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Clam garden bivalve dietary responses and trophic shifts in relation to environment

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Clam garden bivalve dietary responses and trophic shifts in relation to environment

By

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Master's Thesis

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Octavio Cruz

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Clam garden bivalve dietary responses and trophic shifts in relation to environment

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By
Octavio Cruz
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Abstract

Pacific Northwest Indigenous communities historically managed various terrestrial and marine environments to increase access to traditional foods. Clam gardens are a method of Indigenous aquaculture, involving altering of beach gradients, to increase clam habitat that have been used since pre-contact. Ecological examinations of clam gardens have been increasingly led by First Nations groups, as restoration projects begin on clam gardens aiming to re-establish pre-contact conditions.

The purpose of this study was to measure condition indices of traditionally-harvested littleneck clams (*Leukoma staminea*), dietary parameters and beach-level oceanographic conditions were used to investigate differences between clam gardens from non-walled beaches in Kanish Bay, Quadra Island, British Columbia. I utilized non-metric multidimensional scaling, distance-based redundancy analysis, and Bayesian 3-source isotopic mixing models to examine environmental drivers on bivalve dietary composition and condition at four clam garden sites and four non-walled sites. We aimed to capture differences between site types, to clarify ecological relationships on clam gardens useful for restoration.

There were no significant differences in environmentally-available food biomarkers, based on environmentally available fatty acid methyl ester (FAME) profiles. *L. staminea* adductor muscle tissue FAME biomarkers suggest available dietary items were primarily dinoflagellates, followed by diatoms, and a much smaller number of bacterial signatures.

Bivalve FAME proportions differentiated site types indicating increased saturated fatty acids (SFAs) and decreased monounsaturated fatty acids (MUFAs) in clam garden sites compared to non-walled beaches. Stable isotope dietary proportion estimates indicated that site types were not different proportions of food particulates, with a majority of all site diets originating from oceanic food sources (POM).

Stable isotope trophic positioning indicated that clam garden *L. staminea* bivalves feed at a higher at a tropic level with a narrower dietary compared to non-walled clams. This indicates either a major difference in dietary particulate intake, increased physiological stress driving differences between site types, or a combination of both.

Correlations in a distanced-based redundancy (db-RDA) analysis indicted that both changes in feeding behavior, increases in primary productivity over clam garden sites, and decreased stress could be driving observed trophic shifts and fatty acid differences.

We conclude that clam garden *L. staminea* bivalve trophic shifts and fatty acid changes are consistent with stress-alleviated bivalve responses, and matched correlated variables in our db-RDA. I theorize that more stable oceanic parameters lead to increased bivalve growth rates in clam gardens, as decreased stressors were correlated with filtration feeding behavior indicated by FAME profile shifts, increased condition indices, and trophic shifts observed in this study.

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Introduction

Coastal intertidal environments in British Columbia are gathering points for coastal Pacific Northwest Indigenous communities where traditional foods are stewarded through interactive management activities (Berkes, 2012; Groesbeck et al., 2014; Mathews & Turner, 2017). Indigenous communities of the Pacific Northwest possess deeply rooted management systems for coastal resources spanning from mountaintop (Deur et al., 2005; Turner et al., 2011), to mid-elevation coastal rainforests and meadows (Turner & Turner, 2008), in coastal estuaries (Deur et al., 2013) and intertidal regions (Augustine & Dearden, 2014; Lepofsky & Caldwell, 2015).

Clam gardens are one particularly visible example of Indigenous resource management. Clam gardens are modified intertidal beaches created by Indigenous communities spanning from Alaska (Moss, 2018) to the Salish Sea, along the Northeast Pacific Coast (Groesbeck et al., 2014; Deur et al., 2015). There is extensive literature on the archeological histories and ethno-ecological uses of clam gardens in the Kanish Bay region, in the traditional territories of the Northern Coast Salish and Southern Kwakwaka'wakw First Nations, including specifically the We Wai Kai First Nation and the Laich-qwil-Tach Treaty Society (Groesbeck et al., 2014; Neudorf et al. 2017; Toniello, 2017; Smith et al., 2019). The clam gardens within Quadra Island have been carbon dated to approximately 3500 years before present (Smith et al., 2019), with various forms built around naturally occurring landforms within the bay. Clam garden shapes vary according to locations along the Pacific Northwest (Lepofsky et al., 2015), and similar variation occurs within Kanish Bay (Smith et al., 2019).

Clam gardens are alike by possessing a main wall (Lepofsky et al., 2015) built in the lower intertidal by Indigenous practitioners. Sedimentation rates are consistently higher in clam gardens compared to non-walled beaches, as found by Neudorf et al. (2017) and increase the optimal growth area for targeted clam species by reducing the slope of the beach (Lepofsky et al., 2015). The invertebrate communities within clam gardens have been shown to have more abundant bivalve species overall (Groesbeck et al., 2014; Jackley et al., 2016; Cox et al., 2020), in addition more diverse infaunal communities (Cox et al., 2020) compared to non-modified

mudflat beaches. Bivalves within clam gardens show increased biomass, densities, and growth rates (Groesbeck et al., 2014) despite lower instances of traditional management today (Deur et al., 2015; Cox et al., 2020). Traditional knowledge holders have discussed management techniques as a key component to the system, promoting deposition of shell hash materials, tilling of clam garden sediments increasing aeration, and tending clam garden walls (Deur et al., 2015). Unfortunately, most clam gardens have seen decreasing management and disuse, as intertidal spaces are currently contended with settler usage (Deur et al., 2013; Silver et al., 2014) and removal of Indigenous communities from these coastal areas.

Nevertheless, there are increasing opportunities for Indigenous communities to re-connect with traditional practices and apply traditional ecological knowledges today in scientific monitoring programs and joint community-management efforts (Berkes et al., 2000). There have been recent joint efforts within British Columbia, Canada, to introduce integrated management programs for clam gardens, specifically in the Gulf Islands National Park Reserve that combine Indigenous community management with scientific input for a co-operative strategy to restore the clam gardens in that region (Augustine & Dearden, 2014). There are increasing benefits to both First Nations communities and conservation as traditional practices are re-introduced (Augustine & Dearden, 2014).

As clam garden restoration and management practices increase by First Nations communities in the Southern Gulf Islands, there is an increased need to examine the current state of the restored ecosystem as well as understand main drivers to examine that affect target species within that ecosystem. Bivalves represented a traditional food with deep cultural ties to coastal First Nations (Reid, 1980) and are central to understanding restoration progress of clam gardens. But, clam garden bivalves are currently growing at lower rates broadly, compared to when they were actively managed by First Nations communities (Toniello, 2017). Bivalve restoration in clam gardens has focused primarily on restoration of the rock walls, and there are increasingly questions about whether conditions are changing at the site-level for bivalves, such as new dietary shifts, impacts from increasing winter storms, and increased anthropogenic impacts due to increased coastal populations by settlers. I aimed to examine the bivalves in

clam gardens and their current dietary relationships to the modern environment, as well as correlations with modern-day environmental parameters that could be impacting them.

Fatty acids have been utilized in the marine environment as qualitative markers for dietary relationships, reviewing both producer and consumer relationships (Dalsgaard et al., 2003; Budge et al., 2006; Iverson, 2009; Kelly & Scheibling, 2012). Distributions of fatty acids of a consumer species can be used to determine spatial and temporal variations in diets, among individuals or larger communities (Budge et al., 2006). Additionally, fatty acid species proportions capture longer dietary timeframes (weeks to months), with limited sampling biases compared to traditional stomach-content dietary analysis (France, 1995; Kang et al., 1999; Iverson et al., 2004). Fatty acids have also been used in marine ecosystems as biomarkers for different primary producers (Shin et al., 2008; Zhao et al., 2013), which have specific fatty acid profiles that are transferred relatively unchanged to consumers (Shin et al., 2008). Different bivalve dietary markers have been determined previously for plankton (Dalsgaard et al., 2003; Zhao et al., 2013), as well bacterial signatures (Ying et al., 2012; Zhao et al., 2013), and terrestrial organic matter (Budge & Parrish, 1998).

Stable isotopes can give information on trophic structure of target organisms (Layman et al., 2007), such as food web complexity, trophic levels for bivalves, and overall evenness and range of dietary metrics in a system. Multiple stable isotope mixing models are increasing in use to estimate dietary proportion of source signals in ecological studies (Parnell et al., 2013; Stock et al., 2018). I utilized a 3-source mixing model, comparing high-tide detritus, low-tide detritus and oceanic food sources from various positions in clam garden and non-walled beaches to better understand the deposition of food sources onto each beach type. Additionally, I examined trophic positioning between beach types as well as dietary proportion data, to gain a snapshot of the trophic metrics associated with stable isotopic proportions in clam gardens.

I examined the main differences in dietary proportion, fatty acid profiles, and environmental variability within clam gardens and non-walled beaches in Kanish Bay, Quadra Island, British Columbia, Canada (Figure 1). I hypothesize that the clams will vary in condition based on differences in dietary characteristics, proportion of dietary items, and potential differences in

abiotic environments associated with stress. I examined fatty acid profiles and stable isotope signatures for littleneck clams (*Leukoma staminea)* on four clam garden beaches in Quadra Island, and four non-walled beaches in the same bay, to review potential differences between the sites. I additionally compared the relative health indices of bivalves according to differences found in each site, to determine if there is a relationship between clam condition and site types.

Methods

Bivalve Sample Processing and Analysis

Field sampling occurred during the low tides from May 18th to May 22nd, 2019, and from July 13th to July 20th, 2019. A total of eight beach sites were sampled based on presence of *L. staminea* and accessibility throughout Kanish Bay (Figure 1). We sampled along 10-meter transects, running parallel to the water line, at ranked low (0.5 – 0.7 meters above Mean Sea Level) and high (1.0 to 1.5 meters above Mean Sea Level) tidal heights per beach associated with ideal clam habitat (Groesbeck et al. 2014), for a total of two transects per beach. Transect placement was based on closest available sampling area near optimal clam habitat between 0.5 to 1.5 m (Groesbeck et al., 2014).

