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UNIVERSITY OF CALIFORNIA, MERCED

The utility of marine neogastropod *Californiconus californicus* as a
model system for investigation of the venom microbiome

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

Doctor of Philosophy

in

Quantitative and Systems Biology

by

Sabah Ul-Hasan

Committee in Charge

 Professor J Michael Beman, Chair
 Professor Suzanne Sindi
 Professor Eric W Schmidt
 Professor Thomas F Duda
 Professor Tanja Woyke
 Professor Clarissa J Nobile

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University of California, Merced
2019

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Acknowledgments

I used to sit in front of the television and absorb everything PBS (Public Broadcasting Service) had to offer on the venomous animals of Australia. I became obsessed with the idea of diving and exploring the ocean, dreaming of one day being able to contribute to positive change for the benefit of conservation and humanity. I cannot believe I did all of this in my PhD, and that I was able to include my family in the process of how much these things matter to us and our culture just as much as becoming a medical doctor or a lawyer or an engineer.

I was once told not to do a PhD for my family, but for myself. But it's because of my family that I figured out why this degree is important to me. They represent generations of sacrifices made for me to live a better life in the United States of America, and they took a big risk not knowing what that life would look like here. This knowledge I have now is the fruit of their labor and it is meant to be shared so that another kid, and another, and another, can one day see possibilities for themselves and their contributions to society that I see in me now.

I am so grateful to have met so many inspiring people throughout this journey, who have taught me by example how to go forward in completing this PhD, and how to carry myself with more equanimity than when I started – even, and especially, during the times when the appropriate response was to outcry at the inequity. Thank you for existing. Thank you to my co-advisors, Tanja and Clarissa. I sometimes sit back and think, "Wow. I am working for science rockstars who are also incredibly good people. Is this real?". Thank you for choosing me, and for your constant investment and support. Thank you to my partner Nicholas, Levi, and Jess for your motivation and inspiration. Thank you to my committee members, mentors, collaborators, colleagues, peers, students, and friends. I began writing out each person's name and this alone took up several pages, then I went into a "What if I forget someone?!" rabbithole. If you read this, I hope you know just how much each of you matter to me in this world.

Yes. I put in a lot of hard work, but it is all of you who got me – and everyone like me – here. We are so very lucky for the many people like you in this world. You show us how to be better humans than we were the day before. And that is what matters. That is how I learned to do good science.

Thank you to the people of Merced and Yokut for welcoming me here as a visitor. I hope UC Merced and the people within it go forward treating each of you with the utmost respect.

Progress is consistent, balanced dialogue between people who vehemently disagree.
That is how theories are born and discoveries are made.

For my brother, Saood.
You are so kind and have done so much despite how unfair life has been to you.
You're the most impressive person I know.
Yup, you're eternally acknowledged in a public piece of writing now.

Curriculum Vita

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Ph.D. University of California, Merced, CA 95343 Jan 2015 – Aug 2019
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S Ul-Hasan*, E Rodríguez-Román, AM Reitzel, RMM Adams, V Herzig, SA Trim, AJ Saviola, CJ Nobile, EE Stiers, SA Moschos, CN Keiser, D Petras, Y Moran, TJ Colston. The emerging field of venom-microbiomics for exploring venom as a microenvironment, and the corresponding Initiative for Venom Associated Microbes and Parasites (iVAMP). *In Review* (Toxicon:X)

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studies in the pituitary of adult sea lamprey, *Petromyzon marinus*. (2014) University of
New Hampshire: Master of Science Thesis.

Posters and Presentations

Sep	2019	National, **invited** - Reclaim STEM (Irvine, CA) Multimedia SciComm
Jun	2019	International, poster - ASM (SF, CA) Metabolites and microbes in venom
Jan	2019	International, talk - SICB (Tampa, FL) Microbes in venom and iVAMP
Oct	2018	Local, poster - NCASM (Santa Clara, CA) Microbes in venom
Oct	2018	Local, talk - NCCB (SF, CA) Coastal microbes of Baja California
Aug	2018	Stanford, **invited** - SLAC Lab, DIYE (Palo Alto, CA) On Intersectionality
Mar	2018	International, poster - JGI User (SF, CA) Coastal microbes of Baja
Jan	2018	International, **invited** - SICB (SF, CA) BIOTA: A Mixed-media approach
Jan	2018	International, talk - SICB (SF, CA) Impacts on *C. californicus*
Nov	2017	International, poster - CERF (Providence, RI) Impacts on *C. californicus*
Oct	2017	International, talk - Venoms to Drugs (Brisbane, OZ) Microbes in venom
Jun	2017	International, talk - Evolution (Portland, OR) Microbes in *C. californicus*
May	2017	International, talk - YSW (Wawona, CA) Microbes in *C. californicus* venom
Mar	2017	National, talk - GWS (Norfolk, VA) Revisiting the Dual Mandate
Mar	2017	Local, talk - GradSLAM! (Merced, CA) Microbes *C. californicus* venom
Mar	2017	International, poster - JGI User (Walnut Creek, CA) Microbes in venom
Nov	2016	International, **invited** - New England Biolabs, Biota: Symbiosis in Action
Nov	2016	International, talk - WSN (Monterey, CA) Microbes in *C. californicus*
Oct	2016	Local, **invited** - UC Merced (Merced, CA) "Pick Your Advisor" Panelist
Sep	2016	Local, **invited** - MCSBA (Monterey, CA) Improving K-12 science literacy
Jun	2016	International, talk - Evolution (Austin, TX) MEX evolution in *P. aeruginosa*
Jun	2016	International, attendee - Google Earth Engine Summit (Mountain View, CA)
Apr	2016	Local, talk - GradSLAM! (Merced, CA) Science + Community
Jan	2016	International, poster - SICB (Portland, OR) Marine host-microbe symbioses
Sep	2016	Local, **invited** - JGI All-Hands (Walnut Creek, CA) Microbes in plant roots
Mar	2015	Local, talk - GradSlam (Merced, CA) Germs, genomes, and jellies
Jul	2014	Local, talk (defense) - UNH (Durham, NH) Lamprey β, A2, and B5 GpH
Apr	2014	Local, poster - UNH (Durham, NH) *P. marinus* hormone subunit GpB5
Feb	2014	Local, talk - UNH (Durham, NH) *P. marinus* hormone subunit GpB5
Nov	2012	Local, talk - UNH (Durham, NH) Lamprey GnRH and *Vibrio glnD*
Apr	2012	Local, talk - U of U (SLC, UT) PAM enzyme from *Conus bullatus*
Apr	2012	Local, poster - U of U (SLC, UT) PAM enzyme from *Conus bullatus*
Apr	2011	State, talk - UCUR (Weber, UT) PAM enzyme from *Conus bullatus*
Apr	2011	Local, poster - U of U (SLC, UT) PAM enzyme from *Conus bullatus*

Mar 2011   National, talk - NCUR (Ithaca, NY) PAM enzyme from *Conus bullatus*
Feb 2011   State, poster - Capitol Hill (SLC, UT) PAM enzyme from *Conus bullatus*
May 2010   National, poster - HHMI (Bethesda, MD) Sar1 specificity in mammals
Apr 2010   Local, poster - U of U (SLC, UT) PAM enzyme from *Conus bullatus*
Aug 2009   Local, poster - UC Berkeley (Berkeley, CA) Sar1 specificity in mammals
Apr 2009   Local, poster - U of U (SLC, UT) Conotoxins and NMDA receptor binding
Aug 2007   Local, poster - U of U (SLC, UT) Chytridiomycosis in amphibians

Abstract of the Dissertation

The California Cone Snail as a System for Venom Microbiomics

by
Sabah Ul-Hasan
Doctor of Philosophy, Quantitative and Systems Biology
University of California, Merced, 2019
Advisors: Dr. Tanja Woyke and Dr. Clarissa J. Nobile

The primary question of my dissertation is, "Does venom possess a microbiome specific to it as an ecosystem, and why?" Given the limited amount of literature on venom microbiomes, I selected the California Cone Snail, *Californiconus californicus* as a proposed, wild model system for studying venom-microbe interactions in the process of investigating hypothesized microbial interactions with host venom.

First, I present a data-driven approach for how sampling sites of venomous animals of interest can be selected in conjunction with the current trend to rely on anecdotal information. This work integrates curated museum collections, crowd-sourced data through digital mediums, knowledge through scientific literature, and personal research. This segment delves deeper into our understanding of *C. californicus* across space and time, dating back to the Pleistocene. We identify relationships between shell morphology and temperature, contributing foundational knowledge for this species and prospective context of venom microbiome coevolution.

Second, I characterize the seawater and sediment coastal microbial ecology of the initial known sampling site (Puerto Nuevo, Mexico) in which this species is commonly found. We sampled several sites along a gradient of exposure to urbanization (0.45 km) and characterized the core microbial communities for archaea, bacteria, and microbial eukaryotes using 16S and 18S amplicon sequencing. While only representing one time point and location, our experimental design allows us to demonstrate consistency in the literature in that we identify functionally relevant microbial taxa specific to different environmental types and distance. This work contributes as a preliminary example for the determination of how and where microbes in the venom may be sourced from the wild.

Third, I outline the main findings of the venom microbiome for *C. californicus*. Model organisms used today are common, simplified points of reference for downstream application. The California Cone Snail is a commonly found neogastropod along the California-Baja coast by the 100s. It can be cultured and maintained in a laboratory, acting well for experiments in the wild and *in vivo* or *in vitro*. We sampled *C. californicus* for three major sites for geographical variation: Puerto Nuevo, San Diego, and Monterey. We sampled summer, winter, and summer again, as well as three consecutive days in Puerto Nuevo for temporal variation. We sampled adult and eggs to compare microbial communities across life stage. We then compared venom microbial communities in a lab setting by testing for different hydrostatic pressures, axenic conditions, and exposure to prey. In summary, we find a specific microbial community (16S and 18S) found in and along the venom gland when compared to other environments, tissues, and conditions.

Finally, I tie extensions of science outreach together with scientific practice through communication, education, policy, and the first venom-microbe consortium. These initiatives act as proof-of-concept for strengths in democratically practiced open-source, interdisciplinary research through inclusion across demographics and educational and professional stages.

Chapter 1

Bergmann's Rule across the Pleistocene, Holocene, and Anthropocene in an Eastern Pacific gastropod, *Californiconus californicus* (Reeve, 1844) (Gastropoda: Conidae)

In preparation for submission; Authors: ME Malloy*, S Ul-Hasan*, LL Lewis, JH Hofmeister, S Crickenberger, CA O'Leary, A Hendy, CJ Nobile, WF Gilly, S Sindi, T Woyke, LT Groves, J Vendetti.

1.1 Abstract

Bergmann's Rule posits that taxa are larger in body size or mass at high versus low latitudes as a function of climate. In many taxonomic groups, including marine gastropods, clines of body size throughout a species' range may follow or violate Bergmann's Rule. To test Bergmann's Rule in an intertidal Eastern Pacific gastropod, the California cone snail species known as *Californiconus californicus* (Reeve, 1844) was chosen for its 10 degree latitudinal span along a north-south coastline and its abundant and accessible shell data. For this study, we sampled *C. californicus* along the coast, accessed a wealth of natural history museum collections and took advantage of data from the online citizen science platform, iNaturalist. Moreover, because of this species' presence in historical and paleontological collections, its adherence to Bergmann's Rule was tested through the Pleistocene, Holocene (1892–1941), and Anthropocene (1946–2018). Adult shell size (as width) and shape (as globosity) were also tested against the specimens' mainland or island habitat as well as proximity to substantial anthropogenic habitat alteration and development (i.e. cities) at the time of collection. Results show that *C. californicus* shell size (as width) is consistent with Bergmann's Rule in the Pleistocene, Holocene, and Anthropocene, but its shape (as globosity) is inconsistent across epochs. Size and shape are significantly different on Northern California islands versus the coastal mainland, and small shell size but not shell shape is significantly correlated to coastal development as it relates to human population density over the past century. In addition to providing insight into the applicability of Bergmann's Rule to marine gastropods and ectotherms, this study highlights the importance and utility of museum collections and citizen scientist observations for species-level analyses.

1.2 Introduction

Bergmann's Rule (1847, in James, 1970) proposes that taxa within a clade, e.g. birds and mammals, tend to increase in body size or mass with increasing latitude and decreasing temperature (McNab, 1971; Meiri & Dayan, 2003; Berke *et al*., 2013). Interpretations and applications of this rule vary widely (Meiri, 2010; Vinarski 2014), and are inconsistent within and across taxonomic groups (Ray 1960, Geist 1987; Watt *et al*., 2010; Olalla-Tarraga, 2011, Chattopadhyay & Chattopadhyay, 2019). In one interpretation, Bergmann's Rule only applies to the relationship between latitude and body size intraspecifically (James, 1970; Vinarski, 2014). In invertebrates, body size within a species can be consistent with Bergmann's Rule in what is referred to as James's Rule (Van Voorhies, 1996; Mousseau 1997; Blackburn *et al*. 1999; Arnett & Gotelli 1999; Heinze *et al*., 2003), Converse Bergmann's Rule (Mousseau, 1997; Bidau and Martí 2007), or show clines that are sawtooth-shaped, step-wise, u-shaped, or absent (Johansson, 2003; Linse *et al*., 2006; Ho *et al*., 2009; Shelomi, 2012). Within marine gastropods species spanning at least 14 degrees of latitude, tests of intraspecific Bergmann's Rule have revealed linear (Lee & Boulding, 2010) and stepwise clines (Ho *et al*., 2009), as well as no relationship between shell or animal size and latitude (Olabarria & Thuston, 2003; Linse *et al*., 2006). In shelled marine gastropods, assessments of shell size and shape have been tested as morphological changes over time and geography (Hellberg *et al*., 2001), local adaptation (Tirado *et al*., 2016), and anthropogenic influences (Roy *et al*., 2003).

