Influence of Physicochemical Water Parameters on the Total Weight of the Slipper-shaped Oyster *Crassostrea iredalei* in Visayas, Philippines

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Annual assessments of the total weights of the most abundant oyster species in the Philippines, *Crassostrea iredalei,* for two consecutive years were examined across ten different sampling sites in Visayas, Philippines. The ANCOVA model was used to investigate the effects of the different sampling sites and the different physicochemical water parameters as covariates on the log total weight. The ANOVA model was also used to examine site differences in the log total weight without taking into account the effects of the covariates. ANOVA and ANCOVA results were compared to distinguish site differences with and without the covariates in the model. The results from the ANOVA model revealed that there were significant differences in the mean log total weight between sites. In the final ANCOVA model, there were still significant differences in the mean log total weight between sites above and beyond the significant positive covariate effect of temperature. The observed variations in the total weight of oysters is most likely due to the varied underlying internal and external factors that affect oyster culture in their respective ecological habitat. The study also reflects both vulnerability and coping mechanism of the Philippine *C. iredalei* with the variations in temperature which are critical for developing tolerance for positive growth and survival. The findings of this study could promote patterns of selective breeding and culture practices with the additional consideration of environmental factors that would lead to a better understanding of the changing

environmental conditions operating in the different culture sites that would help ensure better culture management and harvest.

Keywords: analysis of variance (ANOVA), analysis of covariance (ANCOVA), Tukey-Kramer method, physicochemical water parameter, slipper-shaped oyster Crassostrea iredalei

1. Introduction

The slipper-shaped oyster, *Crassostrea iredalei* locally known as "tsinelas na talaba" is the most abundant oyster species in the Philippines. It is ranked as one of the important oyster species worldwide third only to *C. gigas* and *C. virginica* in 2004 enlisting the country as the only producing source (Laing, 2009). Oysters (Family Ostreidae), as a whole, are among the most extensively studied shellfish (Liu et al., 2011). They have been an important food source since Neolithic times (Laing, 2009); and the expansion of oyster farming industry over the last 30 years was due to the need for greater self-sufficiency in marine food production (Gallardi, 2014). Although oysters are regarded as a challenging group for taxonomists and phylogenetics for its high levels of morphological variation in terms of shape or phenotypic plasticity (Liu et al., 2011), at least 18 of its living species are consumed by humans including the *C. iredalei* (Carriker and Gaffney, 1996). In 2004, worldwide production of oysters accounts for 4.6 million tons (Laing, 2009) and continue to expand in a substantial fraction of the world's overall intensive aquaculture production (de Alvarenga et al., 2012). In the Philippines, the increase of oyster farming in 2001, 2007, 2010 and 2013 were attributed to the availability of better quality spats and good water condition that resulted in full grown and bigger sizes of oysters harvested in the wild. However, downtrend production after those years were traced in culture areas with phenomenon of red tide, water pollution due to poor sanitation, as well as poor water quality and easily-destroyed farms due to frequent typhoons. This yielded lesser spatfalls, slower spat growth and smaller market sizes resulting to unstable production. Moreover, financial constraints and lack of supply of materials prevented the reconstruction and replacement of bamboo poles among damaged oyster farms (BAS 2000-2016).

Oysters thrive in locations with high spatial and temporal changes in the quantity and quality of food supply, salinity and temperature (Bayne, 2017). Moreover, oysters do not regulate their body temperature as well as their body fluid salinity wherein their physiological activity is closely reliant to the temperature and salt content of the surrounding ambient water (Shumway, 1996). And since oysters feed on a mixture of suspended materials or seston (Griffiths and Griffiths, 1987), the physicochemical factors including temperature, pH, dissolved oxygen, and salinity were found to have considerable effect with the quality and quantity of seston available in the surrounding environment (Lodeiros and Himmelman,

2000; Beukema et al., 2014). These factors which particularly regulate the rates of ingestion, absorption, reproduction and oxygen uptake can either positively or negatively alter the growth of oysters (Thompson and MacDonald, 2006; Lomovasky et al., 2011).