We collected clams from each transect, by digging a 1-meter by 10-meter trench, to a depth of 0.25 to 0.5 meters below the surface on the lower-transect. I had a sampling goal of 10 *L. staminea*, and a realized sample size of seven per transect at each site. Clams were frozen and processed within 30 days for fatty acid methyl ester (FAME) extractions and $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis. A total of 224 clams were collected and processed for all sites. A shell-weight condition index was calculated per clam as in Filgueira et al. (2013), and in Mann (1992). A shell-weight condition index was picked due to decreased variation between samples, in addition to less variation related to clam size, limiting effects from juvenile to adult variation in tissue-to-shell ratios. This was calculated as follows:

Shell-Weight Condition Index = 1000 (Dry Tissue Weight (g)/ Dry Shell Weight (g))*

Clams with a higher condition index indicate increased tissue mass, compared to total shell weights, allowing us to create a generalized scale of bivalve health, by site type.

Fatty acid methyl ester (FAME) extraction was completed on each bivalve sample on the anterior adductor muscle utilizing a one-step extraction-transesterification as in Lewis et al. (2000). Extracted FAME samples were stored at -20 C° and analyzed by gas chromatography-mass spectrometry (GS-MS) on a Thermo Finnigan Trace GC/MS with a TR-5ms 60 m x 0.32 µm I.D. column in positive-ion mode. All sample chromatograms were corrected for any peaks that appeared in the blank. This generated a FAME profile, measured as relative percent area, per clam sample, associated with both a bivalve condition index, a sampling site, and associated environmental parameters governing each site.

Bivalve stable isotope samples were completed by removing a small subsample of desiccated clam muscle tissue from the posterior adductor muscle. If posterior adductor muscle was not found due to small clam size, foot muscle tissue was substituted. Bivalve muscle tissues were stored in pressed tin capsules, within target weight for marine invertebrates (1.50 mg), as per University of California, Davis, Stable Isotope Facility (UC Davis SIF) instructions. Samples were sent to UC Davis SIF and processed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

Water column Parameter Collection

I collected oceanographic data at 14 stations, with eight inshore sites (over inundated clam beaches) and six in the nearshore within in Kanish bay (Figure 1). I sampled at four depths at each nearshore site, from the surface (0.1 m), one-meter, two-meter, and three-meter depths. I sampled at the surface (0.1 m) and bottom (variable by tidal height) at inshore sites above sampling beaches. I measured water temperature (C°), salinity (PSU), dissolved oxygen (mg/L), and dissolved oxygen (percent saturation) using a calibrated YSI Pro-2030 with a polar-graphic membrane.

We filtered seawater (1 L) for environmental FAME and δ13C-δ15N stable isotope samples using a combusted GFF filter from a 2 m depth at nearshore sites, and surface and bottom of inshore.

Chlorophyll-*a* samples were collected by filtering 500 ml of seawater through glass fiber filters (GF/F) at each sampling station depth. Chlorophyll-*a* was extracted using 100% acetone, from GF/F filters and freeze-dried sediment samples. Extracted chlorophyll-*a* was processed using acid-verification chlorophyll and phaeopigment analyses, described by Caspers (1985), measured a using a calibrated Turner Designs TD700 fluorometer with a daylight white lamp, 340-500 nm bandpass excitation filter and >665 nm sharp cut emission filter. Fluorescence values from the chlorophyll-*a* extractions were converted into µg per milliliter of chlorophyll-*a*, as well as µg per milliliter of phaeopigments present in each sample.

All filter samples were stored separately and frozen at -20 C° and processed within 30 days of sample collection. Stable isotope filters were freeze-dried, ground, and weighed to approximately 125-150 µg according to UC Davis SIF processing protocols Samples were processed for $\delta^{13}C$ and $\delta^{15}N$ isotope using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. Samples were run against in-house standards with a mean absolute accuracy for ^{15}N of ±0.06 ‰ and ^{13}C of ±0.04 ‰ (Sercon Ltd., Cheshire, UK).

Beach Parameter Collection

Transect level temperature was determined by use of Hobo Tidbit V2 temperature loggers programed to record 6-minute intervals with an accuracy of +/- 0.2 (°C). Loggers were deployed and recorded from May 24th to July 13th, 2019. Observed sea level data were retrieved from the nearest Department of Fisheries and Oceans Campbell River tidal station (DFO, 2020). Tidal heights were used to calculate inundation events for each transect. Seawater temperature was measured per inundation period per transect for range comparisons to estimate temperature during *L. staminea* feeding times.

Site-specific algae cover was collected using a ranked-quadrat method (Staehr et al., 2000) at 3 randomly determined points placed on the upper-half of the 10-meter transect per site, away from the water line. Quadrat sub-sections were ranked (0 = 0%, 1 = 1-24%. 2 = 25-49%, 3 = 50-74%, 4 = 75-100%), and summarized by median algae cover per transect, per site.

Sediment samples were collected at 3 randomly determined points on the 10-meter transect, opposite of the algae cover quadrats, to reduce disturbance, per site. Sediment size collected for the May sampling period, and sediment chlorophyll-*a* samples collected at each site for all sampling periods. Sediment grain size samples were collected using a coring tube (98 mm in height, 65 mm in diameter) to collect a core with a volume of approximately 300 ml of sediment. Grain size samples were dried at ambient laboratory temperatures for a 3-week period and stored dry prior to lab analysis.

Sediment grain-size analysis was completed according to Poppe et al. (2000), on a randomly quartered section of re-hydrated sample. Samples were separated from coarse and fine fragments, by wet-sieving through 63-µm mesh. Fine sediment size fractions were measured using known-settling velocity sampling, for size fractions of size 5 to 9 φ. Coarse sediments were dried and processed using 7 sieves (size φ=-2, -1, 0, 1, 2, 3, 4) and a sediment catch for any remaining <63--µm sample.

All sediment size categories were converted into percentages utilizing the GRADISTAT statistical package from Blott and Pye (2001). Samples from NW-04 were not processed due to limitations in physical lab access due to the COVID-19 pandemic and are stored for future completion. NW-04 sediment size profiles were therefore excluded from this analysis. Additionally, during processing, if final sample size categories exceeded initial sample weights, they were treated as a laboratory error, and removed from the final sample count. A total of 32 sediment sample size profiles were completed, for sediment grain size analysis.

Sediment chlorophyll- *a* samples were collected at the three randomly determined points along transects, utilizing a de-capped 5 ml syringe as a coring device, and stored frozen at -20 C°, to collect 2 ml sediment samples. I collected a total of 96 sediment chlorophyll- *a* samples. Samples were freeze-dried and processed using acid-verification chlorophyll and phaeopigment

analyses using the same fluorimeter and protocols from Caspers (1985). Fluorescence values from the chlorophyll-*a* extractions were converted into µg per milliliter of chlorophyll-*a*, as well as µg per milliliter of phaeopigments present in each sample.

Statistical Analysis

I utilized exploratory ordination methodologies to compare differences between clam garden sites and non-walled sites. Physiological clam parameters including FAME profiles, and condition indices, as well as environmental parameters such as oceanic parameters, and site parameters were included.

Non-metric multidimensional scaling (nMDS) was used to assess grouping according to site types for both extracted FAME profiles, and sediment profiles, using Bray-Curtis Dissimilarity. Bray-Curtis semi-metric dissimilarities were used to quantify the differences in variable profiles between each site, in order to account for percentage measurements for FAME profiles and sediment grain size profiles, as well as account for abundances of variables in the ordination. Fatty acid methyl esters that occurred less than 5 times in all clam samples (rare FAMEs) were removed from the FAME nMDS analysis, to limit undue weighting of rare species (Warton et al., 2012).

A permutation multivariate analysis of variance (PERMANOVA) (Anderson, 2001; McArdle & Anderson, 2001) was run to test for significant statistical differences between site types, according to FAME profiles and clam condition indices, utilizing the Bray-Curtis dissimilarity matrix from the FAME Non-metric multidimensional scaling (nMDS). This was additionally done for sediment grain size, by site type. This was done to determine if there was a statistically significant separation between site types, and ordinated variable groups.

I utilized the Mann-Whitney U-test to test for differences between clam gardens and non-walled sites in relevant fatty acid biomarker groups' relative percent areas, as a non-parametric alternative to a Student's T-test (McKnight & Najab, 2010). This was done for main fatty acid biomarkers (bacterial markers) and groupings by type (saturated fatty acids,

monounsaturated fatty acids, and polyunsaturated fatty acids) to examine main profile differences that relate to diet and overall FAME profile shifts by site type.

I used distanced-based redundancy analysis (db-RDA) to assess how physiological and environmental variables correlated to condition indices. The RDA model had 137 clam samples, wherein individual samples included FAME profiles, shell-weight condition indices, and shell thickness. Environmental variables were minimum chlorophyll-*a*, minimum salinity, minimum dissolved oxygen (mg/l), median ranked percent algal cover (percentage), sediment chlorophyll-*a* range (µg/ml), mean sediment chlorophyll-a (ug/l), and mean phaeopigments (µg/ml). Distance-based redundancy analysis was run using Gower's distance, which generated a distance matrix that was run through a principal coordinates analysis (PCoA), from which eigenvalues can be extracted and run in an RDA (Legendre and Legendre, 2012). The resulting RDA shows ordinations of environmental and physiological variables in the model, and can be examined for significance for axis and environmental variables.

Stable Isotope Trophic Level Analysis

Trophic structure and dietary patterns for clams were determined using six Layman et al. (2007) metrics. However, Layman metrics have known shortcomings including sensitivity to sample size, inaccuracies in unbalanced sample size, and lack of incorporation of natural variability in natural systems (Jackson et al., 2011).

We utilized the Stable Isotope Bayesian Ellipses in R (SIBER) method from Jackson et al. (2011) to address known shortcomings of Layman metrics. The SIBER method is more optimal among data sets of differing sample sizes, in addition to being unbiased by sample size (Jackson et al., 2011). Metrics under the SIBER method are similarly named as Layman metrics, with mode, true population values, and credible intervals demarcated.

Two isotope metrics calculated were $\delta^{15}N$ range (NR) for most enriched to most depleted $\delta^{15}N$ values and $\delta^{13}C$ range from most enriched to most depleted $\delta^{13}C$ values. Total area (TA) encompassed by stable isotope species in $\delta^{13}C$ - $\delta^{15}N$ biplot space is akin to niche space occupied per grouping variable and is related to food web trophic diversity (Layman et al.,

2007). Mean Euclidean distance of each species to the $\delta^{13}C$ - $\delta^{15}N$ centroid (CD) per grouping variable relate to trophic diversity in samples. Nearest neighbor distance (NDD) indicates species density by grouping variable and standard deviation of nearest neighbor distance (SDNND) relates to evenness of species distributions (Layman et al., 2007).