The north-south geography of the California-Baja Sur coastline offers a defined, mostly linear study site (Blanchette *et al.,* 2008) within which to test the effects of latitude and temperature-associated spatiotemporal variation in shell size and shape (Sagarin & Gaines, 2002). Marine diversity in this region is under pressure from a rapidly growing human population (Johnson & Baarli, 1999) that has increased approximately 18-fold in the past century to over 18 million people in the present day (State Census Data Center 2011). Gastropods in populations adjacent to intensely exploited regions of the coast tend to be smaller in shell/body size when compared to populations experiencing reduced human activity (Keough *et al.,* 1993; Murray and Bray 1994; Pombo & Escofet 1996; Murray *et al*., 1999; Roy *et al.*, 2003). The California Channel Islands along this coastline represent a region where comparisons between mainland and island in relation to marine gastropod shell shape (Conde-Padín et al. 2009) can be made.

The California cone snail, *Californiconus californicus* (Reeve, 1844), is an intertidal to subtidal neogastropod endemic to the northeast Pacific that spans approximately 10 degrees of latitude along a mostly linear north-south coastline from San Francisco Bay, California to Baja California Sur, Mexico (Biggs *et al*., 2010; Peters et al. 2013). It is the only conid in its range, which includes two to three marine ecoregions (Spalding, 2007; Peters et al. 2013) and the cities of San Francisco, Los Angeles, and San Diego/Tijuana, regions with large human populations and substantial coastal impact. Notably, the habitat of *C. californicus* is substantially cooler than that of most other conids (Peters *et al*., 2013), making it potentially vulnerable to anthropogenic habitat perturbations and climate warming (Moore *et al*., 2011). For these reasons, and its accessibility within modern, historical, and Quaternary-age museum collections (Supplemental

Table 2), *C. californicus* is a unique candidate for the study of Bergmann's Rule through time and to assess anthropogenic influences on shell size and shape.

The proximity of a large, roughly north-south coastal range and a large island chain in the range of *C. californicus* allows for comparisons between individuals from both regions. While we do not have extensive knowledge of the history of *C. californicus*' colonization of these islands, preliminary morphological differences, if present, could help characterize the extent to which these populations are distinct island-to-island and island-to-coast. Gastropods are shown to experience morphological shifts where shallow-water individuals colonize deep-sea regions, roughly equivalent to the established "island rule" for mammals, although the causes and extent of this trend are in question (Welch 2010; McClain *et al*. 2006).

Herein, shell size and shape of 2935 adult *C. californicus* shells from six museums, the citizen science platform iNaturalist, and two authors' collections are used to test if:

(i) shell size follows Bergmann's Rule; i.e. that body size increases with latitude and/or sea surface temperature during the Pleistocene, Holocene, and Anthropocene.

(ii) shell shape follows Bergmann's Rule in the Pleistocene, Holocene, and Anthropocene.

(iii) shell size and/or shape varies significantly by ecoregion, coastal proximity (islands vs. mainland-collected), and/or coastal urbanization.

1.3 Methods and Materials

1.3.1 Sample Selection

Californiconus californicus shells were measured from museum specimens at the Academy of Natural Sciences of Drexel University (ANSP; n=27), California Academy of Sciences (CAS; n=301), Museum of Comparative Zoology at Harvard University (MCZ; n=199), Natural History Museum of Los Angeles County (LACM-M for Malacology and LACM-IP for Invertebrate Paleontology; n=1272 and 331 respectively), Santa Barbara Museum of Natural History (SBNHM; n=274), Scripps Institute of Oceanography (SIO; n=19), samples collected by S. Ul-Hasan (PR; n=474), and digital photos from iNaturalist observations (iNat; n=99). Each museum specimen lot represents a discrete spatiotemporal sampling of shells and a variety of collection, collector, and other data (Supplementary Tables 1–2). The resulting dataset approximates *C. californicus* size and shape from the Pleistocene through the Anthropocene along the California–Baja coastline (Figure 1.1).
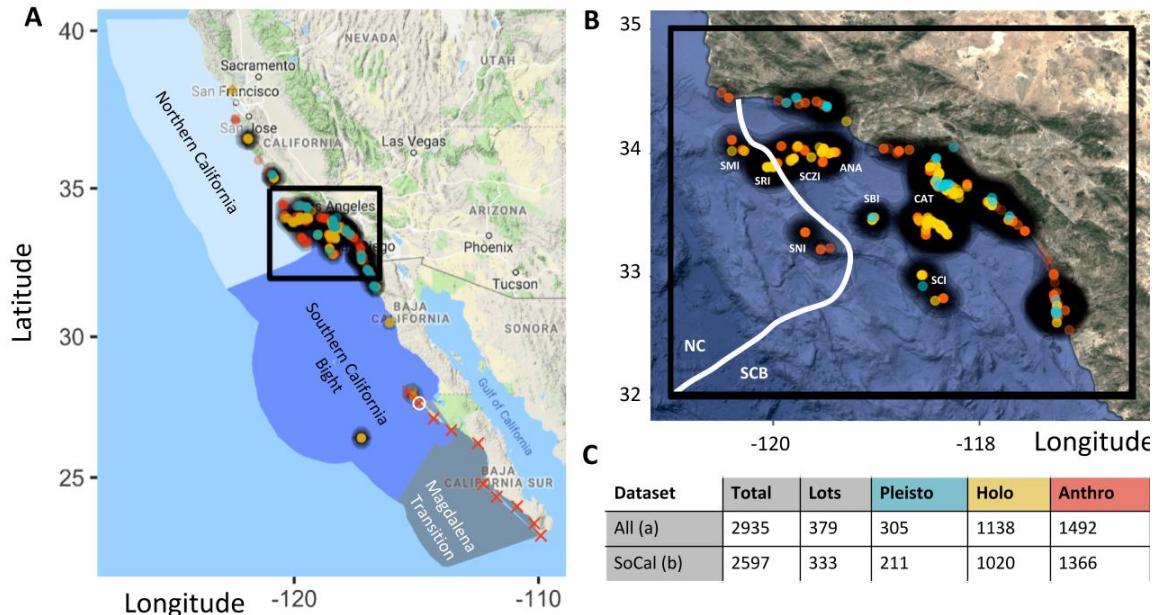
Figure 1.1 Map of specimen localities of adult *C. californicus* shells included in this analysis.

Dots in aqua are Pleistocene, gold are Holocene, and red-orange are Anthropocene. (a) Distribution of specimens included in this analysis, with individuals represented as dots, along the California and Baja coastline. Snail population density is shaded in black. Red Xs denote where *C. californicus* has been searched for, but not found, in the 21st century. The white circle represents a location where *C. californicus* has been found in the 21st century that overlaps with an absence marker. (b) A close up of the Southern California Bight including the California Channel Islands (ANA=Anacapa, SCZI=Santa Cruz, SRI=Santa Rosa, SMI=San Miguel, SCI=San Clemente, SNI=San Nicolas, SBI=Santa Barbara, and CAT=Santa Catalina). The white line indicates the boundary between the Northern California (NC) and Southern California Bight (SCB) ecoregions (see Supplemental Table 1). (c) A table summary of specimens across all locations (a) and specimens in the SoCal or Southern California region (b) included in the analyses of this study.

1.3.2 Shell Measurements

Shells were measured in millimeters for length (L), width (W), aperture width (A), and shell thickness (T) (Figure 1.2, Supplemental Tables 2–4) (Kitching and Lockwood, 1974; Kemp and Bertness, 1984). Measurements were made using Neiko 01407A digital vernier calipers with a resolution of 0.01mm. Shells 5mm long or longer, from shell apex/protoconch to the anterior siphonal notch/aperture, were considered mature adults and measured for analyses (Hellberg *et al.,* 2001).

Shell length, width, aperture width, and thickness were measured in 2935 adult *C. californicus* shells (Supplemental Table 2): 305 from the Pleistocene, 1138 from the Holocene (1892–1941) including the Channel Islands to test for mainland versus island size and/or shape

differences (Porcasi et al., 1999), and 1492 from the Anthropocene (1946–2018, with a gap between 1992-2009), the time interval most heavily impacted by humans (Dirzo *et al*. 2014; Figure 1.1, Supplemental Table 3). Shell width and length were used to calculate shell shape as globosity, or aspect ratio, (G = W/L) (Melatunan et al, 2012, Kemp and Bertness, 1984) and geometric mean (Olabarria and Thurston 2003, Berke et al. 2012; Klingenberg 2016) (Supplemental Tables 2–4). Damaged shells (e.g. with crushed spires or broken apertural lips) and those from lots with fewer than 3 specimens (Teske et al. 2007), with the exception of *C. californicus* observations from iNaturalist, were excluded from this study. Adult *C. californicus* shells from iNaturalist observations (n=99 of ~200 total available) were measured digitally using ImageJ2 (Reuden et al. 2017). Images were selected for measurement only if the shell was displayed horizontally. Globosity was then recorded as the distance from the base of the aperture to the tip of the apex according to the pixel ratio of the shell for an animal.

We identified and removed outliers outside of 95% confidence for all specimens as length > 20 mm, width < 2 mm or > 15 mm, aperture < 2 mm, thickness < 2 mm, and globosity < 0.71 or > 0.42 ratio (Figure 1.2, Supplemental-Results.rmd). We assume that shells measured for this study, from the Holocene and Anthropocene, were collected as recently dead, and in the Pleistocene, represent comparable populations across time.

Californiconus californicus is dioecious but lacks sexually dimorphic shell characters (Shaffer 1986), thus the sex of sampled shells was not recorded. Evidence of predation and/or predation attempts on shells such as scars, boreholes or crushed whorls, and the presence of any epibionts were recorded though not included in the analyses of this study (Shanks 2001; Supplemental Tables 1–2). Measurements were not made from shells in which epibionts altered shells length, width, or thickness.
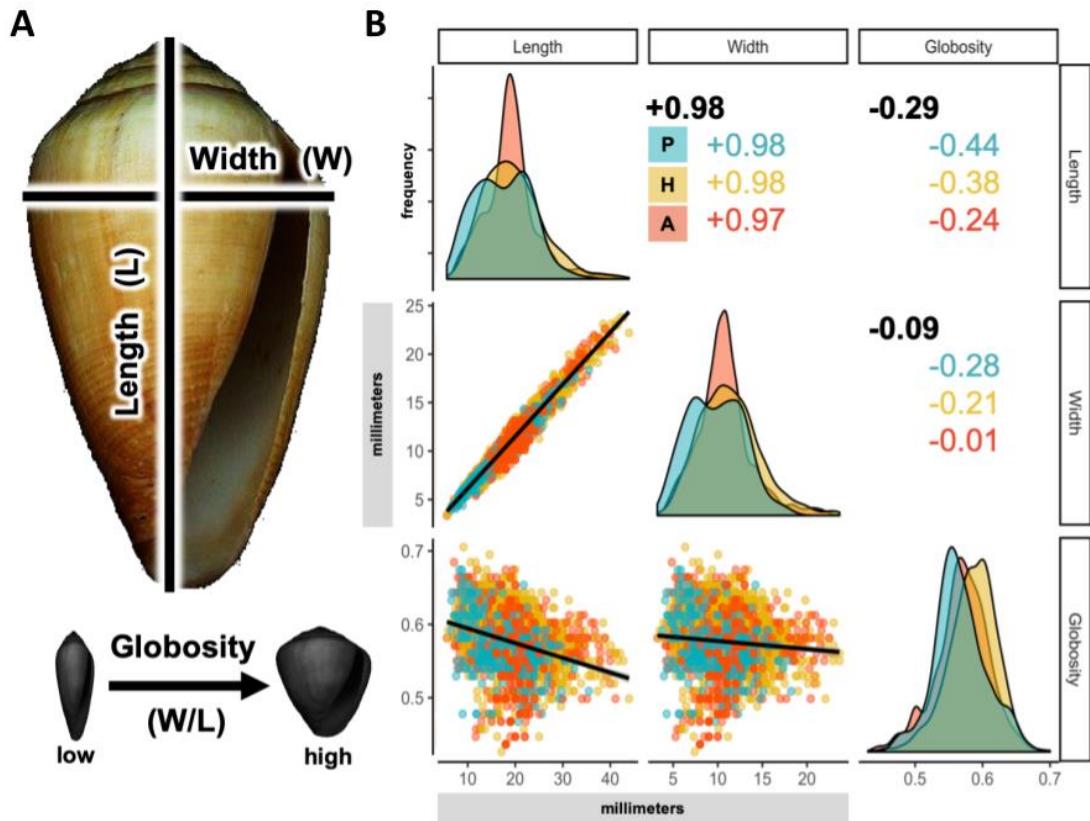
Figure 1.2. Measures of adult *C. californicus* shell length and width and tests of correlation between shell size and shape in the Anthropocene, Holocene, and Pleistocene. Blue indicates Pleistocene, yellow: Holocene, and orange: Anthropocene. (a) Shell measurements of length (L),width (W), and globosity (G, as W/L). (b) Frequency distributions and correlations of and between shell length, width, and globosity for 2897 *C. californicus* shells in the A = Anthropocene, H = Holocene, and P = Pleistocene, after removal of outliers (Supplemental-Results.Rmd). p-values are for each epoch and the combined data from all epochs (in black). Shell image by G. and Ph. Poppe.

1.3.3 Epoch Designations, Geographic Range, and Data Bins

The epochs used herein are defined as follows: Pleistocene: any fossil shells from museum collections labeled as Pleistocene (2.5MYA–1892); Holocene: from the earliest human-collected specimens in museum collections to the start of the atomic era (1892–1941); and Anthropocene:from 1942 to the present (though no museum shell collections for this species spanned 1942–1945) following Zalasiewicz et al. 2010, Zalasiewicz *et al*. 2015, and Pettetier and Coltman 2018, with the knowledge that this epoch designation is used unofficially (Zalasiewicz *et al*., 2019).

Shell collection localities were divided into geographical regions to compare morphological variation within and between ecoregions spanning the range of *C. californicus* (Supplemental Tables 2–4). The marine ecoregion boundaries defined by Spalding et al. (2007) were used to align with previous studies of mollusks in coastal California (Hellberg et al., 2001), and to test for differences between populations north and south of Point Conception (Santa Barbara County), a well-known biogeographic boundary (Wares et al. 2001, Payne et al. 2012). Shells from the California Channel Islands were grouped into two regions: the Northern Channel Islands (San Miguel, Santa Rosa, and San Nicolas) and the Southern California Channel Islands (Santa Cruz, Anacapa, Santa Barbara, San Clemente, and Santa Catalina).

