INVESTIGATION OF THE INFLUENCE OF MODIFIED SEDIMENT ON pH USING RAPID

pH ASSESSMENT METHODS

By

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A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In

ENVIRONMENT AND MANAGEMENT

We accept this thesis as conforming
to the required standard

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Abstract

Ocean acidification is changing the chemistry of the ocean and has the potential to result in large-scale ecosystem changes. Observations of dissolved shells and low survivorship rates of juvenile bivalves are a concern for the aquaculture industry. This research conducted in Baynes Sound, assessed the variability of sediment pH measurements between farmed and non-farmed beaches; evaluated the use of an Orion low maintenance pH triode and a Honeywell Durafet to rapidly assess sediment pH; examined existing shell content between farmed and non-farmed beaches; determined if there is a relationship between shell content and sediment pH; and determined if there is a difference in sediment pH and/or shell content in spatially separate sites. Sediment pH was highly variable, both between and within sites and no buffering effect was observed from existing shell content in the sediment. Any influences of aquaculture practices and shell content were prevailed by regional differences.

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Acknowledgements

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- My Thesis Supervisor, Dr. Sarah Dudas for her knowledge, support and guidance throughout my research and writing;
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- My workplace and colleagues for supporting me on this adventure.

Introduction

Atmospheric carbon dioxide (CO_2) emissions are a global concern and recognized as a driver of climate change. Over the past 200 years, the ocean has absorbed one-third of the total atmospheric CO_2 emissions, acting as a CO_2 oceanic sink (Sabine et al., 2004). The CO_2 emissions dissolve in seawater and form carbonic acid (H_2CO_3). The H_2CO_3 then dissociates rapidly and releases a hydrogen ion (H^+); resulting in bicarbonate (HCO_3^-) and carbonate ions (CO_3^{2}) (Doney, Fabry, Feely, & Kleypas, 2009). The addition of H^+ ions results in an increase in acidity of the marine environment. The changes to the water chemistry from this reaction are collectively referred to as ocean acidification.

By year 2100 it is estimated pH will further reduce by 0.3-0.5 units from a current ocean surface water pH of 8.1 (Intergovernmental Panel on Climate Change [IPCC], 2013; Caldeira & Wickett, 2005). This decrease in pH corresponds to a change in atmospheric partial pressure of CO_2 (PCO_2) from approximately 392 ppm to 650-970 ppm by year 2100 (IPCC, 2013; Caldeira & Wickett, 2005). Ocean acidification is an emerging field and a global problem with documented impacts to marine biota and oceanographic processes. The effects of ocean acidification have resulted in environmental and economic concerns globally as well as on a local level in British Columbia (BC).

The effects of ocean acidification are a primary concern of the aquaculture industry. BC has an established shellfish aquaculture industry that produced 8,800 tonnes of oysters and clams in 2012, with a wholesale value of $26.4 million (Ministry of Agriculture, 2013). Organisms that require calcium carbonate ($CaCO_3$) for shell or skeleton growth are at high risk due to the H^+ ions from the chemical reaction reducing the saturation state of calcite and aragonite, two forms of $CaCO_3$ (Doney et al., 2009). Studies have reported negative effects on the growth,

development, shell integrity and physiology of calcifying organisms due to elevated oceanic CO_2 levels (Timmins-Schiffman, O'Donnell, Friedman, & Roberts, 2013; Barton, Hales, Waldbusser, Langdon, & Feely, 2012; Doney et al,. 2009). BC's local shellfish aquaculture industry will need to be prepared for increased ocean acidification pressures.

Bivalves exposed to ocean acidity conditions predicted for later this century have been observed with malformed and/or eroded shells as well as a lower survival rate of juveniles (Talmage & Gobler, 2010). Ocean acidification has also been correlated to decreased larval survival of *Crassostrea gigas* and is associated with the multiple years of low recruitment in the Pacific Northwest (Barton et al., 2012). Starting in 2007, reports of mortalities of *C. gigas* larvae have been associated with ocean acidification (National Oceanic and Atmospheric Administration [NOAA], 2013); resulting in economic stress to the shellfish industry (Barton et al., 2012). This has led to interest in determining if something can be done to mitigate this type of catastrophic recruitment failure in the future.

A recent study on the East coast in Maine, United States, examined the influence of buffered sediments using crushed *Mya arenaria* shell and its influence on the survivorship of *M. arenaria* (Green, Waldbusser, Reilly, Emerson, & O'Donnell, 2009). This study found that the addition of crushed shell increased the alkalinity, pH and aragonite-saturation state of sediment relative to a control site (Green et al., 2009). Modification of marine sediment through the addition of shell and/or gravel at aquaculture sites is a common practice in BC. This practice has been found to increase the stability of the sediment and interstitial spaces as well as providing protection to juvenile clams and increases their rate of survival (Thompson, 1995). If similar effects are observed in BC as on the East Coast, this addition of shell may also serve as a buffer against rising ocean acidity. Currently, there is no information regarding the existing shell

content on farmed and non-farmed beaches. This study was conducted to address these

information gaps and examine if existing shell content on shellfish farms could provide a buffer

against ocean acidification.

A fundamental component of ocean acidification research is marine pH (Martz, Connery,

& Johnson, 2010). Currently there are challenges to accurately measure oceanic pH as well as an

absence of pH data both temporally and spatially (Rérolle et al., 2012; Martz et al., 2010). This

information gap is also present for sediment pH.   A primary challenge is that marine pH is

highly variable both spatially and temporally. Fluctuations can occur daily, seasonally and is

influenced by oceanographic and biological processes (Andersson & Mackenzie, 2012; Martz et

al., 2010). To accurately forecast the impact of ocean acidification on marine organisms it is

important to assess the variability of carbonate chemistry (Andersson & Mackenzie, 2012).

When conducting measurements on marine carbonate chemistry at least two of the following

variables should be measured: total dissolved inorganic carbon, partial pressure of CO_2, total

alkalinity and pH, ideally in addition to in situ temperature, macronutrients and salinity (Rérolle

et al., 2012; Martz et al., 2010). The measurement of two of these variables allows for the

calculation of the remaining two. Assessment of multiple carbonate-chemistry provides more

robust measures and makes it possible to check if the variables are consistent with one another

(Ribas-Ribas et al., 2014). Rérolle et al. (2012) recommend measuring more than two marine

carbonate-chemistry variables due to inconsistent results when using different pairs of the marine

carbonate-chemistry variables.

This study focused on measuring sediment pH and determining the variability of

environmental pH. Measurements were conducted at six sites; surveying an area of

approximately 41,000 m^2. Due to the time required to measure pH over this large area, pH was

the sole focus of this study. A limitation of this study is pH was the only carbonate-chemistry

variable measured; therefore, pH values will be used for comparative analysis rather than

determining an accurate portrayal of the carbonate-chemistry. This research is a preliminary

investigation, providing a first look at how variable pH is in Baynes Sound and the different

instruments that can measure it. This study will facilitate future studies with additional

carbonate-chemistry variables and allow the additional variables to be targeted appropriately.

There are a variety of instruments to assess pH; however, they vary in terms of accuracy,

precision, portability and cost. The two instruments that may be suitable for the shellfish

industry application are glass electrodes and/or ion-sensitive field-effect transistors (ISFETs)

such as the Honeywell Durafet. Glass electrodes are relatively inexpensive, portable and

durable. However, they require frequent calibration and typically have a maximum accuracy of

0.01 pH units (Rérolle et al., 2012). The Durafet shows promise for in field pH measurements

(Rérolle et al. 2012; Martz et al., 2010). The Durafet has a short-term precision of ±0.0005

(Rérolle et al., 2012) and provides a pH reading within milliseconds (Martz et al., 2010). The

cost of the glass electrode and Durafet is approximately $700 USD (glass electrode and pH

meter) and $1300 USD, respectively. This study used both the glass electrode and Durafet to

measure sediment pH in Baynes Sound and assessed the application of both instruments for use

by the shellfish aquaculture industry. The findings of this research may provide insight on tools

and techniques to develop pH baseline data. This information will be valuable for future

monitoring and forecasting of ocean acidification. This research may also provide a potential

management strategy for the shellfish industry to maintain production with increased ocean

acidification pressures using a naturally occurring by-product of shellfish production.

Research Objectives and Hypotheses

The objectives of this research were to assess the variability of sediment pH between farmed and non-farmed sites; evaluate the use of two pH instruments to rapidly assess sediment pH; examine existing shell content between farmed and non-farmed sites; determine if there is a relationship between shell content and sediment pH; and determine if there is a difference in sediment pH and/or shell content in spatially separate sites.

pH Measurements by Site Type

H_{01}: There will be no statistically significant difference in sediment pH between farmed and non-farmed sites.

pH Measurements by Intertidal Zone

H_{02}: There will be no statistically significant difference in sediment pH between intertidal zones between farmed and non-farmed sites.

pH Instrument

H_{03}: There will be no statistically significant difference in the glass electrode and Durafet pH measurements.

Shell Content

H_{04}: There will be no statistically significant difference in mid-intertidal shell content between farmed and non-farmed sites.

Shell Content and pH Measurement Relationship

H_{05}: There will be no statistically significant linear relationship between shell content and sediment pH.

Sediment pH by Site

H_{06}: There will be no statistically significant difference in sediment pH between sites.

Shell Content by Site

H_{07}: There will be no statistically significant difference in shell content in spatially separate sites.

Research Methodology

Site Selection

This research was conducted in Baynes Sound using a paired site design. This area produces over 50% of the cultured shellfish in the province (Ministry of Sustainable Resource Management Coast and Marine Planning Branch, 2002). A total of three pairs of sites were sampled, each consisting of a farmed shellfish beach and a reference site on a non-farmed beach for a total of six sites. The reference sites were chosen due to their proximity and similar characteristics, i.e., slope and substrate, to their paired shellfish tenure. The following pairs were used in this study: Reid and Deep Bay (reference site); Taylor and North of Taylor (hereafter, N. of Taylor) (reference site); and Tran and Reserve (reference site) (*Figure 1*). It should be noted that the reference sites have likely been affected by aquaculture practices. Of particular significance is Deep Bay. This site was previously a lease over six years ago. In addition, the paired sites are adjacent to one another with the exception of Reid and Deep Bay which are approximately 1,200 m apart. The proximity of sites to one another may result in the reference sites being affected by aquaculture practices. Although the Reserve site is considered a reference site it is an open recreational shellfish reserve and subject to shellfish harvest from the general public.
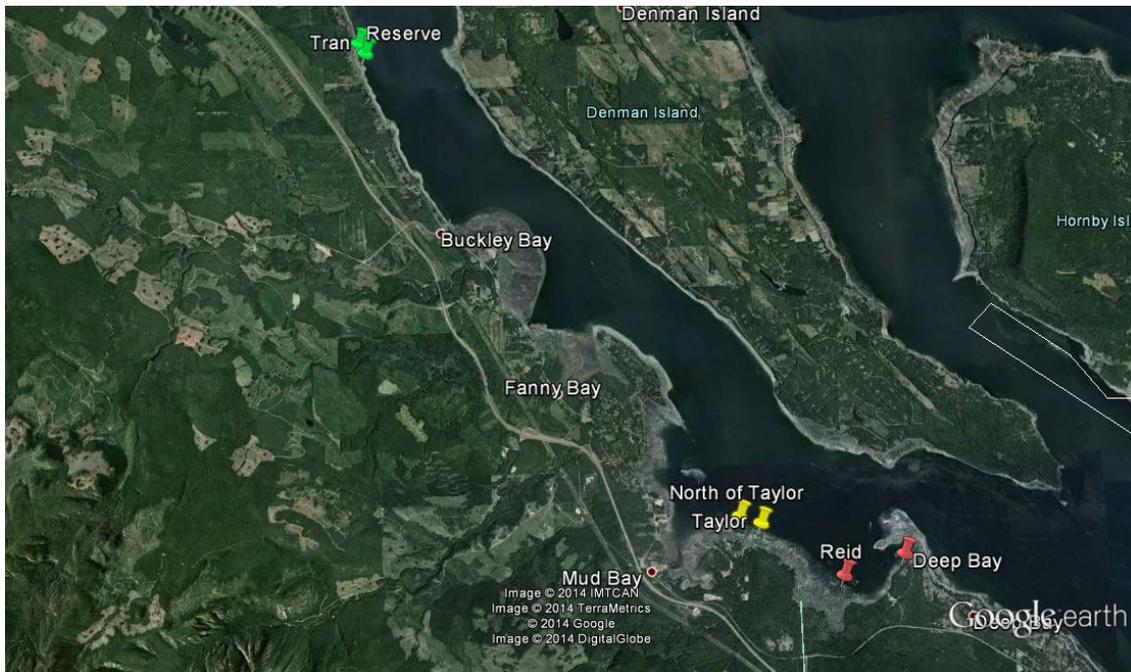
Figure 1. Paired site design in Baynes Sound, British Columbia, Canada (Google Earth, 2014). Coloured pins designate a paired site: Reid and Deep Bay (🚩); Taylor and North of Taylor (📍); and Tran and Reserve (📍).

