

## Influences of Physico-Chemical Water Parameter on The Natural Distribution Site of Cokcles (*Tegillarca granosa* and *Anadara antiquata*) in Coastal Areas of Kuala Kedah, Kedah, Malaysia

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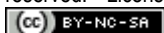
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**Keywords:** *Tegillarca granosa*, *Anadara antiquata*, Site Assessment, Cockle Culture, Physico-Chemical Water Parameter

**Abstract:** This study has deduced the influences of physico-chemical parameter towards the natural distribution of cokcles (*Tegillarca granosa* and *Anadara antiquata*) in coastal areas of Kuala Kedah, Kedah, Malaysia. Several studies on cockle culture sites were conducted throughout West Coast of Malaysia's waters. Kuala Kedah has been identified to have similarities in physical characteristics for cockle culture thus determining the best way to explore the coastline of Kuala Kedah of cockle culture. Cockles nowadays are one of the biggest contributions in Malaysia's aquaculture landings. This is due to cockle's ideal characteristics for aquaculture industries for its high growth rate, availability throughout most time of the year and highly demanded by the locals. The study used 48 hours observation on physical parameters on one point at the river mouth of Kuala Kedah River which water quality of physical and nutrients and bathymetry. The biomass of the cockles was done using swept- using a core made to harvest cockles. The 48 hours observation suggested that each of the physical parameters were homogenous while the stations physical parameter and nutrients overall showed that temperature ranged 27.90 to 28.47 °C, salinity 28.15 to 33.92 ppt, DO 3.92 to 7.03, ph 7.62 to 8.34, turbidity 4.58 to 158.17 NTU, TSS 6.82 to 344.83 mg/L and chlorophyll a 3.17 to 18.65 µg/L in addition that study area was in a slope condition. Both *T. granosa* and *A. antiquata* were found. Present study recommended the study site can be proposed as a cockle culture site.

## INTRODUCTION

Cockles are widely distributed in Asia such as in China as early as the 17<sup>th</sup> century and Japan previously had been successfully culturing *Tegillara granosa* as early as 1859 (Cahn, 1951, Cockles, *T. granosa* and *A. antiquata* are few of the several bivalve

species found in the Peninsular Malaysia. Today, cockles had huge role and considered as an economic importance to Malaysia. Cockles are also the main bivalve being cultured in Malaysia (Fariduddin et al, 2017). Cockles production contribution was more than 50% to the national aquaculture total production which Malaysia was placed as the 5<sup>th</sup> ranked in Asia Pacific with production of 78,712 tons in 2002 (Sugiyama et al., 2004). Cockle culture in Malaysian northern state like Penang and Perak makes the northern area to be the third largest cockles' producer in the west coast of peninsular Malaysia (Hassan, 2004).

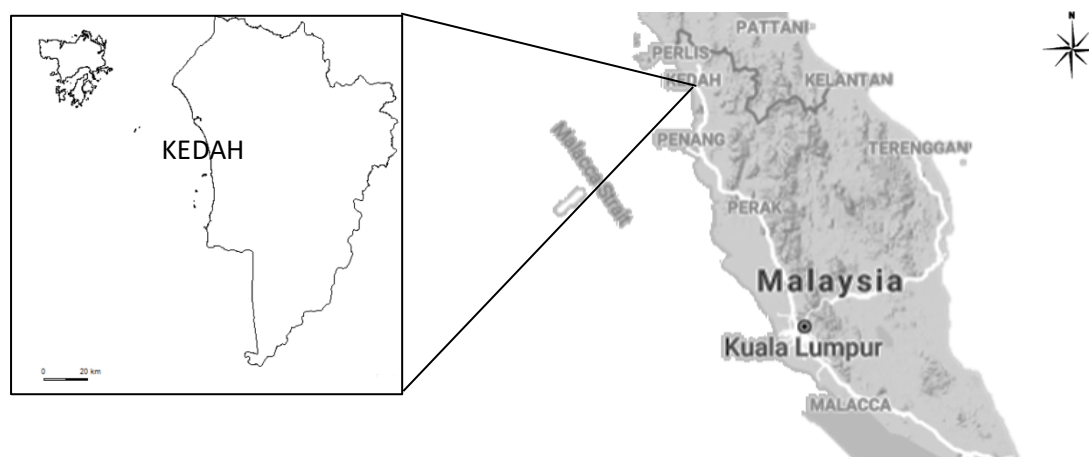
Constant harvest of *T. granosa* is dreaded to give a negative impact on the sustainability of this species population in the future (Ruth et al, 2014). Although most of cockles are well known to be farmed or cultured close to the river mouth area (Wibowo, 2012), we must take into consideration of all the nearby industrial activities that are highly able to produce waste pollution (Alfionita et al, 2018) that is harmful to the cockles population survival (Komala et al 2011). In order to increase the landings of cockles in Malaysia through farming, more studies on the habitat of naturally distributed cockles need to be done. Furthermore, the mass mortality reported in Malaysia (Yurimoto et al., 2014) had triggered the need to increase the culture area for cockles. Due to this, more understanding of how physical parameters such as salinity, pH temperature and along with other parameter affect the cockles as some shellfish like *Mytilus edulis* and *Arctica islandica* are Interactively affected by temperature and salinity (Hiebenthal et al, 2012).