Stable Isotope Dietary Proportion Analysis

L. staminea δ13C - δ15N stable isotope ratios allowed for estimation of dietary proportion of generalized bivalve dietary sources. Samples with similar stable isotope ratios were grouped for increased clarity in our dietary proportion model, as well as tidal height where algae were found, to maintain ecological relevance (Fry, 2006). Dietary food sources were grouped into 3 ecological groups, based on location of algae detritus and seawater samples in the inshore and nearshore environments interacting with sampling beaches. Algae samples were measured at the same proportions estimated by UC Davis guidelines, and were ground thoroughly using an ethanol-sterilized glass mortar and pestle. High tide algae were a combination of bladder wrack (*Fucus* sp.) and sea lettuce (*Ulva* sp.). Low tide algae sources were a combination of bull kelp (*Nereocystis luetkeana*), seabrush (*Odonthalia floccosa*), and *Laminaria nigripes.* Oceanic samples were grouped inshore and nearshore GFF-filter isotopic samples in Kanish bay, aiming to capture phytoplankton and detritus contributions.

Trophic enrichment factors (TEFs) were estimated in two different scenarios, as per Lefebvre et al. (2009a). Scenario one used a whole body TEF estimate, and scenario two used a muscle-tissue specific TEF estimate, available for a filter-feeding bivalve (*Crassostrea gigas*) within Puget Sound, Washington, (Strom et al., *In Press*). Fractionation of stable isotopes are enriched in consumers relative to food sources and are a large source of uncertainty when estimating consumer dietary proportion in dietary mixing models (Lefebvre et al., 2009). Factors that could influence isotopic fractionation are individual physiology, environmental temperatures, growth rates, and food availability (Fry, 2006). By utilizing two scenarios, I aimed to capture the range of dietary proportion in each mixing model, focusing on the similar trends between scenarios as main dietary proportion results.

Littleneck clam bi-plot values that fell outside one standard deviation range of food source polygons were removed for the food proportion model, resulting in variation in total samples per scenario. Scenario one contained a total of 196 viable clam samples, and scenario two included a total of 72 viable clam samples. The trophic enrichment factors for scenario two lead to most samples from site NW-01 to fall outside of the standard deviation food source polygon, indicating appropriate dietary sources were not captured for that site under this scenario, or TEF estimates were out of range for *L. staminea* compared to the dietary source data from *C. gigas* in Strom et al. (*In Press*). The site NW - 01 was therefore dropped from scenario two.

Estimated dietary proportions were run using Stable Isotope Mixing Models in R (SIMMR), using site type bivalve isotopic data and aggregated sources per TEF scenario within a Bayesian framework (Markov Chain Monte Carlo), to give estimated contributions to littleneck clam (*L. staminea*) dietary proportions. Markov Chain Monte Carlo iterations for each scenario, per bivalve group, were checked for proper model creation using convergence diagnostics, which should all be close to or equal to 1. All models were examined using a posterior predictive check, by reviewing that data points **y** fell into the fitted value intervals ($\mathbf{y}_{\mathbf{rep}}$ = 50% interval), which indicates good fit. Data priors for dietary sources were not altered and left as generalist prior distributions, as I did not have data to suggest a different prior distribution. Dietary proportion trends are expected to fall within the ranges shown, assuming each scenario is on the extreme ranges of variation due to enrichment factor differences within our target species.

Results

Environmentally available fatty acid biomarkers

Environmentally available fatty acids, which relate to potential dietary items for bivalves in both clam gardens and non-walled sites, did not differ greatly across Kanish Bay. Inshore seawater samples were dominated by dinoflagellate fatty acid signatures, determined by C16:1(n-7) and C16:0 ratios less than 1 (Zhao et al., 2013), with the C16:1(n-7)/C16:0 ratio for clam gardens being 0.12 and non-walled ocean filter C16:1(n-7)/C16:0 ratio being 0.02. Mean bacterial FAME relative percent areas in clam garden surface waters (1.44%), clam garden bottom waters

(1.06%), non-walled bottom waters (1.04%) and non-walled site surface waters (0.24%) were all relatively small overall. There was also a large lack of lack of terrestrial FAME C18:2(n-6) and C18:3(n-3) biomarkers with all site types having less than 1% relative percent area.

Site level environmental variable differences and similarities

Site types were not differentiated additionally by seawater temperatures, with daily mean temperatures for both site types staying between 13 and 16 degrees Celsius, for inundated periods. Average dry times, where sites were exposed, were calculated to be 1.67 hours with a maximum dry time of 6.0 hours in a 50-day period. Inundated beach temperature means were not shown to vary significantly and were determined utilizing a 95% confidence interval around smoothed daily temperature means per day, over the total beach temperature sampling period (Figure 2).

Site types were not differentiated by sediment grain size profile ordinations despite moderate fit (nMDS using Bray-Curtis; k=2, Stress = 0.11). There was no statistically significant difference in sediment grain size profiles by site type (PERMANOVA $_{(1,37)}$, $p = 0.68$, $R^2 = 0.011$, out of 999 randomized iterations).

Sediment grain size profiles in clam gardens showed minor increased levels of grain sizes smaller than 500 µm, down to 2 µm (Figure 3), and were shown to drive minor differences by site type (SIMPER SD grain size ratios >1, for all below 500 µm except grain size 31.3 µm), though these grain sizes made up a relatively small percentage of total grain size categories on both beach types (Figure 3). Lack of differences in sediment grain size indicate relatively similar beach substrate sizes by site type, but does not capture differences in sediment composition.

Bivalve FAME profile differences by site types, by ordination and significant group shifts

We found that site type FAME differences were statistically significant (PERMANOVA $_{(1,136)}$, $p = 0.032$, $R^2 = 0.12$, out of 999 randomized iterations). Clam garden bivalve FAME profiles were significant differences to non-walled bivalve FAME profiles overall, indicating different dietary or physiological stressors impacting bivalves at each site type in Kanish Bay.

Bivalve fatty acid profiles were differentiated visually (Figure 4a, 4b) by ordination (nMDS using Bray-Curtis; k=3; Stress = 0.091). Fatty acid profile ordinations (Figure 4a) showed more consistent FAME profiles in clam gardens, compared to the increased variation in non-walled sites.

Site type fatty acid dissimilarities, determined by SIMPER analysis, were consistently driven by increased saturated fatty acid species, and decreased monounsaturated fatty acid species. Clam garden bivalve fatty acid profiles showed decreased C16:1(n-7), C15:1, C18:0, and C18:1(n-9) FAME species and increased in C16:0, i16:0, i17:0 and C17:0 FAME species as main drivers for ordination differences (SIMPER, FA species SD ratios >1).

Saturated fatty acids were found to be significantly different in clam garden bivalves compared to non-walled sites (Mann-Whitney U-Test, W = 3447, P = <0.01, α = 0.05), with higher averages occurring in clam gardens (Table 2). Monounsaturated fatty acids were found to be significantly lower in clam garden bivalves compared to non-walled bivalves (Mann-Whitney U-Test, W = 1202, P = <0.01, α = 0.05). These differences by fatty acid groups are important to overall fatty acid profile differences by site type, seen by ordination.

I found no significant differences in bacterial fatty acid species between site types (Mann-Whitney U-Test, W = 2144, P=0.35, α = 0.05). There were no consistent signatures from terrestrial fatty acid biomarkers, as I expected from lack of environmentally available species, so no test was conducted on the low (less than 2%) levels of polyunsaturated fatty acid terrestrial biomarkers detected.

Bivalve stable isotope dietary proportion and trophic niche differences by site type

Stable isotope dietary proportions were similar between both site types (Table 3). Stable isotope analysis of both muscle and whole tissue scenarios indicated that oceanic food sources were the primary diet of bivalves with supplements from intertidal detritus (Figure 5). Scenario 1 (whole-tissue) was a stronger model for visualization, and scenario 2 (muscle-tissue) indicated that increased food sources would be beneficial in the model as some food sources occupied

similar isotopic spaces. Overall, each scenario indicated similar trends, with oceanic food particulates forming the majority of bivalve dietary proportions (Table 3) in Kanish Bay.

Total area differences, from stable isotope Laymen metric calculations, indicate that clam garden *L. staminea* and non-walled *L. staminea* occupy different niche spaces and have different trophic food web diversity (Figure 6, Figure 7). Clam garden *L. staminea* had an increased $\delta^{15}N$ (‰) range and $\delta^{13}C$ range compared to non-walled *L. staminea* samples (Figure 6). Differences in $\delta^{15}N$ (‰) relate to clam garden bivalves sitting at a higher trophic position and $\delta^{13}C$ (‰) indicates changes food web complexities than non-walled bivalves.

Bivalve condition indices and Site Type

We did not find a significant difference between clam garden condition indices versus non-walled ranked mean condition indices (Mann-Whitney U-Test, U = 2061, P = 0.255). This suggests that there are significant overlaps in condition indices alone, which are not clearly defined by site types. Clam garden bivalves had a smaller range of condition indices (150.61) compared to non-walled bivalves (207.93). Means were relatively similar by site type, with clam garden bivalves averaging 161.54, and non-walled bivalves averaging 167.79.

We included condition indices in later models, to determine factors correlated with increased condition indices regardless of site type. We aimed to determine which factors were related to increased condition indices, at both site types.

Correlations between Bivalve condition indices & FAME profiles in relation to potential environmental drivers

The db-RDA showed that there was overlap within bivalve profiles by site type, in relation to environmental variables measured, but overall clam garden bivalves ordinated in less variable groupings that were more correlated to increased condition indices. Distance-based redundancy analysis (Figure 8a, 9b) was found to generate a statistically significant model (*Pseudo-*$F_{5, 131}$ = 2.84, P = <0.05) between bivalve condition indices, FAME species, and shell thickness against minimum salinity (PSU), minimum dissolved oxygen (mg/l), mean sediment chlorophyll-*a* (ug/l), and mean sediment phaeopigments (µg/l). Largest variation significantly

explained by environmental variables were minimum dissolved oxygen (*Pseudo-*$F_{1, 131}$ = 4.12, P = <0.05), mean sediment phaeopigments (*Pseudo-*$F_{1, 131}$ = 3.98, P = <0.05), minimum salinity (*Pseudo-*$F_{1, 131}$ = 2.57, P = <0.05) and mean sediment chlorophyll-*a* (*Pseudo-*$F_{1, 131}$ = 2.4241, P = <0.05). Bivalve condition index was relatively small, indicating less pull in the ordination, but was correlated in the ordination with higher minimum dissolved oxygen, higher minimum salinity, mean sediment phaeopigments, and higher minimum seawater chlorophyll-*a*. Overall, condition indices overlapped by site type, represented in figure 8, but increased towards the centroid of clam garden bivalve species scores in the db-RDA.

