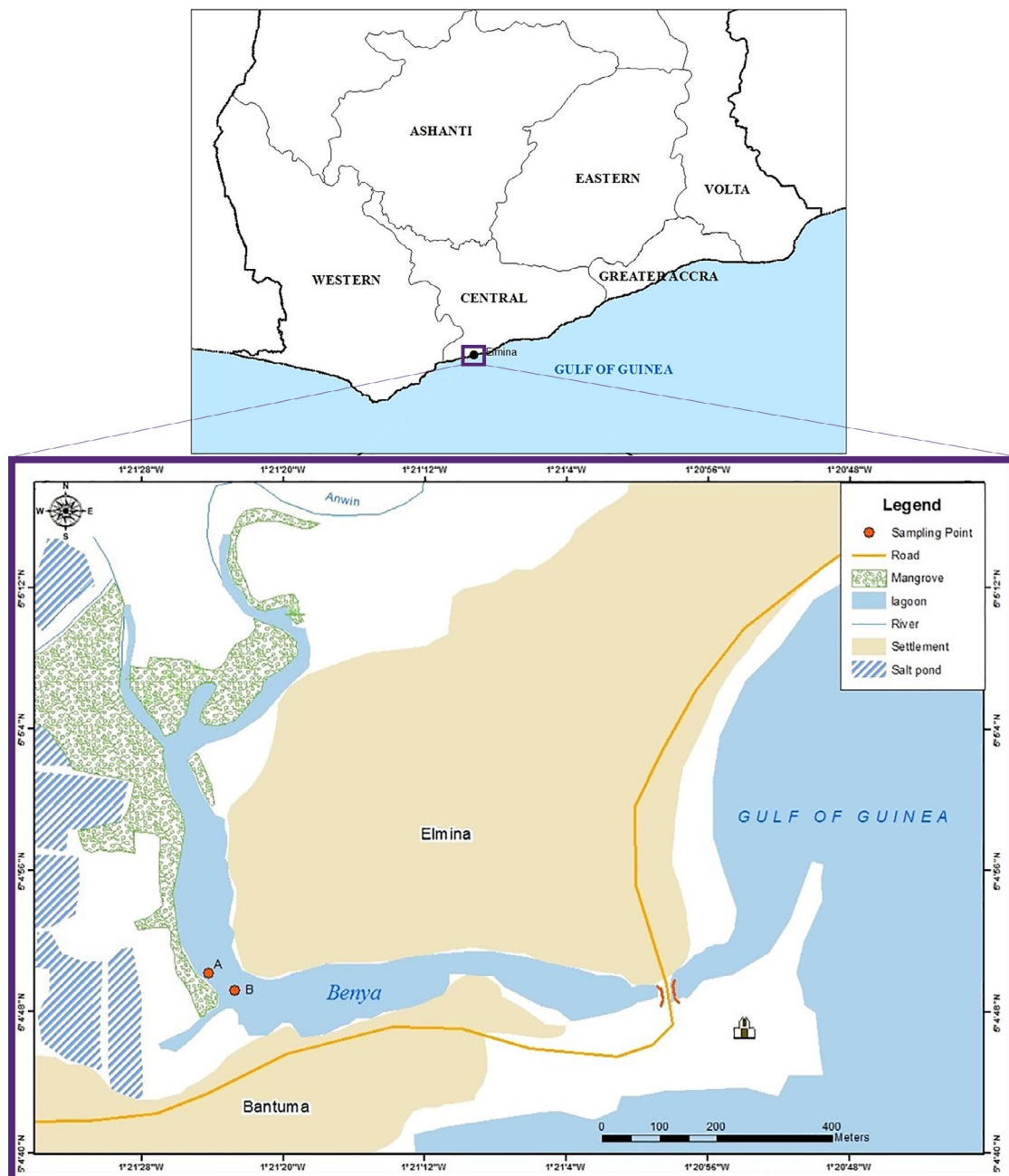


Figure 1: Map of Southern Ghana showing location of sampling sites 1 and 2 on the fringes of Benya lagoon, Elmina, Ghana

equal height (of ≈ 13 cm) above MLW. Five different colonies were randomly selected from each of the two sites.