Sampling Design

        Fifteen quadrats per site were measured using a stratified random sampling design. A grid was laid out at each site (*Figure 2*). The grid was completed by laying out three, 50 m reference lines parallel to the water at 1.5 m, 2.2 m and 3 m. This marked the boundaries of the three intertidal zones: low = 0.8-1.5 m, mid = 1.6-2.2 m and high = 2.3–3 m. Five perpendicular transects were then laid, one every 10 m along the 50 m reference lines. The intertidal zones were marked by placing flags labelled with the corresponding intertidal zone at the upper end of the zone. One quadrat per intertidal zone per transect was randomly placed; resulting in three quadrats per transect for a total of 15 quadrats. Each quadrat was marked with a numbered flag and the quadrat (0.5 m x 0.5 m) was placed with the flag in the upper left hand corner.
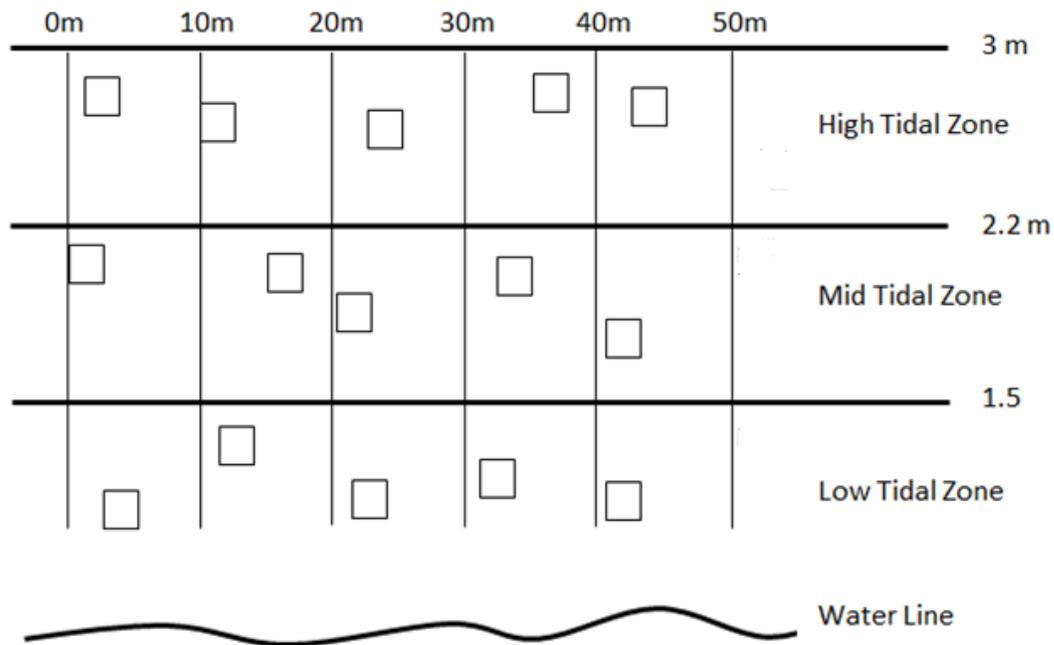
Figure 2. Sampling design developed by Dr. Sarah Dudas, Vancouver Island University, for biodiversity surveys conducted in Baynes Sound. The horizontal lines represent the reference lines for the upper end of each intertidal zone: mid, low and high. The vertical lines represent the five transects that were placed every 10 m along the reference lines. The squares represent a quadrat. One quadrat (0.5 m x 0.5 m) per transect was placed randomly in each intertidal zone.

Sediment pH

Two instruments were used to measure the pH of the sediment. The first was an Orion™ 4-Star portable pH/ISE meter with an Orion low maintenance pH triode (referred to as glass electrode hereafter) and the second a Honeywell Durafet pH electrode (referred to as Durafet from hereafter). The Durafet was modified to accommodate an external power pack. Four pH measurements per quadrat were measured. Deep Bay and Reid were measured using the glass electrode, Taylor and N. of Taylor were measured using both instruments to provide a comparison of the two instruments, and Tran and Reserve were measured with the Durafet.

To complete the pH measurements, four samples were taken randomly from the surface of each quadrat. Each sample was obtained by taking two 10 ml measures from the surface of the quadrat using an open-ended syringe for a total sample of 20 ml. Each sample was

transferred into a plastic cup that was rinsed with deionized water. If the sample was dry, 5 mL

of deionized water was added. If the sediment was moist, no deionized water was added. The

sample was then mixed with a plastic spoon that had been rinsed with deionized water. The

sensor of the pH instruments were inserted into the sample until the reading stabilized and then

the measurement was recorded.

Sediment Core Samples

Three core samples were collected in the mid-intertidal zone for each site. One intertidal

zone was selected, as processing time constraints did not permit all intertidal zone analyses to be

completed. The mid-intertidal zone was selected as this zone contains the highest densities of

clams and is where sediment modification efforts would have been focused. The results from

this zone will also be of greatest interest to industry. Samples were collected by obtaining a core

sample (5 cm diameter x 10 cm length) adjacent to a randomly selected quadrat and placed into a

labelled Ziploc bag. Samples were stored in the freezer at -5 °C.

Sediment Shell Content

The amount of shell content in the sediment was determined for each of the core samples

obtained in the mid-intertidal zone. The samples were dried at 100 °C for 48 hours and then

shaken in sieves for 15 minutes to remove the size fractions greater than 2 mm. The shell

content was manually removed from the sediment size fractions greater than 2 mm. The

remaining samples less than 2 mm in size were processed using a carbonate removal process

(Department of Geo-Information Science and Earth Observation, n.d.). The samples were

treated with hydrochloric acid (HCl) diluted using demineralized water to remove the carbonate.

For each sample 25 ml HCl 1 M was added plus an additional 1 ml for each estimated 50 mg of

carbonate.   The amount of carbonate was estimated by calculating the percentage of shell

content in the sediment size fractions greater than 2 mm and applying this percentage to the

remaining sample weight of the size fractions less than 2mm. The suspension was then placed in

a water bath at approximately 80 °C for 15 minutes, and stirred occasionally. The suspension

was removed from the water bath and left to stand overnight. The supernatant was then decanted

and the sediment was washed with demineralized water until the electrical conductivity of

supernatant was less than 0.4 mS/cm. The samples were dried a second time at 100 °C for 48

hours.

Following the second drying of the samples there was a minor amount of shell left in

some of the samples. A subsample of approximately 5 g was taken from each homogenized

sample. The shell content was manually removed from the subsamples, weighed, and the shell

percentage in the subsample was then applied to the overall sample.

Statistical Analysis

The assumptions of normality and homogeneity of variance were checked prior to

conducting all statistical tests. The assumptions were met for all tests unless otherwise stated

below. Statistical analyses were completed using Statistica (version 7.0).

pH measurements by site type and intertidal zone –glass electrode.

To determine if there is a significant difference in sediment pH between farmed and non-

farmed sites and between intertidal zones of farmed and non-farmed sites a two factorial analysis

of variance (ANOVA) was completed. The fixed factors in this analysis were site type (farmed

and non-farmed) and intertidal height (low, mid and high) and the random factor was glass

electrode pH. The data was transformed from a positively skewed distribution to a normal

distribution using a reciprocal-exponential transformation ($-1/5^Y$).

Normality for all intertidal heights was met with the exception of the high intertidal height of the non-farmed sites. This analysis is fairly robust against the violation of non-normality when the homogeneity of variance assumption is met (Quinn & Keough, 2002). A post-hoc analysis was completed using the Tukey's test.

pH measurements by site type and intertidal zone –Durafet.

Non-parametric tests were used to analyze the effect of site type and intertidal zone on Durafet pH measurements as the data could not be transformed to meet assumptions of normality or homogeneity of variances. The difference in sediment pH measured with the Durafet analyzer, between farmed and non-farmed sites and between intertidal zones was analysed using the Mann-Whitney U and the Kruskal-Wallis test respectively. The non-parametric tests are unable to analyze if there is an interaction between site type and intertidal zone as the factorial ANOVA does; therefore, it was decided to run a factorial ANOVA as well. The most fitting transformation applied was a reciprocal transformation (-1/Y). The results of the factorial ANOVA were compared to see if they were consistent with the non-parametric results. This was to provide an indication of how confident we may be in the results because of potential interactions between site type and intertidal zone.

pH instrument.

To determine if there was a significant difference in the glass electrode and Durafet pH measurements a one-way ANOVA was completed. The data was first transformed from a positively skewed distribution to a normal distribution using a reciprocal transformation ($-1/Y^4$).

Shell content.

To determine if there is a significant difference in mid-intertidal shell content between farmed and non-farmed sites a one-way ANOVA model was completed.

Shell content and pH measurement relationship.

A linear regression was conducted to determine if there is a relationship between shell content and pH measurements for both the glass electrode and Durafet. An exponential transformation (Y^2) was applied to the Durafet data to meet the assumptions of normality and homogeneity of variances.

<div align="center">

Results

</div>

pH Measurements by Site Type and Intertidal Zone – Glass Electrode

There was no interaction between site type (farmed and non-farmed) and intertidal zones. However; there was a significant difference in the glass electrode pH measurements between farmed and non-farmed sites as well as between intertidal zones (Table 1). The farmed sites had a higher average pH of 7.71 relative to the non-farmed sites with an average pH reading of 7.62. The pH measurements ranged from 7.25-8.41 for farmed sites (Reid and Taylor) and 7.28-8.33 for non-farmed sites (Deep Bay and N. of Taylor) (*Figure 3*). There was a significant difference between the low-intertidal zone of non-farmed sites compared to all intertidal zones of farmed sites and the mid and high-intertidal zone of non-farmed sites (Table 2). The farmed sites had a higher pH in all intertidal zones relative to non-farmed sites, though not all differences were significant (*Figure 4*).

Table 1

Factorial ANOVA results testing for effect of site type (farmed or non-farmed) and intertidal zone (low, mid or high) on glass electrode pH measurements.

Variable	df	F	*p*
Site type	1	7.09	**.01**

Tidal zone	2	5.33	**.01**
Site type*Tidal zone	2	2.55	.08
Error	223		

Table 2

Results from Tukey's analysis, testing for significant difference in pH measurements using a glass electrode between intertidal zones between farmed (F) and non-farmed sites (NF).

	F: Low	F: Mid	F: High	NF: Low	NF: Mid	NF: High
F:Low	-	1.00	.97	**.01**	1.00	1.00
F: Mid	1.00	-	1.00	**.00**	1.00	1.00
F: High	.97	1.00	-	**.00**	1.00	1.00
NF: Low	**.01**	**.00**	**.00**	-	**.03**	**.01**
NF: Mid	1.00	1.00	1.00	**.03**	-	.99
NF: High	1.00	1.00	1.00	**.01**	.99	-
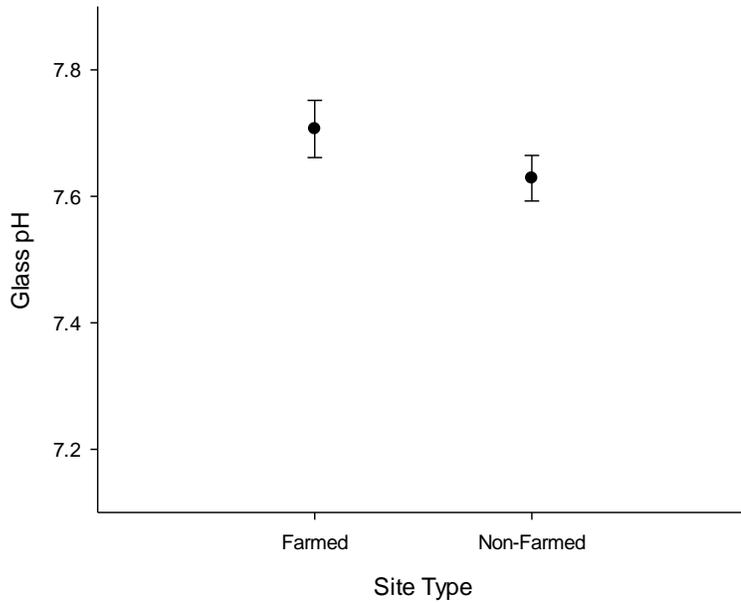
Figure 3. Mean glass electrode pH measurements of farmed (Reid and Taylor) and non-farmed sites (Deep Bay and N. of Taylor). Vertical bars denote 0.95 confidence intervals.
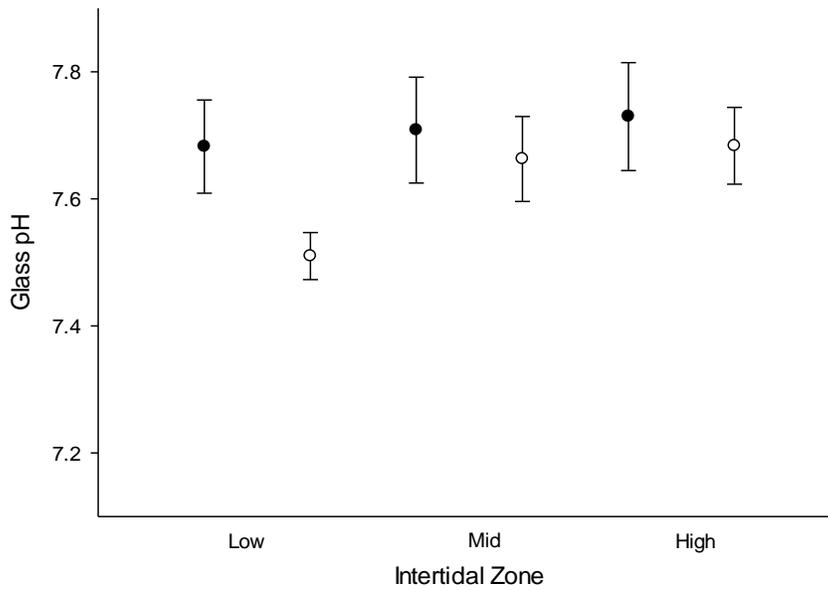
Figure 4. Mean glass electrode pH measurements for farmed (Reid and Taylor) (●) and non-farmed (Deep Bay and N. of Taylor) (○) sites by intertidal zone. Vertical bars denote 0.95 confidence intervals.