1.3.4 Sea Surface Temperatures and Urbanization Impact as Human Population Density and MPAs

Sea surface temperatures (SSTs) were extracted from the Hadley Centre SST data set (HADISST; Rayner et al. 2003, 2005; HADISST accessed 01 March 2019) and used to calculate the mean annual temperature and its standard deviation at each *C. californicus* locality during the Holocene and Anthropocene. Data were processed using R programming language (R 3.5.0 R Core Team 2018, Supplemental Tables 2–4). Pleistocene mean annual SST and annual variance were extracted from the Last Glacial Maximum (21 ka) ensemble of all climatological models from the MARSPEC dataset (Braconnot et al. 2007; Sbrocco 2014) in ArcGIS 10.5. For these datasets, the value in the nearest SST pixel was used for locations where *C. californicus* were found that did not overlap with SST data.

Human population density was extracted from model 5 in the dataset of Fang and Jawitz (2018) using ArcGIS 10.5 from the coastal pixel nearest to each location of *C. californicus* in the decade of collection between the Holocene and Anthropocene, except from 1960–1970 due to lack of data. All data processing and analyses were conducted using R (code available at https://github.com/MichaelMalloy/C_californicus_Morphology). Unrealistic outlier values in size or shape outside of a 95% confidence interval were removed (Supplemental Tables 2-3, Supplemental-Results.rmd, Figure 1.2).

Data from shells collected in the Southern California Bight marine ecoregion (Figure 1.1a, 1.1b), were subdivided into coastal and island bins and assigned levels of protection based on the year a Marine Protected Area (MPA) was established. For example, if specimens were collected from an area that was established as an MPA in 2012 then any specimens collected prior to that year were designated as "not protected" and any specimens collected after were designated as "protected". MPAs in this region have not been established sufficiently long or frequent enough for us to conduct analyses with statistical power for the significant difference between specimen lots designated as MPA or non-MPA over time.

1.3.5 Statistical Analyses

Shell thickness and aperture width were tightly correlated to shell length and width, and thus were not analysed separately (Supplemental Table 2, Supplemental-Results.rmd). Geometric mean was calculated as a proxy for shell shape as in other studies (Olabarria and Thurston 2003, Berke et al. 2012), and showed similar results as shell width and length. Because length showed

some minimal correlation to globosity whereas width showed little to no correlation to globosity, width was used as a proxy for size (Supplemental Tables 2-4, Supplemental-Results.rmd, Figure 1.2b). Pearson correlation coefficients were used to compare relationships between shell morphometrics and their variation between epochs ($\alpha = 0.05$, Figure 1.2). For each epoch, linear regressions were used to compare size and shape against latitude and annual mean SST (Figure 1.3). Slope values from generalized linear models (Figure 1.3) denoted with an asterisk indicate a p-value < 0.05; an overall trend was determined to be of greater importance than the r^2 values because of the small scale for size and shape measurements (i.e. we would not expect the globosity ratio to change from 0 to 1 from the Pleistocene to Anthropocene, an $r^2 = 1$, as this would be unrealistic). Tukey's test of Honest Significant Differences, analysis of variance or ANOVA, and Spearman's rho rank correlation were used to determine if data could be compared linearly or non-parametrically, and to identify correlative relationships between oceanographic and biological covariates and *C. californicus* shell descriptors (Supplemental-Results.rmd). General additive models were used to examine the variance in shell width and globosity explained by different nonlinear terms (k, distribution) such as epoch, mean SST (linear, in some instances), latitude (linear, in some instances), ecoregion, coastal proximity, and human population density (Tables 1.1–1.2, Supplemental-Results.rmd).

1.4 Results

1.4.1 Distribution of Adult *C. californicus* Shells

Consistent with the range stated in McLean (1978), the specimens examined herein (from museum collections and iNaturalist) were found with the greatest density within the Southern California Bight ecoregion within the Pleistocene, Holocene, and Anthropocene, and not in the Magdalena Transition as stated by some authors (Peters et al. 2013) (Figure 1.1).

The abundance of *C. californicus* in the Southern California Bight appears consistent across time (Figure 1.1, Supplemental-Results.rmd). That *C. californicus* specimens were not found within the Magdalena Transition ecoregion could be due to collector bias or that collections in this study were restricted to museums in the United States. However, no *C. californicus* specimens were found in the Magdalena Transition ecoregion by two independent research groups between 2004 and 2018. These locations are shown by red "Xs" in Figure 1.1 (Supplemental Table 1). Likewise, research vessels Velero III, Velero IV, and SEARCHER also ventured south of Punta Abreojos, Baja, Mexico with expeditions ranging from 1940–1970 and did not collect *C. californicus* shells (Supplemental Table 2). The "O" located at Punta Abreojos, a location in the Southern California Bight ecoregion, denotes that *C. californicus* was observed at that location in the 21st century by W. Gilly (Supplemental Table 1).

Length, width and globosity for adult *C. californicus* shells (Figure 1.2a) all displayed relatively normally (Figure 1.2b) despite the Pleistocene sample size (n=305) being smaller than that of the Holocene (n=1138) and Anthropocene (n=1492). Linear regressions indicate size (length or width) and shape (globosity) are independent of each other in each time epoch (Pleistocene, Anthropocene, Holocene) ($r^2 = 0.085$, $p < 0.0001$ for Length~Globosity and $r^2 = 0.007$, $p < 0.0001$ for Width~Globosity). The correlation between length and width was nearly $+1.0$ in all three epochs (Figure 1.2b). Length and width were treated as equivalent because of

their strong correlation, and shell aperture width and shell thickness were strongly dependent on shell length and/or width (Supplemental-Results.Rmd).

1.4.2 Latitude and Sea Surface Temperature (SST)

Overall, the California-Baja coastline can be treated as geographically linear with a strong negative correlation between temperature and distance to the equator (Supplemental Figure 1.1, Supplemental-Results.Rmd). Most specimens analyzed in this study are within the Southern California region, where there is shell size and shape vary for specimens from mainland versus island coastlines (Figure 1.4, Supplemental Figure 1.2). As expected, the sea surface temperature is higher in the Holocene and Anthropocene epochs than the Pleistocene epoch. Mean SST was not closely correlated to shell shape or size (Figure 1.3, Table 1.1, Supplemental Figure 1.2). There is a significant difference ($p < 0.05$) between Pleistocene, Holocene, and Anthropocene mean SST, with the sea surface temperature being higher in the Holocene and Anthropocene epochs than during the Pleistocene. We tested the relationship between size, shape, and mean SST by epoch, and found that size increases with latitude across all epochs and significantly ($p < 0.05$) decreases with temperature for the Holocene , and that shape significantly decreases with latitude for the Pleistocene but increases for the Anthropocene and increases with temperature for the Holocene by decreases for the Pleistocene and Anthropocene (Figure 1.3, Supplemental-Results.Rmd).
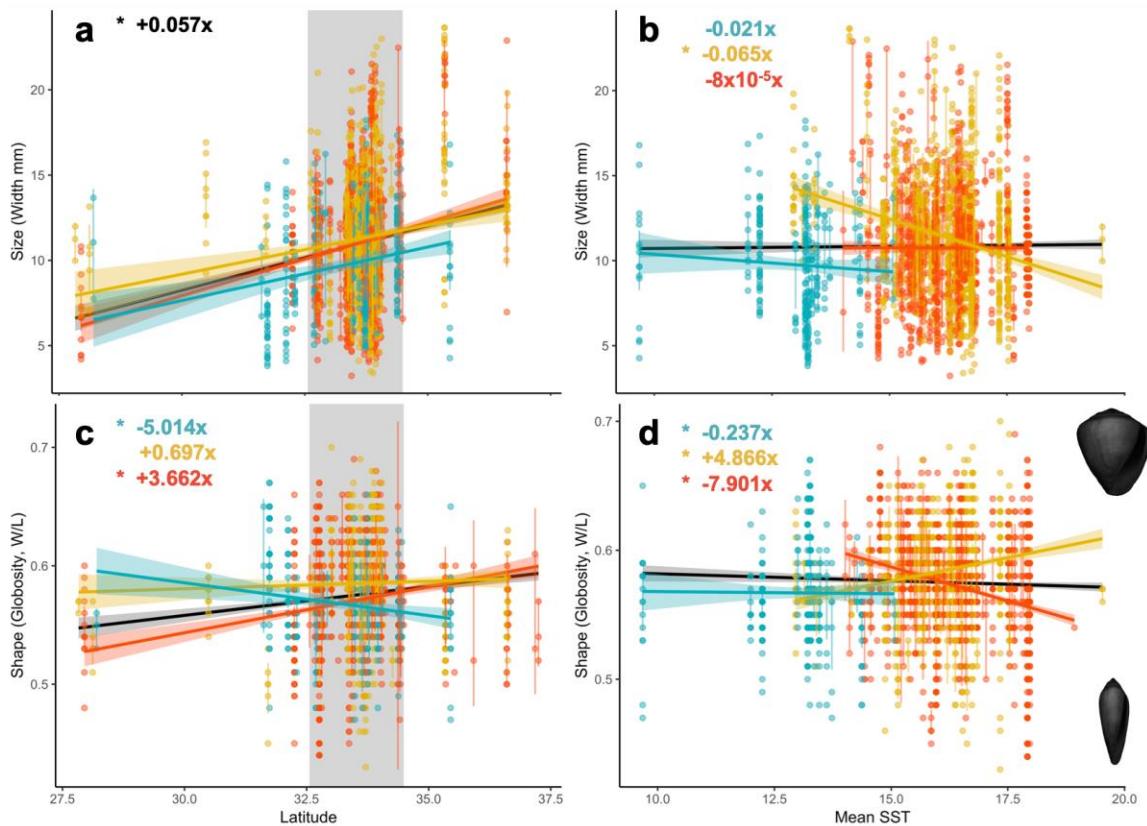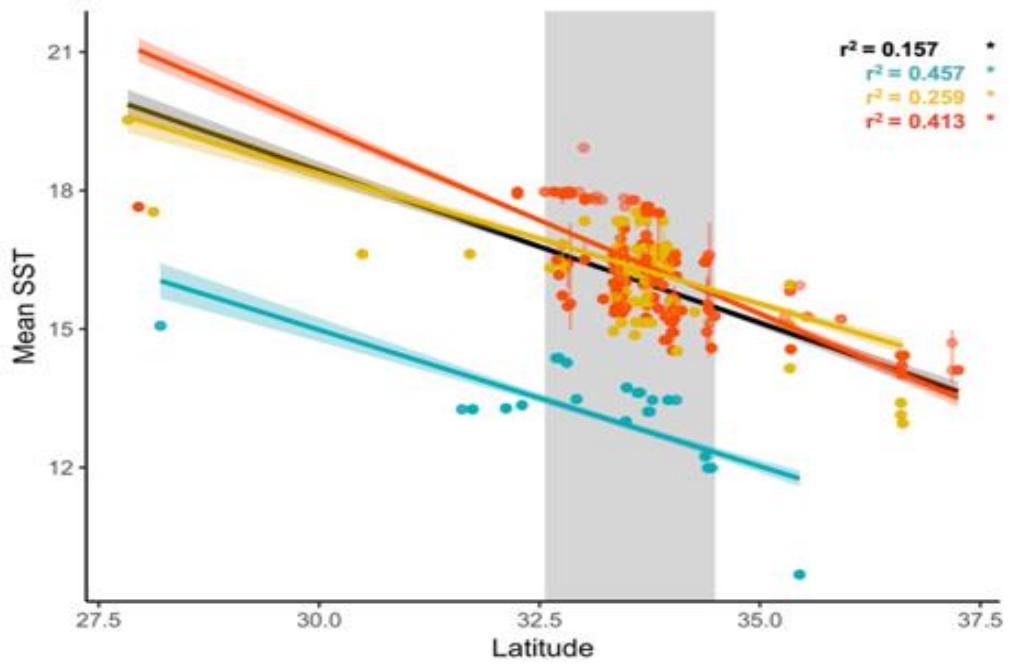
Figure 1.3. General linear regressions of adult *C. californicus* shell width and globosity plotted against latitude and mean SST in the Anthropocene, Holocene, and Pleistocene. Dots indicate Pleistocene (aqua), Holocene (gold), and Anthropocene (red-orange). Values of *C. californicus* specimens from Southern California region corresponding to Figure 1b, including the California Channel Islands, are shaded in gray (latitudes 32.56–34.49). Numeric values in aqua, gold and red-orange indicate slopes by overall trend (black) or by epoch, with asterisks designating significance ($p < 0.05$).

Table 1.1. Generative interactive models examining relationships between size (width) and shape (globosity) and latitude and sea surface temperature across epochs.
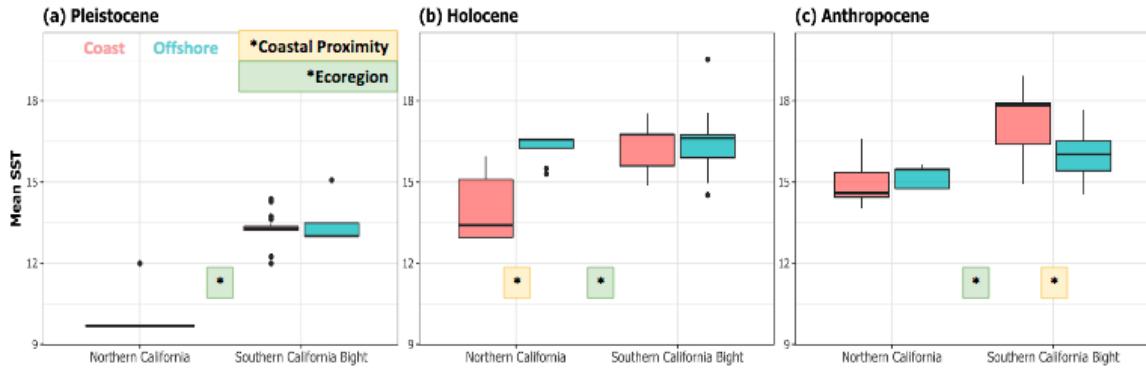
Term	Coefficients (β)	SE (stnd error)	t-value	P (> | t |)
Width ~ Latitude + Epoch + Latitude * Epoch				
Latitude	1.008	0.174	5.783	< 0.0001
Epoch	3.970	3.059	1.298	0.194
Latitude * Epoch	-0.127	0.092	-1.388	0.165
The simplest test of Bergmann's Rule: size changes with latitude.				