Temperature, for example, triggers spawning and larval development of bivalves (Thorson, 1950). These reproduction cycles are also varied among populations and among species distributed in different geographical areas (Bayne, 2017). Moreover, embryos and larvae are more vulnerable than adults to environmental stress that slight increase in temperature beyond the temperature spawning range will negatively correlate with growth and survival rates (Talmage and Gobler, 2011). Since oysters are also euryhaline (Shumway, 1996), they can survive with pronounced fluctuations in salinity. And salinity, like temperature, can be population and species dependent. This was observed in *Crassostrea* species which can tolerate wider range of salinity from 1 up to 40 psu (Practical Salinity Unit) (Nascimento, 1991; Shumway, 1996; Shatkin et al., 1997; Yoon et al., 2008) as compared with *Ostrea edulis* with salinity range of 30-32 psu only (Hutchinson and Hawkins, 1992). The interactive and triggering effects of temperature with other environmental factors including salinity, oxygen uptake, occurrence of competitors and disease as well as food supply and availability were found to have intensive consequences than its effect alone (Gosling, 2003). Exposed thermal fluctuations in habitats trigger oyster tissue oxygen levels to change rapidly affecting oxygen consumption rates (Dunphy et al., 2006). Excessive or sustained low dissolved oxygen in oysters could lead to reduced growth, reduced feeding, and increased susceptibility to diseases (Patterson et al., 2014). Moreover, stress to thermal fluctuations also impairs the resilience of oysters to reduced pH or acidification (Goncalves et al., 2017). Hence, it is typical that oysters exhibit high levels of plasticity for their growth and physiological activities (Levins, 1968). And with these differences in environmental conditions in different geographical areas, different mechanisms may have evolved to control growth in those areas (Lodeiros and Himmelman, 2000).

In the Philippines, oyster production is most affected by environmental conditions since staking and hanging culture techniques are still being employed relying intensively on spats available in the wild (BAS, 2000-2016). Investigating the influence of physicochemical factors on the growth of oysters as governed by site differences may define significant environmental conditions of which the species thrives that is relevant as an underlying basis of sustainability and conservation (Lowe et al., 2017). Hence, the objective of this study was to statistically examine site differences in the total weight and evaluate the influence of physicochemical water parameters including temperature, salinity, dissolved oxygen and pH on the total weight of the slipper shaped oysters *C. iredalei* collected in Visayas, Philippines. The specific objectives were: (1.) to determine whether or not site differences in the total weight of the slipper shaped oysters

exist; and, (2.) to examine the effects of physicochemical water parameters on the total weight of slipper shaped oysters coupled with the different site effects.

Statistical studies on the impact of environmental conditions in the culture of oysters can be vital in illustrating oyster growth, its vulnerability and coping behavior with the respective environmental conditions in that particular area. Being economically important oysters of the country, this study can be an initial step to build habitat models along with better understanding of the environmental factors governing in the different sites. Most importantly, the present study, being the first documented investigation on the effect of physicochemical water parameters on *C. iredalei* farmed in different locations in the Philippines, may help to further facilitate oyster culture site management and practices especially in the effect of changing environment to improve production and sustainability.

2. Method

2.1. Sampling and sample processing

Oyster samples of the same age (6-8 months) were collected from ten different culture sites in Visayas, Philippines (Table 1). It includes four sampling sites from Aklan, three from Capiz, two from Negros Occidental and one from Samar. Most of the samples were taken from Panay particularly in Aklan and Capiz for these sites contributed larger scale production of oysters compared to other areas in the region (BAS, 2000-2016). Annual sampling was done in ten culture sites for two consecutive years: 2015 and 2016.

Individual oysters were removed from bamboo stakes and spat collectors with the help of the oyster farmers themselves. Oysters cultured in bamboo stakes practice the staking method, while those being employed using spat collectors like old vehicle tires and empty oyster shells which were hanged in lines of bamboo stakes utilize the hanging technique. Oysters cultured in Samar were attached with hanged coconut husks employed in bamboo long lines and rafts. The collected oysters were manually cleaned removing any encrusting materials and fouling organisms. The cleaned oysters were maintained in cool temperature in styro box and/or coolers with ice packs to trigger the oysters to tightly close its shells. In this way, the oysters were maintained alive. On-site measurements of water physicochemical parameters including temperature (°C), salinity (ppt) and dissolved oxygen (mg/L) were determined using Hach Sension6® multiparameter while pH was measured using Merk®pH indicator strips in each respective sampling area. These on-site measurements were done by dipping the probe of the multiparameter with the culture site water following the equipment's manual. Three readings of each temperature (°C), salinity (ppt) and dissolved oxygen (mg/L) were measured and recorded for each respective site. Same procedures were conducted in measuring the pH. Sampling period in each sampling site of oyster collection and on-site measurements of water physicochemical parameters

were scheduled from eight to eleven o'clock in the morning. The forty-five to sixty oysters collected from each site were brought to the laboratory to determine the total weight. Individual oyster's total weight from each site was measured to the nearest 0.01g with the use of AND® digital top loading balance.