pH Measurements by Site Type and Intertidal Zone –Durafet

The assumptions of normality and homogeneity of variances were not met for this

analysis using raw or transformed data. The best transformation to meet these assumptions was a

reciprocal transformation (-1/Y). This transformation normalized the distribution of the farmed

sites; however; the assumption of normality was not met for the non-farmed sites. The primary

interest of the factorial ANOVA was to determine if there is an interaction between site type and

intertidal zone that cannot be detected by a comparable non-parametric test (Kruskal-Wallis test).

Therefore, the results of the factorial ANOVA are being reported as well.

The factorial ANOVA showed no interaction between site type (farmed and non-farmed)

and intertidal zones; no significant difference in the Durafet pH measurements between farmed

and non-farmed sites or between intertidal zones (Table 3). The Mann-Whitney U and the

Kruskall-Wallis test results were consistent with those of the ANOVA and reported no

significant difference in the Durafet pH measurements between farmed and non-farmed sites (U

=6321.50, p=.10) or between intertidal zones χ^2(2, N = 240) = 1.50, p = .47). The consistency

between the results of the parametric and non-parametric tests provides support that the output of

the factorial ANOVA is correct, that there is no significant interaction between site type and

intertidal zone. The risk of not meeting the assumptions for this test is a type I error; the null

hypothesis would be rejected when it is true.

Though insignificant, farmed sites had a higher average pH than non-farmed sites, 7.45

and 7.38 respectively (*Figure 5*). The pH range was 6.51-8.33 for farmed sites (Taylor and Tran)

and 6.7-8.33 for non-farmed sites (N. of Taylor and Reserve).   The pH values for the three

intertidal zones of the farmed sites were also higher, though not significantly, than non-farmed

sites with the exception of the mid-intertidal zone where non-farmed sites had a higher pH than

farmed (*Figure 6*).

Table 3

Results from the factorial ANOVA testing for an effect of site type (farmed or non-farmed) and intertidal zone (low, mid or high) on Durafet pH measurements.

Variable	df	F	*p*
Site type	1	1.70	.19
Tidal zone	2	0.61	.54
Site type*Tidal zone	2	2.94	.05
Error	234		
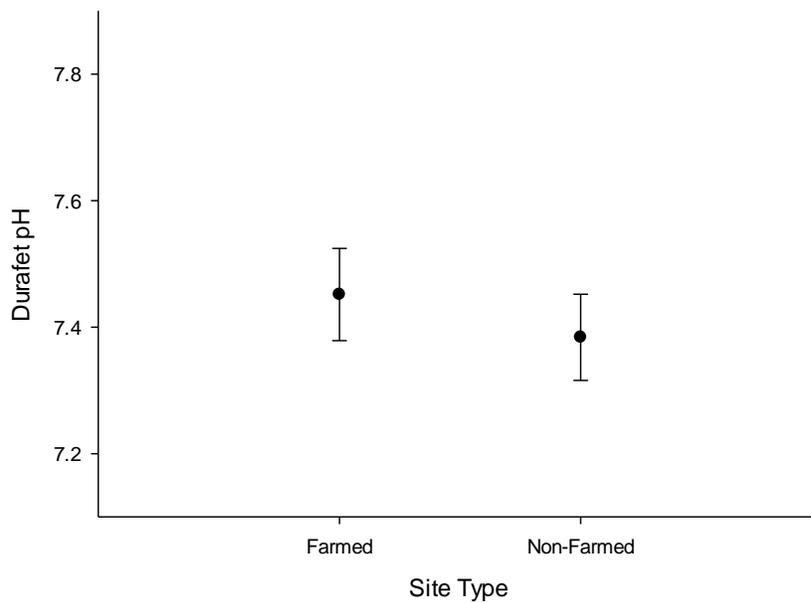
Figure 5. Mean Durafet pH measurements of farmed (Taylor and Tran) and non-farmed sites (N. of Taylor and Reserve). Vertical bars denote 0.95 confidence intervals.
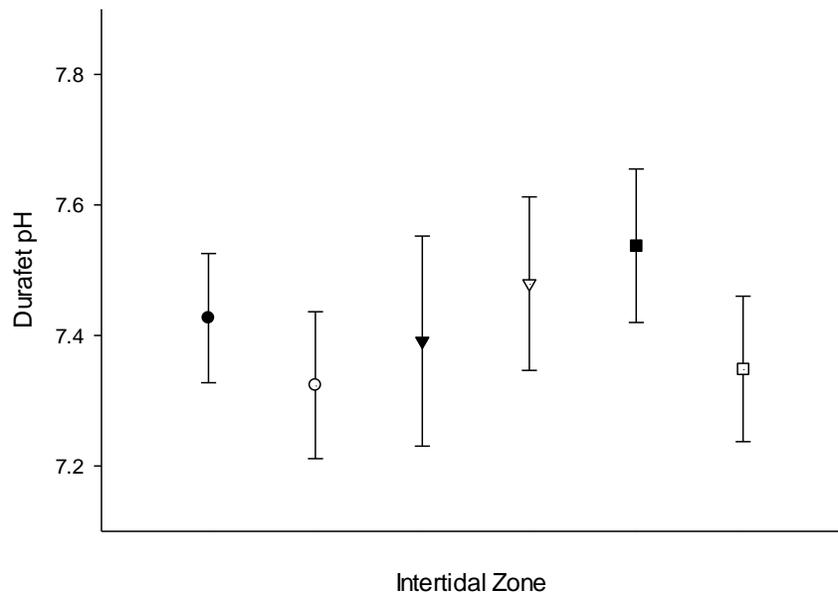
Figure 6. Mean Durafet pH measurements for farmed (Taylor and Tran) (black) and non-farmed (N. of Taylor and Reserve) (white) sites by intertidal zone: low(●/○), mid(▼/▽) and high(■/□). Vertical bars denote 0.95 confidence intervals.

pH Instrument

There was no significant difference between the glass electrode and Durafet instruments ($F_{(1, 238)}$ =3.17, *p*=.08). The average pH measurements were slightly lower for the glass electrode than the Durafet, 7.69 and 7.74 respectively (*Figure 7*). The variability in the pH measurements for the glass electrode and Durafet is seen in the scatterplot for measurements completed at Taylor and N. of Taylor (*Figure 8*). The r-squared value for the linear regression was r^2=.49.
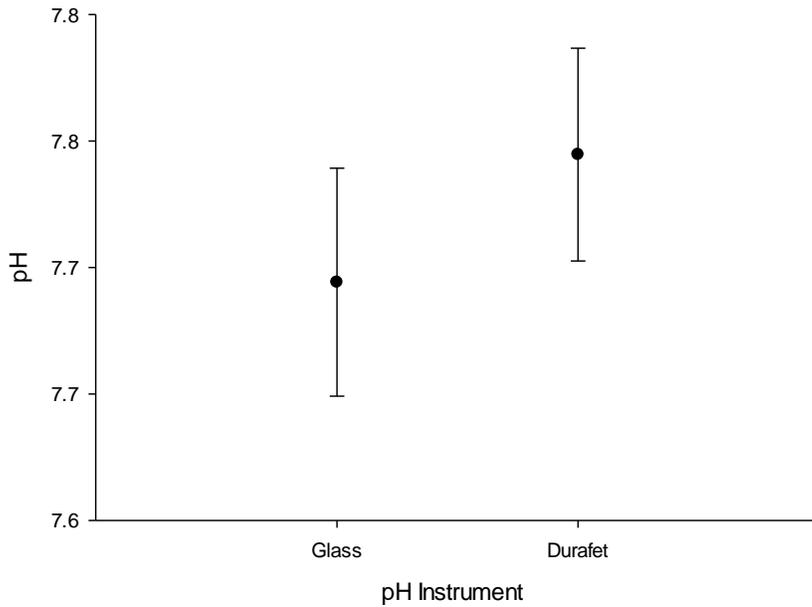
Figure 7. Comparison between mean glass electrode and mean Durafet pH measurements. Vertical bars denote 0.95 confidence intervals.
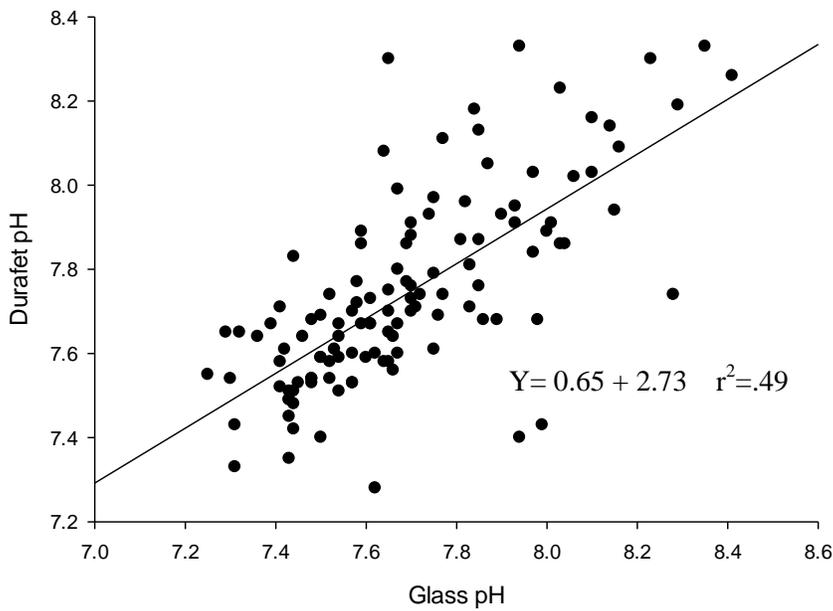
Figure 8. Scatterplot and linear regression of glass electrode versus Durafet pH measurements from Taylor and N. of Taylor sites.

Shell Content

Mid-intertidal shell content was not significantly different between farmed and non-

farmed sites (F (1, 16) =0.02, p =.90). The average shell content was 7.1% with a range of 3.6%-

15.6% on farmed sites and an average of 7.2% on non-farmed sites, with a range of 2.7%-15.7%

(*Figure 9*).
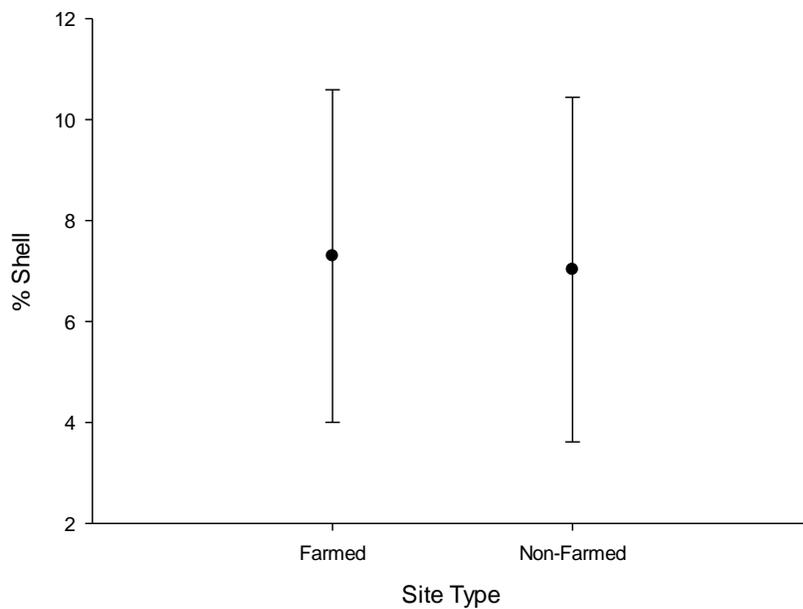
Figure 9. Sediment sample mean shell content percentage from mid-intertidal zone of farmed
and non-farmed sites. Vertical bars denote 0.95 confidence intervals.