Width ~ SST + Epoch + SST * Epoch

SST	-0.558	0.119	-4.694	< 0.0001
Epoch	-4.272	0.828	-5.158	< 0.0001
SST * Epoch	0.265	0.053	5.006	< 0.0001

The relationship between width and mean sea surface temperature varies by epoch.

Globosity ~ Latitude + Epoch + Latitude * Epoch

Latitude	0.015	0.002	7.483	< 0.0001
Epoch	0.207	0.036	5.774	< 0.0001
Latitude * Epoch	-0.006	0.001	-5.662	< 0.0001

The relationship between globosity and latitude varies by epoch.

Globosity ~ SST + Epoch + SST * Epoch

SST	-0.018	0.001	-13.400	< 0.0001
Epoch	-0.136	0.009	-14.520	< 0.0001
SST * Epoch	0.009	0.001	15.220	< 0.0001

The relationship between globosity and mean sea surface temperature varies by epoch.

Supplemental Figure 1.1. Relationship between mean sea surface temperature and latitude throughout the range of *C. californicus*.

Across three epochs (black), mean sea surface temperature (SST) temperature increases as latitude decreases (linear regression, $y = 38.222 - 0.660x$, $r^2 = 0.157$, $p < 0.0001$). An asterisk next to an r^2 value indicates that the p-value for that linear regression was significant (< 0.05). Mean SST was lower in the Pleistocene than in the Holocene and Anthropocene. The shaded gray region indicates latitudinal span of Southern California specimens (32.56 – 34.49 decimal degrees).

Supplemental Figure 1.2. Mean sea surface temperature by coastal proximity (coastal= mainland and offshore= island) and ecoregions as the Northern California ecoregion (NCE) and the Southern California Bight (SCB) in the Anthropocene, Holocene, and Pleistocene. Mean sea surface temperature for the (a) Pleistocene, (b) Holocene, and (c) Anthropocene in NCE and SCB for coastal versus offshore regions. Significant differences between ecoregions are shown in green, and significant differences by coastal proximity within an ecoregion are shown in yellow.

1.4.3 Adult *C. californicus* Shell Size and Bergmann's Rule

Our results indicate small, but significant, variation in adult *C. californicus* shell morphology in time and space (Figures 1.3–1.4). *Californiconus californicus* size, as measured by shell width (W), is independent of shell shape, here calculated as globosity (W/L) (Figure 1.2). Shell specimens were wider at higher than lower latitudes across all epochs (Figure 1.3a), consistent with Bergmann's Rule. Also, similar to predictions of Bergmann's Rule, shell size (as width) decreased with increasing mean SST (i.e. warmer ocean temperatures), but the slope was only significant ($p < 0.05$) for the Holocene (Figure 1.3b). Differences between SST at coastal (mainland) and off-shore (island) locations in the Holocene and Anthropocene may explain the weaker relationship between SST and size due to being a nonlinear region within *C. californicus* range (Supplemental Figure 2, SST ~ Coastal Proximity: $p = 0.923$ across Epochs, $p = 0.03$ for the Pleistocene, $p < 0.0001$ for the Holocene and Anthropocene).

1.4.4 Adult *C. californicus* Shell Shape and Bergmann's Rule

Shell shape only conformed to Bergmann's Rule in the Anthropocene (Figure 1.3c), while the opposite pattern was found in the Pleistocene and no pattern was found in the Holocene (Figure 1.3c). Our results show that specimens were slightly more globose at higher latitudes during the Pleistocene, whereas specimens were narrower at northern latitudes during the Anthropocene, and without any difference during the Holocene (Figure 1.3d). These conflicting clines may indicate that there is little to no correlation between shell shape and temperature or latitude.

1.4.5 Adult *C. californicus* Shell Size and Shape by Marine Ecoregion

For size, differences by ecoregion were significant ($p < 0.05$) for the Holocene and Anthropocene, but not for the Pleistocene (Table 1.2, Figure 1.3a-c). For shape, differences by ecoregion were significant for the Holocene, but not for the Pleistocene or Anthropocene (Figure 1.3d-f). Animals are overall larger in size (significant for Holocene and Anthropocene) and rounder in shape (significant in Holocene only) for the Northern California ecoregion than the Southern California Bight. A significant difference was observed for mean sea surface temperature (SST) between ecoregions across all epochs, showing that the Northern California ecoregion is warmer than that of the Southern California Bight and supporting current ecoregion boundaries outlined by Spalding, 2007 (Supplemental Figure 1.2, Figure 1.1). Further, SST increases across all epochs.

1.4.6 Adult *C. californicus* Shell Shape and Size by Coastal Proximity (islands vs. mainland coastline)

For size, specimens were significantly ($p < 0.05$) larger on the mainland in Northern California for the Holocene and Anthropocene, but not significantly different in size for the Southern California Bight marine ecoregion islands versus mainland (Table 1.2, Figure 1.4a-c). For shape, specimens are less globose (more narrow) near the mainland for the Northern California marine ecoregion with no difference for the Southern California Bight ecoregion in the Holocene and comparatively more narrow in shape for the mainland than islands for both the Northern California and Southern California Bight ecoregions (Figure 1.4d-f). Additionally observed were significant differences in mean SST between for coastal proximity (mainland coast or island) within the Northern California Bight during the Holocene, being cooler on the coast than offshore, and being warmer on the mainland than offshore within the Southern California Bight ecoregion for the Anthropocene (Supplemental Figure 1.2).
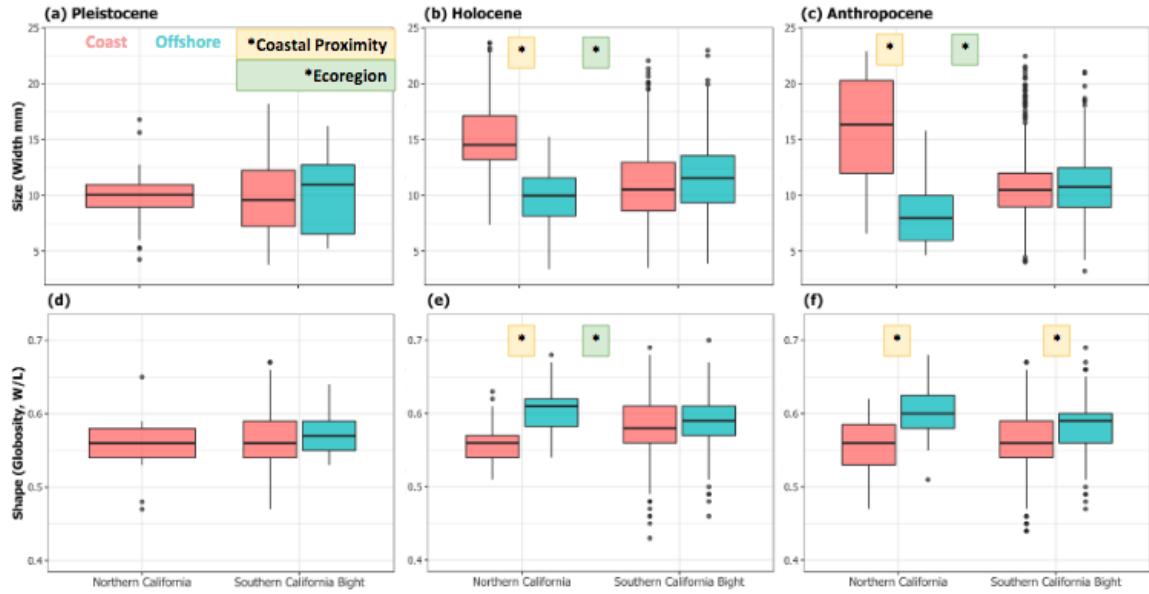
Figure 1.4. Adult *C. californicus* shell size and shape by ecoregion, coastal proximity (coastal= mainland and offshore= island), and epoch in Northern California ecoregion (NCE) and the Southern California Bight (SCB).
Pleistocene, Holocene, and Anthropocene shell size (as width) in NCE and SCB (a–c). Note: no specimens were included for Northern California from the Pleistocene. Pleistocene, Holocene, and Anthropocene shell shape (as globosity) in NCE and SCB (d–f). Significant differences between ecoregions are shown in green, and significant differences by coastal proximity within an ecoregion are shown in yellow.

Table 1.2. Global interactive models examining relationships between size or shape and ecoregion and coastal proximity (island versus mainland) across epochs.
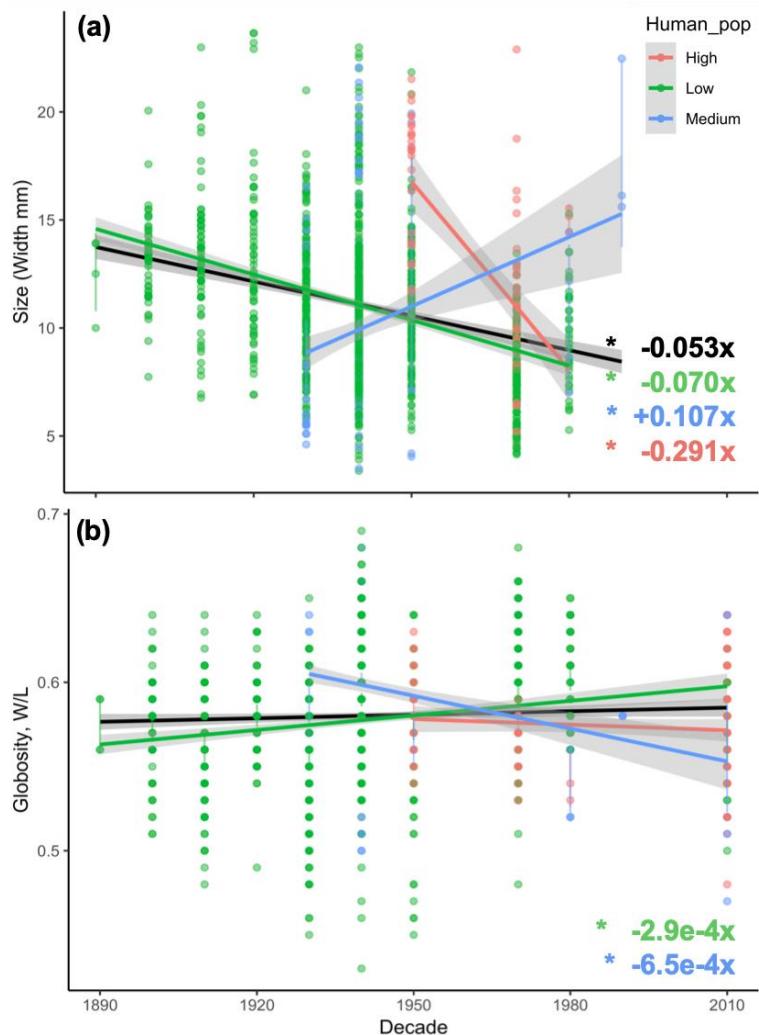
Term	Coefficients (β)	SE	t-value	P (> | t |)
Width ~ Ecoregion + Coastal Proximity + Ecoregion + Coastal Proximity (Pleistocene)				
Ecoregion	-0.266	0.701	-0.379	0.705
Coastal Proximity	0.258	0.774	0.334	0.739
Ecoregion * Proximity	NA	NA	NA	NA
There is no relationship between Width, Ecoregion and/or Coastal Proximity in the Pleistocene.				

Width ~ Ecoregion + Coastal Proximity + Ecoregion + Coastal Proximity (Holocene)				
Ecoregion	-10.137	0.977	-10.370	< 0.0001
Coastal Proximity	-11.216	1.105	-10.150	< 0.0001
Ecoregion * Proximity	5.875	0.583	10.080	< 0.0001
There is a significant relationship for Width, Ecoregion and/or Coastal Proximity in the Holocene.				
Width ~ Ecoregion + Coastal Proximity + Ecoregion + Coastal Proximity (Anthropocene)				
Ecoregion	-12.320	1.022	-12.050	< 0.0001
Coastal Proximity	-14.718	1.202	-12.250	< 0.0001
Ecoregion * Proximity	7.411	0.619	11.970	< 0.0001
There is a significant relationship for Width, Ecoregion and/or Coastal Proximity in the Anthropocene				
Globosity ~ Ecoregion + Coastal Proximity + Ecoregion + Coastal Proximity (Pleistocene)				
Ecoregion	0.009	0.009	1.000	0.318
Coastal Proximity	0.008	0.009	0.805	0.422
Ecoregion * Proximity	NA	NA	NA	NA
There is no relationship between Globosity Ecoregion and/or Coastal Proximity in the Pleistocene.				
Globosity ~ Ecoregion + Coastal Proximity + Ecoregion + Coastal Proximity (Holocene)				
Ecoregion	0.061	0.010	5.950	< 0.0001

Coastal Proximity	0.088	0.012	7.516	< 0.0001
Ecoregion * Proximity	-0.039	0.006	-6.326	< 0.0001
There is a significant relationship for Globosity, Ecoregion and/or Coastal Proximity in the Holocene.				
Globosity ~ Ecoregion + Coastal Proximity + Ecoregion + Coastal Proximity (Anthropocene)				
Ecoregion	0.025	0.012	2.122	0.034
Coastal Proximity	0.065	0.014	4.577	< 0.0001
Ecoregion * Proximity	-0.021	0.007	-2.833	< 0.0001
There is a significant relationship for Globosity, Ecoregion and/or Coastal Proximity in the Anthropocene				

1.4.7 Adult *C. californicus* Shell Shape and Size by Coastal Urbanization & Human Population Density

Trends show different relationships for size and shape in relation to human population density that infer factors influencing size and/or shape outside of urbanization (Supplemental Figure 1.3). For size, correlations over the past century were significant ($p < 0.05$) across all to human population density levels (overall, low, medium, and high). Over time, animals decrease in size for areas where human population density is low or high but increase in size for areas where human population density is medium. For shape, correlations over the past century were significant for low and medium human population density levels. Over time, shells become more globose where human population densities are low but more narrow as human population densities increase. Comparisons of shell size and shape in Marine Protected Areas (MPAs) versus non-MPAs were also tested, though specimen lots were not high enough in number over time for statistical power to determine significance (Supplemental-Results.rmd).