For a basic understanding of geological and oceanographic processes, knowledge of bathymetry is essential (Anne-Cathrin et al. 2019). Early studies on echosounding profiles had enabled us to understand the relationship between mid-ocean ridges and earthquake seismicity that played a major role in one of science's most critical paradigm shifts (Hess, 1962). Studies on user of seabed mapping conducted in 2018 by Geoscience Australia and FrontierSI gathered data from national data had revealed that habitat mapping and hydrographic charting were the most common applications for the use of high-resolution bathymetric data (Amirebrahimi et al., 2019). This shows that a thorough understanding of the coastal bathymetry of a nation is also important for political and commercial purposes since the study region can be determined by bathymetry to be suitable for what kind of activities in terms of boosting the national economic sector such as the aquaculture industry. The main objectives of this study are to do assessment on the water quality, bathymetry study, and observation on natural distribution of cockles in Kuala Kedah coastal.

## 2. Materials and Methods

### *Area's of interest (AOI)*

The AOI started from the Kuala Kedah Mouth River to Kuala Tebengai at the location of 6°04'21.2"N 100°16'50.4"E (Figure 1). The AOI for this study covers around 6,657.64 ha. The location of this study area was also set to overlap with the bathymetry study within the study area (Figure 2).

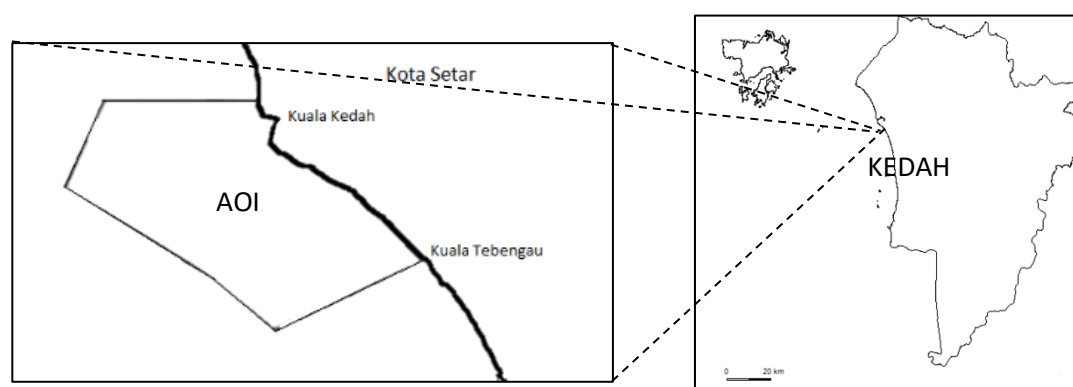


**Figure 1.** Kedah State was chosen as the study site.

### Sampling design

Physical water parameters readings were taken from most of the stations that had been taken from the AOI. The stations were randomly labelled to avoid any bias when doing analyzing. A total of 10 stations were chosen and labelled as KS1, KS1a, KS1B, KS2, KS3, KS5, KS6, KS7 and KS8. Apart from KS1a, all the other stations physical parameters reading was taken only once during high tide due to the surface of the coastal has limited area that could be reach during low tide, while KS1a station was set as the 48 hours observation station for physical water parameters for every hour for 48 hours.

The AOI started from the Kuala Kedah Mouth River to Kuala Tebengai at the location of  $6^{\circ}04'21.2''\text{N}$   $100^{\circ}16'50.4''\text{E}$ . The AOI for this study covers around 6,657.64 ha.



**Figure 2.** Study site (AOI) area ( $6^{\circ}04'21.2''\text{N}$   $100^{\circ}16'50.4''\text{E}$ ) with 9 sampling stations covers from Kuala Kedah to Kuala Tebengau, Kedah.

### Water physical parameters

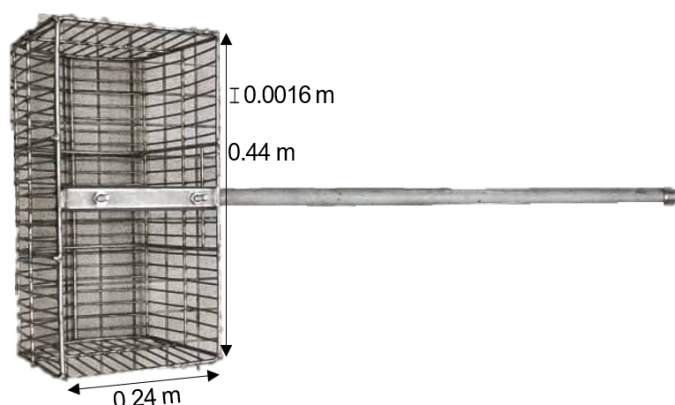
The physical parameters involved in this study are the one the elements that are closely related to the growth and influential to the blood cockles spawning. They are salinity (ppt), dissolve oxygen (DO), pH, total dissolved solid (TDS), turbidity (NTU), total suspended solid, water depth, bathymetry and total chlorophyll a. (Chl a.). All involved equipment were calibrated prior to the sampling activities di avoid and minimizweany possible error during the data collection. For physical parameters, it was measured using YSI multi-probe ProDSS, USA. The results were than compared to the Malaysia Marine Water Quality Standard (MMWQS) set by the Malaysian Department of Environment.