Relationship between Shell Content and pH

Shell content had a very weak positive but insignificant relationship with the glass

electrode pH measurements; r^2 = .01, 43 d.f., p=.53 (*Figure 10*). Shell content and the Durafet

pH measurements had a significant negative relationship r^2 = .55, 43 d.f., p=.00 (*Figure 11*).

$$y= 0.01x + 7.57 \quad r^2 =.01$$
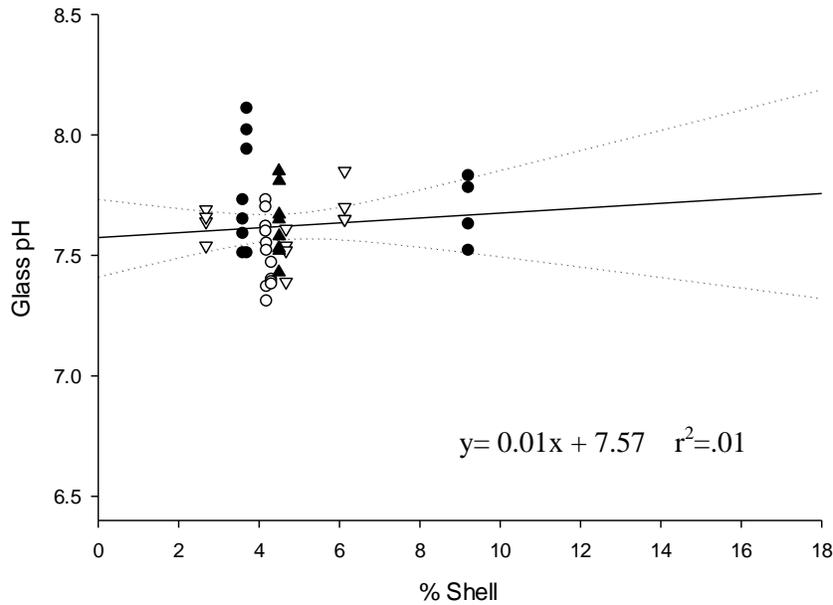
Figure 10. Percent shell versus glass electrode pH of mid-intertidal zone for Reid (⬤), Taylor (▲), Deep Bay (○) and N. of Taylor (▽). Solid line is linear regression. Dashed lines denote 0.95 confidence intervals.

$$y= -0.07x + 7.96 \quad r^2 =.55$$
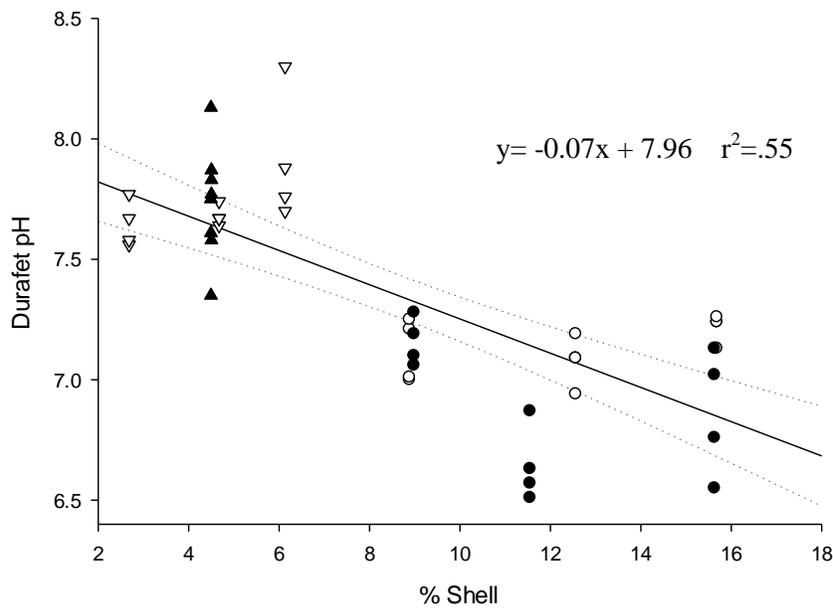
Figure 11. Percent shell versus Durafet pH of mid-intertidal zone for Tran (⬤), Taylor (▲), Reserve (○) and N. of Taylor (▽). Solid line is linear regression. Dashed lines denote 0.95 confidence intervals.

pH Measurements by Site

When reviewing the Durafet data graphically, the farmed and non-farmed sites had a bimodal distribution. There appeared to be a difference between the sets of paired sites which suggested that the geographic area may be more indicative of a site's pH than if it is farmed or not-farmed (*Figure 12*). The glass electrode pH measurements did not exhibit a bimodal distribution, which supported this theory (*Figure 12*). Due to this, additional analyses were conducted to determine if there is a difference in sediment pH in spatially separate sites for each instrument. These analyses were conducted using Kruskal-Wallis tests due to homogeneity of variance and normality not being met.

There was no significant difference in glass pH measurements between sites χ^2(3, N = 229) = 7.91, *p* = .05) (Table 4). For the Durafet pH measurements there was a significant difference between sites χ^2(3, N = 240) = 176.70, *p* = .00); with significant differences between Tran and Taylor; Tran and N. of Taylor; Reserve and Taylor; and Reserve and N. of Taylor (Table 5). Tran, Reserve, Taylor and N. of Taylor had pH ranges of 6.51-7.53, 6.70-7.68, 7.33-8.33 and 7.28-8.33 respectively (*Figure 12).*
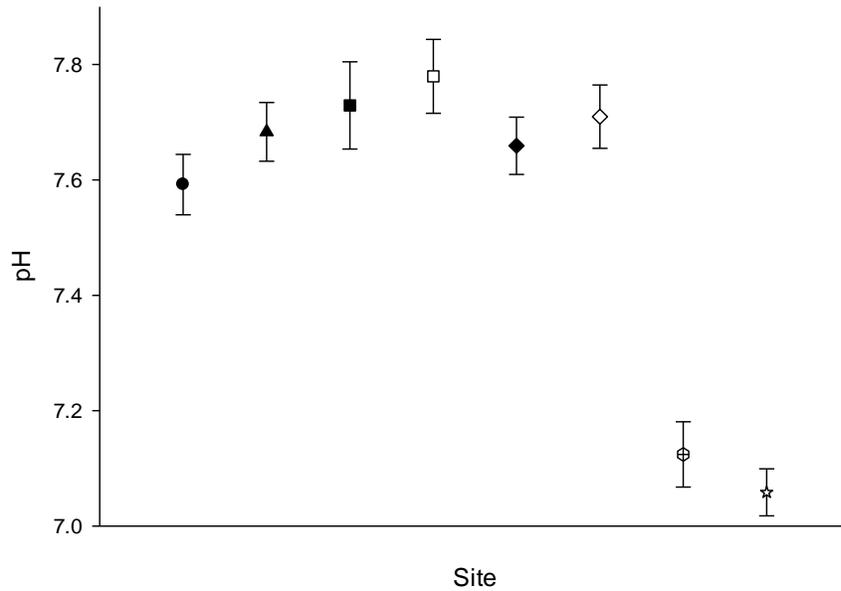
Figure 12. Mean glass electrode measurements (black) and mean Durafet pH measurements (white) for all sites. Sites located in the southern area of Baynes Sound include Deep Bay (●), Reid (▲), Taylor (■/□) and N. of Taylor (◆/◇). Sites located in the northern area of Baynes Sound include Tran (⊕) and Reserve (☆). Vertical bars denote 0.95 confidence intervals.

Table 4

Kruskal-Wallis test results for determining the difference in Glass pH measurements between the four sampled sites (Reid Taylor, Deep Bay and N. of Taylor).

	Reid	Taylor	Deep Bay	N. of Taylor
Reid	-	1.00	.17	1.00
Taylor	1.00	-	.05	1.00
Deep Bay	.17	.05	-	.75
N. of Taylor	1.00	1.00	.75	-

Table 5

Kruskal-Wallis test results for determining the difference in Durafet pH measurements between the four sampled sites (Taylor, Tran, N. of Taylor and Reserve).

	Taylor	**Tran**	**N. of Taylor**	**Reserve**
Taylor	-	**.00**	1.00	**0.00**
Tran	.00	-	**.00**	.91
NoT	1.00	**.00**	-	**.00**
Reserve	.00	.91	**.00**	-

Shell Content by Site

The potential spatial differences in sediment pH also led to a secondary analysis on shell content to determine if there is a significant difference in mid-intertidal shell content between sites. This analysis was completed using the Kruskal-Wallis test. Transformations were completed on the data in an attempt to meet the assumptions of an ANOVA; however, the assumption of normality was not met for all sites. Due to the relatively small sample size, the analysis was vulnerable to the violation of the assumptions. A non-parametric test (Kruskal-Wallis) was chosen for the site comparison for this reason.

There was no significant difference in mid-intertidal shell content between the six sampled sites χ^2(5, N=18) = 10.26, p = .07) (Table 6). Average shell content ranged from a low of 4.2% at Deep Bay to a high of 12.4% at Reserve. The average shell content for Reid, Taylor, N. of Taylor and Tran were 5.5%, 4.3%, 4.5% and 12.1% respectively (*Figure 13*). The average sample dry weight for all sites was 422 g. However, the average sample dry weight at N. of Taylor was 154 g. The average shell content sampled at all sites had some variation with the exception of Taylor and Deep Bay. These sites were relatively consistent across samples. The

average shell content at Taylor and Deep Bay ranged from 4.0%-4.6% and 4.2%-4.3%

respectively.

Table 6

Kruskal-Wallis test results for determining the difference in mid-intertidal shell content between the six sampled sites.

	Reid	Taylor	Tran	Deep Bay	N. of Taylor	Reserve
Reid	-	1.00	.70	1.00	1.00	.58
Taylor	1.00	-	1.00	1.00	1.00	.84
Tran	.70	1.00	-	.58	1.00	1.00
Deep Bay	1.00	1.00	.58	-	1.00	.48
N. of Taylor	1.00	1.00	1.00	1.00	-	1.00
Reserve	.58	.84	1.00	.48	1.00	-
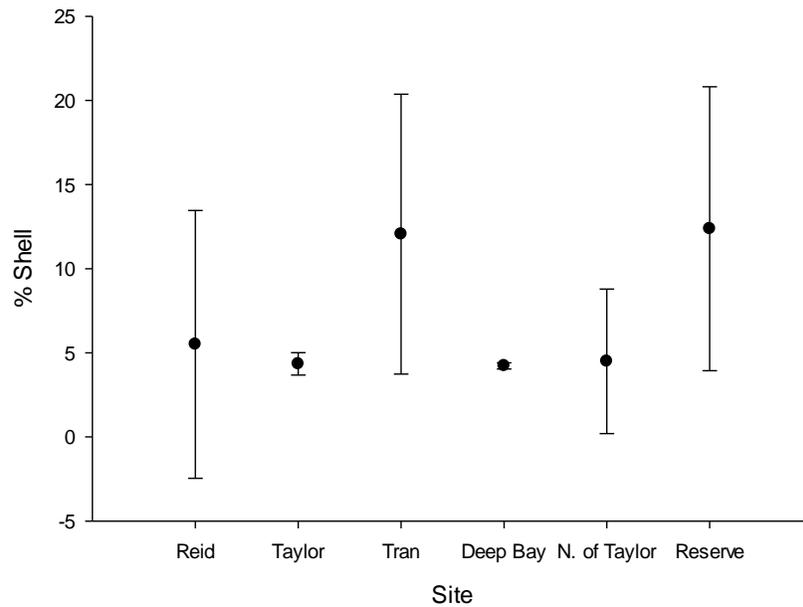
Figure 13. Mean percent shell content of sediment sample from mid-intertidal zone of all sampled sites: Reid, Taylor, Tran, Deep Bay, N. of Taylor and Reserve. Vertical bars denote 0.95 confidence intervals.