(a)

Human_pop
— High
— Low
— Medium

* **-0.053x**
* **-0.070x**
* **+0.107x**
* **-0.291x**

(b)

* **-2.9e-4x**
* **-6.5e-4x**

Supplemental Figure 1.3. Shell size and shape exhibit differing relationships to human population density by decade.

Linear models (± 1 CI) of size or shape with decade versus size or shape across categories of human population density. Shell (a) size or (b) shape values corresponding to their respective collection sites as Low human population densities by decade are highlighted as green < 1 SD or 248 people per square mile, Medium as blue +/- 1 SD or 546 people per square mile, High as pink > 1 SD or 2708 people per square mile, and the overall trend as black. Slope values denoted with an asterisk correspond to a $p < 0.05$ as overall trend was determined of greater importance than the r^2 values.

1.5 Discussion

1.5.1 Distribution and Range of Adult *C. californicus*

Modern *Californiconus californicus* examined in this study ranged from the city of San Mateo in the greater San Francisco area to Isla de Cedros, Baja California, a span shorter than that reported by the IUCN (Peters et al. 2013). Notably, other reports of the southernmost limit of this species include Cabo San Lucas, Baja, Mexico (IUCN 2011) and rarely La Paz, Baja, Mexico in the Gulf of California (Keen 1971). Lugo et al. (2016) reports *C. californicus* prefers an average temperature of 23.3 C, with a range from 21.9 - 24.8 C. This study is based on *C. californicus* in the Puerto Nuevo, Mexico area and thus temperature preferences may vary by population as we have demonstrated *C. californicus* to be found outside of that temperature range though rare south of Isla de Cedros through museum collections, citizen science species observations (via iNaturalist), and personal observations and observations (Supplemental Tables 1–2, Figure 1.3). Population genetic estimates of connectivity for *C. californicus* are unknown, and *C. californicus* is the only conid species to reside north of Baja California Sur, and is likely tolerant of colder temperatures. Shell collections north of Monterey Bay were few in number for a given lot and sparse compared to those south of Monterey (Supplemental Table 2). Use of museum records, iNaturalist observations (as digital images), and anecdotal information, can thus serve towards stronger evidentiary standards for reporting of species ranges (McKelvey et al., 2008, Boessenkool et al, 2019).

Present-day ranges of species are increasingly central to understanding population response and adaptation in the Anthropocene (Schimel et al. 2013, Birks et al. 2016). Unfortunately, there is a limited number of modern specimens available from the last fifty years, the interval during which anthropogenic effects have intensified. These findings add justification for *C. californicus*, a prevalent predator and one of few venomous marine snails along the California-Baja coastline (Kohn 1966, Duda and Palumbi 2004, Stewart and Gilly 2005, Elliger et al. 2011, Gilly et al. 2011), to become a species of interest in marine biodiversity surveys and specimen collection by museums. The tracking of *C. californicus* populations over time for characterizing its range and determining the extent to which it has expanded northward alongside other invertebrates (Sorte et al. 2010, Morley et al. 2018) could be critical as sea temperature continues to rise, adding justification for expansion and wide-practice of citizen science initiatives such as iNaturalist.

1.5.2 *Californiconus californicus* Shell Size and Shape within Bergmann's Rule

Consistent with Bergmann's Rule, there was a size (as specimen width) cline with increasing latitude such that *C. californicus* specimens were larger at higher latitudes in all three epochs (Figure 1.3, Table 1.1). However, the relationships between size and mean sea surface temperature are less clear, and are not consistent across epochs. Similar to interpretations that Bergmann's Rule is a phenomenon based on temperature, size significantly decreases with increasing mean SST (or shell size increases with cooler and higher latitudes), but only during the Holocene. Globosity as a measure of shell shape rather than size was also tested for compliance with Bergmann's Rule, but since larger specimens appear to grow in an allometrically-independent pattern changes in globosity were independent of changes in size. As a result, unsurprisingly, globosity did not follow a consistent latitudinal pattern (Figure 1.3, Table 1.1). It instead varied locally along the range of the species, in all three tested epochs. Shape becomes more globose in the Anthropocene, while the opposite pattern was found in the Pleistocene, and no pattern was found in the Holocene. During the Pleistocene, there was a shell shape change from globose to narrow, from southern latitudes (i.e. Baja) to higher latitudes (i.e. 36 degrees). Conversely, specimens collected during the Anthropocene across the range of this species are more narrow at lower latitudes (i.e. Baja) and more stout at higher latitudes (i.e. Monterey). Since interpretations of Bergmann's Rule, including the various studies measuring molluscs, typically focus on animal size, we believe that width is a better measure for compliance with Bergmann's Rule than globosity.

Our findings add that Bergmann's Rule should be accounted for with respect to geological time as well as clearly defined latitudinal and/or temperature thresholds. One such instance is the Southern California Bight region, in which *C. californicus* is found in high abundance (Figure 1.3). For instance, gastropod *Littorina keenae* demonstrates phenotypic plasticity for its body size in response to the environment across latitudes (Lee and Boulding 2010). Ectotherms are also strongly influenced by the surrounding environmental temperatures (Vinarski 2014, Partridge and Coyne 2017). Results from this study are consistent with studies of molluscan responses to sudden shifts in climate and abiotic conditions. Responses to changing temperatures have been found in the sympatric *Acanthinucella spirata* in a population shift northward (Hellberg et al. 2001), a species similar to *C. californicus* in also preferring the high intertidal, where wave action effects are most significant (Brown and Quinn, 1998). Morphological studies testing for macroecological patterns of size clines such as Bergmann's Rule can face a number of complications and contradictions (Gaston and Blackburn 2008).

1.5.3 Shell Shape and Size by Marine Ecoregion and Coastal Proximity (islands vs. mainland coastline)

Specimens are significantly larger in the Northern California ecoregion than the Southern

California Bight ecoregion for the Holocene and Anthropocene (Figure 1.4). These results are consistent with marine ecoregion boundaries outlined by Spalding, 2007 (Figure 1.1). This observation aligns with known differences between species collected in areas north and south of the traditional "biogeographical break" at Point Conception (Murray and Littler 1981, Murray and Bray 1993). When also accounting for shell shape the majority of changes in intertidal community makeup appear to have occurred over a 60-year period overlapping the Holocene and Anthropocene ending in 1994 (gap in collections, Figure 1.4 and Supplemental Figure 2) as a possible result of increases in SST, possibly blurring the distinction between ecoregions north and south of Point Conception (Barry et al. 1995, Blanchette et al. 2008) and prospectively driving range expansion and morphological evolution of warm-water marine gastropods (Hellberg et al. 2001).

 We do not observe island gigantism (Lomolino 1985) in adult *C. californicus*, but do observe more globose shells for islands specifically in the Holocene and Anthropocene (Figure 1.4). These results may be due to colder temperature differences between island and mainland especially notable in the Anthropocene (Supplementary Figure 1.2). Additional factors may play a role, such as the dynamic currents of the Channel Islands (Conde-Padín et al. 2009) and/or potential impacts from humans (Johnson & Baarli, 1999). Our findings on shell size or shape in relation to urbanization are correlatory (Supplemental Figure 1.3), and thus mechanisms behind these results are unclear.

1.5.4 Dispersal Ability and Larval Development as Confounding Factors

The dispersal ability and larval developmental mode of *C. californicus* are unknown, and we thus cannot be certain that effects reflect local variation with the degree of specificity typically seen in egg-laying species (Shaffer 1986). Due to our lack of knowledge of *C. californicus* life history, we do not know the generation time of populations, which can affect the speed at which morphological responses evolve. This presents difficulties in identifying short term trends in morphology (Bourdeau et al., 2015). Determination of dispersal time as well as differences in growth rates or precise age structure can make short-term studies of local variation difficult. Models of species spawning in the currents surrounding the Southern California Bight, however, have found that the planktonic period does not substantially affect the gene flow of dispersing species (Hohenlohe 2004). To address these short-term dispersal issues, a focus on longer-term trends in temperature over the range of the species was utilized. Determining the effects of warming on this species' range would further inform the effects of critical topics such as human urbanization on the range of gastropods (Rivadeneira 2005, Supplemental Figure 1.3). Evidence indicates that factors which determine the limits of distribution of marine gastropods include the cooling resistance of these species (Pörtner, 2001) and larval dispersal (Gaylord and Gaines, 2000).

1.6 Conclusions

Our findings indicate that (i) adult *C. californicus* does adhere to Bergmann's Rule across epochs for size across but this rule (ii) cannot be applied to shell shape, and (iii) both shell size and shape vary by epoch for either ecoregion or coastal proximity differences. The need for continuing studies of morphological trends requires use and continued growth of museum collections (Wandeler et al, 2007). The paucity of specimens available after 1990 interrupts a continuum of data necessary for understanding species size, range, and abundance in time and space, which is fundamental to studies of extant and extinct biodiversity (Moss et al, 2016). Thus, museum records are essential for species and faunal-level studies of ecological and evolutionary processes (Cook et al., 2014, Suarez and Tsutsui 2004). Today, these collections are complemented by citizen science platforms (e.g. iNaturalist), which contribute real-time data points as species observations generated by the public, as well as large-scale efforts to digitize museum collections for greater accessibility and research applicability (Marshall et al. 2018).

Author Contributions
MEM and SU contributed to experimental design, sampling, and measurements. SU, LLL, MEM, CAO and SS contributed to statistical analyses. JHH, AH, CAO, and SC contributed data for enhancing statistical analyses. MEM, SU, LLL, JHH, SC, CAO, SS, AH, WFG, TW, CJN, LTG, and JEV contributed to the writing of this manuscript.

1.7 References

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Chapter 2

Community ecology across bacteria, archaea and microbial eukaryotes in the sediment and seawater of coastal Puerto Nuevo, Baja California

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2.1 Abstract

Microbial communities control numerous biogeochemical processes critical for ecosystem function and health. Most analyses of coastal microbial communities focus on the characterization of bacteria present in either sediment or seawater, with fewer studies characterizing both sediment and seawater together at a given site, and even fewer studies including information about non-bacterial microbial communities. As a result, knowledge about the ecological patterns of microbial biodiversity across domains and habitats in coastal communities is limited – despite the fact that archaea, bacteria, and microbial eukaryotes are present and known to interact in coastal habitats. To better understand microbial biodiversity patterns in coastal ecosystems, we characterized sediment and seawater microbial communities for three sites along the coastline of Puerto Nuevo, Baja California, Mexico using both 16S and 18S rRNA gene amplicon sequencing. We found that sediment hosted approximately 500-fold more operational taxonomic units (OTUs) for bacteria, archaea, and microbial eukaryotes than seawater (p < 0.001). Distinct phyla were found in sediment versus seawater samples. Of the top ten most abundant classes, Cytophagia (bacterial) and Chromadorea (eukaryal) were specific to the sediment environment, whereas Cyanobacteria and Bacteroidia (bacterial) and Chlorophyceae (eukaryal) were specific to the seawater environment. A total of 47 unique genera were observed to comprise the core taxa community across environment types and sites. No archaeal taxa were observed as part of either the abundant or core taxa. No significant differences were observed for sediment community composition across domains or between sites. For seawater, the bacterial and archaeal community composition was statistically different for the Major Outlet site (p < 0.05), the site closest to a residential area, and the eukaryal community composition was statistically different between all sites (p < 0.05). Our findings highlight the distinct patterns and spatial heterogeneity in microbial communities of a coastal region in Baja California, Mexico.

2.2 Introduction

The identification and description of microbial biodiversity patterns is important for understanding the biological underpinnings of ecosystem function. This is particularly true for coastal microbial communities, as they play important roles in the regulation of biogeochemical cycling at the land-sea interface (Wallenstein et al.; Bauer et al. 2013), and in the ecological dynamics of larger organisms through symbiosis and disease (McFall-Ngai 2014; Ghaisas et al. 2016). Coastal microbial communities are complex and spatially variable (Hollibaugh et al. 2014; Fuhrman et al. 2015; Bowen et al. 2015), consisting of all domains of life interacting with each other in the water column and sediment (Moulton et al. 2016). The heterogeneity of coastal microbial communities thus demands intensive sampling to improve our understanding of microbial ecology and the structure and function of coastal ecosystems. Many studies of coastal microbial communities, however, take place along waters of Western world countries or at somewhat subjective "exotic" locales (Petro et al. 2017). This leaves large swaths of un-sampled/under-sampled coastlines around the world where microbial diversity – and its associated geochemical and physical diversity – is poorly characterized.

A surge in marine microbial community ecology research over the past decade has led to a wealth of new information on the dynamics between microorganisms and their surrounding environments (Fuhrman et al. 2015). As a result, the identification of spatial and temporal patterns of microbial diversity, and how this information correlates to biogeochemical cycling, has been vastly expanded (Jessup et al. 2004; Prosser et al. 2007; Whitton and Potts 2007; Kirchman 2016; Kavagutti 2016; He et al. 2017; Kaestli et al. 2017; Haskell William Z. et al. 2017). A recent commentary by Brussaard and colleagues, for example, highlights the growing roles that "big data" from microbial ecology and biogeochemistry studies play in understanding how microbial communities shape the biogeochemical cycling patterns of coasts and oceans (Brussaard et al. 2016). Such information gathered over time provides a starting point to determining the causes and effects of microbial community disturbances (Hunt and Ward 2015). While these discoveries are innovative by providing new insight into marine microbial ecosystems, much coastal microbial diversity remains uncharacterized (Galloway et al. 2004; Gradoville Mary R. et al. 2017; Angell et al. 2018).

The majority of microbial biodiversity "omics" studies are overwhelmingly focused on bacterial communities using 16S rRNA amplicon sequencing, and are often limited to a specific environment type rather than considering multiple aspects of microbial ecosystems (Röling et al. 2010; Cowan 2018). Coastal microbial communities present a dynamic assemblage to test taxa richness and diversity between two environment types: sediment solids and seawater liquids. As a result of its texture, soil is well known to host high microbial richness across domains (Kuzyakov and Blagodatskaya 2015) and, by extrapolation, this is also likely to be the case for sediment (Hedges and Oades 1997) since sediment also possesses a large surface area for microorganisms to attach (Aleklett et al. 2018). The added value of using next-generation technology with these types of sampling studies is that it provides detailed information on taxa within a larger ecosystem framework.