Discussion

This study was successfully differentiated clam garden bivalves from non-walled bivalves, utilizing both fatty acid profiles and $\delta^{13}C$ - $\delta^{15}N$ stable isotopes. Drivers for differences found in clam garden sites compared to non-walled sites included potential stress alleviation effects as well as potential differences in feeding behaviours. Both stress alleviation and changes in feeding behaviours would explain trophic niche shifts observed, and are correlated with appropriate fatty acids within our distance-based RDA. Exact causes were not defined in this study, but previous estuarine studies of bivalves to suggest potential drivers for the correlations observed in our db-RDA.

Dietary FAME Comparisons and Differences by Site

Dietary differences between clam garden and non-walled sites were significant, according to FAME SIMPER analysis. FAME differences were driven primarily by changes in SFA and MUFA species, with clam gardens increasing in relative proportion of C16:0 and i16:0 SFAs and decreases in C16:1(n-7), C15:1, C18:0, and C18:1(n-7) FAs. Main FA species differentiating between sites are expected common FAME biomarkers in bivalves (Langdon & Newell, 1999; Kharlamenko et al., 2001; Zhao et al., 2013). Clam garden samples were also ordinated more closely with some outliers compared to the separated and spread non-walled sites, within the db-RDA (Figure 8b). This indicates that clam gardens are more similar to each other and generate similar dietary FAME proportions consistently over time, compared to non-walled site

bivalve samples which are highly variable in dietary intakes. It appears that clam gardens are dominated by a more consistent diet and environment, derived from similar FAME profiles across clam garden sites compared to non-walled sites which showed much higher variation in both physiological and environmental parameters affecting bivalve ordinations.

Bacterial markers (sum of odd-branched numbered FAs) were found for both site types. Bacterial FAME signatures were larger in surrounding inshore seawater compared to bivalve tissues. Bacteria attach themselves to ingested particulate organic matter (POM) and create a mechanism for increased supply of dissolved nitrogen from seawater to a species of filter-feeding oyster (Langdon & Newell, 1999). Clam garden inshore seawater had higher FA (17:0 and *iso*-C17:0 bacterial) which is associated with the presence of anaerobic bacteria (Zhao et al., 2013).

There were biomarkers indicating the presence of dinoflagellates in the diet of bivalves (Langdon & Newell, 1999; Kharlamenko et al., 2001; Zhao et al., 2013) from both sites with 16:1(n-7) and C16:0 ratios averaging less than 1, with a ratio in clam garden bivalves of 0.09, and a ratio at non-walled site bivalves of 0.18. The differences in these ratios with increases in C16:1(n-7) towards the non-walled sites could be indicative of a mixed diet of diatoms or other photosynthetically-derived algal food sources, which are considered diatom-dominated when 16:1(n-7) and C16:0 ratios are greater than 1 (Shin et al., 2008; Zhao et al., 2013). However, I do not know which specific food particulates impact the ratios of 16:1(n-7) and C16:0 ratios observed, since 16:1(n-7) and C16:0 are common marine FA species present in many algae and planktonic sources in marine systems (Langdon & Newell, 1999; Viso & Marty, 1999; Dalsgaard et al., 2003).

Overall, there appeared to be overlap between site type dietary FAME profiles (Table 2), with main dietary FAME drivers being primarily dinoflagellates, though other food sources could contribute to the ratio used to estimate dinoflagellate biomarkers, and bacterial signature presences increasing slightly compared to surrounding seawater profiles. However, there were statistical differences between main SFA profiles and MUFA profiles, with clam gardens having higher overall SFAs and lower MUFAs compared to non-walled sites. The presence of

dinoflagellate biomarkers across bivalves from all site types matches observed markers within inshore seawater FAME profiles, and matches known contributions of phytoplankton and flagellates to filter-feeding bivalve diets (Cranford & Hill, 1999; Cranford et al., 2011).

Trophic Level and Dietary Proportion

Overall, both models indicated that ocean-based dietary sources make up a larger proportion of *L. staminea* diets for both site types, compared to tidal algae detritus sources. Dietary proportion is supported by our FAME profile results indicating increased dinoflagellates and limited marine POM signatures (PUFAs), though non-walled sites potentially showed some increased diatoms in the ratio of C16:0 and C16:1(n-7).

There does appear to be a location-based effect, where sites located closer to one another in the bay have closer dietary proportions from each food sources and there does not appear to be a strong site type difference according to food source proportion. Areas within small inlet in Kanish Bay (CG-03, CG-04, and NW-04) had similar ocean food source estimates compared to remaining sites for both scenarios, though site differences are less striking in scenario 2, possibly due to increased variation in the model and lack of differentiation ability between certain food sources per individual sites.

Ocean-derived food sources appear to influence bivalve diets and intertidal algae from both heights play more of a supplemental role in providing food particulates within the summer months in this study. FAME profiles for bivalves support this proportion of food intake, with the majority of food sources being derived from planktonic sources, with limited algae or terrestrial markers. I do not see a site type difference according to food intake, unlike FAME profiles which indicate a strong difference in clam gardens compared to non-walled sites, therefore dietary differences appear to be related to conditions at each site type instead of consumed food sources. I additionally see no difference in available food sources seen in our ocean water filter FAME profiles, indicating that the same foods are being distributed to all sites in the bay, and consumption is occurring at the same proportions, suggesting that FAME profile differences are being altered by bivalve responses to site differences.

Isotopic trophic shifts in marine species have been observed previously, when switching feeding behavior (Fry 2006), changing life stages (Hentschel 1999), physiological constraints (Rossi et al., 2004), and availability of food particulates (Rossi et al., 2004). I observed a difference in trophic niche positioning (Figure 6) in clam garden *L. staminea* samples, compared to non-walled samples. Layman metrics showed an overlap in both $\delta^{15}N$ and $\delta^{13}C$ profiles, though clam garden bivalves appear to be feeding at a higher trophic niche compared to bivalves at non-walled sites as indicated by overall higher ranges in $\delta^{15}N$, and clam garden bivalves consume a smaller number of food particulate types indicated by smaller $\delta^{13}C$ levels.

I selected individuals that were similar in size, avoiding juvenile bivalves at all sites when possible to limit potential effects on $\delta^{15}N$ and $\delta^{13}C$ uptake related to increased turnover of tissues and increased particulate uptake rates. I examined just one species of bivalves to reduce inter-species variation in isotopic ratios of $\delta^{15}N$ and $\delta^{13}C$. Variation of food particulates could create changes in trophic positioning by site types, but available food profiles indicated by FAME profiles of ocean filters and estimated food proportion uptake in our 3-source dietary mixing models does not support different food particulate uptake by site type as a cause for trophic niche differences. Differences in trophic niche positioning between clam garden *L. staminea* samples and non-walled samples to be the result of physiological constraints between site types, differences in feeding behavior, or some combination of each.

Environmental variable correlations and potential mixing effects

Leukoma staminea in clam gardens were able to be differentiated by fame profiles. Distance-based RDA indicated that site differences in FAME profiles additionally correlated with higher minimum salinity (PSU), higher minimum dissolved oxygen (mg/l), higher minimum chlorophyll-*a* (µg/l), and higher mean sediment phaeopigments (µg/l).

Dissolved oxygen explained the most variation within the db-RDA, and whereas dissolved oxygen was below calculated oxygen saturation (using temperature, salinity, and atmospheric pressure on sampling day) at some sites, hypoxia was never observed at any sites. I would generally not expect dissolved oxygen to be positively correlated with salinity, as salinity

negatively correlates with oxygen solubility in seawater (Debelius et al. 2009). Aerobic and anaerobic bacterial fatty acids, such as C15:0, i15:0, and C17:0, were strongly correlated with both salinity and dissolved oxygen in our db-RDA . These bacterial FA species were found in greater levels in bottom water at all sites. I suggest a hydrological effect is creating the correlation between increased salinity and oxygen, where water is more mixed over clam gardens compared to non-walled beaches, as indicated by higher levels of both oxygen and salinity, as well as bacterial signatures being correlated with each.

We expect there to be similar wave dynamics in clam gardens that have been found in armored seawalls in estuary systems (Bozek and Burdick, 2005 ; Tyler 2009; Dugen et al., 2018), though with a smaller overall effect due to reduced wall size and area (Lepofsky et al., 2020). Seawall armouring has been found to alter hydrodynamics of beaches, specifically in relation to dissolved oxygen, as well as differences in nutrient cycling, and trophic shifts (Dugen et al., 2018). Additionally, armoured seawalls have been associated with changes to hydrology on the beach, associated with alterations to wave activity, including increased salinity and oxygen levels (Bozek and Burdick, 2005; Dugen et al., 2018). Clam garden walls sit lower in the intertidal, and can be much smaller in area (Lepofsky et al., 2020). Correlations with salinity and dissolved oxygen due to clam garden wall presence could generate hydrological shifts similar to armoured seawalls.

Correlated environmental stressors, condition indices, and potential feeding mechanisms in clam gardens

Increased trophic positioning in clam garden *L. staminea* samples as well as reductions in food diversity signaled by increased $\delta^{15}N$ and decreased $\delta^{13}C$ levels could suggest both feeding behavioral shifts and decreased physiological stressors in clam garden sites.

Talkington (2015) showed that *L. staminea* reduced key osmolytes involved in osmotic regulation under low salinity stress, related to lower available energy outputs for physiological processes. Salinity stress also has strong behaviourial effects on *L.* staminea, including shell closing (Talkington 2015), which negatively affects optimal feeding and respiration in bivalves (Vernburg et al. 1963; Pierce et al. 1971).

Both salinity and dissolved oxygen vectors in our db-RDA were moderately correlated with condition indices in clam gardens. *Leukoma staminea* has been classified as a stenohaline species previously (Talkington et al., 2015). *Leukoma staminea* has also been previously observed die faster in comparison to other local bivalve species under low oxygen conditions (Allee, 2010), and it has been suggested that synergistic stressors would lower anoxic tolerance (Allee, 2010).