### Nutrient Analysis

In order to classify and measure the quantity of nutrients that may cause eutrophication, testing of nutrients in the water sample from the study area was carried out. The goal is to provide spatiotemporal information to detect short-term status of the nutrient status and to search for any sign of pollution. In order to analyse the samples, Spectrophotometer HACH DR3900 was used. The water samples were process using the respective kits provided for ammonia, phosphate, nitrate and nitrite. The results are than compared to the MMWQS.

### Biomass study

All the sampling activities were done using swept method area of the AOI. Every sampling was done for one minute at the speed of 1.25m/s using modified traditional fishing gear or locally called 'corr' (Figure 3) with the size of 0.44 m X 0.24 m with gap size of 0.0016 m. All the samples found were brought back to land for measurements of total length and weight. Every sampling station was done in triplicate.



**Figure 3.** The corr (fishing gear) that was used in the study of biomass of cockles in Kuala Kedah.

### Statistical analysis

The analysis done using one way anova on SPSS version 20 and significane difference is  $p \leq 0.05$ .

## 3. Results and Discussions

### 48 hours observation in a static station

A fix station for physical parameter profiling had been done at station KS1a for 48 hours from 14-16 January 2020.

**Table 1.** 48 hr observation on physical parameters at station KS1a

	Temperatur e (°C)	SALINITI (ppt)	DO (mg/L )	pH	TURBIDITI (NTU)	TSS (mg/L)	CHL A µg /L
Min	27.2	29.21	3.87	6.23	10.72	16.84	2.22
Max	29.5	34.31	11.22	8.42	191.35	770.5	49.46
Averag e	28.24	33.27	6.18	8.21	48.47	77.99	11.81

Table 1 suggested that at the bottom of station KS1a, the differential value of temperature within 48 hours ranged around 2.3°C in between high and low tide. The

minimum temperature was at 27.2°C and highest at 29.3°C. During the study, no rain had been observed and the results would have more variations if rainfall had occurred. (Rong-Gang and Mark, 2012). The dry season at that time supported the results in Table 1 where salinity maintain above 29 ppt.

The salinity differential between the 48 hours observation is 5.1 ppt with the highest being 34.31 ppt and the lowest at 29.21 ppt. This suggested that this area does not receive a significant volume of freshwater influx despite being close to the river mouth at Kuala Kedah. The observation on pH (6.2 – 8.4) reading was within the standard of aquatic animals in Malaysia Marine Water Quality Standards And Index (Class II). While the DO was observed at optimum level (>5.0 mg/L) during hightide, however during lowtide at 8.00 – 9.00 a.m DO was recorded to be less than 3.0 mg/L. During this hour, the NTU reading was recorded to be 140 - 191.35. This was expected due to the netrification activities brought from the nearest river (Argenal & Gomez, 2006; Heest, Burkhardt, & Curry, 2006; Li, Liu, Li, & Zhang, 2013).

Wide ranged of chl A readings showed at some point of the day, level of Chl. a were not sufficient to cover aquatic life in that area as the minumum reading was at 2.22 µg/l and the highest at 49.46 µg/l. The lower reading of chl (<4 µg/l) was seen during low turbidity (<40 NTU), TSS (<30), temperature >27.2 °C), saliniti (> 33 ppt) and pH (> 8). All of the mentioned happened during the influx of seawater during the peak of high tide.

**Table 2.** Pearson's correlation coefficients between all measured physical parameters within 48 hours

	Temperatur e	Salinity	DO	pH	Turbidit y	Chl a	Depth	Current
Temperatur e							-0.470**	
Salinity				0.464**	-0.405**	-0.403**	0.494**	0.523**
DO				0.445**	-0.558**		0.395**	0.437**
pH					-0.578**	-0.582**	0.697**	0.526**
Turbidity						0.740**	-0.498**	
Chl a							-0.677**	-0.439**
Depth								0.646**
Current								