Table 1. Sampling Sites and the Different Culture Methods Used

Sampling Site	Geographic Positioning System (GPS) Location	Culture Method
Batan, Aklan	11^0 35' 41.30" N 122^0 28' 30.3" E	Hanging
Lawaan, New Washington, Aklan	11^0 38' 01.3" N 122^0 25' 34.9" E	Hanging
Mabilo, New Washington, Aklan	11^0 39' 05.2" N 122^0 25' 32.6" E	Hanging
Pinamuk-an, New Washington, Aklan	11^0 38' 08.9" N 122^0 26' 02.9" E	Hanging/Staking
Ivisan, Capiz	11^0 32' 10.5" N 122^0 38' 44.0" E	Hanging/Staking
Panay, Capiz	11^0 35' 20.2" N 122^0 49' 36.2" E	Hanging
Sapian, Capiz	11^0 31' 00.5" N 122^0 35' 12.5" E	Staking
Himamaylan, Negros Occ	10^0 05' 59.1" N 122^0 52' 02.9" E	Hanging
Hinigaran, Negros Occ	100 16' 36.5" N 1220 51' 15.5" E	Hanging
Tarangnan, Samar	11^0 54' 38.6" N 124^0 48' 08.2" E	Longline/Raft

2.2. Statistical analysis

The response variable in this study was the total weight. The primary factor of interest was the geographical location of the ten different sampling sites. The analysis of covariance (ANCOVA) model using site as primary factor of interest was the statistical model used to analyze differences in total weight. Four water physicochemical parameters measured at each sampling site, namely: temperature, pH, dissolved oxygen, and salinity were included as covariates in the subsequent statistical analyses. Variations in each of these water quality parameters were known to affect the total weight (Bayne et al., 1976; Raffaelli and Hawkins, 1996; Lodeiros and Himmelman, 2000; Gosling, 2003; Beukema et al., 2014; Bayne, 2017).

The following ANCOVA model was used to explain the differences in total weight (Neter et al., 1996; Montgomery, 2001):

$$\log(y_{ij}) = \mu + \tau_i + \beta_1 Temp_{ij} + \beta_2 pH_{ij} + \beta_3 DO_{ij} + \beta_4 Sal_{ij} + \varepsilon_{ij}$$
$$\text{for } i = 1, 2, \ldots, 10 \qquad (1)$$
$$j = 1, 2, \ldots,$$

where y_{ij} represents the total weight of the jth observation at the ith sampling site, the τ_i parameters quantify the 10 distinct site effects, the four β parameters represent the four water quality covariate effects (water temperature, pH, dissolved oxygen, and salinity), and the ε_{ij} error terms are assumed to be normally, independently, and identically distributed with zero mean and a constant variance. The total weight was log-transformed to reduce the skewness in the residual error distribution and induce approximate normality.

The single-factor analysis of variance model (ANOVA) was also used to describe the variations in log total weight where sampling site was the only primary factor of interest and no covariates were included in the model in order to compare site differences with and without the presence of covariates in the model. The ANOVA model is given by (Neter et al., 1996; Montgomery, 2001):

$$log(y_{ij}) = \mu + \tau_i + \varepsilon_{ij}$$
$$\text{for } i = 1, 2, \ldots, 10 \qquad (2)$$
$$j = 1, 2, \ldots,$$

where y_{ij} represents the total weight of the jth observation at the ith sampling site, the τ_i parameters quantify the 10 distinct site effects, and the ε_{ij} error terms are assumed to be normally, independently, and identically distributed with zero mean and a constant variance.

The model building process as well as the analysis for this study was performed using the GLM procedure in the SAS University Edition software package. The Tukey-Kramer method that controls the maximum experimentwise error rate under any complete or partial null hypothesis was used for all pairwise means comparisons. The Tukey-Kramer method is more powerful than the Bonferroni, Sidak, or Scheffe methods for pairwise means comparisons (The GLM Procedure, SAS/STAT User's Guide, Version 8).

3. Results

Data were encoded and converted into a permanent dataset that can be accessed by a free statistical software. Frequency tables were generated and summary measures were produced to check for outlier and influential observations. Spurious outliers were verified and corrected before exploratory techniques were applied on the data.

The results of the current data (Table 2) indicated the total weight varied from 8.26 grams to 296.38 grams based on a total sample of 445 observations. The overall mean total weight was 85.04 grams. The mean total weight across the different sites are also shown in the table. The highest mean total weight in the sample came from Batan, Aklan (136.54 grams) and the lowest mean total weight came from Himamaylan, Negros Occidental (24.66 grams).