Discussion

This research assessed the variability in sediment pH between farmed and non-farmed sites; evaluated the use of two pH instruments to rapidly assess sediment pH; examined existing shell content between farmed and non-farmed sites; determined if there is a relationship between shell content and sediment pH; and determined if there is a difference in sediment pH and shell content in spatially separate sites. The four sites measured with the glass electrode had a significant difference in pH between farmed and non-farmed sites. There was also a significant difference between the low intertidal zone of non-farmed sites relative to other intertidal zones of farmed and non-farmed sites for glass electrode pH measurements. This result was not found for the four sites measured with the Durafet; no significant difference was found between farmed and non-farmed sites or between intertidal zones. It was expected that farmed sites would have a higher concentration of shell content than non-farmed sites and would therefore have a higher pH

due to the shell providing a buffering effect. This research found no difference in shell content

between farmed and non-farmed sites and no relationship indicating a buffering effect between

shell content and pH. A significant difference was found when comparing Durafet pH

measurements between sites; the sites that were farther apart (i.e., 12 km) were significantly

different while the close sites (i.e., 0.5 km) were not. The following subheadings expand on

these findings.

pH Measurements by Site Type and Intertidal Zone

The results of the statistical analysis were not consistent between the glass electrode and

Durafet. However, two of the four sites sampled for each of the instruments differed, so they are

not directly comparable. When looking at the grouped site data by farmed and non-farmed sites,

the farmed sites had a higher pH than the non-farmed sites for both instruments. This was

consistent across intertidal zones of farmed and non-farmed sites with the exception of the

Durafet measurements of the mid-intertidal zone. The mid-intertidal zone of non-farmed sites in

this instance had a higher pH than farmed sites. The Taylor and N. of Taylor sites had a similar

mean pH in the mid-intertidal zone; whereas, the Reserve site had a higher mean pH for this

intertidal zone by 0.15 compared to Tran (Appendix C, *Figure 23*). A contributing factor to this

inconsistency may be that the Tran site appeared to have finer grained sediment and to be more

anoxic than the Reserve site. Anoxic environments may be more acidic because of by-products

of organic decomposition (Ben-Yaakov, 1973).

The low intertidal zone of non-farmed sites had a significantly lower pH relative to all

intertidal zones of farmed and non-farmed sites measured with the glass electrode (*Figure 17*).

The mechanisms that explain the low-intertidal difference at non-farmed sites are unclear and are

likely similar to those that contribute to the general variability of sediment pH. This involves

multiple interacting variables; including physical, biological and chemical interactions occurring within each site. Drivers of lower pH may be increased respiration, decreased photosynthesis, release of CO_2 from decaying organic matter and/or increased salinity (Alenius & Munguia, 2012). In addition, the availability of $CaCO_3$, such as the presence of shell, would increase pH. The low intertidal zone of Reid is relatively basic compared to Taylor, Deep Bay and N. of Taylor sites. Reid had a high concentration of *C. gigas* growing in the low intertidal zone. It is possible the presence of oysters, which are found on the surface of the sediment, contribute to a more basic environment due to the $CaCO_3$ in their shells. Other influences that were observed in the field were small freshwater streams at Taylor and N. of Taylor that passed through the low and mid-intertidal zone study area. Freshwater inputs increase pCO_2 and would result in a decrease in pH (Bates & Mathis, 2009). Developing an understanding of the causes of variability in pH in Baynes Sound would assist in forecasting future implications of ocean acidification for intertidal organisms and the potential impacts to the shellfish industry at both local and regional scales.

With shellfish exposed to relatively acidic environments in Baynes Sound there is concern about negative impacts on growth, development, shell integrity and physiology (Timmins-Schiffman et al.; 2013; Barton et al., 2012 & Doney, et al., 2009). Many studies have focused ocean acidification research on the early life stages of juvenile bivalves when calcification is a fundamental process of growth and survival (Barton et al., 2012; Parker et al., 2012; Timmins-Schiffman, 2012; Talmage & Gobler, 2010; & Green et al., 2009). Negative growth and survival of juvenile larvae have been documented for *C. gigas, M. arenaria, Mercenaria mercenaria, Argopecten irradians, Saccostrea glomerata* from exposure to elevated pCO_2 conditions predicted to occur later this century (Barton et al., 2012; Parker et al., 2012;

Timmins-Schiffman, 2012; Talmage & Gobler, 2010; & Green et al., 2009). The above

mentioned studies were conducted on larvae less than 1mm in length. However, Metzger (2012)

found larvae (less than 1mm in length) survival and growth rates were similar between current

and elevated pCO$_2$ conditions for *Ruditapes philippinarum*, a common species cultured in BC.

The BC shellfish industry seeds their beaches with *R. philippinarum* ranging in length from 5

mm to 10 mm (Munroe & McKinley, 2007). This species may be more robust to ocean

acidification pressures and the seed size results in dissolution being a lesser concern.   Acidic

conditions may pose a concern for members of the industry that rely on natural recruitment as

well as natural populations for species other than *R. philippinarum*.

pH Instrument

The pH measurements were quite variable for both the glass electrode and Durafet

instruments. This variability was seen at multiple spatial levels, including variability within a

quadrat, intertidal zone and site (Appendix A, *Figure 14*,*Figure 15*). The variability of pH

readings from both instruments is likely due to high spatial variability in pH within each site.

The methodology to measure sediment pH may also have contributed to this variability. The

addition of deionized water to dry sediment samples may have influenced the pH; raising or

lowering the sample's pH towards a pH of seven which is the pH of deionized water at 20 °C

(HANNA Instruments, 2012). A small test was conducted to determine if deionized water

influenced the pH measurement of the glass electrode and the Durafet while in the field. The

addition of 5 mL of deionized water resulted in an average increase in the glass electrode pH by

0.05 units and an average decrease of 0.09 pH units for the Durafet. This test was conducted on

a small set of sediment samples that were above a pH of seven. There was no pattern of lower

acidity values observed in the high intertidal zone that were typically drier and required the

addition of deionized water.

Other factors contributing to the sediment pH variability may have been changes in the

pH during the tidal cycle. Measurements were conducted at various times during the tidal cycle

which resulted in the sample locations being exposed to the air for different durations. An

increase in aerial exposure may result in warming of the sediment and cause a higher pH

measurement (Wootton, Pfister, & Forester, 2008). However, the instruments used in this study

automatically compensated for temperature and adjusted the reading to 25 °C. Therefore,

temperature driven pH changes would not have been observed in this study. This is supported by

the findings of this study. The low intertidal quadrats were sampled first and there is no strong

pattern of decreased acidity with an increase in intertidal zone (Appendix B, *Figure 18*;

Appendix C, *Figure 27*). A possible solution to avoid any potential influence of air exposure

would be to take measurements starting at high tide and sampling as the tide dropped. However,

this method may limit the number of samples obtained. The instruments themselves will also be

variable. The variability of the two instruments was similar. The standard deviation for the glass

electrode and Durafet measurements was 0.25 and 0.23 respectively. The glass electrode is

prone to sensor drift which may account for some of the variability; however, this is a less likely

cause for the variability of pH readings for the Durafet.

This study indicates that the glass electrode may be sufficient for industry to monitor

sediment pH due to the lack of a significant difference between pH measurements when

comparing the two instruments. However, this method is not well supported by scientists active

in the ocean acidification field due to the instrument's tendency to drift and the requirement for

more frequent calibration (Rérolle et al., 2012; Martz et al., 2010). The glass electrode has a

daily drift of up to 0.02 pH units per day and is sufficient for observing pH variations greater

than 0.01 pH (Rérolle et al., 2012). To detect the average annual change in the pH of the ocean's

surface the precision of the chosen instrument needs to be greater than 0.002; the glass electrode

is not recommended for this purpose (Rérolle et al., 2012). Within a quadrat the average

difference between the maximum and minimum pH reading was 0.25 and 0.33 pH units for the

glass electrode and Durafet instruments respectively. Impacts to juvenile bivalve recruitment

have been observed with exposure to pH levels 0.3-0.5 units lower than current atmospheric

conditions (i.e. 7.6) (Barton et al., 2012; Parker et al., 2012; Timmins-Schiffman, 2012; Talmage

& Gobler, 2010; & Green et al., 2009). There is limited data available to conclude if smaller

changes in pH affect recruitment. Due to the high spatial variability within a site the glass

electrode will likely provide industry with adequate information for monitoring. However, the

data collected may not be easily comparable across the industry due to the challenges in

calibration; where two identifcal electrodes may produce different results (Martz et al., 2010).

This was not identified as an issue for the Durafet instrument. To obtain the most accurate and

precise data the Durafet should be used for pH measurements along with the measurement of

other carbonate-chemistry variables, to meet the science community's standards and to provide

data that can be shared between industry members.

The high variability of pH readings in Baynes Sound also indicates there is a need for

additional samples. The challenge of this is the limitation of the number of samples that can be

completed within a tidal cycle for each site, which was prohibitive in this research. Both

instruments were relatively quick in producing a pH reading; however, the Durafet was faster

overall and does not require frequent calibration. In addition, other carbonate variables will need

to be assessed which will provide a more accurate assessment of the carbonate-chemistry. The

trade-off with the inclusion of additional carbonate-chemistry variables is fewer samples will be

assessed due to the increased cost and time required to complete them.

Shell Content and Relationship to pH

The lack of significant differences in shell content indicates that shell content between

farmed and non-farmed sites in Baynes Sound is fairly consistent. A challenge with this study is

there is no historic data on shellfish practices in Baynes Sound to inform how shellfish practices

differ among sites and operators. It has been documented that adding crushed shell or gravel is a

common practice in shellfish aquaculture in BC; however, there is no specific data on this

practice such as operators that employ this procedure or the amount of shell introduced to a site.

This study is the first to quantify shell content in Baynes Sound. The results indicate that

shellfish aquaculture operators at the sampled sites may not add crushed shell as originally

thought or there are external factors influencing the transport of shell off the site. It cannot be

assumed that non-farmed sites have had no external additions of shell. For instance, shell may

have been added by members of the public. This is a probable scenario for the Reserve site as it

is an active shellfish reserve open for public shellfish harvest. Sediment modifications may be

conducted by individuals that utilize this site for personal shellfish harvest and consumption.

With no difference found in shell content between site types it was anticipated that there

would be no buffering effect from the shell found in this study. The results were consistent with

what was expected. The relationship between shell content and sediment pH were inconsistent

between the glass electrode and the Durafet. The glass electrode pH measurements had a very

weak positive relationship with shell content, whereas; the Durafet pH measurements had a

relatively strong negative relationship. The results of the two analyses of the different

instruments are not directly comparable because different sites were sampled by the two instruments.

The results of these analyses contrast with Green et al.'s (2009) findings that a higher presence of crushed shell increases the alkalinity of the sediment, resulting in a higher pH. The study by Green et al. (2009) applied 1.2 kg/m^2 of crushed shell to experimental plots resulting in approximately 45% of the surface being covered by shell. This application of crushed shell would be in addition to any existing shell. This study found an average of 7% shell in the core samples across all sites; this equates to approximately 1.5 kg/m^2 within the top centimetre of the sediment. This suggests that a higher volume of shell may be required to see an effect of sediment buffering in Baynes Sound.

In addition, there may be physical, chemical and/or biological factors that have a greater influence on sediment pH than the single factor of crushed shell. Andersson & Mackenzie (2012) state that no buffering effect will be observed in most shallow water environments that have a high presence of naturally occurring $CaCO_3$ due to the rate of physical mixing and uptake of CO_2. This is inconsistent with Green et al.'s (2009) findings of a significant increase in sediment pH in a study that was conducted over 16 days.

There are four main considerations when researching the buffering effects of seawater from $CaCO_3$ dissolution including (Andersson & Mackenzie, 2012):

- What is the $CaCO_3$ availability that may be reactive to changes in ocean chemistry?
- Is there evidence that dissolved $CaCO_3$ provide a buffer?
- What are the rates and mechanisms for $CaCO_3$ dissolution?
- What is the hydrography of the environment of interest?

This study addressed the first two points by quantifying the shell content to determine the $CaCO_3$ availability and performing a linear regression to model the relationship between shell content and sediment pH. The rates and mechanisms for $CaCO_3$ dissolution as well as the hydrography of the environment are also important considerations. However, they were not addressed in the scope of this research.

pH Measurements and Shell Content by Site

The pH was spatially variable within and between sites; however, there were interesting regional patterns observed. Tran and Reserve are located in the northern area of Baynes Sound and are approximately 12 km apart from Taylor and N. of Taylor, located to the South. The glass electrode measurements were conducted in the four southern sites (Reid, Taylor, Deep Bay and N. of Taylor); the greatest distance between these sites was 3.3 km apart. There was no significant difference in pH between these sites. The northern sites had a statistically significant lower pH than the southern sites for the Durafet measurements. The difference in Durafet pH values between the northern and southern sites was 0.66 pH units; this is higher than the anticipated pH change to occur over the next century. This difference in pH may have been influenced by freshwater inputs from the Courtenay River located near the northern sites. The relatively acidic environments of the northern sites are consistent with what is expected of freshwater influences, causing a decrease in pH (Bates & Mathis, 2009). An additional influence may have been a historic coal mine, the Tsable River mine. The mine site was located approximately 6,000 m from the Tran and Reserve sites compared to approximately 14,000 m from the Taylor and N. of Taylor sites. Coal has been found to contribute to more acidic environments (Vorres, n.d.). Areas that have a lower pH may be susceptible earlier to the effects of ocean acidification relative to sites with a higher pH and may be a focus for management

efforts related to ocean acidification. The design of this study does not allow for additional

spatial analyses to be completed due to the small sample size. Future research should focus on

the spatial differences in sediment pH rather than the effect of aquaculture activities which don't

appear to be a primary influence.