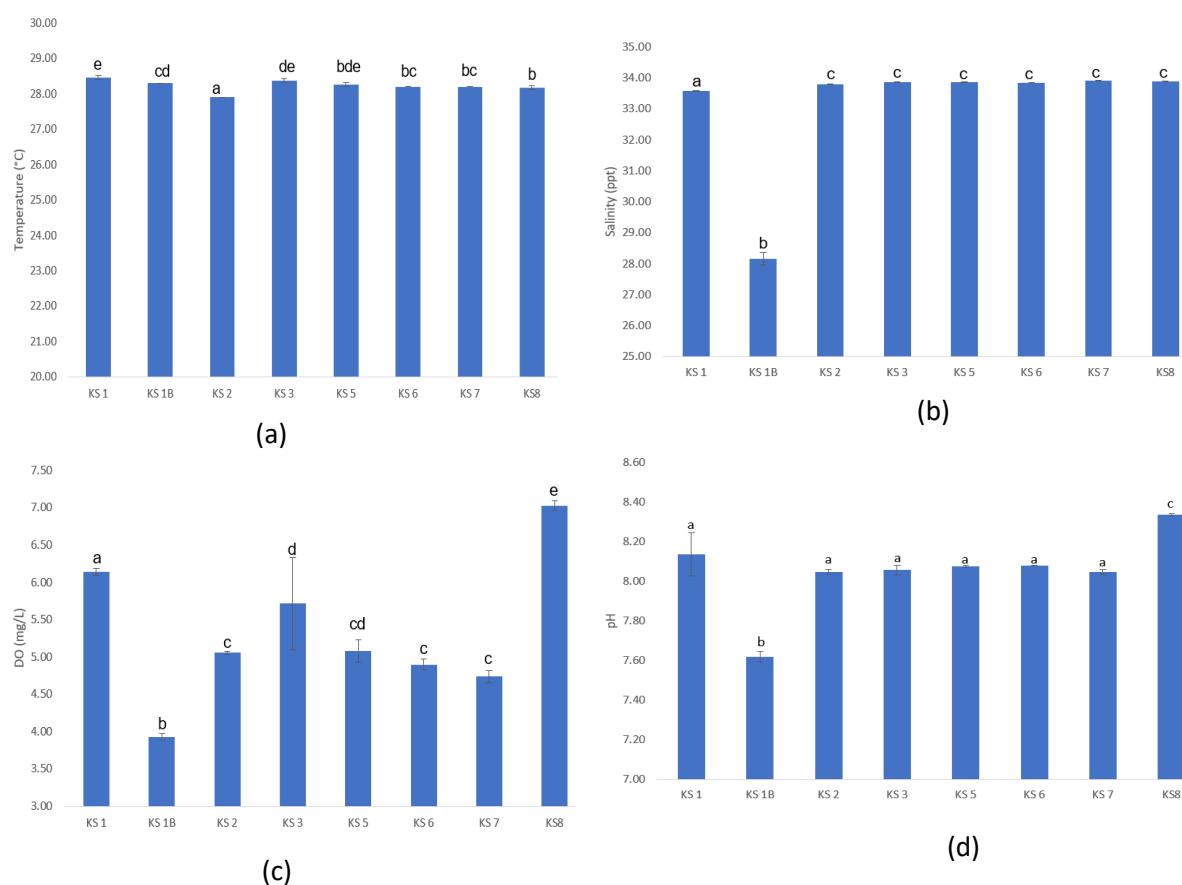
(\*\* Correlation is significant at the 0.01 level (2-tailed); Empty white box showed no significant difference when  $p > 0.01$ )

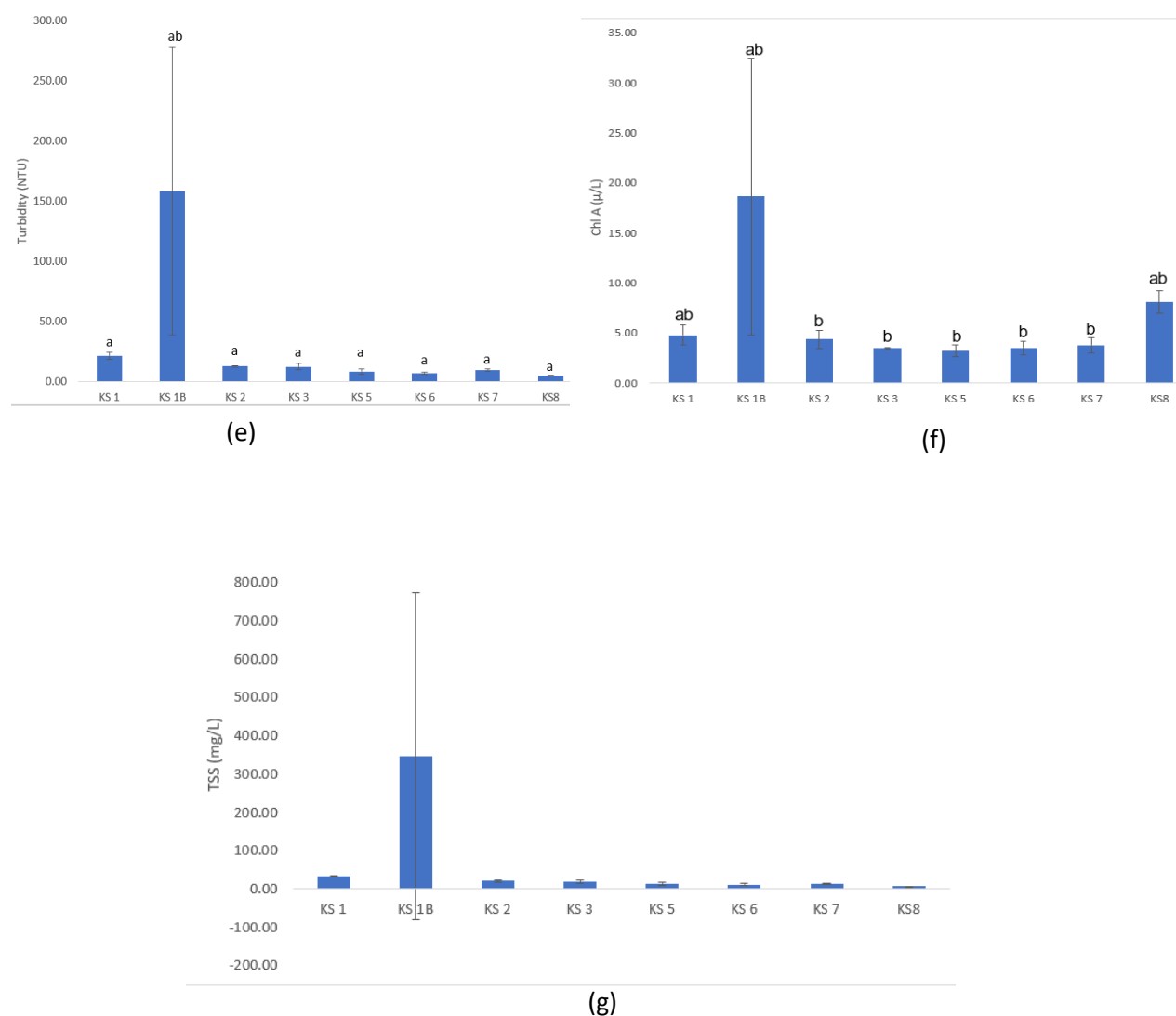
From the results, statistical analysis of Pearson's correlation was done. Based on correlation analysis (Table 2), the pH of the water was positively correlated with salinity ( $r = 0.464$ ) and DO ( $r = 0.445$ ). The turbidity showed negative correlation with salinity ( $r = -0.405$ ), DO ( $r = -0.558$ ) and pH ( $r = -0.578$ ). The correlation showed that the concentration of chlorophyll a was negatively correlated with salinity ( $r = -0.403$ ) and pH ( $r = -0.582$ ) and was positively correlated with turbidity ( $r = 0.740$ ). The temperature ( $r = -0.470$ ), turbidity ( $r = -0.498$ ) and Chl a ( $r = -0.677$ ) decreased with depth, whereas parameters such as salinity ( $r = 0.494$ ), DO ( $r = 0.395$ ), pH ( $r = 0.697$ ) and current ( $r = 0.646$ ) increased as the depth increases. The current of the water was positively correlated with salinity ( $r = 0.523$ ), DO ( $r = 0.437$ ), pH ( $r = 0.526$ ) and was negatively correlated with Chl a ( $r = -0.439$ ).