A previous report suggested that within oyster colonies, individual animals are subjected to varying degrees of tidal exposure depending on their position along the colony – shorter inundation periods for those higher up in the colony and longer inundation periods for those at the bottom (Angell, 1986). We, therefore, determined the level of tidal exposure experienced by individual oysters by considering their position along the length of their respective colonies. The lengths of colonies were based on the vertical distance between individual oysters closest to the MLW and those farthest away at the topmost part of each colony. The length of each colony was then divided into three equal portions: the bottom part closest to the water surface, the middle denoting the mid portion, and top denoting the portion farthest away from the water surface. These three portions were referred to as bottom, middle and top clusters respectively in this report. They were taken to represent separate levels of tidal exposure experienced by the animals.

Laboratory analysis of oysters

In the laboratory the oysters were removed from mangrove root, cleaned with fresh seawater and measured for their sizes, condition index, and gonadal stage of development. The sizes of the oysters were based on the shell height of the animals. This is because shell height is strongly related to the biomass of oysters (Dame, 1972). The heights were measured using callipers, based on the parameters set by Quayle (1980).

The condition index (CI) of the oysters was estimated using the approach adopted by Yankson et al. (1991):

$$CI = \frac{\text{Dry meat weight}}{\text{Inner shell volume}} \times 100$$

The dry meat weight was determined by first removing the soft meaty parts of the oyster into pre-weighed pans, and then drying the meat in an oven at 100°C to a constant weight. Weights of the intact oysters were measured before the animals were dissected for their meat. All weights were measured using an electronic balance (model: FEL-500S). The inner shell volume of the oysters was determined using an approach recommended by Lawrence and Scott (1982). First, empty shells obtained after the dissection of the oysters were air-dried for ≈ 24 hours and weighed. The inner shell volume was then calculated by subtracting the air-dried weight of the empty shells from the weight of the intact animal.

Gonadal development was determined from histological sections of gonadal tissues extracted from

20 individual oysters randomly selected from a pool of oysters collected from each of tidal heights. The sections (8 – 10 μm in diameter) were prepared following gonad tissue histology method developed by Diadhiou and Le Pennec (2000). They were stained with hematoxylin and eosin, and examined under light microscope in order to determine the gonadal development stage of the animal. Five stages – early development, developing, ripe, spawning and redevelopment – of gonadal development were assessed based on an amalgamation of criteria from previous works (Yankson, 1982; Lee, 1988; Shafee, 1989; Diadhiou & Le Pennec, 2000). Identifying features for each of these stages were the same as those described by Krampah et al. (2016). All organisms analysed were placed in one of these categories.

Data analysis

Effect of tidal exposure on the size of the oysters was investigated by comparing the modal shell height of animals located at different tidal heights. The modal shell heights were determined via a frequency distribution analysis.

A nested two-way analysis of variance (ANOVA) recommended by Sokal and Rohlf (1995) for unequal sample size was used to compare condition indices of oysters collected from the different locations. For this analysis, sampling site (1 or 2) was the fixed factor constituting the highest level of classification. Oysters studied per tidal level constituted the subordinate classification nested within the sites. The critical p-value was taken to be 0.05 for all analyses. Whenever differences were found between any set of data, the Tukey's HSD ($\alpha = 0.05$) procedure was used as a post hoc test to determine which pairs of means were significantly different.

Results

A total 333 individual oysters from 10 different colonies, randomly selected from 2 sampling locations were analysed; 169 of these were collected from site 1 located ≈ 600 m away from the sea at neap tide, the remaining 164 were collected from site 2, which was ≈ 100 m closer to the sea than site 1. Average length of the colonies taken from sites 1 and 2 was 40.3 ± 4.6 cm and 39.3 ± 4.2 cm (mean $\pm$ standard deviation), respectively. There was no significant difference between the lengths of the colonies from the two sites (t-test, $\alpha = 0.05$, $df = 18$; t-value = 0.5794). Based on this result, and given that only colonies with bottom-most individual(s) exposed at ≈ 13 cm above MLW were sampled, the top, middle and bottom clusters can be said to have been collected at tidal heights of 13 – 22 cm, 23 – 32 cm and 33 – 41 cm relative to the MLW, respectively. The results are therefore displayed using these tidal height values.