Our results within the FAME profile shifts of SFAs, and MUFAs, are consistent with those seen in typical salinity response studies of estuarine mollusks (Navarro & Winter, 1982; Navarro & Gonzalez, 1998; Tomanek, 2012; Gonçalves et al., 2017).

Alleviation of stressors by increasing salinity and dissolved oxygen are expected to generate different feeding behaviours in clam gardens, as a response to more optimal conditions. I saw that increased levels in minimum chlorophyll-*a* in seawater and mean sediment phaeopigments correlated more strongly with condition indices, and with saturated fatty acids commonly found in marine plankton. The two strongest fatty acid species positively correlated with minimum chlorophyll-*a,* mean sediment phaeopigments, and increased condition indices were C16:0, and C18:0 SFAs. Both are general planktonic fatty acid biomarkers, though C16:0 is associated specifically to dinoflagellates or diatoms, according to the ratio of C16:1(n-7) over C16:0. Clam gardens have been found to increase residence times for seawater (Salter, 2018), which was related to increased growth rates in transplanted bivalves. Decreased salinity stress behaviours seen in *L. staminea*, such as shell closing (Talkington et al., 2015), would allow them to feed for longer with greater efficiency, in waters with higher residence times.

We saw that clam garden bivalves had decreased ratios of C16:1(n-7) over C16:0 in relation to non-walled sites, suggesting more dinoflagellate intake at clam gardens, and a mixed intake of dinoflagellates and diatoms at non-walled sites. In non-walled sites, which negatively correlated with lower salinity and low oxygen levels, I see significant correlations with increasing sediment chlorophyll-*a* levels associated with benthic photosynthetic algae such as diatoms, as well as increases in C16:1(n7) FA species. These correlations could be indicative of less optimal feeding

behaviours, potentially related to increased stress as well as lower residence times observed previously (Salter, 2018).

Both increasing stressors in non-walled bivalves, and increasing optimal feeding behaviours in clam garden bivalves would appear as a trophic shift noted in this study. I suggest that clam gardens are more environmentally stable, allowing for less variability in dietary intake, by decreasing known stressors for *L. staminea*.

Conclusion

My survey of environmental and physiological differences in *L. staminea* bivalves of Kanish bay indicated strong differences between clam garden and non-walled sites, driven by decreased stressors and potential behavioral shifts in clam garden bivalves compared to non-walled bivalves. There were similar bivalve dietary proportions and food particulate fatty acid biomarkers, as well as sediment grain size across all site types. Main differences in fatty acid profiles were increased saturated fatty acid content and decreased unsaturated fatty acid content in clam garden bivalves compared to non-walled bivalves. FAME profile shifts of SFAs and MUFAs are consistent with salinity stress responses in bivalves and potentially indicate changes in feeding behaviors by site.

Mean ranked condition indices in clam gardens were not statistically different than non-walled sites, but did show to have smaller ranges overall. Condition indices were strongly correlated with increased chlorophyll-*a,* increased sediment phaeopigments and were moderately correlated with higher minimum salinity and dissolved oxygen. Correlated relationships with increased condition indices occurred specifically on clam garden sites, compared to non-walled sites, suggesting increased primary productivity, and lower salinity stressors on clam gardens compared to non-walled sites. These related to previous physiological studies of clam gardens under stress, as well as observed increased seawater retention time in clam gardens in previous studies.

We found reasonable correlations to suggest both feeding behaviour shifts, as well as releaved stress factors in clam garden bivalves compared to non-walled clams, which is supported by fatty acid shifts and trophic shifts matching previous literature. Overall, correlated relationships

to significant environmental drivers within our distance-based RDA provide plausible avenues to explain observed fatty acid shifts in saturated fatty acids, and monounsaturated fatty acids in clam gardens, as well as trophic shifts in $\delta^{13}C$ and $\delta^{15}N$ stable isotopes.

Future restoration programs should consider measurements of algae levels over clam gardens, in relation to dietary availability, as well as trophic shift measurements as ways to examine progress of restoration. Utilizing fatty acid measurements for dietary biomarkers as well as stable isotope ratios for dietary proportion and trophic positioning measurements on bivalves was shown to be effective in identifying correlated environmental drivers in this study. These tools are effective strategies for relating environmental and physiological variables within bivalves, and provide avenues to explore for future research, which in clam gardens should examine overall cycling of nutrients as well as controlled experiments for bivalve feeding behaviour to examine what changes are occurring at the feeding level leading to increased condition indices. As restoration continues on clam garden sites, ecological examination of driving forces leading to observed shifts in this study, as well as other mechanisms for clam stress, feeding, and recruitment strategies should be examined, in order to determine efficacy of restoration policies as well as create target restoration goals. Ecological examinations such as this study should be tied into community-led restoration targets, and can help inform on modern conditions on these traditional Indigenous aquaculture sites.

Tables

Table 1. A) Summary of Layman metrics calculated between Clam Garden and Non-Walled beaches, indicating trophic niche positioning and aspects of food web structures. Nitrogen isotope ($\delta^{15}N$) range indicates broad trophic level. Carbon isotope ($\delta^{13}C$) indicates resources at base of the food web. Total area (TA) relates to niche space and food web trophic diversity (Layman et al. 2007). Centroid grouping (CD) relates to trophic diversity, mean nearest neighbor distance relates to density of species per grouping variable and standard deviation of nearest neighbor distance (SDNDD) relates to evenness of species distributions within $\delta^{13}C$ - $\delta^{15}N$ biplot space (Layman et al. 2007). B) Bayesian ellipse metrics for individual sites, indicating total area of ellipse (relating to grouping), Standard Ellipse Area (as an alternative to TA for niche space and food web trophic positioning estimates), and Standard Ellipse Area Corrected (Correction of SEA with sample size variation per group) (Jackson et al., 2011).

A)

Layman Metrics	Clam Garden	Non-Walled
δ15N (‰) Range	0.82	0.12
δ13C (‰) Range	0.15	0.29
Total Area (TA)	0.06	0.02
δ13C-δ15N Centroid Grouping (CD)	0.29	0.11
Mean Nearest Neighbour Distance (MNND)	0.24	0.12
Standard Deviation of Nearest Neighbour Distance (SDNND)	0.28	0.09

B)

Bayesian Ellipse Metrics	CG-01	CG-02	CG-03	CG-04	NW-01	NW-02	NW-03	NW-04
Total Area (TA)	1.16	1.71	2.49	2.09	2.01	1.12	1.31	2.57
Standard Ellipse Area (SEA)	0.36	0.53	0.83	0.71	0.52	0.38	0.4	0.81
Standard Ellipse Area Corrected (SEAc)	0.37	0.55	0.86	0.74	0.54	0.39	0.41	0.84

Table 2. FAME profile summaries for clam garden and non-walled beach *L. staminea* bivalves, grouped by fatty acid group, and relevant biomarkers, showing sums and Mann-Whitney U-Test significances for important groupings between site types.

Group; Biomarker	FA Species	CG	NW
		Relative Percent Area (%)	Relative Percent Area (%)
SFA; -	C14:0	0.01 ± 0.03	0.09 ± 0.24
SFA; -	C18:0	6.51 ± 1.21	6.22 ± 1.28
SFA; - Diatoms/Dinoflagellate	C16:0	22.49 ± 2.99	20.85 ± 2.71
SFA; Diatoms/Dinoflagellate	i16.0	3.65 ± 1.47	2.04 ± 1.83
SFA; Bacterial	C15.0	0.05 ± 0.15	0.11 ± 0.20
SFA; Bacterial	C17:0	0.25 ± 0.39	0.30 ± 0.36
SFA; Bacterial	i15:0	0.13 ± 0.42	0.27 ± 0.51
SFA; Bacterial	i17:0	1.88 ± 0.89	1.88 ± 0.56
	Sum Bacterial FAs*	2.31 ± 1.86	2.56 ± 1.65
	Sum SFAs***	34.96 ± 7.56	31.76 ± 7.71
MUFA; Zooplankton	C14:1	0.01 ± 0.09	0.11 ± 0.32
MUFA; Zooplankton	C15:1	0.47 ± 1.36	1.96 ± 1.95
MUFA; Diatoms/Dinoflagellate	C16:1(n-7)	2.10 ± 1.32	3.61 ± 2.26
MUFA; Zooplankton	C18:1(n-9)	0.84 ± 0.64	1.20 ± 0.99
MUFA; -	C20:1	0.06 ± 0.37	0.09 ± 0.38
	Sum MUFAs***	3.48 ± 3.78	6.97 ± 5.9
PUFA; Terrestrial	C18:3(n-3)	0.84 ± 0.64	1.2 ± 0.99

*Means were compared with Mann-Whitney U-Test

***Means were compared and found to be statistically significant ($P < 0.01$, $\alpha = 0.05$) with Mann-Whitney

Table 3. Summary *L. staminea* food source proportion percentage by individual site, determined by ^{13}C and ^{15}N isotope ratios in a 3-source mixing model, estimated trophic enrichment factor values, with scenario 1 utilizing whole body tissue TEFs and scenario 2 utilizing muscle tissue TEFs for a filter-feeding bivalve (*Crassostrea gigas*) within Puget Sound, Washington (Strom et al. *In Press*).