Investigating the sediment and seawater at one coastal point using biological replicates is advantageous because it allows for the comparisons of species richness estimates and abundance

profiles across sample types (Hill et al. 2003). Taking these measurements into account, an ecological study of microbial mats, for example, observed that bacterial and archaeal mat biodiversity in intertidal, hypersaline, and hot spring environments was influenced by mat chemistry and spatial location, more so than by temporal changes (Bolhuis et al. 2014). These variations between locations can correspond to variations in function and/or recovery after perturbation (Lu et al. 2017), and thus emphasize the importance of simultaneously characterizing both richness and abundance measurements in microbial ecology studies.

While the Baja California coastline shares the same marine ecoregion with the United States (Spalding et al. 2007), its microbial biodiversity is surprisingly understudied relative to the Southern Californian coastline (Martiny et al. 2006). The Southern California Bight ecoregion of Baja California experiences intense upwelling events that are predicted to increase with climate change (Bakun 1990; Bakun et al. 2015), and thus undergoes substantial nutrient flux that could affect microbial composition (Capone and Hutchins 2013). The handful of existing microbial biodiversity next-generation sequencing studies on the Baja California coast are largely centered on the hypersaline environments throughout Guerrero Negro, which differ considerably from coastal environments in terms of community composition (López-Cortés et al. 2001; Martini et al. 2002; Omoregie et al. 2004b, a; Orphan et al. 2008; Reimer and Huerta-Diaz 2011; Huerta-Diaz et al. 2011, 2012; Valdivieso-Ojeda et al. 2014). We selected the coastal site of Puerto Nuevo in Baja California, which is close to the United States-Mexico border, for the following reasons. First, this region experiences strong upwelling events that are associated with nutrient fluxes. Such upwelling events also lead to marine organism habitat loss, and are increasing with climate change (Bakun 1990; Bakun et al. 2015). Second, this region shares overlapping coastal physical features with Southern California and is thus likely to share similarities in microbial ecosystems. Third, this location is unrepresented in terms of coastal microbial community sampling, thus its study would expand our existing knowledge of microbial diversity. With these reasons in mind, the primary goal of our study is to obtain information on coastal microbial diversity across domains and environment types in Puerto Nuevo to set the precedent for additional microbial ecology studies along the Baja California coastline.

Using high-throughput sequencing, we characterized the bacterial, archaeal, and eukaryal microbial diversity in the sediment and seawater of three sites along a 0.45 km range in Puerto Nuevo in Playas de Rosarito, Baja California. Our goals were to determine (1) the differences in coastal microbial community richness and/or abundance between seawater and sediment environment types, (2) the alpha diversity within a sampling site versus the beta diversity among a 0.45 km range, and (3) the shared versus unique patterns between bacterial, archaeal and eukaryal microbial communities.

2.3 Materials and Methods

2.3.1 Study Area and Sampling

The necessary field permit for this study (permit # PPF/DGOPA-009/17) was issued from the Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA), complying with all relevant regulations.

The coastal Puerto Nuevo site is a fishing community near Playas de Rosarito that is frequently visited by tourists and covered in *Zostera* eel grass beds. We selected three sampling sites at low tide (~1 m in depth each) on the Puerto Nuevo coastline with gradient exposures to human impact along a 0.45 km range between 32.248 N, -116.948 E and 32.246 N, -116.944 E (Figure 1). We refer to the most North-facing site at point 0.0 km as the Sheltered (SH) site, the site at point 0.15 km as the Minor Outlet (MN) site, and the site at point 0.3 km as the Major Outlet (MJ) site. The SH site is facing a 5-7 m cliff at point 0.0 km, the MN site is near a small run off outlet or scour at point 0.15 km, and the MJ site is near a large run off outlet and residential area at point 0.3 km. Four replicates of surface seawater samples and sediment core samples were collected at each site according to previously described methods (Walsh et al. 2015). Salinity, temperature (°C), pH, ammonia (ppm), nitrite (ppm), and nitrate (ppm), were measured for each site using the API Saltwater Master Test Kit.

Site	km	pH	Ammonia ppm	Nitrate ppm	Sed 16S,18S	Sea 16S,18S
SH	0.00	7.8	0.25	0.00	4, 3	4, 3
MN	0.15	8.2	0.00	0.00	3, 3	4, 3
MJ	0.30	7.8	0.00	5.00	4, 3	4, 4
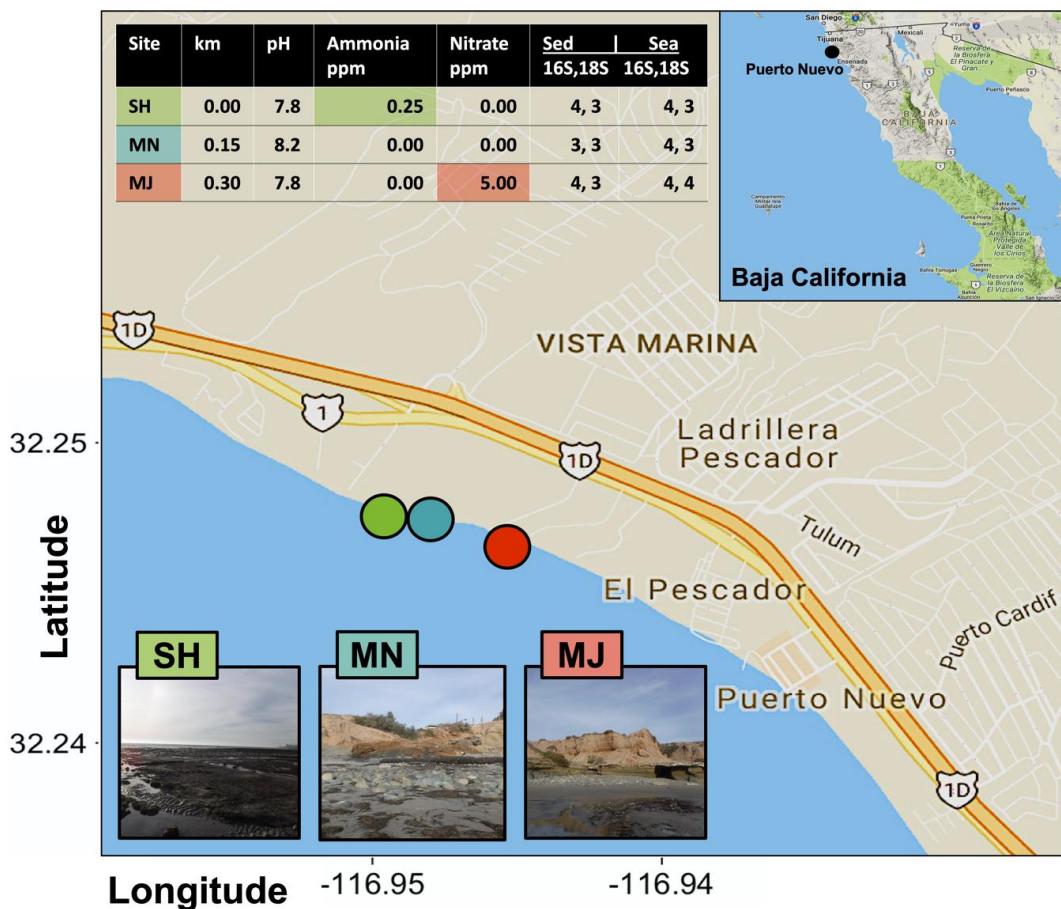
Figure 2.1 Location and metadata information of sampling sites.
The three sampling sites are denoted in lime green (SH or sheltered), cyan (MN or Minor Outlet) and red (MJ or Major Outlet) circles. Sequenced samples based on seawater or sediment are displayed in the right-hand table columns for a total sequence output of 42 out of 48 samples submitted. The inset illustrates the approximate sampling location within Baja California, as denoted with a black circle. Chemical differences unique to sites are highlighted using colored

boxes in the upper inset table and km refers to the distance in kilometers that MN and MJ are relative to SH.

Seawater samples (200 mL) were filtered on-site using sterile 60 mL syringes with 25 mm hydrophilic polyethersulfone 0.1-micron membrane filters (Supor-200 PES; Pall Laboratories) at an approximate rate of 15 mL/min. Filters were then transferred into individual, sterile 2 mL Eppendorf tubes, immediately frozen on dry ice, and stored at -80 °C until further processing. For sediment cores, the tips of sterile 8.5 cm length x 1.5 cm diameter syringes were cut using sterile razor blades prior to being vertically inserted into the sediment. Sediment samples were then kept in their respective syringes and wrapped with Parafilm, immediately frozen on dry ice, and stored at -80 °C until further processing. All samples were handled with sterile nitrile gloves both on- and off-site.

2.3.2 DNA Extraction, PCR Amplification for Validation and Illumina Amplicon Sequencing
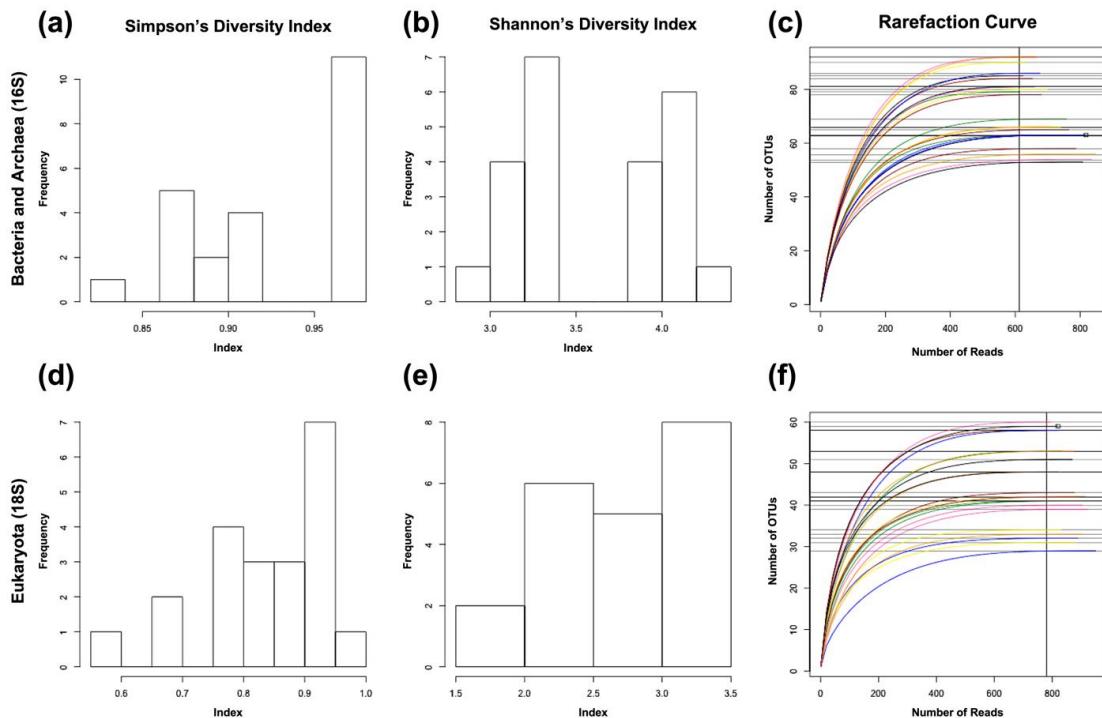
DNA from the filters of 200 mL seawater samples was extracted using the QIAGEN DNeasy Blood & Tissue Kit (Qiagen™, Valencia, CA, United States) following the manufacturer's protocol. Filters were cut into 2 mm strips using sterilized scissors and the microbial content on the filter was homogenized using the Omni Bead Ruptor homogenizer (Omni International™, Kennesaw, GA, United States) with a mixture of 0.1, 0.5, and 1.4 micron beads to maximize retrieval of DNA from all microbial domains. DNA from sediment samples was extracted from 0.5 g of field-moist sediment using the MoBio PowerSoil DNA Isolation Kit (MoBio™, Carlsbad, CA, United States) following the manufacturer's protocol. All extracted DNA from seawater and sediment samples was diluted to a final concentration of 5 ng per µL each.

Ribosomal RNA gene amplification was performed for all samples, including a variable 12 bp barcode sequence to ensure that samples were uniquely identifiable, following a standard protocol from the Department of Energy Joint Genome Institute (JGI) (Quast et al. 2013). The V4-V5 region for 16S rRNA of bacteria and archaea (FW 515 F 5'-GTGYCAGCMGCCGCGGTAA-3', RV 926R 5'- CCGYCAATTYMTTTRAGTTT-3') and the V4 region for the 18S rRNA of eukaryotes (FW 5'- CCAGCASCYGCGGTAATTCC-3', RV 5'-ACTTTCGTTCTTGATYRA-3') were targeted, with sample validation amplifications to assess extraction quality (Stoeck et al. 2010; Quince et al. 2011; Bates et al. 2011; Tremblay et al. 2015; Parada et al. 2016). Stocks of 2x AccuStart II PCR SuperMix containing Taq DNA Polymerase (Quantabio™, Beverly, MA, United States) and 10 mg/mL bovine serum albumin (BSA) (ThermoFischer Scientific™, Waltham, MA, United States) were used during PCR amplification validation checks, conducted prior to amplicon sequencing. A final concentration of 1x SuperMix and 10 µg BSA was used for each 25 µL PCR reaction containing 10 ng DNA, 500 nM each for a given forward and reverse primer (1 µM total), and the remaining PCR reaction volume was made up to 25 µL with PCR grade nuclease-free water. The 16S rRNA region was amplified by denaturation at 94 °C/3 min, followed by 30 cycles of denaturation 94 °C/30 sec, annealing at 50 °C/30 sec, elongation at 72 °C/1 min, and a final elongation 72 °C/10 min. The 18S rRNA region was amplified by denaturation at 94 °C/3 min, followed by 30 cycles of denaturation 94 °C/30 sec, annealing at 60 °C/30 sec, elongation at 72 °C/1.5 min, and a final

elongation 72 °C/10 min. After validation, 250 ng of extracted DNA in 50 μL total volume was used for plate-based next-generation 16S and 18S amplicon sequencing at the JGI using a KAPA Biosystem library qPCR kit and a Roche LightCycler 480 real-time PCR instrument with the same primers; a MiSeq Reagent kit using a 2x300 nt indexed protocol was used for sequencing on the Illumina MiSeq platform (Illumina™, San Diego, CA, United States) (Caporaso et al. 2012). Additional details for similar 16S and 18S sequencing protocols can be found on protocols.io: dx.doi.org/10.17504/protocols.io.nuudeww (Thompson) and dx.doi.org/10.17504/protocols.io.nuvdew6, respectively (Thompson).