Table 2. Summary Statistics for Total Weight and Mean Total Weight by Site (n=445)

Site/Measure	Mean Total Weight (g)	No. of Samples
Site		
Batan, Aklan	136.54	40
Lawaan, Aklan	82.99	40
Mabilo, Aklan	108.97	40
Pinamuk-an, Aklan	90.66	40
Ivisan, Capiz	57.41	55
Panay, Capiz	98.71	45
Sapian, Capiz	113.67	45
Himamaylan, Negros Occ	24.66	40
Hinigaran, Negros Occ	37.25	45
Tarangnan, Samar	103.65	55
Overall		
Min	8.26	
Max	296.38	
Mean	85.04	
Std Dev	46.50	

The summary statistics for the different water parameters are shown in Table 3. The mean values of these water parameters for the different sites are also shown in the table.

The total weight was log-transformed to reduce the influence of large body mass index readings. Exploratory techniques such as the use of scatterplots, boxplots, stem-and-leaf display, and histogram were used as preliminary steps in exploring the data. The basic univariate summary statistics for the log transformed total weight are shown in Table 4. The analysis of the total weight only used 442 observations since three (3) outlier observations were removed due to its influence on the observed distribution of the total weight.

The histogram of the log-transformed total weight is shown in Fig 1. The graph shows near symmetry and approximate normal distribution.

Table 3. Summary Statistics for Water Parameters and Mean Water Parameter by Site (n=445)

Site/Measure	Mean pH	Mean Temp °C	Mean DO mg/L	Mean Salinity Ppt
Site				
Batan, Aklan	7.05	31.41	10.42	29.00
Lawaan, Aklan	7.00	31.60	11.74	28.50
Mabilo, Aklan	7.70	31.75	9.69	30.00
Pinamuk-an, Aklan	7.05	31.10	11.40	31.00
Ivisan, Capiz	7.41	30.31	5.24	28.09
Panay, Capiz	7.13	29.42	7.44	33.56
Sapian, Capiz	7.28	30.70	5.44	25.36
Himamaylan, Negros Occ	7.15	29.00	10.20	24.50
Hinigaran, Negros Occ	6.59	29.09	9.97	10.11
Tarangnan, Samar	7.09	31.46	56.80	30.00
Overall				
Min	6.5	28.2	5.22	9.0
Max	31.0	31.9	56.84	34.0
Mean	7.15	30.58	14.78	27.01
Std Dev	1.16	1.08	16.00	6.22

Table 4. Summary Statistics for Log-transformed Total Weight (n=442)

Characteristic	Log Total Weight (g)
Mean	1.85
Minimum	0.92
Maximum	2.47
Std Dev	0.29

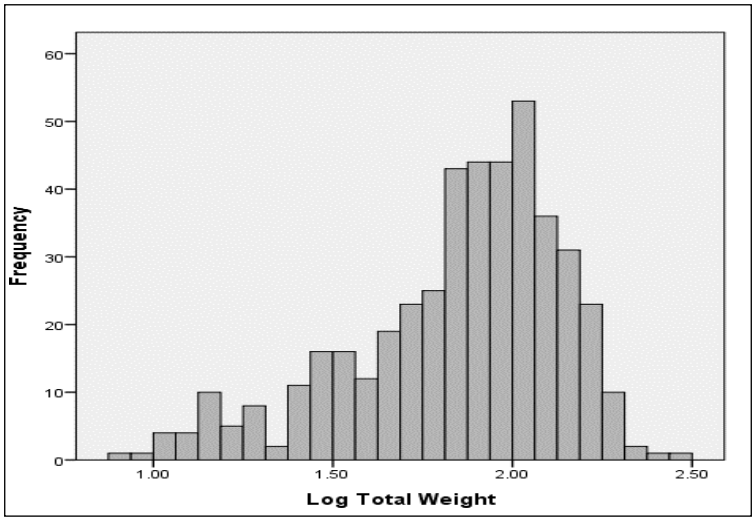


Figure 1. Histogram of the Log-transformed Total Weight

The summary statistics for the ANCOVA model together with the results of Shapiro-Wilk and Levene's test are shown in Table 5. The results revealed that the proposed ANCOVA model for the log total weight was highly significant (Model F statistic = 62.77, $p < 0.0001$). The results indicated that the model was appropriate for the data. The assumptions about the model were also satisfied. Investigation of the residuals revealed that the normality assumption was satisfied (Shapiro-Wilk statistic = 0.994, $p = 0.0574$). The homoscedasticity assumption was also satisfied (Levene statistic = 1.550, $p = 0.214$). The residuals for year 1 and year 2 were also uncorrelated for all the sampling sites. The R-squared values suggested that the model can substantially explain about 65.60% of the total variation in log total weight.