#### *Physico-chemical parameters observation at sampling stations*

The station that their physical parameter was taken were KS1, KS1B, KS2, KS3, KS5, KS6, KS7 and KS8. The location of KS4 is very near to KS5 hence only KS5

parameters were taken. The results (Figure 4) were recorded during high tide as the vessel used for the sampling activities could not reach the target areas during low tide. The temperature was range from 27.9 to 29.5 °C which are the within the range of tropical climate temperature (Carol and Timothy, 1997). For salinity, KS1B showed the lowest level of salinity at 28.15 ppt while the other stations were range from 33.58 to 33.87 ppt. For DO levels stations KS1B, KS6 and KS7 showed level of less than 5.00 mg/L which is less than optimum level for aquaculture activities and it is potentially to become hypoxia if the DO level are maintain in that level for sometimea (Suzuki et al, 2012). For pH the level for each of the stations suggest that all of the stations comply and suitable for both aquaculture activities and aquatic life (6.5-8.5). Turbidity data showed that only KS1B had the data that had high turbidity when the study was done (158.17 NTU). Similarly as the turbidity, TSS was also high with the average of 344.83 mg/L of concentration. The readings of chlorophyll a was high at station KS1B and KS8 suggesting these areas received high nutrient from the river run-off (Furnas, 2011). The high reading of chlorophyll is also believe had influenced the readings of turbidity (Yihe et al, 2019). It has also been observed that the chlorophyll a concentration in KS1B was higher compared to the deeper level of the water. Similar observation was one in a study and it believe that the microphytobenthos was the source of the chlorophyll a higher concentrations (Henning et al, 2007). Overall, all of the station during the time of the study was done, were mostly within the suitable condition for aquatic life. KS1, KS1B and KS8 were located near the river mouth that had been installed with flood gates. This type of location are known to influence the water quality since the floodgates are closed most of the time while trapping pollutants and during having rain the floodgates are open and would discharge these pollutants into the river mouth (Renjit et al., 2017).





**Figure 4.** Levels of physical water parameters observed and recorded at Kota Setar, Kedah coastal. (a):Temperature, (b): Salinity, (c):Dissolved oxygen, (d):pH, (e): Turbidity, (f); Chlorophyll a, (g): Total suspended solid.

### *Spatial variations of physico-chemical parameters*

The normality test for temporal variations showed that all parameters did not meet the null hypothesis ( $p < 0.05$ ), thus one-way ANOVA with permutation was conducted to meet the assumption that the data were normally distributed ( $p > 0.05$ ). Figure 5 shows the reading of physico-chemical parameters at eight sampling stations of Kota Setar.

Mean water temperature had the lowest values of  $27.9 \pm 0.0$  °C (KS 2) and highest  $28.5 \pm 0.1$  °C (KS 1) with a significant difference between stations ( $p < 0.05$ ). Water conductivity values had significant difference between stations ( $p < 0.05$ ) ranged from  $43.77 \pm 0.27$  ms/cm (KS 1B) to  $51.74 \pm 0.03$  ms/cm (KS 7). Mean TDS had significant difference among stations ( $p < 0.05$ ) with the lowest values of  $28.3 \pm 0.6$  ppt (KS 1B) and highest values of  $34.0 \pm 0.0$  ppt (KS 2,3,4,5,6,7,8). Mean salinity had a significant difference among stations ( $p < 0.05$ ) with the lowest values of  $28.15 \pm 0.21$  ppt (KS 1B) and the highest values of  $33.92 \pm 0.02$  ppt (KS 7). Mean concentration of DO ranged from  $3.93 \pm 0.05$  mg/L (KS 1B) to  $7.03 \pm 0.07$  mg/L (KS 8) with significant difference among stations ( $p < 0.05$ ). Mean values of pH showed significant difference among stations ( $p < 0.05$ ) with the values ranged  $7.62 \pm 0.03$  (KS 1B) –  $8.34 \pm 0.01$  (KS8). Mean values of turbidity showed significant differences among stations ( $p < 0.05$ ) with the values ranged  $4.58 \pm 0.25$  NTU (KS 8) –  $158.17 \pm 119.28$  NTU (KS 1B). Although mean concentrations of TSS were fluctuated, their values had no significant difference among stations ( $p > 0.05$ ), where the mean concentrations showed the lowest values of  $6.82 \pm 0.13$  mg/L (KS 8) and highest values of  $344.83 \pm 427.12$  mg/L (KS 1B).