Regional differences were also found for shell content. The shell content at the northern

sites was higher than at the southern sites, though not significantly. The physical sediment

characteristics of the two northern sites were distinctly different; one with a high component of

gravel and the other comprised primarily of fine sandy sediment; this did not appear to influence

the shell distribution or pH. The amount of shell content is likely influenced by anthropogenic

influences, such as external additions of shell as well as digging that would re-suspend and move

shell content. Transportation rates will vary depending on the characteristics of the shell or

sediment, such as size, shape and density (De Meijer, 2002). Other factors influencing shell

content may include abrasion, temperature and pH causing shell degradation (Faulkner, 2011).

Conclusions and Recommendations

Monitoring of ocean acidification will be fundamental in determining the potential effects

ocean acidification may have on shellfish aquaculture. This research indicates that both pH

instruments are sufficient for use by the shellfish industry for coarse measurements of pH.

However, use of the Durafet is recommended to meet standards in the ocean acidification

research community, to have more comparable results between industry members and is more

durable for application in the field.

The assessment of sediment pH has provided insight into identifying the range of pH

values intertidal organisms are exposed to in Baynes Sound. The range of pH tolerance of

intertidal organisms is an important consideration in the assessment of ocean acidification

implications. Further research is required to assess site pH and consider potential temporal

variations that may occur. In addition to temporal variation considerations, this study indicated

that the spatial separation between sites influences its physical and chemical properties. The

results of this research suggest that monitoring activities of ocean pH should have a spatial focus

rather than focus on the influence of aquaculture on ocean acidification. Monitoring activities

for ocean pH may also assist in prioritizing management efforts to areas with higher acidity

levels. In addition to continuing to monitor sediment pH, additional carbonate-chemistry

variables will need to be assessed. This will provide a more accurate portrayal of the changes

occurring in ocean chemistry.

The predicted decrease in ocean acidity due to changes in the ocean chemistry will put

pressure on marine ecosystems and organisms. This study investigated a potential management

strategy of buffering sediments using crushed shell. The finding that there was not a significant

difference in shell content between farmed and non-farmed sites does not rule this out as a viable

strategy. An opportunity for future research is to follow the work of Green et al. (2009) where

pH measurements are conducted at experimental sites that have had additional shell added

relative to control sites. Site selection for this research should focus on areas with lower physical

oceanography energy to minimize the movement of shell off the site. Additionally, the

concentration of shell required to provide a buffer against ocean acidification would need to be

determined as well as how frequently the shell would need to be applied to maintain a buffered

environment. This would inform if it is more cost effective to add shell to a site or to purchase

larger seed that is more resistant to acidic conditions.

The shellfish industry has experienced negative impacts from ocean acidification in

recent years and will need to be prepared for future changes. Monitoring carbonate-chemistry

variables will be a fundamental component to forecast ocean acidity changes as well as monitor

the effectiveness of management strategies. This information will be valuable to environmental

managers and the shellfish industry to maintain and improve environmental quality to ensure the

sustainability of the industry.

Works Cited

Alenius, B., & Munguia, P. (2012). Effects of pH variability on the intertidal isopod, *Paradella dianae*. *Marine and Freshwater Behaviour and Physiology*, *45*(4), 245–259. doi:10.1080/10236244.2012.727235

Andersson, A. J., & Mackenzie, F. T. (2012). Revisiting four scientific debates in ocean acidification research. *Biogeosciences*, *9*(3), 893–905. doi:10.5194/bg-9-893-2012

Barton, A., Hales, B., Walkbusser, G., Langdon, C., & Feely, R. (2012). The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: implications for near-term ocean acidification effects. *Limnology & Oceanography, 57*(3), 698-710.

Bates, N. R., & Mathis, J. T. (2009). The Arctic Ocean marine carbon cycle: evaluation of air-sea CO2 exchanges, ocean acidification impacts and potential feedbacks. *Biogeosciences*, *6*(11), 2433–2459. doi:10.5194/bg-6-2433-2009

Ben-Yaakov, S. (1973). pH Buffering of Pore Water of Recent Anoxic Marine Sediments. *Limnology and Oceanography*, *18*(1), 86–94. Retrieved from http://www.jstor.org/stable/2834527

Brink, K.H., & Chameides, W.L. (Eds.). (2010). *Ocean acidification : a national strategy to meet the challenges of a changing ocean*. Washington, D.C.: National Academies Press.

Caldeira, K., & Wickett, M. E. (2005). Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Journal of Geophysical Research: Oceans*, *110*(C9), C09S04. doi:10.1029/2004JC002671

Department of Geo-Information Science and Earth Observation. (n.d.). *Particle-size analysis.*

    Enschede, Netherlands: University of Twente. Retrieved from

    http://www.itc.nl/PDF/Organisation/Our%20Institute/GeoScience%20Lab/SOP-Particle-

    Size-Determination.pdf

Doney, S. C., Fabry, V. J., Feely, R. A., & Kleypas, J. A. (2009). Ocean acidification: the other

    CO2 problem. *Marine Science*, *1*(1), 169-192.

    doi:0.1146/annurev.marine.010908.163834

Dupont, S., Dorey, N., Stumpp, M., Melzner, F., & Thorndyke, M. (2013). Long-term and trans-

    life-cycle effects of exposure to ocean acidification in the green sea urchin

    Strongylocentrotus droebachiensis. *Marine Biology*,*160*(8), 1835–1843.

    doi:10.1007/s00227-012-1921-x

Faulkner, P. (2011). Quantifying shell weight loss in archaeological deposits. *Archaeology in*

    Oceania, *46*(3), 118–129.

Google Earth. 2014. Baynes Sound. Accessed on July 12, 2014.

Green, M. A., Waldbusser, G. G., Reilly, S. L., Emerson, K., & O'Donnell, S. (2009). Death by

    dissolution: Sediment saturation state as a mortality factor for juvenile bivalves.

    Limnology & Oceanography, 54(4), 1037-1047.

HANNA Instruments. (2012). *Deionized Water Safety Data Sheet*. Retrieved from

    http://www.hannainst.com/sds/SDS_Deionized%20Water_2012-04-19.pdf

Intergovernmental Panel on Climate Change. (2013). Climate Change 2013: The Physical

    Science Basis, Cambridge University Press, New York.

Martz, T. R., Connery, J. G., & Johnson, K. S. (2010). Testing the Honeywell Durafet for

    seawater pH applications. *Limnology and Oceanography: Methods*, *8*, 172–184.

    doi:10.4319/lom.2010.8.172

Metzger, D. C. (2012). *Characterizing the effects of ocean acidification in larval and juvenile

    Manila clam, Ruditapes philippinarum, using a transcriptomic approach* (M.S.).

    University of Washington, United States -- Washington. Retrieved from

    http://search.proquest.com.ezproxy.royalroads.ca/docview/1034896944/abstract?accounti

    d=8056

Ministry of Agriculture. (2013). British Columbia seafood industry: Year in review 2012.

    Victoria: Agriculture Science and Policy Division, Ministry of Agriculture.

Ministry of sustainable resource management coast and marine planning branch. (2002). The

    Baynes Sound coastal plan for shellfish aquaculture (Issue Brief No. 960208-4). Victoria,

    Canada: Truscott, J., Deegan, R., Winterbottom, G., Askew, C., Cruickshank, D.,

    Williams, D., Carswell, B., Russell, J., Hamilton, C., Davis, J., Hubbard, B., Webb, R.,

    Marcus, K., & Brock, K.

Munroe, D., & McKinley, R. S. (2007). Commercial Manila clam (Tapes philippinarum) culture

    in British Columbia, Canada: The effects of predator netting on intertidal sediment

    characteristics. *Estuarine, Coastal and Shelf Science*, *72*(1–2), 319–328.

    doi:10.1016/j.ecss.2006.10.025

National Oceanic and Atmospheric Administration. (2013). *State of the Science Fact Sheet:*

    Ocean Acidification. Retrieved from

http://www.noaa.gov/factsheets/new%20version/SoS%20Fact%20Sheet_Ocean%20Acidi

fication%2020130306%20Final.pdf

Parker, L. M., Ross, P. M., O'Connor, W. A., Borysko, L., Raftos, D. A., & Pörtner, H.-O.

(2012). Adult exposure influences offspring response to ocean acidification in

oysters. *Global Change Biology*, *18*(1), 82–92. doi:10.1111/j.1365-2486.2011.02520.x

Quinn, G. P., & Keough, M. J. (2002). *Experimental Design and Data Analysis for Biologists*.

Cambridge University Press.

Rérolle, V. M. C., Floquet, C. F. A., Mowlem, M. C., Connelly, D. P., Achterberg, E. P., &

Bellerby, R. R. G. J. (2012). Seawater-pH measurements for ocean-acidification

observations. *TrAC Trends in Analytical Chemistry, 40*, 146–157.

doi:10.1016/j.trac.2012.07.016

Ribas-Ribas, M., Rérolle, V. M. C., Bakker, D. C. E., Kitidis, V., Lee, G. A., Brown, I., Tyrrell,

T. (2014). Intercomparison of carbonate chemistry measurements on a cruise in

northwestern European shelf seas. *Biogeosciences*, *11*(16), 4339–4355. doi:10.5194/bg-

11-4339-2014

Sabine, C.L., Feely, R.A., Gruber, N., Key, R.M., Lee, K., Bullister, J.L., Wanninkhof, R.,

Wong, C.S., Wallace, D.W.R., Tilbrook, B., Millero, F.J., Peng, T-H., Kozyr, A., Ono,

T., & Rios, A.F. (2004). The oceanic sink for anthropogenic CO2. *Science*, *305*(5682),

367-371.

Talmage, S.C. & Gobler, C.J. (2010). Effects of past, present, and future ocean carbon dioxide

concentrations on the growth and survival of larval shellfish. *Proceedings of the

National Academy of Sciences of the United States of America*, *107*(40), 17246-17251.

Thompson, D.S. (1995). Substrate additive studies for the development of hardshell clam habitat in water of Puget Wound in Washington State: An analysis of effects of recruitment , growth, and survival of the Manila clam, Tapes phillippinarium, and on the species diversity and abundance of existing benthic organisms. *Estuaries, 18* (1A), 91-107.