2.3.3 Sequence Processing

Raw sequences were de-multiplexed and clustered into Operational Taxonomic Units (OTUs) using the iTagger v1.2 (Tremblay et al. 2015) and QIIME2 (Bokulich et al. 2017) pipelines for quality control and sequence analyses. Taxonomy was assigned by 97% identity or higher via the Silva database SSU for the 16S marker and SSU for the 18S r108 marker (Quast et al. 2013; Tremblay et al. 2015). Identified and matched sequences were additionally filtered to remove mitochondrial DNA sequences. All remaining 16S and 18S rRNA gene sequences, with the sample having the lowest number of reads being 141944, were then rarefied at 1,000 reads per sample (23 output x 1,000 = 23,000 for 16S rRNA total rarefied reads and 21 output x 1,000 = 21,000 for 18S rRNA total rarefied reads; Figure 1 and Supplemental Figure 1). In sum, we submitted 24 samples for 16S and 18S sequencing (12 for seawater and 12 for sediment, containing 4 biological replicates per site), with an output of 23 datasets for 16S and 21 datasets for 18S.

Supplementary Figure 2.1 Metrics and rarefaction curves on read abundance.
Read abundance histograms of prokaryotic 16S (a) and eukaryotic 18S (d) Simpson's diversity, histograms of prokaryotic 16S (b) and eukaryotic 18S (e) Shannon's diversity, and rarefaction curves of all prokaryotic 16S (c) and eukaryotic 18S (f) operational taxonomic units versus number of reads.

2.3.4 Data Analyses and Statistics

Singleton and doubleton reads were removed before creating the two datasets per rRNA region (four in total). The four datasets include read abundance or presence-absence data, with 16S and 18S for each. The first dataset created was read abundance and the second was a conservative "presence=1" or "absence=0" assignment of rarefied reads (GitHub Supplemental-Results.Rmd code available at https://github.com/sabahzero/Puerto-Nuevo_Coastal-Microbial-Ecology_16S-18S-Workflow_UlHasan-etal). These metrics were then used to determine the biodiversity of each site (alpha diversity) and among sites (beta diversity). Abundant phyla and classes were classified and ranked into respective taxonomic groups. For diversity, we utilized Shannon's and Simpson's diversity indices based on read abundance. For abundance, we compared rarefied OTU reads of taxa by log fold. For richness, we assigned taxa as present or absent, then compiled taxa by phylogenetic group (i.e. phyla, class, order). For core taxa as indicators of the community, we took a DESeq2-like approach and compared taxa richness 16S or 18S across all samples versus sediment and seawater environment types versus SH, MN, and MJ site locations in order to define core taxa between three total categories: Puerto Nuevo core taxa, core taxa of environment type, and core taxa of location.

All statistical tests and visualizations were conducted in R (Core R Team 2011) with all code and package citation information available at https://github.com/sabahzero/Puerto-Nuevo_Coastal-Microbial-Ecology_16S-18S-Workflow_UlHasan-etal (Supplemental Tables 1-8). Changes in microbial community structure among sites were analyzed using permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) with Bray-Curtis distances (Bray and Curtis 1957a) for the abundance datasets and Jaccard indices (Jaccard 1912) for the richness datasets. A Bonferroni p-value correction was used to determine pairwise differences between sites. Beta diversity differences in community structure and associated statistics were visualized using Venn diagrams and proportion of variance for principal components analysis (PCA) along two axes, grouped by environment type (sediment or seawater) versus location. For all univariate data, we used analysis of variance (ANOVA) to determine significant differences among sites, environment type, and site*environment type interactions. We used q-q plots and scale-location plots to inspect normality and homoscedasticity, respectively. Where significant differences were detected, Tukey's Test of Honest Significant Differences was used to determine the range of differences among the sites and interactions.

2.4 Results

2.4.1 *Coastal Puerto Nuevo sample site metadata*

We sampled four replicates of sediment and seawater from three sites within a 0.45 km range off the Puerto Nuevo coastline (Figure 1) and collected associated metadata at the interface where seawater meets sediment. The pH (7.9 ± 0.2), ammonia (0.08 ± 0.14 ppm) and nitrate (1.7 ± 2.9 ppm) levels, as well as temperature (15.8 ± 0.3 °C) varied between sites during sampling in June 2016, whereas salinity (1.02 ± 0.00 psu), nitrite (0.0 ± 0.0 ppm), and depth (1.0 ± 0.0 m) were constant (Supplemental Table 6).

2.4.2 *Microbial community diversity richness and abundance*

A total of 14,137,026 raw reads were recovered from 23 of the 24 submitted seawater and sediment samples with median lengths of ~380 bp, publicly accessible upon free registration at the Joint Genome Institute Genome Portal, ID 502935 (Supplemental Table 5). 16S rRNA gene sequences were recovered for 11 of the 12 sediment samples (1,960,774 reads) and all of the 12 seawater samples (2,156,286 reads) for a total of 4,117,060 raw reads. 18S rRNA sequences were recovered for 9 of the 12 sediment samples (3,682,950 reads) and all of the 12 seawater samples (6,337,016 reads) for a total of 10,019,966 raw reads.

Shannon and Simpson diversity indices were produced from rarefied reads (Supplemental Figure 1), and all rarefied datasets passed the Shapiro-Wilk normality test (see GitHub Supplemental-Results.Rmd code available at https://github.com/sabahzero/Puerto-Nuevo_Coastal-Microbial-Ecology_16S-18S-Workflow_UlHasan-etal). The environment type (sediment or seawater) was found to be statistically significant for all 16S and 18S richness and abundance datasets ($p < 0.005$). Location was not statistically significant for any of the datasets, meaning that SH, MN or MJ did not significantly vary, although there was a correlation between environment type and site location observed for the 16S abundance dataset ($p = 0.06$). Focusing on environment type (sediment or seawater), analyses of reads by taking into account either raw or normalized sample mass indicated that microbial communities for sediment were orders of magnitude richer (approximately 500-fold) relative to those of seawater (Figure 2), regardless of how the data were analyzed. Taxa across domains are 2 fold richer and abundant in the sediment compared to seawater environment type. The sediment had 5.0×10^2 fold greater bacterial and archaeal taxa richness and 3.9×10^2 greater eukaryal taxa richness relative to seawater after normalization by mass. The sediment had 3.0×10^2 fold greater bacterial and archaeal taxa abundance and 2.7×10^2 greater eukaryal taxa abundance relative to seawater after normalization by mass.
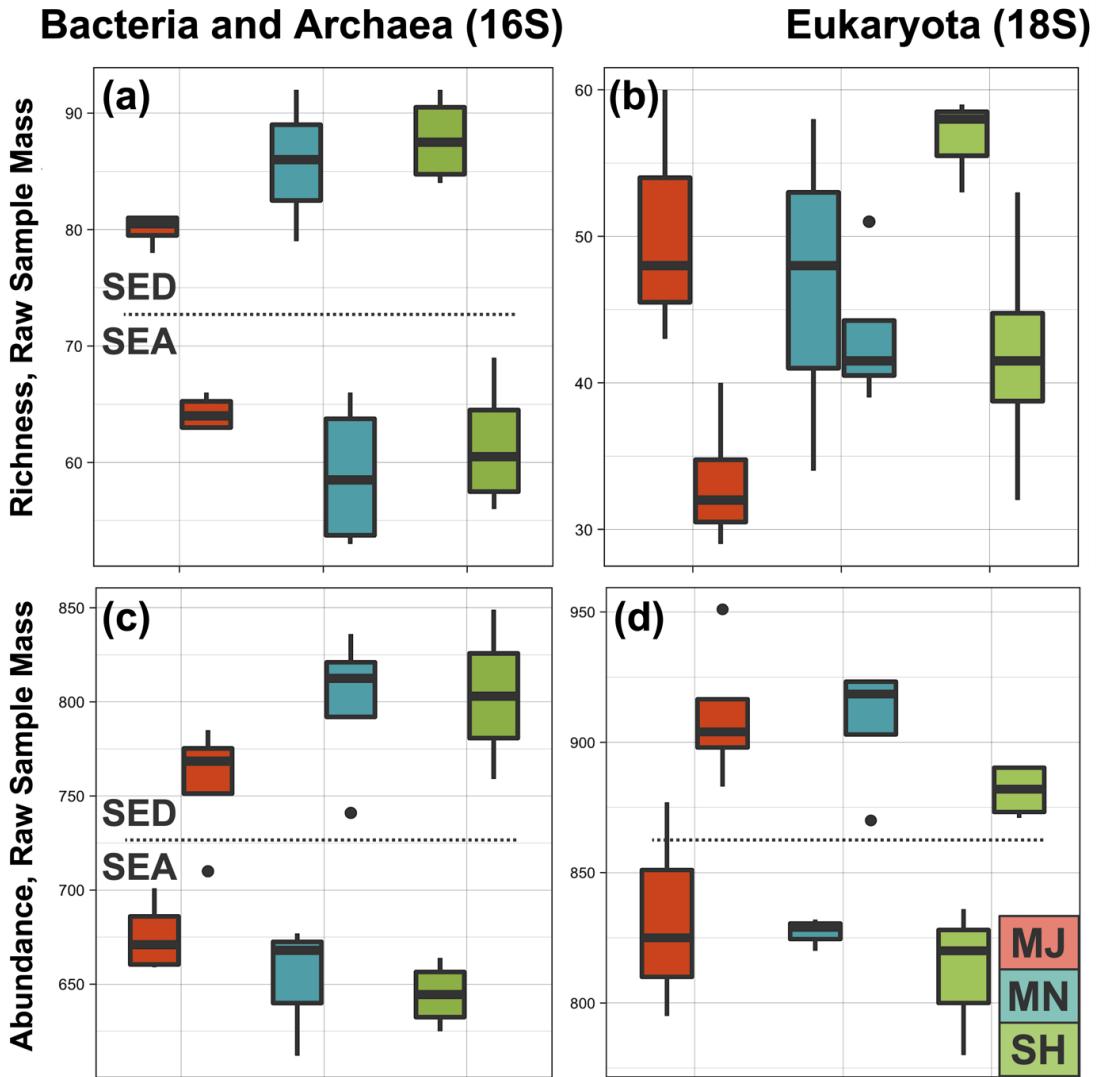
Figure 2.2 Microbial community richness and abundance.
(a-b) Boxplot comparisons of rarefied bacterial and archaeal (16S) and eukaryal (18S) richness estimates for different environment types (sediment and seawater) and by sampling site (major outlet/MJ, minor outlet/MN, sheltered/SH), with a p value ($p < 0.001$) for environment type. (c-d) Boxplot comparisons of rarefied bacterial and archaeal (16S) and eukaryal (18S) abundance estimates for different environment types (sediment and seawater) and by sampling site (major outlet/MJ, minor outlet/MN, sheltered/SH), with a p value ($p < 0.001$) for environment type.

2.4.3 *Microbial community composition*

In total, the Puerto Nuevo microbial community composition during the time of sampling was comprised of 3 domains: Archaea, Bacteria, and Eukarya. For prokaryotes, there were 50 phyla, 130 classes, 240 orders, 441 families, and 859 genera represented. For eukaryotes, there were 30 phyla, 56 classes, 130 orders, 165 families, and 317 genera represented. Microbial communities

revealed specific taxonomic assemblages associated with sediment versus seawater samples collected from the same sites (Figures 3-4, Table 2). Similar to OTU richness and abundance, PERMANOVA statistics indicated that microbial community composition differed by environment type (p = 0.001, f = 68.06 for prokaryote 16S and f = 25.09 for eukaryote 18S).