Mean concentration of Chl a showed significant difference among stations ( $p < 0.05$ ) with the values ranged from  $3.17 \pm 0.59$  µ/L (KS 5) to  $18.65 \pm 13.83$  µ/L (KS 1B). Mean concentration of ammonia had the lowest values of  $0.01 \pm 0.01$  mg/L (KS 8) and the highest of  $0.96 \pm 0.06$  mg/L (KS 1B) with significant difference between stations ( $p < 0.05$ ). Mean concentration of phosphate ranged from  $0.05 \pm 0.01$  mg/L (KS8) to  $0.18 \pm 0.01$  mg/L (KS 6) with significant difference among stations ( $p < 0.05$ ). The concentration of nitrate had significant difference between stations ( $p < 0.05$ ) ranged from  $0.04 \pm 0.01$  mg/L (KS7) to  $0.13 \pm 0.02$  mg/L (KS 1B). Mean concentration of nitrite ranged from  $0.1 \pm 0$  mg/L (KS 5,6,7,8) to  $0.06 \pm 0.01$  mg/L (KS 1B) with significant difference among stations ( $p < 0.05$ ).

The depth where the samples were taken ranged from  $0.4 \pm 0.1$  m (KS 1B) to  $1.4 \pm 0.9$  m (KS 8) with no significant difference among stations ( $p > 0.05$ ). The depth at all sampling stations varied from  $0.7 \pm 0$  m (KS 1B) to  $2.7 \pm 0.0$  m (KS 8), and the current ranged from  $0.1 \pm 0$  M/S (KS1B,2,3,4,5,6,7) to  $0.2 \pm 0$  M/S (KS 1,8)

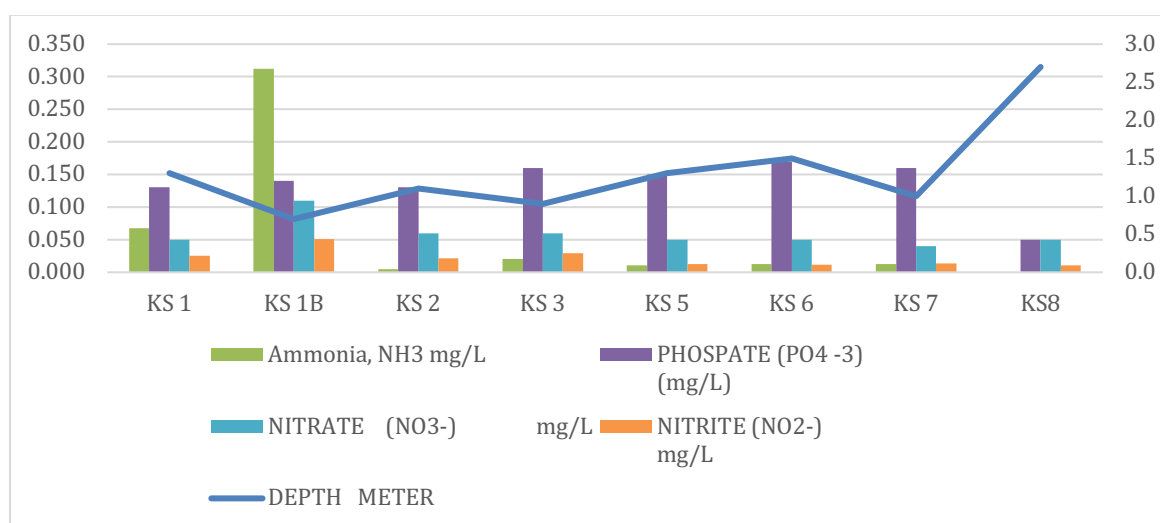
### *Nutrient analysis*

Figure 5 showed that KS1B had the highest ammonia level (0.031 mg/L), though KS1B had the highest level from all of the sampling stations, it is still complied with the MMWQCS range ( $< 0.07$ mg/L) endorsed by Department of Environment (DOE) Malaysia. For phosphate, only station KS8 with level of 0.05 mg/L was the only one that comply with the MMWQCS which is less than 0.075 mg/L while the other stations were range from 0.13 to 0.17 mg/L. Only KS1B had the level of nitrate (0.11mg/L) above 0.06 mg/L while the other stations were lower than KS1B. For Nitrite all the stations had level less than 0.055mg/L and the highest was at 0.051 mg/L at station KS1B. These results support the data of chlorophyll a obtained at KS1B where ammonia, phosphate, nitrate and nitrite at this station were sufficient to boost the high



level of chlorophyll a to be more than 18  $\mu\text{g/L}$ . Phytoplankton greatly affected by light conditions and also mainly influenced by nutrient conditions offshore (Yihe et al, 2019). The study by also mentioned that phosphate essentially determined the biomass of phytoplankton however, it could not be confirmed that if this study accept that findings as the phosphate concentrations were similar for all the stations except for KS8 that the second highest chlorophyll a concentration but had the lowest level of phosphate.