Timmins-Schiffman, E., O'Donnell, M. J., Friedman, C. S., & Roberts, S. B. (2013). Elevated pCO2 causes developmental delay in early larval Pacific oysters, Crassostrea gigas. *Marine Biology*, *160*(8), 1973–1982. doi:10.1007/s00227-012-2055-x

Vorres, K.S., (n.d.). Coal Slurry pH Studies. Argonne, Illinois: Argonne National Laboratory. Retrieved from https://web.anl.gov/PCS/acsfuel/preprint%20archive/Files/40_2_ANAHEIM_04-95_0228.pdf

Wootton, J. T., Pfister, C. A., & Forester, J. D. (2008). Dynamic patterns and ecological impacts of declining ocean pH in a high-resolution multi-year dataset. *Proceedings of the National Academy of Sciences*,*105*(48), 18848–18853. doi:10.1073/pnas.0810079105

Appendix A: pH Instrument

The two sites measured with both the glass electrode and Durafet were Taylor and N. of Taylor. The figures below provide a graphical representation of the variability of pH measurements between intertidal zones and quadrats for both pH instruments.
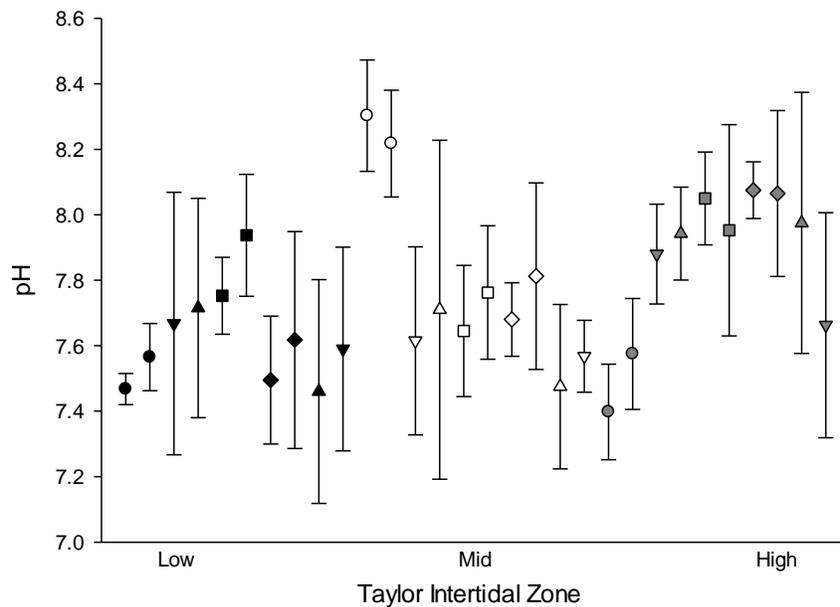

Figure 14. Comparison of mean glass electrode and mean Durafet pH measurements by quadrat at Taylor site. Each quadrat pair is designated by a unique symbol with the glass electrode measurement first and the Durafet following. The intertidal zones are distinguished by colour; black represents the low-intertidal zone, white represents the mid-intertidal zone and grey represents the high-intertidal zone. Vertical bars denote 0.95 confidence intervals.
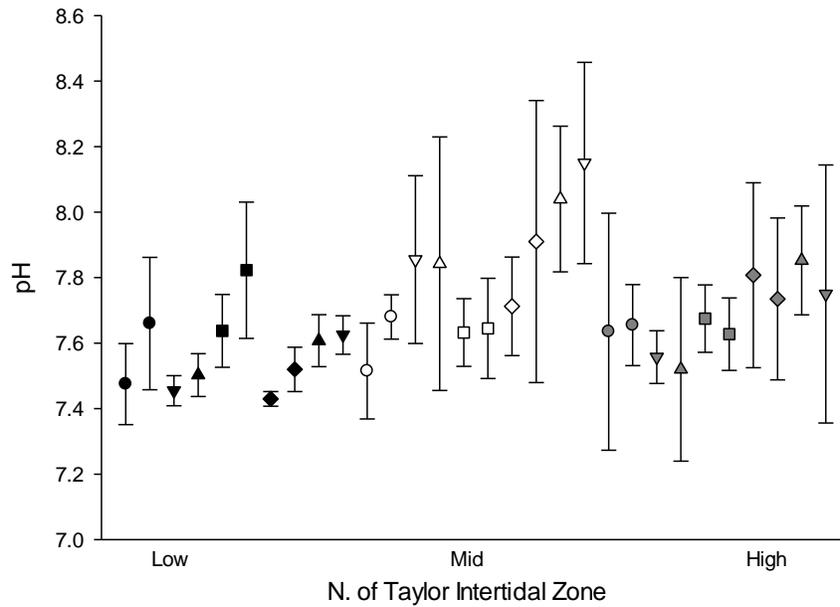
Figure 15. Comparison of mean glass electrode and mean Durafet pH measurements by quadrat at N. of Taylor site. Each quadrat pair is designated by a unique symbol with the glass electrode measurement first and the Durafet following. The intertidal zones are distinguished by colour; black represents the low-intertidal zone, white represents the mid-intertidal zone and grey represents the high-intertidal zone. Vertical bars denote 0.95 confidence intervals.

Appendix B: Glass Electrode pH

This research is the first work to assess the sediment pH in Baynes Sound. The following figures may be beneficial to industry for future research as well as to see the variability of pH measurements at multiple spatial scales for pH measurements conducted using the glass electrode.
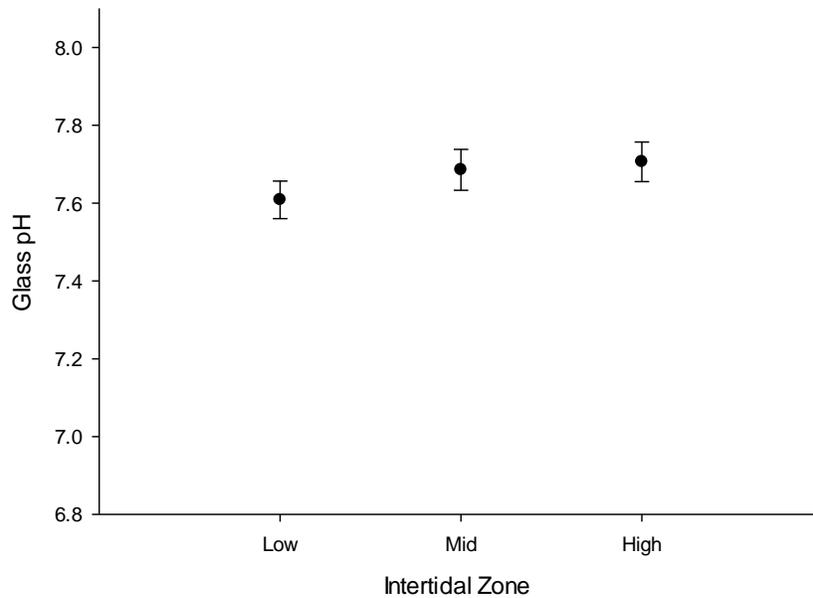
Figure 16. Mean glass electrode pH measurements for intertidal zones of farmed and non-farmed sites combined (Reid, Taylor, Deep Bay and N. of Taylor). Vertical bars denote 0.95 confidence intervals.
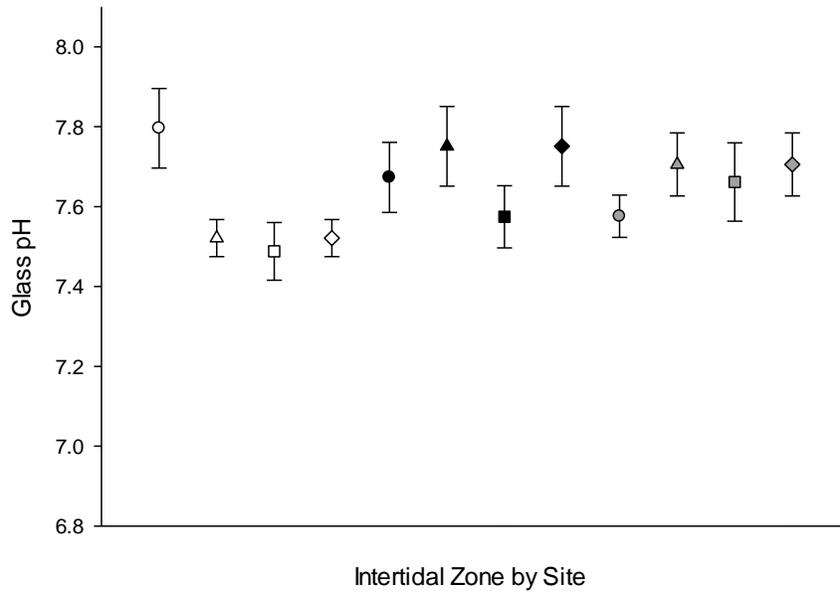
Figure 17. Mean glass electrode pH measurements by intertidal zone (low: white, mid: black, high: grey) for Reid (⬤), Taylor (▲), Deep Bay (■) and N. of Taylor (◆) sites. Vertical bars denote 0.95 confidence intervals.
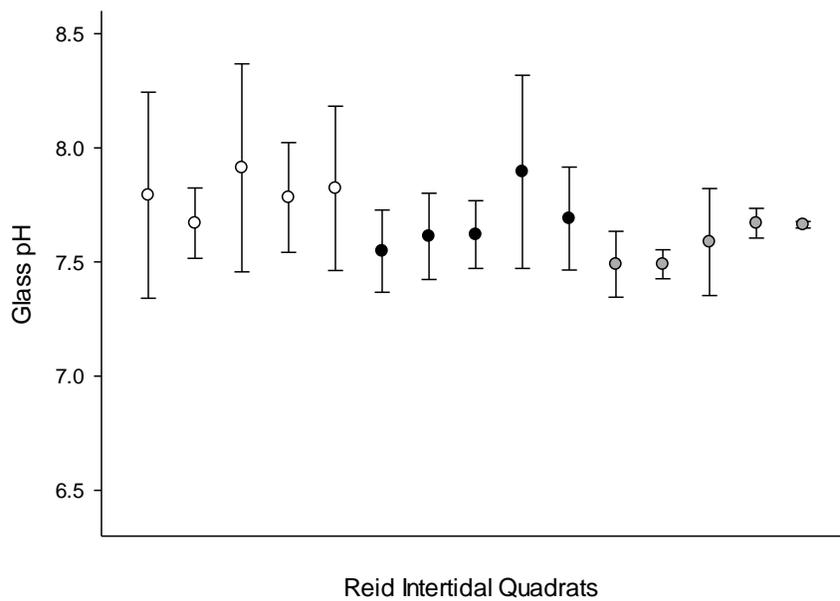
Figure 18. Reid mean glass electrode pH measurements for quadrats: low (○), mid (⬤) and high (◉). Vertical bars denote 0.95 confidence intervals.
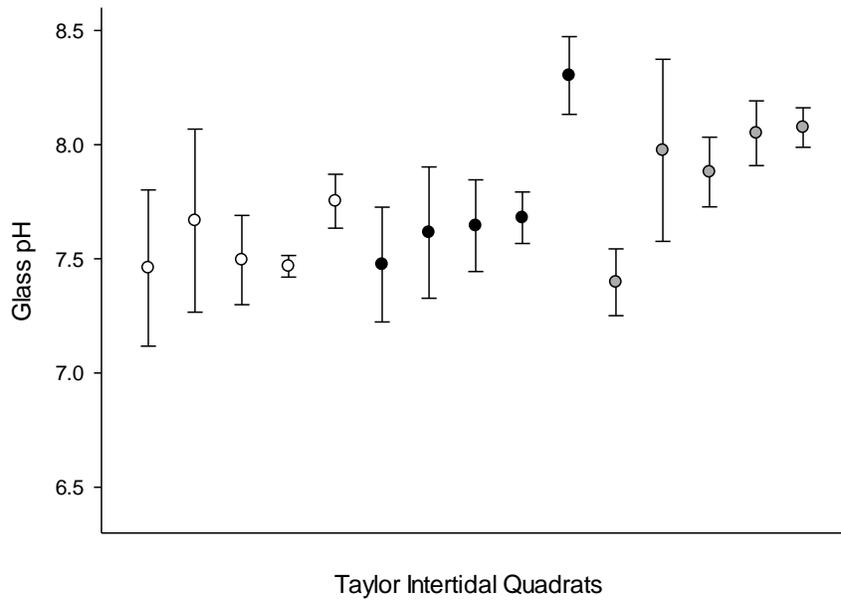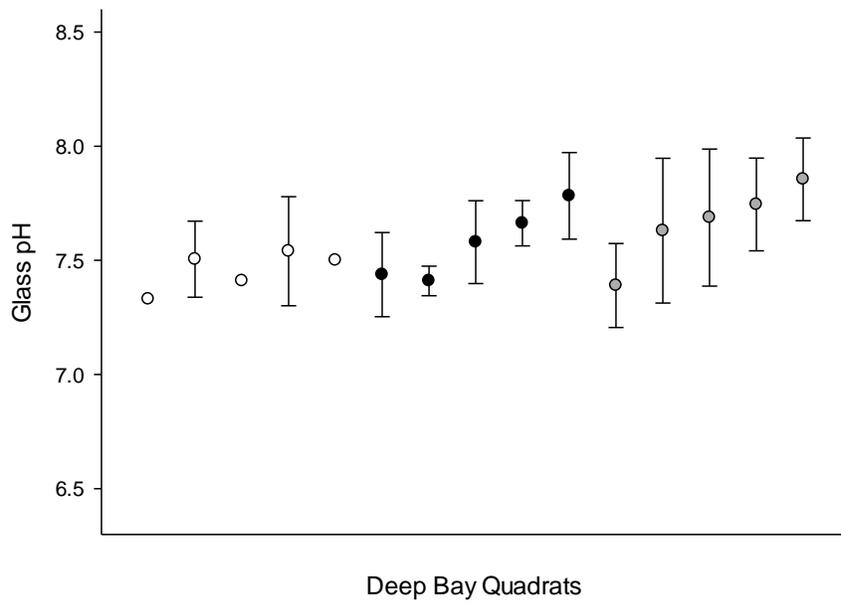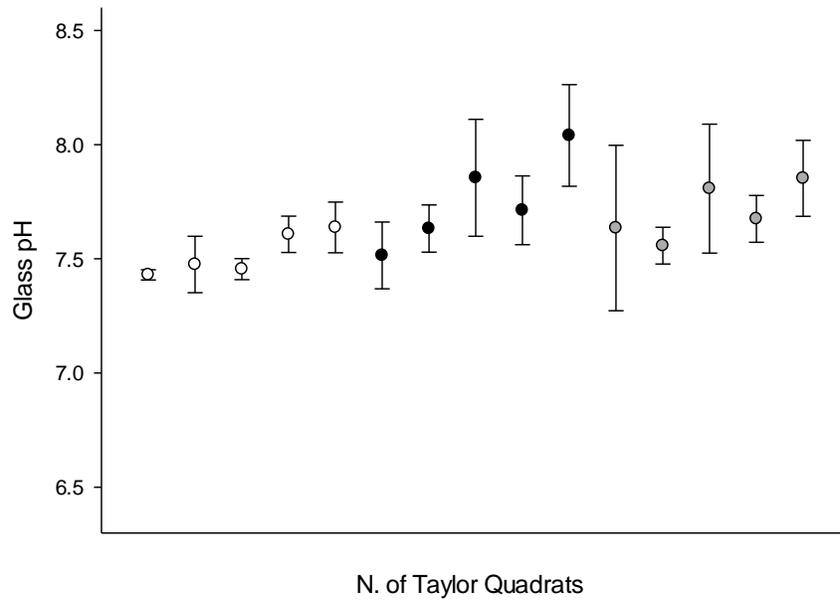
Figure 19. Taylor mean glass electrode pH measurements for quadrats: low (○), mid (◆) and high (◉). Vertical bars denote 0.95 confidence intervals.