Scenario Type	Food Source (%)	CG-01	CG-02	CG-03	CG-04	NW-01	NW-02	NW-03	NW-04
	High Tide Algae (%)	0.17 ± 0.06	15.9 ± 0.06	11.9 ± 5.3	12 ± 5.4	17.8 ± 6.8	17.1 ± 6.5	15.3 ± 6.6	11.1 ± 5
Scenario 1	Low Tide Algae (%)	21.2 ± 10.6	29.7 ± 9.7	16.5 ± 8.3	16.8 ± 8.4	22.2 ± 11.7	26.2 ± 10.9	21.3 ± 11	19.1 ± 7.9
	Ocean Particulates (%)	61.5 ± 7.2	54.5 ± 5.8	71.6 ± 6.4	71.2 ± 6.4	60 ± 8.6	56.7 ± 6.9	63.4 ± 8.3	69.8 ± 5.7
	High Tide Algae (%)	8.9 ± 5	9 ± 4.8	8.9 ± 6	9.1 ± 5.7	-	10.9 ± 5.6	11.8 ± 7.8	7.1 ± 4.3
Scenario 2	Low Tide Algae (%)	12.6 ± 7.9	15 ± 7.8	11.8 ± 8.6	12.9 ± 8.8	-	17.1 ± 9.1	16.7 ± 12.1	10 ± 6.4
	Ocean Particulates (%)	78.5 ± 8	75 ± 6.7	79.2 ± 10.1	78 ± 9.5	-	72 ± 7.9	71.5 ± 13.3	83 ± 7

Figures

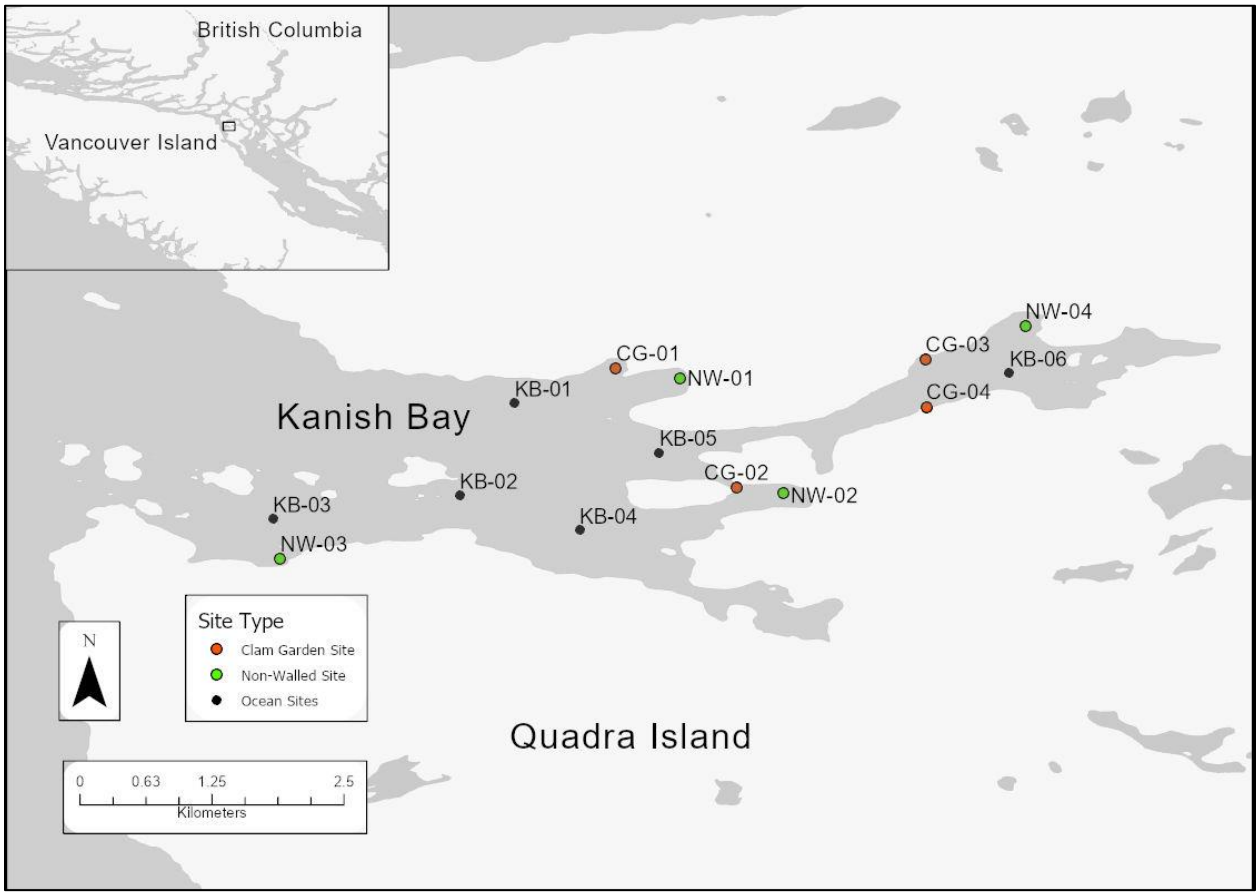

Figure 1. Map of sampling beaches and beach types in Kanish Bay, Quadra Island, Canada, from May and July sampling times in 2019.

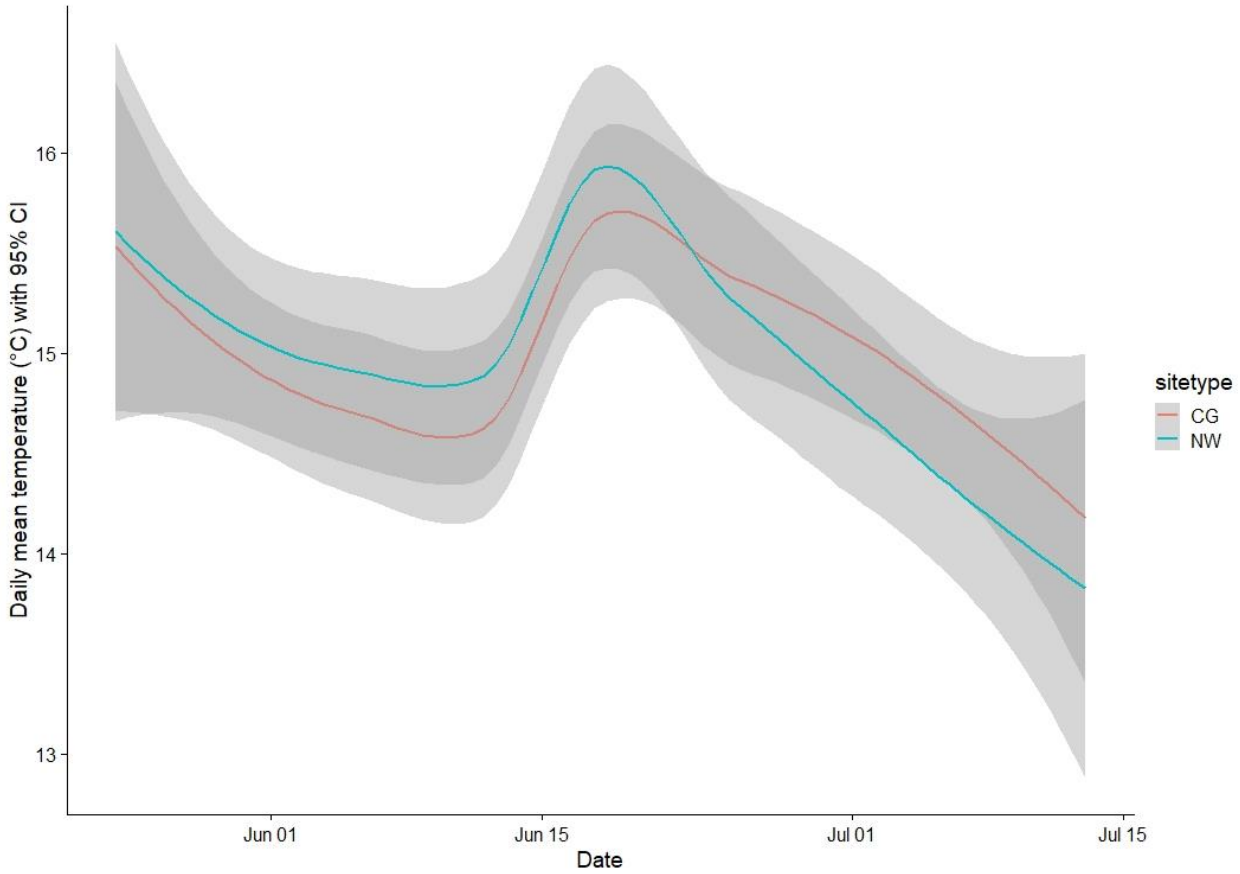

Figure 2. Mean daily inundated beach surface temperature profiles, taken in May 2019 to July 2019, during a total of 50 days. Strong overlap of 95% confidence intervals for period show no major changes in temperature profiles over sites during inundation times, when clams are below ocean level at all tidal heights.