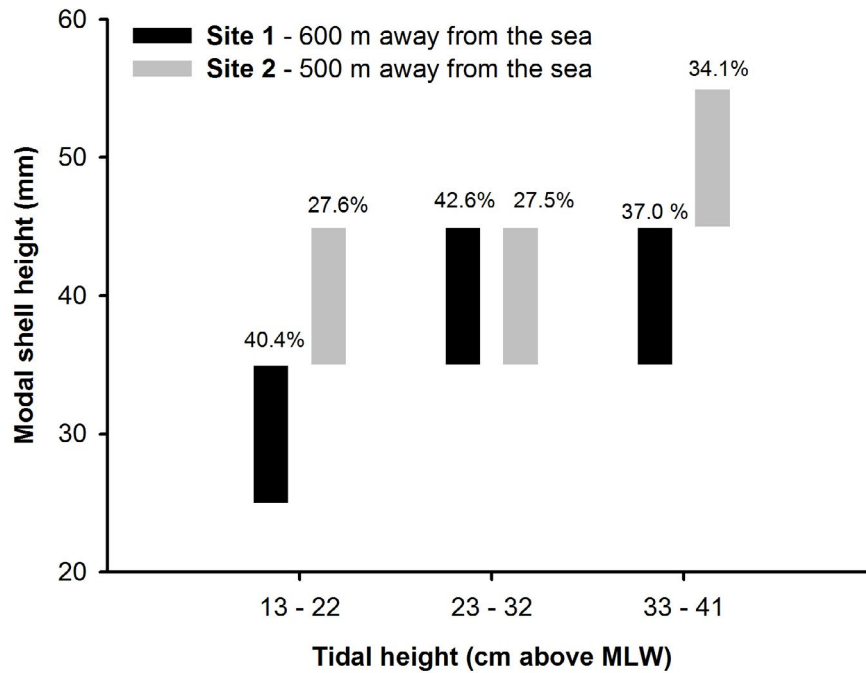


Figure 2: Modal shell heights of *Crassostrea tulipa* located at different heights above the mean low water (MLW) mark of Benya lagoon, Elmina, Ghana. Indicated on top of the bars are the frequency of occurrence of the modal size classes.

Figure 2 shows modal shell height of *C. tulipa* collected from different locations in the study area. Shell height of the oysters generally increased with the tidal elevation of the clusters. Furthermore, at the bottom and upper sections of the colonies, the oysters in terms of their modal size were generally bigger when the animals were located closer to the sea (site 2). Consequently, clusters at the top of colonies at site 2 were about 20 mm bigger (modal size = 45 – 54.9 mm) than those located at the bottom of colonies at site 1 (modal size = 25 – 34.9 mm).

At the site located farther away from the sea (site 1), the modal size of *C. tulipa* was biggest (size = 35 – 44.9 mm) among the top and middle clusters of the colonies. These individuals constituted 37% and 42.6% of the top and middle clusters, respectively. At the site located a bit closer to the sea (site 2), the modal size was biggest (size = 45 – 54.9 mm) only at the top of the colonies; the frequency of occurrence of this size class was $\approx 34\%$ (Figure 2).

Condition indices of the oysters are shown in Figure 3. At each sampling station, the index increased with tidal heights: oysters located at the top of their colonies had the highest condition index; those located at bottom of their colonies had the lowest condition index. On average, the increase in CI from the bottom to top cluster of the colonies was $\approx 30\%$; it was $\approx 17\%$ from middle to the top cluster of the colonies. The tidal level at which the oyster were located was therefore a significant determinant of condition index among *C. tulipa* (Table 1). This effect was most significant when

oysters located at the top of the colonies in upland areas at site 1 were compared with those at the bottom of the colonies located closer to the sea at site 2 (Tukey's HSD post hoc test, $p < 0.05$). Condition index of oysters at the top of colonies at site 1 was $\approx 38\%$ higher than those located at the bottom of colonies at site 2 (Figure 3).