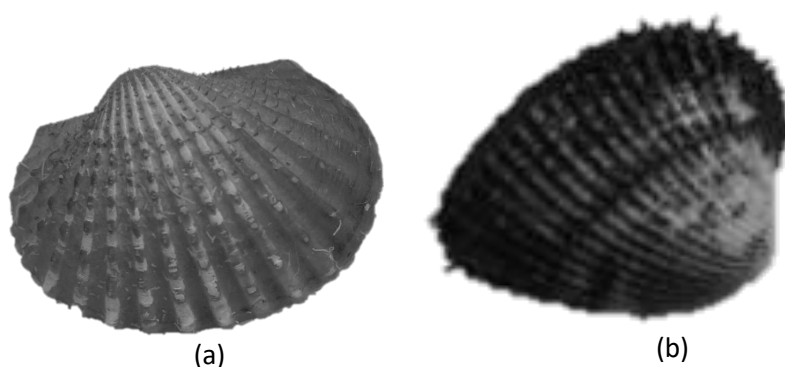
For ammonia, phosphate, nitrate and nitrite, all of them were significantly different between sampling stations group ( $p < 0.05$ ). For ammonia, the analysis showed that within groups the level of ammonia is significant among the sampling stations ( $p < 0.05$ ) the most significant stations were station KS1B, KS3 and KS8. These stations were significantly difference against each other including all the other sampling stations. Station KS1 and KS2 were not significant with each other while station KS5, KS6 and KS7 were also not significant with each other. Phosphate also showed between the sampling stations, the level of phosphate is significant during the study is done ( $p < 0.05$ ). Only KS8 was seen to be very significant from each other. While most of the stations were not significant with each other, except for KS6 and KS7 that were only significant with each other. For nitrate level the significant difference between the sampling stations was  $p < 0.05$  and only KS1B was significant from all the other stations while the other stations were not significant between the other sampling stations. Nitrite concentrations also showed significant difference of  $p < 0.05$ . KS1, KS2 and KS3 were not significant with each other while KS1B is significantly difference from all the other sampling stations. KS2 nitrite level was also not significant with KS5, KS7 and KS8. KS6 showed it is not significant with KS 5, KS7 and KS8.



**Figure 5.** Nutrient analysis from water sampled from the sampling stations in Kuala Kedah, Kedah.

### Distribution and the biomass of naturally found cockles

The sampling activities done at the AOI showed there are two type of cockles species which are dominant in certain areas using swept method area. These cockles (Figure 6) which were *T. Granosa* and *A. antiquata* are naturally found in certain area of the AOI except for stations KS7 and KS8. Both of these stations does not have any naturally available natural cockle. Results showed presence of cockles with size bigger than 30mm and there was very little empty shells found shown that the area where the blood cockles found is very suitable for cockle culture or farming.



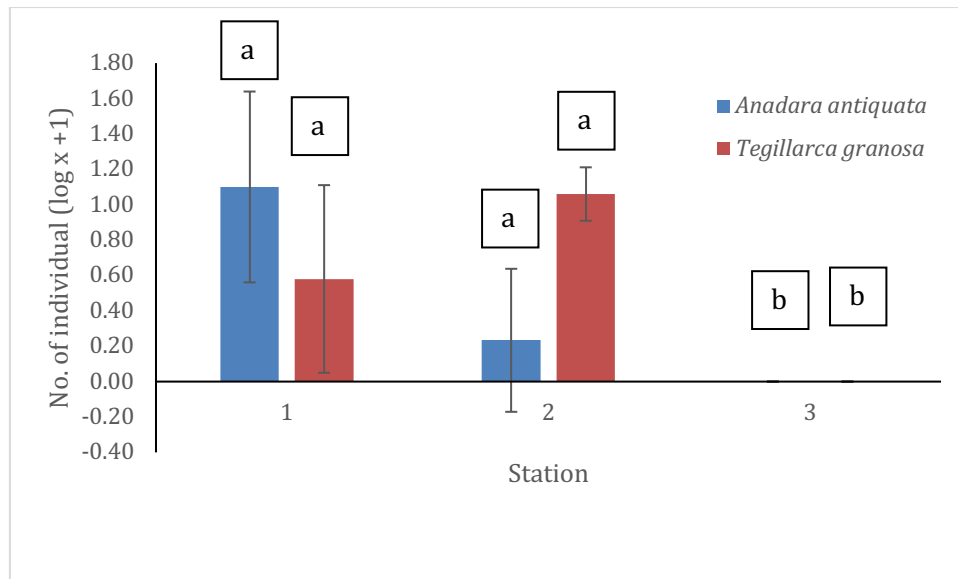
**Figure 6.** The species of cockles that were naturally distributed at Kuala Kedah; (a) *T. granosa* and (b) *A. antiquata*.