Table 5. ANCOVA Model Summary Statistics, Shapiro-Wilk and Levene's Test

Measure/Statistic	Value
Model F statistic	62.77
Model p-value	<0.0001
S-W statistic	0.994
S-W p-value	0.0574
Levene statistic	1.550
Levene p-value	0.214
R-squared	0.656

The result of the test of fixed effects in the ANCOVA model is shown in Table 6. The site effect was highly significant ($F = 34.36$, $p < 0.0001$) after taking into account the effects of the covariates. The only covariate that had significant effect was water temperature ($F = 25.67$, $p < 0.0001$).

Table 6. Test of Fixed Effects for the ANCOVA Model

Factor/Covariate	F-statistic	p-value
Site	34.36	<0.0001
Temperature	25.67	<0.0001
pH	0.92	0.337
Dissolved Oxygen	0.01	0.932
Salinity	0.69	0.406

The ANCOVA model was then re-estimated with site as the primary factor of interest and the only covariate used was temperature. The results of the re-estimated ANCOVA model are shown in Table 7. The F-statistic of the new ANCOVA model was still highly significant (F value = 81.693, $p < 0.0001$) and indicated that the model was appropriate for the data. The major assumptions of normality and homoscedasticity of the residuals were still satisfied (Shapiro-Wilk statistic = 0.993, $p = 0.05$; Levene's statistic = 1.545, $p = 0.215$). The R-squared value practically remained the same (65.50%). The values of the F-statistics and the corresponding p-values for site and temperature are shown in Table 8. Sampling site was highly significant in the re-estimated ANCOVA model (F statistic = 41.931, $p <0.0001$). Temperature was still a significant covariate in the model ($F = 25.874$, $p < 0.0001$).

Table 7. Summary Statistics for the Final ANCOVA Model, Shapiro-Wilk and Levene's Test

Measure/Statistic	Value
Model F statistic	81.693
Model p-value	<0.0001
S-W statistic	0.993
S-W p-value	0.05
Levene statistic	1.545
Levene p-value	0.215
R-squared	0.655

Table 8. Test of Fixed Effects for the Final ANCOVA Model

Factor/Covariate	F-statistic	p-value
Site	41.931	<0.0001
Temperature	25.874	<0.0001

The solution for fixed effect in the final ANCOVA model showed that water temperature had a highly significant positive effect on the mean log total weight (parameter estimate for temperature = 0.102, $p < 0.0001$), above and beyond the effect of site.

The Tukey-Kramer method for all pairwise means comparisons was used to determine significant differences in the mean log total weight between sites. The overall experiment wise error rate was set at 0.05. Pairwise means comparisons were also performed in the ANOVA model for log total weight without covariates and site as the only primary factor of interest in order to compare site differences with (ANCOVA) and without the presence of covariates in the model (ANOVA, Equation 2). For the ANOVA model, the site effect was also significant (results not shown).

Table 9 presents the least-square means from the ANOVA and ANCOVA models of the log total weight. The differences observed in the table were the manifestations of the effect of the covariate (temperature) in the adjustment of the least-square means.

Table 9. Least Square Means from the ANOVA and ANCOVA Models

Site	L-S Means from the ANOVA Model	L-S Means from the ANCOVA Model
1. Batan, Aklan	2.10469	2.02002
2. Lawaan, Aklan	1.83899	1.73384
3. Mabilo, Aklan	2.01832	1.89821
4. Pinamuk-an, Aklan	1.93745	1.88350
5. Ivisan, Capiz	1.73664	1.76402
6. Panay, Capiz	1.98398	2.10181
7. Sapian, Capiz	2.02916	2.01617
8. Himamaylan, Negros Occ.	1.33111	1.49218
9. Hinigaran, Negros Occ.	1.53409	1.68606
10. Tarangnan, Samar	1.99861	1.90742

Table 10 provides the p-values for all pairwise means comparisons between sites. The results in (a) were the different p-values of pairwise means comparisons using the Tukey-Kramer method from the ANOVA model while the results in (b) were from the ANCOVA model. Pairwise means comparisons with p-values greater than 0.05 were declared not significant.

Figure 2 summarizes the results of all pairwise means comparisons between sites from the ANOVA (a) and ANCOVA models (b). In both (a) and (b), the sites were arranged in decreasing order of the mean log total weight where the means in (a) were the observed and unadjusted site means and the means in (b) were the site means adjusted for the covariate (temperature). Sites that were not significantly

different belonged to the same group and sites that did not belong to the same group were significantly different.