Figure 20. Deep Bay mean glass electrode pH measurements for quadrats: low (○), mid (◆) and high   (◉). Vertical bars denote 0.95 confidence intervals.
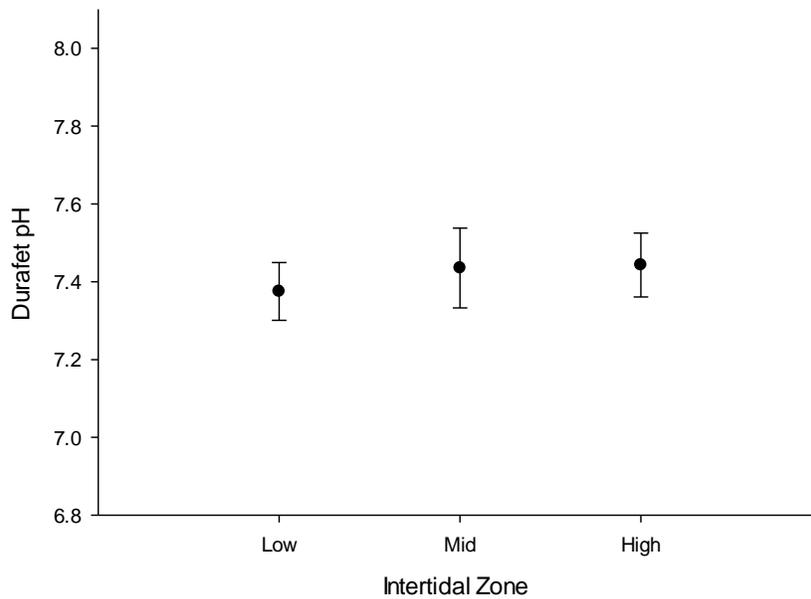
Figure 21. N. of Taylor mean glass electrode pH measurements for quadrats: low (○), mid (✦) and high (◓). Vertical bars denote 0.95 confidence intervals.

Appendix C: Durafet pH

pH measurements were conducted using two instruments. As above, the following figures may be beneficial to industry for future research as well as to see the variability of pH measurements at multiple spatial scales for pH measurements conducted using the Durafet.

Figure 22. Mean Durafet pH measurements for intertidal zones of farmed and non-farmed sites combined (Tran, Taylor, Reserve and N. of Taylor). Vertical bars denote 0.95 confidence intervals.
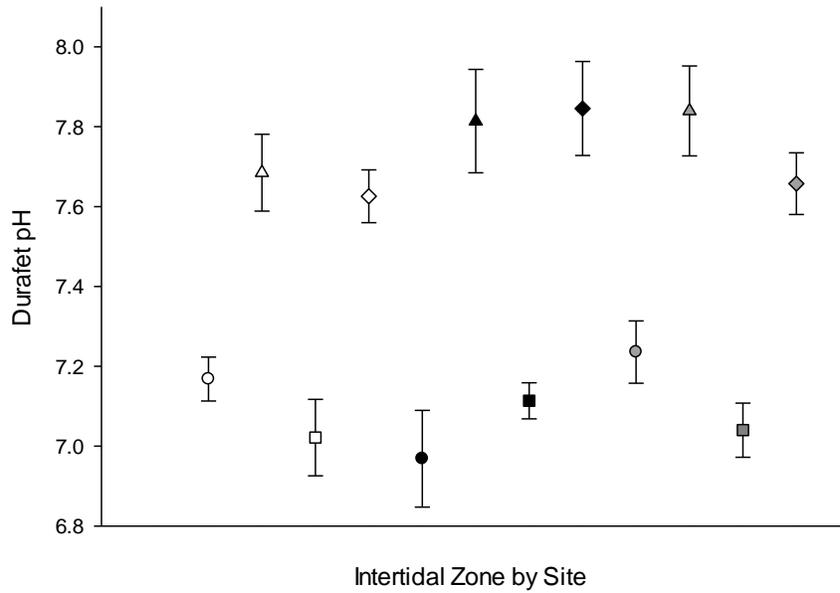
Figure 23. Mean Durafet pH measurements by intertidal zone (low: white, mid: black, high: grey) for Tran (✱), Taylor (▲), Reserve (■) and N. of Taylor (◆) sites. Vertical bars denote 0.95 confidence intervals.
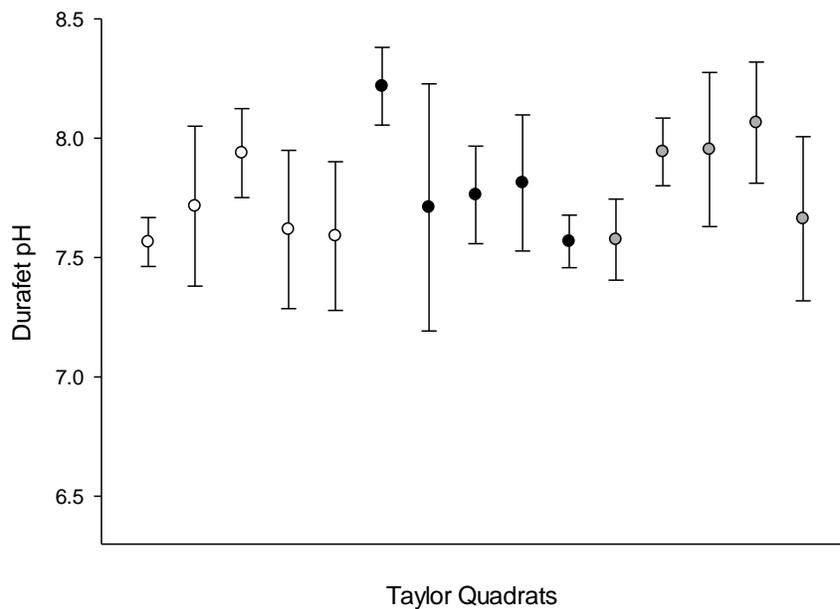

Figure 24. Taylor mean Durafet pH measurements for quadrats: low (○), mid (✱) and high (◉) intertidal zone. Vertical bars denote 0.95 confidence intervals.
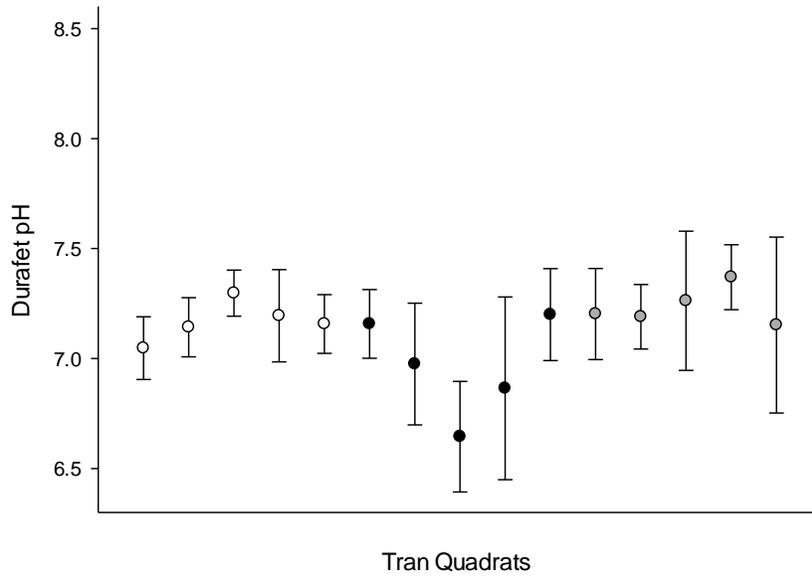
Figure 25. Tran mean Durafet pH measurements for quadrats: low (○), mid (◉) and high (◉) intertidal zone. Vertical bars denote 0.95 confidence intervals.
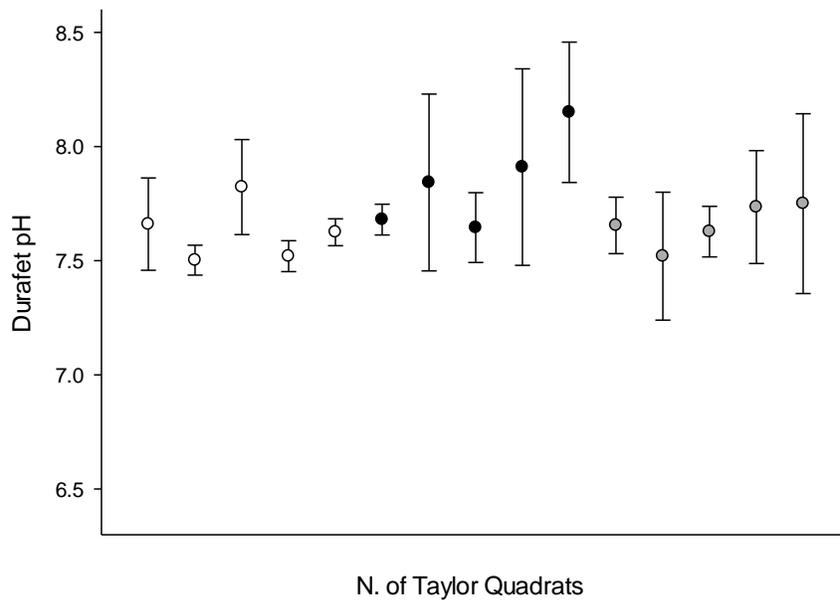

Figure 26. N. of Taylor mean Durafet pH measurements for quadrats: low (○), mid (◉) and high (◉) intertidal zone. Vertical bars denote 0.95 confidence intervals.
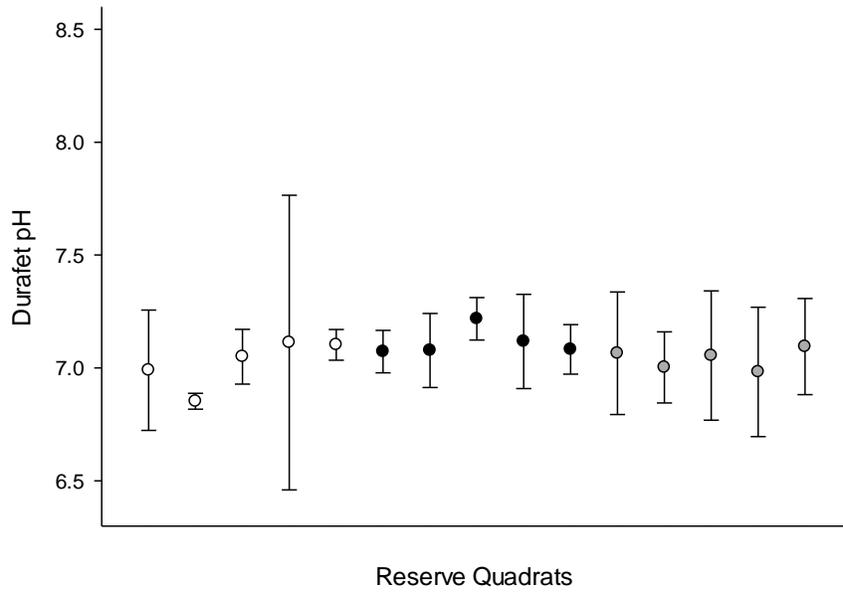
Figure 27. Reserve mean Durafet pH measurements for quadrats: low (○), mid (◉) and high (◉) intertidal zone. Vertical bars denote 0.95 confidence intervals.