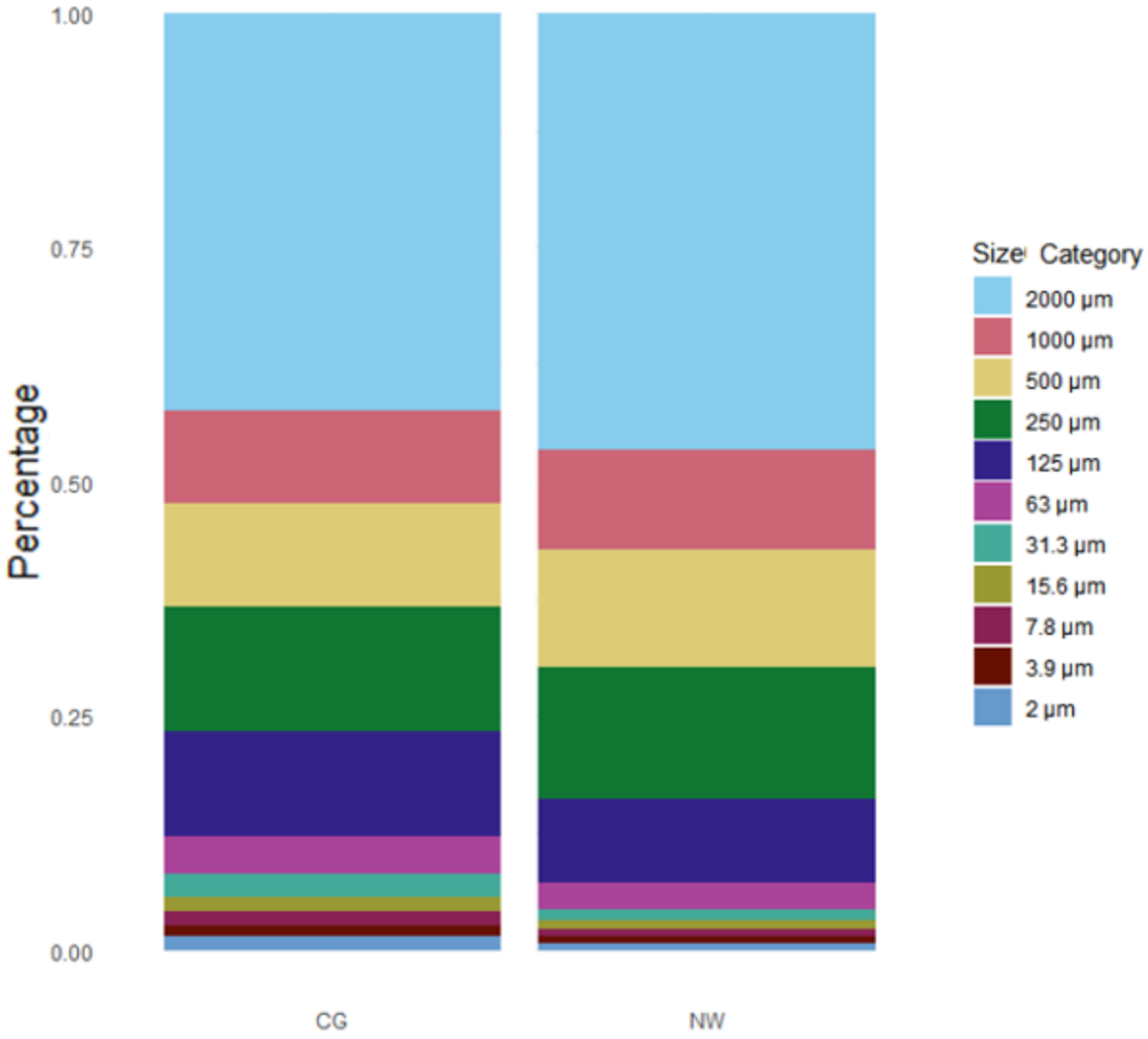

Figure 3. Mean sediment grain size profile percentages for clam garden beaches and non-walled beaches in Kanish Bay, with grain sizes from 2000 µm to 2 µm in size.

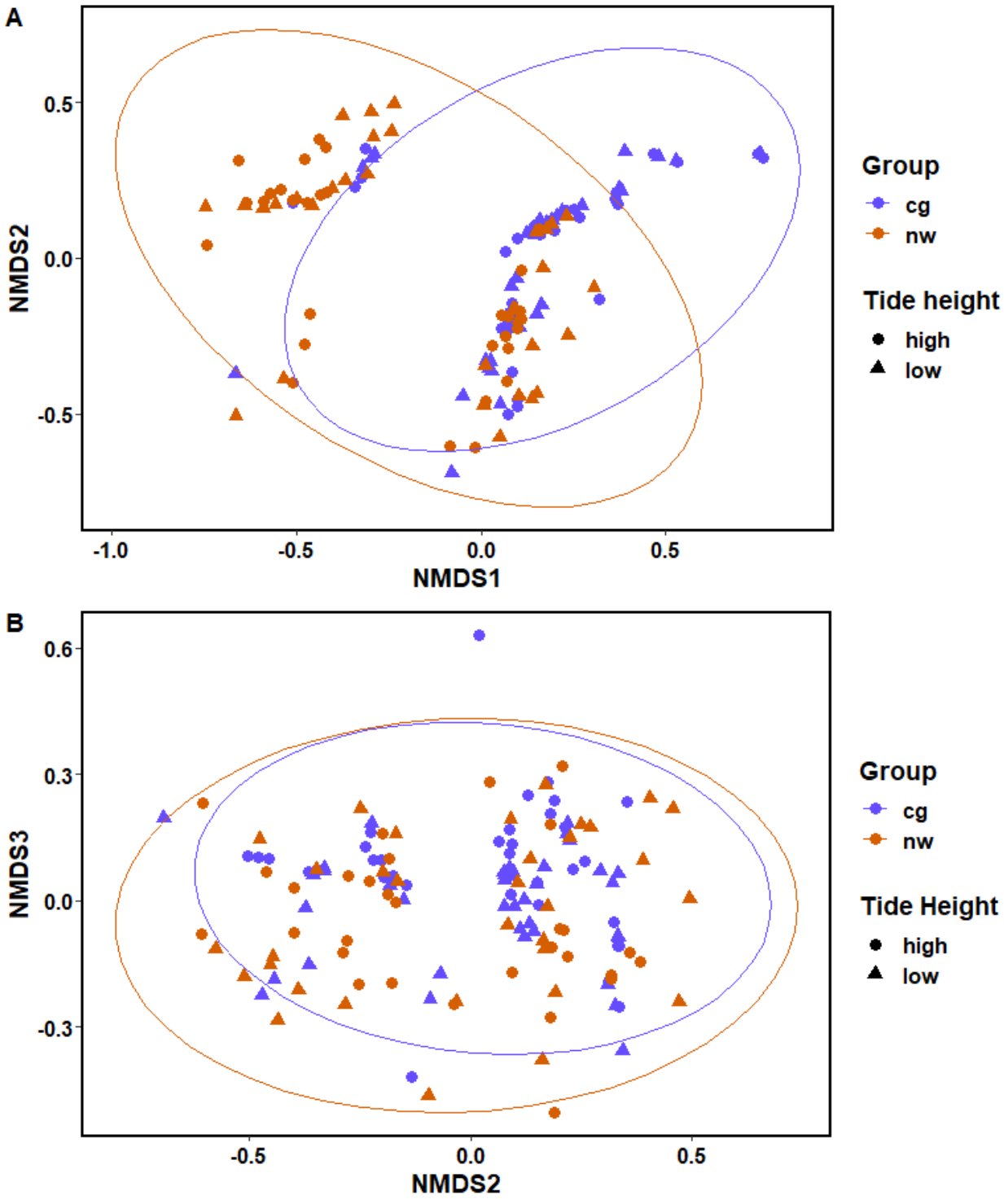

Figure 4. Fatty Acid Methyl Ester (FAME) Non-metric multidimensional scaling ordination along 3-dimensions (k = 3), utilizing Bray-Curtis semi-metric dissimilarity scores to ordinate percentages of FAME profiles with **a)** visualization of nMDS dimension 1 and nMDS dimension 2, indicating increased grouping in that direction between site types, and **b)** visualization of nMDS dimension 2, and nMDS dimension 3.

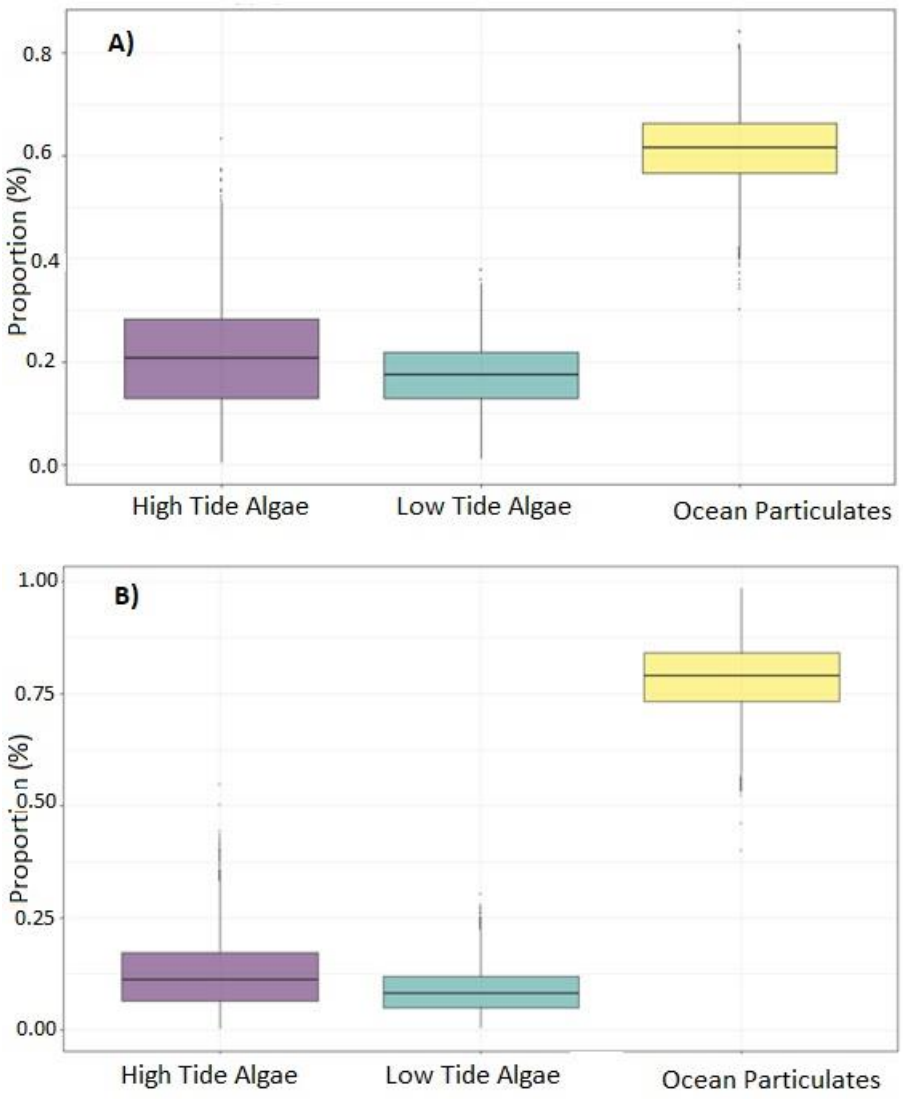

Figure 5. *Leukoma staminea* bivalve dietary proportion, resolved utilizing a 3-source mixing model with C_{13} and N_{15} stable isotope ratios for Scenario 1 (A) utilizing an estimated TEF score from whole body tissues and Scenario 2 (B) utilizing an estimated TEF score for bivalve muscle tissues.