Table 10. P-values of Pairwise Means Comparisons Between Sites from the ANOVA and ANCOVA Models

(a)										
i/j	1	2	3	4	5	6	7	8	9	10
1		<.0001	0.4797	0.0012	<.0001	0.0563	0.6246	<.0001	<.0001	0.1129
2	<.0001		0.0004	0.2775	0.1531	0.0071	<.0001	<.0001	<.0001	0.0007
3	0.4797	0.0004		0.5776	<.0001	0.9968	1.0000	<.0001	<.0001	0.9999
4	0.0012	0.2775	0.5776		<.0001	0.9706	0.3373	<.0001	<.0001	0.8154
5	<.0001	0.1531	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001
6	0.0563	0.0071	0.9968	0.9706	<.0001		0.9703	<.0001	<.0001	1.0000
7	0.6246	<.0001	1.0000	0.3373	<.0001	0.9703		<.0001	<.0001	0.9975
8	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001
9	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
10	0.1129	0.0007	0.9999	0.8154	<.0001	1.0000	0.9975	<.0001	<.0001	

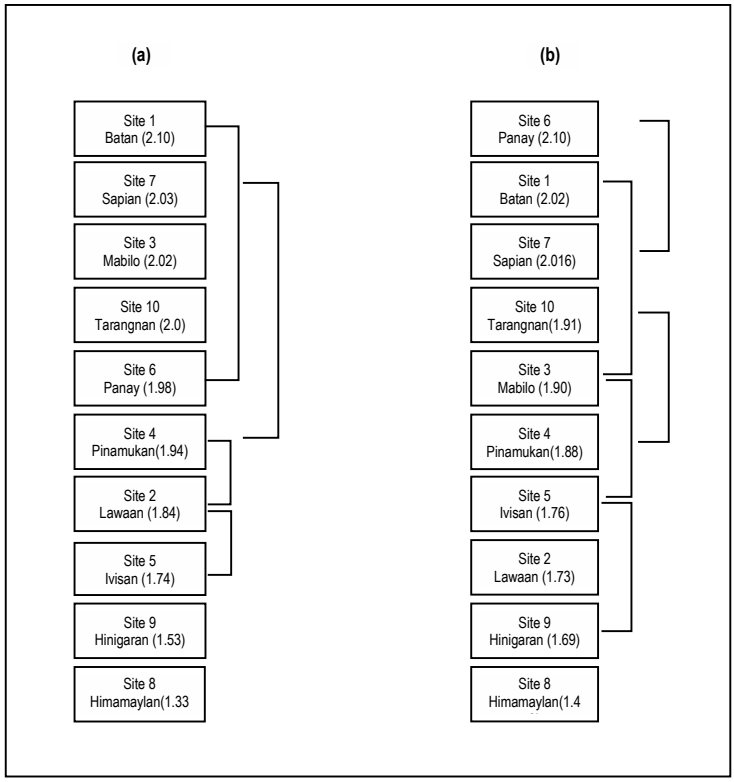
(b)										
i/j	1	2	3	4	5	6	7	8	9	10
1		<.0001	0.0638	0.0179	<.0001	0.8928	1.0000	<.0001	<.0001	0.0551
2	<.0001		0.0012	0.0072	0.9996	<.0001	<.0001	0.0085	0.9990	<.0001
3	0.0638	0.0012		1.0000	0.1120	0.0261	0.1631	<.0001	0.0414	1.0000
4	0.0179	0.0072	1.0000		0.0767	0.0008	0.0202	<.0001	0.0138	0.9997
5	<.0001	0.9996	0.1120	0.0767		<.0001	<.0001	<.0001	0.7162	0.0159
6	0.8928	<.0001	0.0261	0.0008	<.0001		0.6512	<.0001	<.0001	0.0121
7	1.0000	<.0001	0.1631	0.0202	<.0001	0.6512		<.0001	<.0001	0.1164
8	<.0001	0.0085	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001
9	<.0001	0.9990	0.0414	0.0138	0.7162	<.0001	<.0001	<.0001		0.0076
10	0.0551	<.0001	1.0000	0.9997	0.0159	0.0121	0.1164	<.0001	0.0076	

(a) P-values of pairwise means comparisons between sites from the ANOVA and (b) ANCOVA models for the log total weight

From the results of the ANOVA model, there were no significant differences in the unadjusted mean log total weight between sites 1 (Batan), 7 (Sapian), 3 (Mabilo), 10 (Tarangnan), and 6 (Panay); there were no significant differences between sites 7 (Sapian), 3 (Mabilo), 10 (Tarangnan), 6 (Panay), and 4 (Pinamukan); there were no significant differences between sites 4 (Pinamukan) and 2 (Lawaan); and, there were no significant differences between sites 2 (Lawaan) and 5 (Ivisan). All the other pairwise means comparisons were significant.