Table 1: Two-way nested ANOVA testing for differences between condition indices of *Crassostrea tulipa* located at different sampling sites and tidal levels within Benya lagoon, Elmina, Ghana

Source of variation	SS	df	MS	F	P
Sampling site	1364.99	1	1364.99	2.06	0.1609
Tidal level	7505	2	3752.50	5.67	0.0078
Sampling site $\times$ Tidal level	497.6	2	248.8	0.38	0.6869
Error	21195.38	32	662.36		
Total	30562.97	37			

Figure 4 shows five stages of gonadal development identified among the *C. tulipa* in this study. All the five stages were found among clusters at the bottom tidal levels (13 – 22 cm above MLW) at both sampling stations. However, there were marked changes in the gonadal development of the animals with tidal height. At both sampling stations, the number of gonads at early developmental stage decreased with increasing tidal height; in contrast, the numbers of ripe gonads increased with increasing tidal height. Sixty-seven percent of the gonads found at the highest tidal level

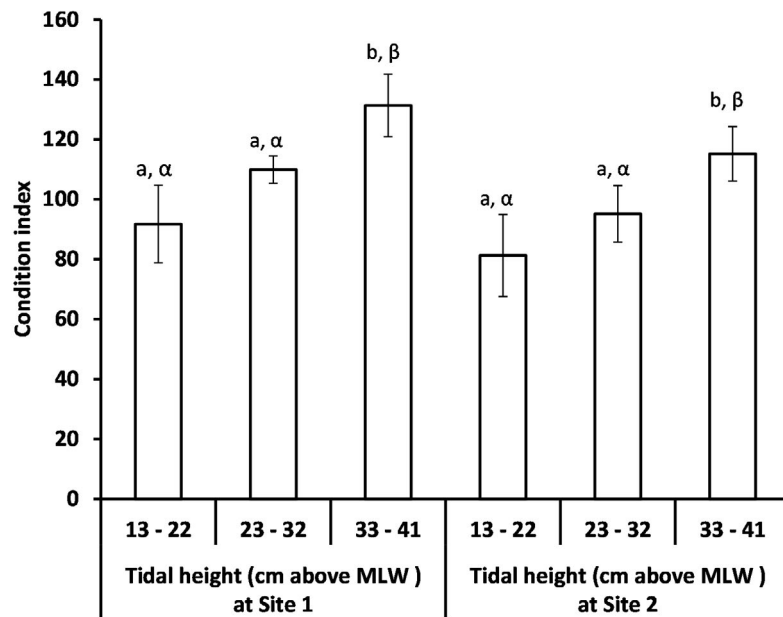


Figure 3: Condition index (mean $\pm$ standard errors) of *Crassostrea tulipa* located at different tidal height above the mean low water (MLW) mark of Benya lagoon, Elmina, Ghana. Sampling sites are the same as described under Figures 1 and 2. English and Greek alphabets on top of the bars indicate Tukey's HSD ($\alpha = 0.05$) post hoc comparison of results within and between sampling sites, respectively; bars not sharing a common alphabet are significantly different ($p < 0.05$).