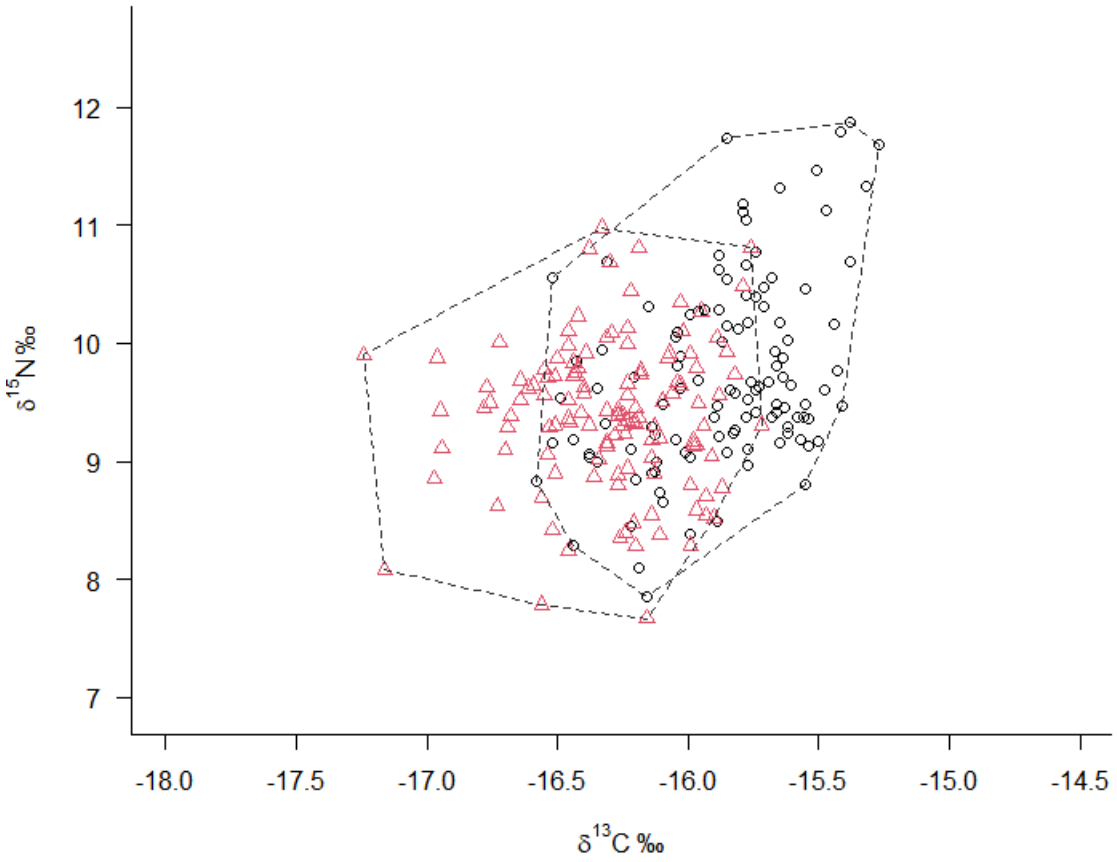

Figure 6. A) Clam Garden $\delta^{15}N$- $\delta^{13}C$ distribution (Red; n = 115) and Non-walled bivalve $\delta^{15}N$-$\delta^{13}C$ distribution (Red; n = 123) for littleneck clam (*Leukoma staminea*) samples, showing a shift in isotopic space within clam garden bivalves compared to non-walled bivalves.

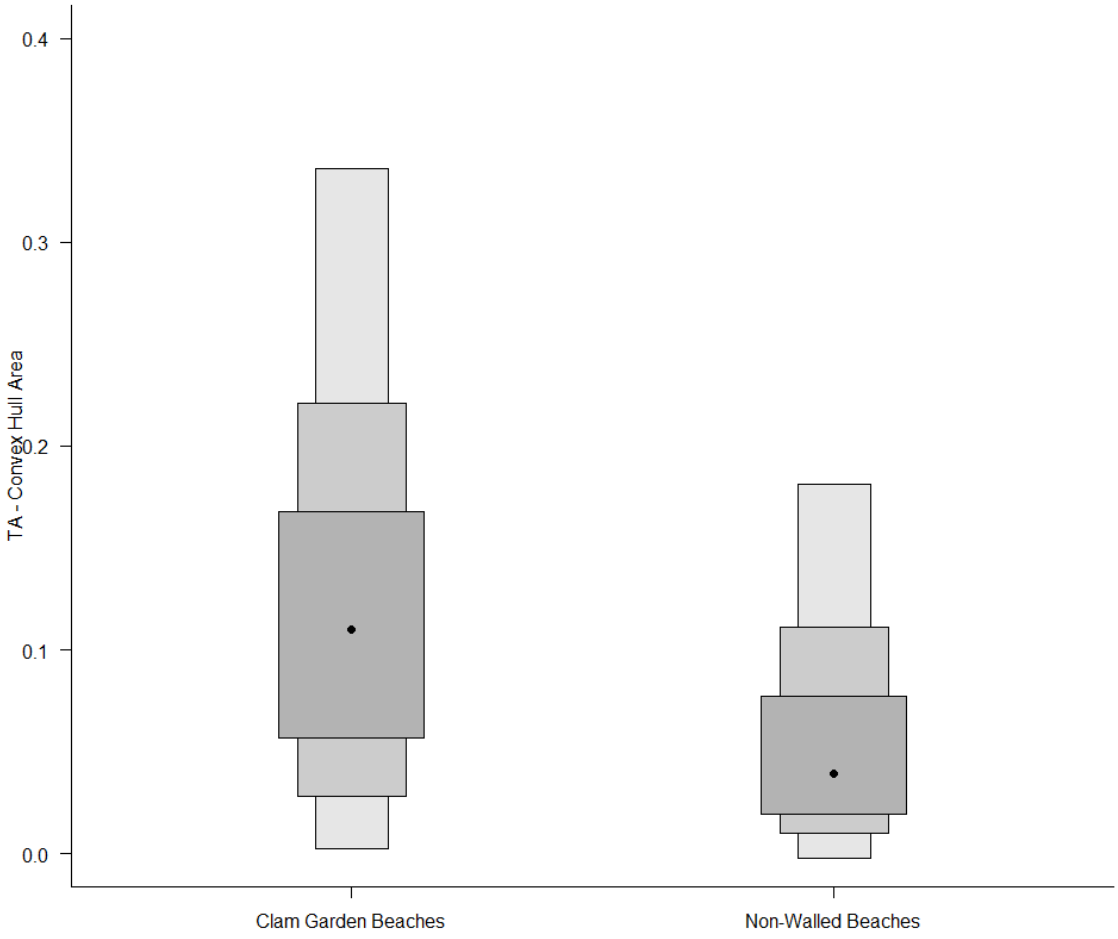

Figure 7. Community comparison of Total Area – Convex Hull area with shaded boxes representing the 50%, 75%, and 95% credible intervals from dark to light gray, for clam gardens and non-walled beaches.

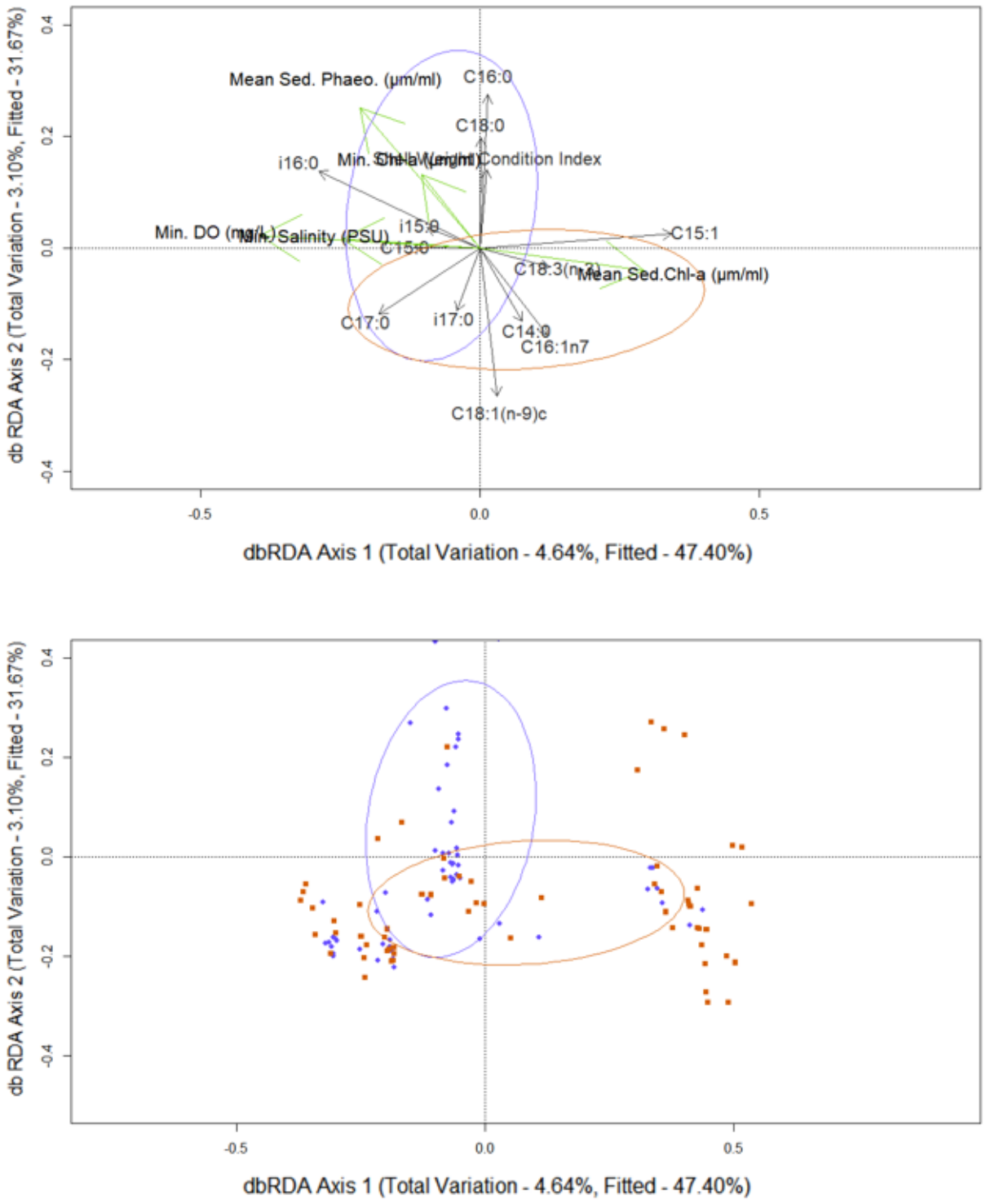

Figure 8. Distance-based Redundancy Analysis ordination for **a)** environmental (green) and physiological (black) driving factors for RDA analysis, for RDA axis 1 (4.64% of variance explained) and RDA axis 2 (3.10% of variance explained), where RDA 1 and 2 were found statistically significant (p = <0.01), and **b)** Individual site scores for canonical redundancy analysis, indicating differences between clam garden (violet) sites compared to non-walled sites (orange).

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