From the results of the ANCOVA model, there were no significant differences in the adjusted mean log total weight between sites 6 (Panay), 1 (Batan), and 7 (Sapian); there were no significant differences between sites 1 (Batan), 7 (Sapian), 10 (Tarangnan), and 3 (Mabilo); there were no significant differences between

sites 10 (Tarangnan), 3 (Mabilo), and 4 (Pinamukan); there were no significant differences between sites 3 (Mabilo), 4 (Pinamukan), and 5 (Ivisan); and, there were no significant differences between sites 5 (Ivisan), 2 (Lawaan), and 9 (Hinigaran). All the other pairwise means comparisons were significant.



(a) Summary of pairwise means comparisons between sites from the ANOVA and (b) ANCOVA model for the log total weight

Fig 2. Summary of Pairwise Means Comparisons Between Sites from the ANOVA and ANCOVA Models

4. Discussion

This study examined the annual assessments of the total weights of the most abundant oyster species in the Philippines, the slipper-shaped oyster *Crassostrea iredalei*, for two consecutive years across ten different sampling sites in Visayas, Philippines. Site differences in the mean log total weight of oysters over time were investigated. In addition, site differences in the mean log total weight were compared with and without the presence of covariates in the model.

The ANCOVA model was used to investigate the effects of the different sampling sites and the different easily measurable physicochemical water parameters as covariates on the log total weight of oysters over time. The ANOVA model was also used to examine site differences in the log total weight without taking into account the effects of the covariates. ANOVA and ANCOVA results were compared to distinguish site differences with and without the covariates in the model. The Tukey-Kramer method was used to determine significant differences in the mean log total weight between all pairs of sampling sites.

The results indicated that both the ANOVA and the ANCOVA models were appropriate for the data. The models generated moderate R-squared values of 0.63 and 0.66, respectively. All the model assumptions were met and no major model violations were observed.

The results from the ANOVA model revealed that there were significant differences in the mean log total weight between sites. Such distribution pattern in terms of total weight may involve larval-substrate settlement that initiates competition of resources affecting the growth of individual oysters (Weins, 1989; Bayne, 2017). This is most likely due to the varied underlying internal and external factors that could affect oyster culture (Lodeiros and Himmelman, 2000). However, the results also showed no significant differences and clustering of sites coming from Aklan, Capiz and Samar located at the upper portion of Fig. (2a), as well as those two sites from Negros which were at the bottom portion. Aklan and Capiz site locations were almost at relative geographical area but revealed comparative total body weights of oysters with Tarangan, Samar than those cultured from Negros sites. This was observed regardless of the culture technique being employed. According to BAS (2000-2016), Aklan and Capiz have great contributions in the bulk of oyster production in the Visayas region while Hinigaran and Himamaylan culture sites revealed unstable production. Moreover, most Aklan and Capiz sites were almost at neighboring junctions in the coastal area and that larval stages of these oysters are waterbone allowing its dispersal through water currents. This can lead the oysters to colonize new locations resulting to genetic mixing (Yonge and Thompson, 1976). The finding of no significant differences between Aklan, Capiz and Tarangnan, Samar sites implies that Tarangnan, Samar, with the utilization of their culture method, has a high economic potential for a major source of heavy oysters.

When the physicochemical factors were added to the model as covariates on top of the effect of the different sampling sites, there were still differences in the mean log total weight between sites and it was only temperature that had significant positive covariate effect. This follows the majority of influential impacts of temperature compared with other environmental factors (Bayne et al., 1976). This is evident because oysters rely in the temperature of their surroundings, when the environment temperature changes, their body temperature is also affected (Shumway, 1996; Bakken and Angellitta, 2014). Such site

differences with the effect of temperature on the log total weight of oysters reflect the gradients and discontinuities of temperature (Hochachka and Somero, 2002) in each of the culture sites. Remarkably in each site, temperature acts both directly on the organism's performance such as physiological responses and survival as well as indirectly through influence on food supply (Bayne et al., 1976; Bayne, 2017), hence creating variations in the total weight depending on the condition of the respective ecological habitat. And the responses from those important physiological activities which include feeding, metabolism and reproduction were integrated to growth itself (Rafffaelli and Hawkins, 1996; Lodeiros and Himmelman, 2000; Gosling, 2003, Beukema et al., 2014; Bayne, 2017).