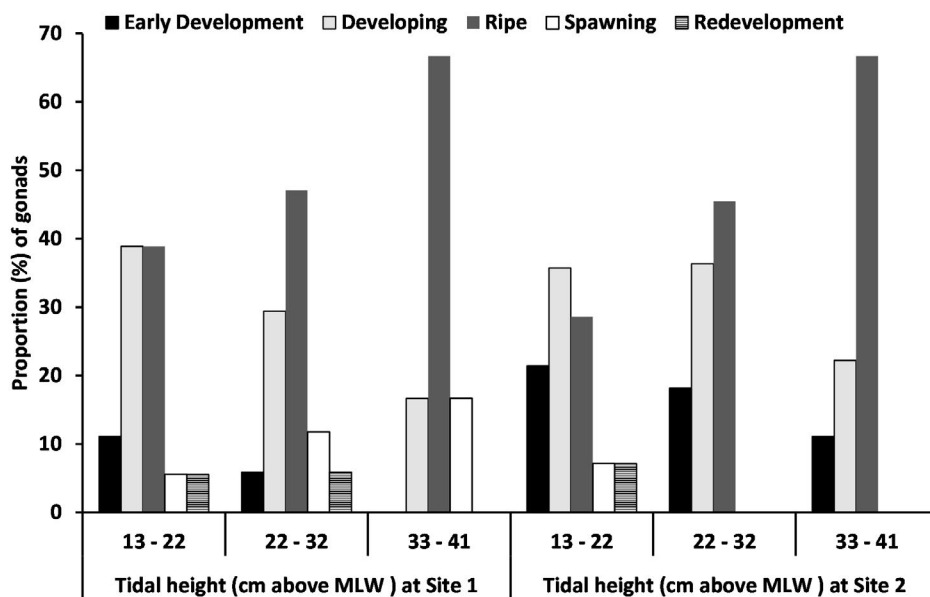


Figure 4: Distribution of *Crassostrea tulipa* gonadal stages at different tidal heights above the mean low water (MLW) mark of Benya lagoon, Elmina, Ghana. Sampling sites are the same as described under Figures 1 and 2. Sample size = 20 at each tidal height.

(32 – 41 cm above MLW) were ripe at both sampling stations (Figure 4).

Discussion

It is well established that sessile organisms inhabiting different tidal heights are affected differently by tidal exposure: those located at lower tidal levels are submerged longer and are therefore able to spend more time engaging in crucial physiological activities such as

feeding and respiration for growth; sessile organisms at higher tidal levels do not get submerged as much, hence, they spend less time engaging in crucial physiological activities for growth (Kosch et al., 1963; Southward, 1965; Roegner & Man, 1995). We investigated the effect of tidal exposure on the shell size, condition index and gonadal development of *C. tulipa* recognizing that tidal inundation also increases the access of natural predators and fouling organisms to intertidal areas with negative

consequences for the physiological condition of sessile intertidal organisms (Barillé et al., 2000). Over three hundred oysters located at three tidal heights (bottom cluster: 13 – 22 cm above the mean low water (MLW); middle cluster: 23 – 32 cm above MLW and top clusters: 33 – 40 cm above MLW) from two different sites within a mangrove forest were investigated. One of the sites (site 2) was located $\approx$ 500 m close to the sea; the other (Site 1) was located approximately 100 m away from Site 2 in an upland area (Figure 1). Hence, colonies at site 2 were likely to be inundated first and exposed last by tidal flows; in contrast, the colonies at site 1 were likely to be inundated last and exposed first by tidal flows.

The results indicate that the shell height of *C. tulipa* generally increased with tidal elevation (Figure 2). Furthermore, clusters at the top of colonies located closer to the sea (site 2) were $\approx$ 20 mm bigger (modal size = 45 – 54.9 mm) than those located at the bottom of colonies farther away at site 1 (modal size = 25 – 34.9 mm). This observation compares with earlier reports. Littlewood (1988) for example reported that *Crassostrea rhizophorae* grows faster in mid-intertidal areas than in habitats several centimetres lower in elevation. The body size of limpet also increases with tidal height (Hobday, 1995). Given these previous observations and our results, it can be said that bigger individuals of *C. tulipa* are likely to occur at higher tidal levels.