### Biomass of cockles

The study suggested that the AOI generally is barely dominant by *A. antiquata* compared to *T. granosa*. This is due to *A. antiquata* were found in sampling stations were 54.39% from the total samples. Overall, *T. granosa* average size was between 33.12 mm to 42.37 mm while *A. antiquata* was ranged from 21.71 mm to 53.91 mm. The wide range of variation *A. antiquata* suggested that the area is indeed a natural occurrences for the cockles (Silva et al, 2016). The patches of naturally found cockle are normal when for cockles natural distribution in one area due to the water current movement that brought the planktonic stage of the cockles (Caddy and Defeo, 2003). There was no significant difference of number of individuals at both Station 1 and Station 2 ( $p>0.05$ ). This show that the number of individuals of two species recorded at Station 1 and Station 2 are relatively similar. However, two-way ANOVA showed a significant interaction between the effects of station and species on number of individuals ( $p<0.05$ ). There were 52 and 14 individuals of *Anadara antiquata* and *Tegillarca granosa* recorded at Station 1, respectively. At Station 2, four individuals of *A. antiquata* and 33 individuals of *T. granosa* were recorded. There was no individual of cockles recorded at Station 3 (Table 3). There was no significant difference of number of individuals at both Station 1 and Station 2 ( $p>0.05$ ) (Figure 7). This shows that the number of individuals of two species recorded at Station 1 and Station 2 are relatively similar. However, two-way ANOVA showed a significant interaction between the effects of station and species on number of individuals ( $p<0.05$ ).

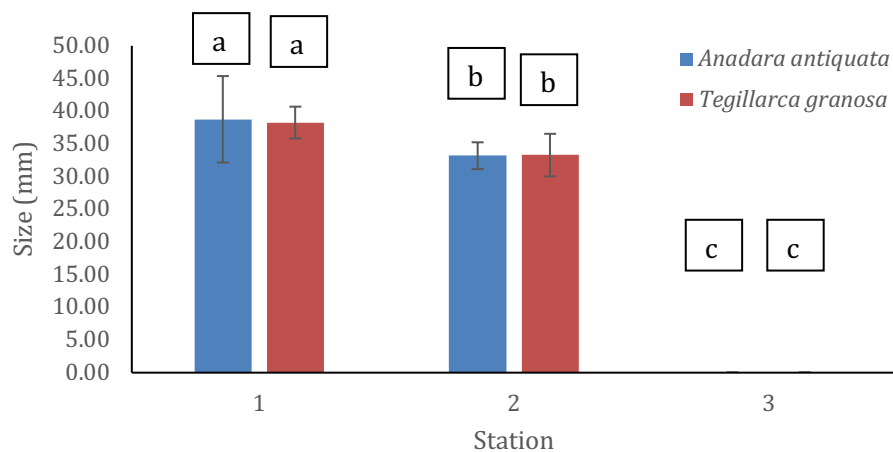
**Table 3.** The number(n) of individual cockles recorded at three stations of Kota Setar

Species	1	2	3
<i>Anadara antiquata</i>	52	4	0
<i>Tegillarca granosa</i>	14	33	0



**Figure 7.** Number of individuals ( $\log x + 1$ ) of *Anadara antiquata* and *Tegillarca granosa* recorded at three stations of Kota Setar.

The *Anadara antiquata* at Station 1 size ranged from  $38.75 \text{ mm} \pm 6.61$ , whereas at Station 2, it ranged from  $33.19 \text{ mm} \pm 2.05$  (Figure 10). For *Tegillarca granosa*, the size ranged from  $38.26 \text{ mm} \pm 2.43$  at Station 1, whereas at Station 2, the size ranged from  $33.29 \text{ mm} \pm 3.25$  (Figure 8). There was no significant difference of cockle sizes between two species at each station ( $p > 0.05$ ). Two-way ANOVA showed no significant interaction between the effects of station and species on the cockles sizes ( $p > 0.05$ ) (Figure 10).



**Figure 8.** Sizes (mean  $\pm$  s.d) of *Anadara antiquata* and *Tegillarca granosa* recorded at three stations of Kota Setar.

## 5. Conclusion

The outcome of this study assessment suggested that the sampling area in the AOI in Kuala Kedah, Kedah waters were presence with naturally distributed cockles. The physical and nutrient analysis suggested that the current status can support the cockles and it is recommended that in the future, areas and its surrounds will be taken into consideration as the natural site for blood cockle's distribution will not be converted into aquaculture area for the sake of resources sustainability. The naturally existence of two cockles *T. granosa* and *A. antiquata* had proved cockles can survive in those areas. However, prior to that, research regarding the stock assessment should take into deeper approach to account the differences in growth parameters of cockle populations. Furthermore, due to its economic importance, we recommend that future management should include a monitoring system in order to collect size composition data of the cockles.

**Patents:** Not applicable

**Author Contribution:** For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, M-F.H. and H.H.; methodology, M-F.H. and H.H.; software , H.H.; validation, M-F.H, H.H., K.K.K.Y.; formal analysis, M.A.H.; investigation, M-F.H. and H.H.; resources, H.H.; data curation, M-F.H., M.A.H. and H.H.; writing—original draft preparation, ; writing—review and editing, M-F.H., M.A.H., M.I and H.H.; visualization, X.X.; supervision, X.X.; project administration, K.K.K.Y; funding acquisition, H.H. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** Not Applicable as no animals were sacrificed in this study.

**Informed Consent Statement:** Not applicable

**Data Availability Statement:** In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study.

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