Temperature influences in the bivalve feeding mechanism are associated with the combination of physiological (gill ciliary action) and mechanical (water dynamics) effects (Jørgenson et al., 1990 ; Podolsky, 1994). In the study of Galtsoff (1927), the increasing gill ciliary activity of *Ostrea virginica* (presently *C. virginica*) was affected by an increasing temperature ranging from 10°C up to 25°C but decreases suddenly after the latter temperature limit. The same positive trend of clearance rate also occurred in the marine pearl oyster *Pinctada fucata* along with increasing temperature of 18°C to 28°C but also declined with further increase of temperature to 31°C (Mondal, 2006). Relating to mechanical effects, water viscosity is inversely related to temperature such that high viscosity hinders water flow within the shellfish's ciliary action at low temperatures which limits their maxium clearance rate (Jorgenson et al., 1990 ; Podolsky, 1994; Cranford et al., 2011). Therefore, as active suspension or filter feeders (Bayne, 2017), oysters' capacity to initiate gill activity depicted in the clearance rates was revealed to have a positive effect in its feeding mechanism or food acquisition (Cranford et al., 2011) following the increase in temperature at particular range in the above mentioned studies.

Moreover, metabolic activities are also temperature-dependent which greatly affects organism's oxygen consumption rates that regulate energetic requirements of individuals (Bayne, 1973, Dunphy et al., 2006; Kheder et al., 2010). This was illustrated in the increase rates of oxygen consumption of the subtidal flat oyster *Ostrea chilensis* as temperature increase from 10°C up to 20°C (Dunphy et al., 2006) as well as in P. fucata with increasing temperature of 18°C to 31°C (Mondal, 2006). Temperature accelerates these metabolic functions that also triggers increased ingestion or feeding (Rico-Villa et al., 2009; Kheder et al., 2010). And faster feeding is coupled with high rates of growth (Tamayo et al., 2011).

Spawning and larval development are among the reproductive cycle patterns that were influenced greatly by temperature (Eversole, 2001; Barber and Blake, 2006). Spawning optimum temperature is both species-specific and influenced by habitat location (Bayne, 2017). This is depicted by *O. edulis* with different temperature spawning range in South Africa (12-14°C) as compared to England (20-21°C). These patterns were also narrower than the Pacific oyster *C. gigas* with

a general optimum temperature spawning range of 20-25°C (Bayne, 2017). On the other hand, larval growth is positive as temperature increases but larval survival is high at low temperatures (Douroudi et al., 1999; Kheder et al., 2010). This was observed in the larval rearing of Malaysian *C. iredalei* which had high survival rate at temperatures 20°C to 27°C but reduced at 34°C. Such reduction of survival rate was coupled with observed increase in growth rate as temperature increases (Teh Chiew Peng et al., 2016). This was explained by Gosling (2003) on the variations of energy requirements in each life cycle stage and that the temperature required for spawning is normally higher than the minimum temperature required for growth. Temperature for positive growth of the most investigated oyster species such as the American eastern oyster *Crassostrea virginca* generally ranged from 5-34°C (Shumway, 1996) while *C. gigas* varies among the area being cultured. Taiwan (5-25°C), Korea (6.5-26.5°C), New Zealand (12-22°C) and Argentina (7.5-21.5) have nearer temperature range for positive growth compared with USA (4.5-27°C) and Atlantic France (3.5-24.5°C) (Bayne, 2017).

The effect of temperature in the log total weight of slipper shaped oysters from the different sites in the study also follows the positive growth trend for increasing temperature. Such positive growth trend is governed by temperature limit which is species-specific (Gosling, 2003). In this study the positive covariate effect of temperature in the log total weights of oysters can be attributed to the oysters' wide tolerance range to thermal fluctuations wherein the measured temperatures in the study are still at the range for the slipper-shaped oysters' positive growth.

Growth is considered significant in terms of production and sustainability since it is the major economic target (Bayne, 2017). Aside from its internal or endogenous factors like genetic differences (Tamayo et al., 2011), growth is integrated by the responses of the organism's physiological activities to its habitat as governed by the different environmental conditions (Bayne, 2017). The study follows other investigations suggesting that temperature is a vital factor that regulates food availability and acquisition, as well as spawning and larval development that mediates growth and survival particularly of oysters. Although, predicting growth and survival rates of economically important bivalve species remains difficult (Lowe et al., 2017), the study reflects both vulnerability and coping mechanism of the Philippine *C. iredalei* with the variations in temperature which are critical in the development of tolerance for positive growth and survival. The findings of this study illustrate the performance in terms of total weight of oysters cultured in different geographical areas in Visayas region which could promote patterns of selective breeding and culture practices with the additional consideration of environmental factors. Furthermore, such breeding innovations will lead to a better understanding of the changing environmental conditions operating in those culture sites that would help ensure better culture management and harvest.

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