Condition index of *C. tulipa* also increased with tidal elevation (Figure 3). In particular, CI of oysters located at the bottom in areas that are likely to be inundated first and exposed last (tidal height = 13 – 22 cm at site 2) was $\approx$ 38% lower than that of those located at the top of colonies in upland areas at site 1. This result, considered together with the increase in modal shell height with tidal elevation (Figure 2), suggest that *C. tulipa* located at lower tidal heights are likely to contain less amount of meat, and *vice versa*. This can be attributed to excessive concentration of heavy metals, bacteria and fungi in the Benya lagoon (Obodai et al., 2010; Obodai et al., 2011; Vowotor et al., 2014). The reason is that excessive concentration of heavy metals, bacteria and fungi interfere with the production of new biomass by oysters (Pridmore et al., 1990; Rebelo et al., 2005; Wargo & Ford, 1993). The exposure of the oysters to these pollutants may vary with tidal inundation of the study area, which will limit the meat content of *C. tulipa* that are likely to be inundated relatively longer at lower tidal heights. On the other hand, effect of the pollutants on meat content may be reduced among oysters that receive less inundation at higher tidal heights (Figure 3).

Loss of biomass during spawning may also reduce the meat content of oysters (Nascimento & Pereira, 1980). For example, females of *Crassostrea gigas* catabolise 43 – 62 % of their biomass to provide energy for gametogenesis; in males, the value is 18 – 56 %

(Deslous-Paoli & Héral, 1988). Among *C. rhizophorae*, spawning is the most important biological factor that significantly reduce CI (Rebelo et al., 2005). Consistent with these observation, lowest CI values were found at low elevations (13 – 22 cm above MLW) where clusters held all five stages of gonadal development (Figure 3 and 4). This wide spread of developmental stages at lower tidal heights suggests continuous breeding of the oyster at those levels (Quayle, 1980). It is likely because oysters at low-lying areas may receive increased inundation allowing them to frequently access food and undertake respiration for growth (Kosch et al., 1963; Southward, 1965; Roegner & Man, 1995). In contrast, only 3 of the 5 gonadal stages identified in this study occurred at the topmost elevations (33 – 41 cm above MLW) where oysters are likely to experience less inundation (Figure 4). This apparent lack of continuous breeding at high tidal levels can be attributed to thermal stress as found in other brackish water bivalves in the tropics (e.g., *Anadara senilis*; Yankson, 1982). In our observation, two-thirds of the oysters at these elevations had ripe gonads (Figure 4). Such gonads, occurring around the visceral mass of the oyster, contributes to filling the inner shell space and therefore increase CI values as observed among oysters located at the top of their colonies (Figure 3).

Based on these observations, it can be concluded that (i) aquaculture zones for the production of oyster shell and meat may be located at relatively higher tidal levels (33 – 41 cm above the MLW; Figure 2 and 3) where limits on inundation is likely to reduce overcrowding and its associated competition for food and space particularly when spat settle on parent oysters to form reefs with generations layered one above the other (Kamara, 1982), and (ii) habitats for maintaining reproductive stock of the species may be located at lower tidal heights (13 – 22 cm above the MLW) where oysters are likely to receive enough nutrition to power gametogenesis and continuous reproduction (Figure 4; Southward, 1965; Roegner & Man, 1995). We view our study as a preliminary snap-shot that needs to be extended to include other ecosystems (e.g., estuaries) and environmental factors vital for the growth of *C. tulipa*. In particular, the sampling protocol presented in this study is recommended as it is similar to previously documented studies (e.g., Hobday 1995; Mercado-Silva, 2005). In addition, sampling sites should be located wider apart in order to cover the extremes of the tidal exposure conditions experienced by the organisms. With only 100 m between sampling sites in the present study, effects arising from possible differences in hydrographic conditions may be moderated by reduced differences in inundation and exposure times between the sites. We also recognise that oyster gonadal development could be seasonal (Diadhiou & Le Pennec, 2000). Thus, sampling over a year may be needed in

order to fully establish the link we observed between reproductive state and CI of the oyster at different tidal heights (Figures 3 and 4). As part of the sampling, temperature and salinity conditions associated with dry/wet seasons of the study area may be collected as these factors affect the condition, gametogenesis and survival of oysters (La Peyre, 2013; Shpigel et al. 1992).

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