Bacterial Proteobacteria and eukaryal Florideophycidae displayed higher richness in seawater, whereas Bacterial Bacteroides and Planctomycetes and eukaryal Ciliophora and Annelida had higher richness in sediment; richness of all other archaeal-, bacterial-, and eukaryal phyla did not differ substantially between environment types (Figure 3, Supplemental Figure 2). Cytophagia (bacterial) and Chromadorea (eukaryal) were abundant classes specific to the sediment environment, whereas Cyanobacteria and Bacteroidia (bacterial) and Chlorophyceae (eukaryal) were specific to the seawater environment. A further breakdown of taxa richness by pairwise comparisons revealed that the microbial community taxa richness was the same across domains and locations for sediment (p > 0.1, f < 1.25). For seawater, archaeal and bacterial community composition (p = 0.017, f = 2.09 for 16S seawater subset; MJ-SH p = 0.084 / f = 2.45 and MJ-MN p = 0.021 / f = 2.80) was distinct for the MJ site and all sites were distinct for eukaryal community composition (p = 0.003, f = 2.28 for 18S seawater subset; MJ-SH p = 0.054 / f = 2.06, MJ-MN p = 0.031 / f = 2.69, SH-MN p = 0.025 / f = 2.02) (Figure 2.4, Supplemental Figure 2.3).
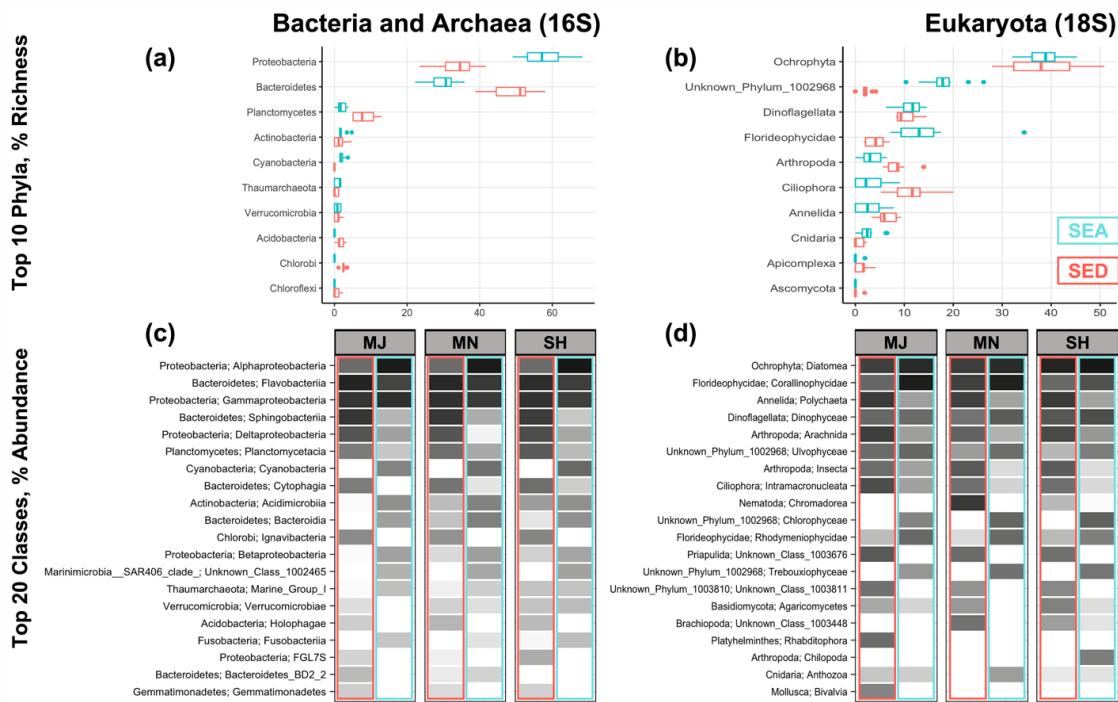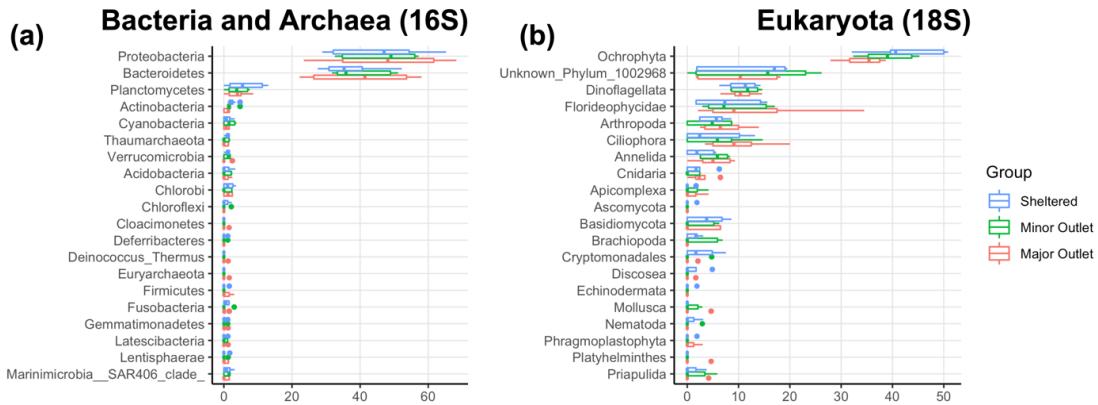
Figure 2.3 Microbial phyla by richness and abundance.

Top phyla by richness differ by environment type, with some abundance specificity by site location. (a-b) The top 10 bacterial and archaeal as well as eukaryal phyla across biological replicates of site locations for both seawater (SEA) and sediment (SED) environment types from most to least richness in the sediment in order of highest relative abundance to lowest. (c-d) The top 20 bacterial and archaeal as well as eukaryal classes, demonstrating variation in abundance by environment type and site location from a gray-scale gradient of white (0) to black (100). Common or rare phyla and classes can be additionally viewed by comparison of OTU tables.

Supplementary Figure 2.2 Top 20 phyla per site location.
Boxplots display top 20 (a) bacterial and archaeal 16S phyla and (b) eukaryal 18S phyla by richness.
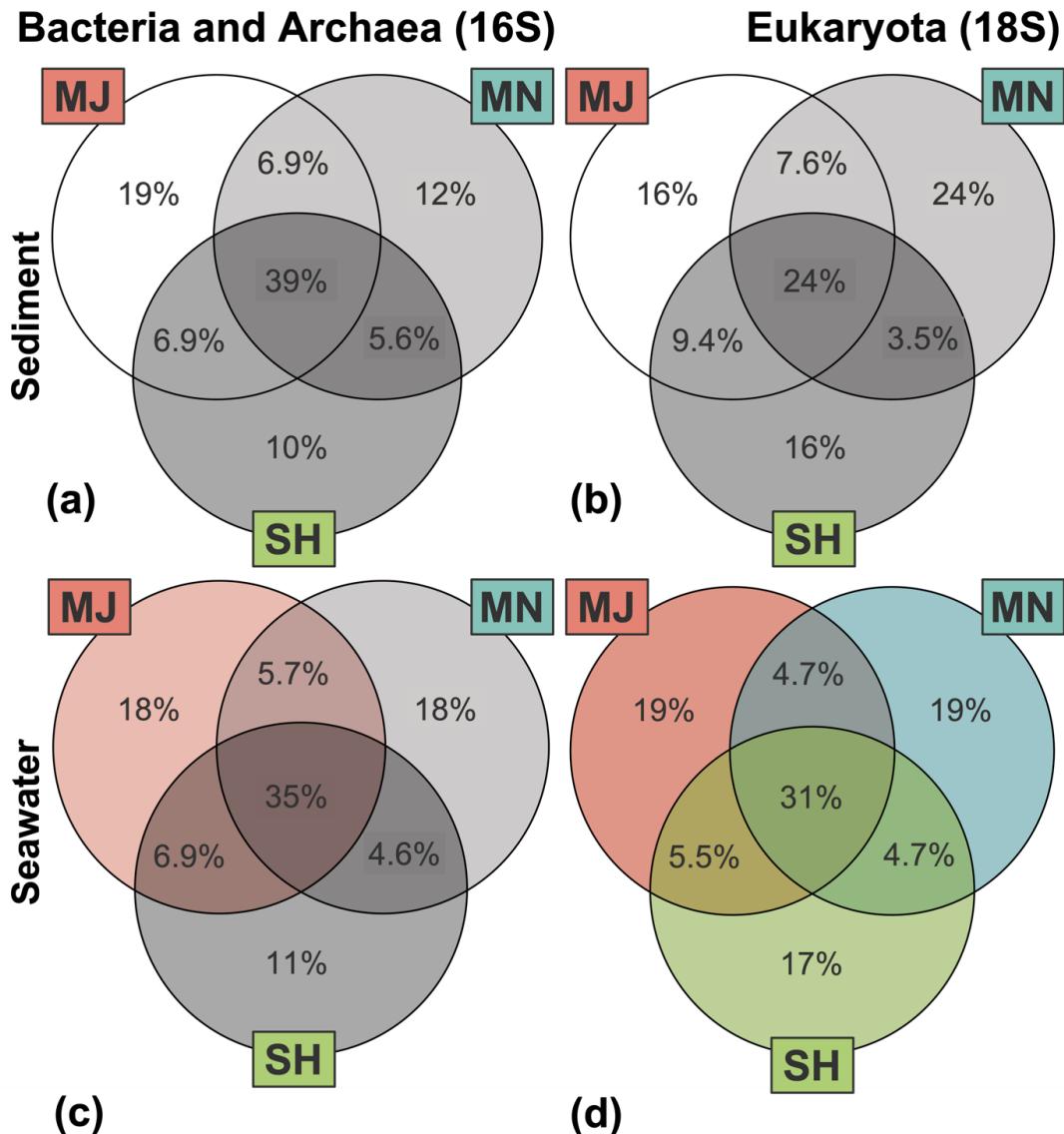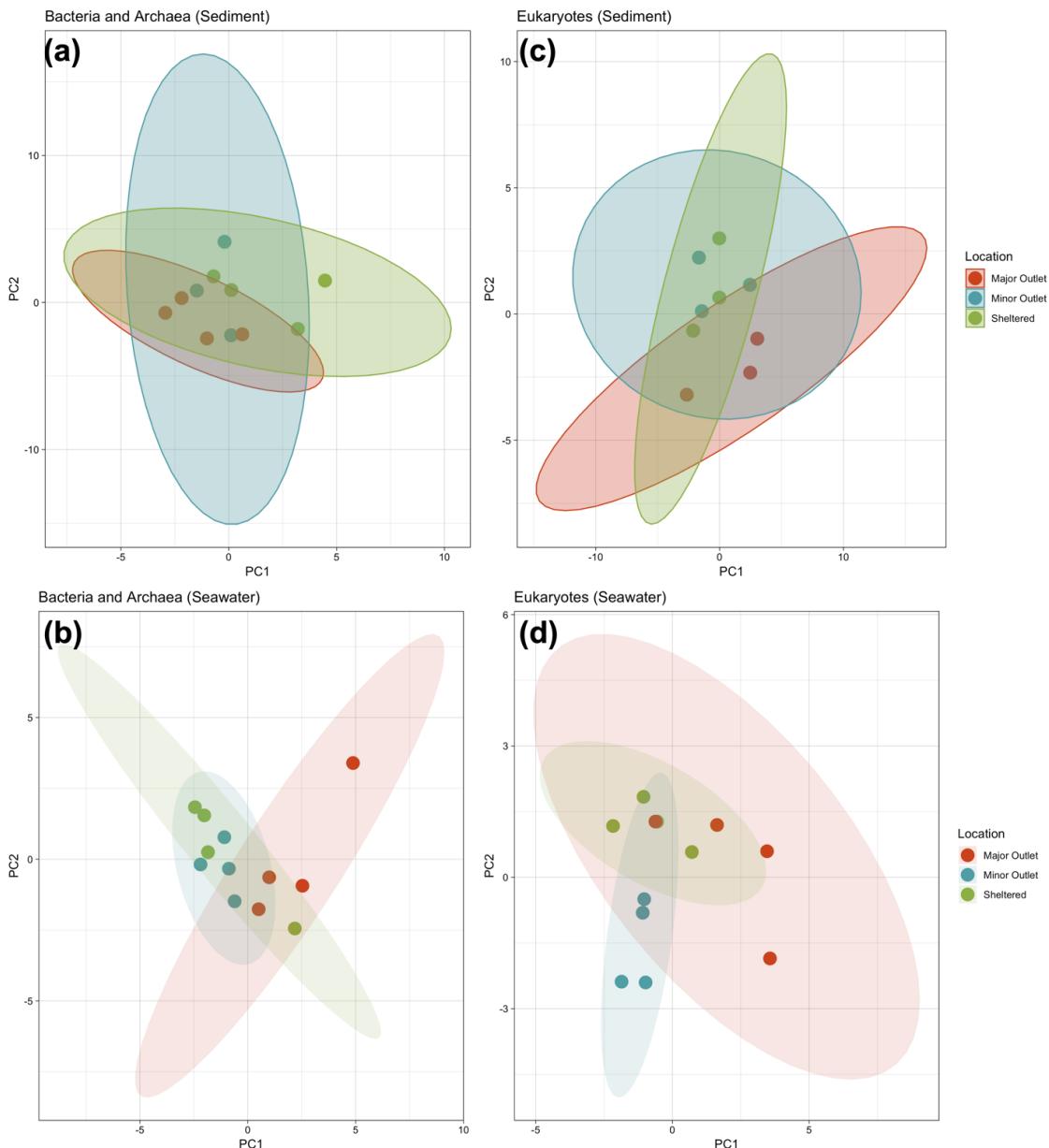
Figure 2.4 Microbial community composition and beta-diversity.
Venn diagrams of percentage overlapping OTUs for bacterial and archaeal as well as eukaryal microbial communities based on richness, with site location colors corresponding to Figure 1. Statistically significant variation by PERMANOVA and pairwise comparison tests is shown in color whereas similarities (no variation) are shown as gray-scale.

Supplementary Figure 2.3 Microbial community variations per site location.
Principal component analysis (PCA) plots of PC1 x PC2 show variation versus similarity of
microbial communities between site locations for (a) bacterial and archaeal 16S in the sediment
and (b) seawater and (c) eukaryal 18S in the sediment and (d) seawater.

Investigation of the Puerto Nuevo 'core' taxa – those consistently found across
environment types (sediment or seawater) and locations – resulted in 47 genera and 50 unique
OTU identifications (Table 1, Supplemental Tables 7-8). For prokaryotic domains, only Bacteria
were part of the Puerto Nuevo core taxa – no core Archaea were observed. Across the two
domains (Bacteria and Eukarya), 13 phyla were observed. Actinobacteria, Cyanobacteria, and
Phyla 1002968 were core phyla specific to the seawater environment, whereas Chlorobi,
Arthropod, Ciliophora, and Phyla 1003810 were specific to the sediment environment.

Proteobacteria, Orchophta, Dinoflagellata, and Bacteroides were core phyla shared between both environment types. Three bacterial classes and one eukaryal class were core to seawater, and four bacterial classes and three eukaryal classes were specific to sediment. Bacterial Alphaproteobacteria, Flavobacteriia, and Gammaproteobacteria and eukaryal Diatomea and Dinophyceae were shared core classes between sediment and seawater. Three genera were core taxa specific to the SH and MJ site locations, with two of the three genera specific to other categories (Puerto Nuevo core community taxa and sediment core community taxa). No core taxa were specific to the MN site location.

Domain	Phylum	Class	Order	Family	Genus	Core		
Eukaryota	Florideophycidae	Corallinophycidae	Unknown 1003045	Unknown 1003046	Mesophyllum	ALL		
Eukaryota	Ochrophyta	Diatomea	Coscinodiscophytina	Fragilariales	Licmophora	ALL		
Eukaryota	Ochrophyta	Diatomea	Bacillariophytina	Bacillariophyceae	Psammodictyon	ALL		
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	uncultured 1216	ALL		
Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	OM1 clade	Candidatus Actinomarina	WAT		
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amylibacter	WAT		
Bacteria	Proteobacteria	Alphaproteobacteria	SAR11 clade	Surface 1	Candidatus Pelagibacter	WAT		
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Lentibacter	WAT		
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Sulfitobacter	WAT		
Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	SAR116 clade	Unknown 1000731	WAT		
Bacteria	Proteobacteria	Alphaproteobacteria	SAR11 clade	Surface 2	Unknown 1000744	WAT		
Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	OM43 clade	WAT		
Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	Synechococcus	WAT		
Eukaryota	Ochrophyta	Diatomea	Bacillariophytina	Bacillariophyceae	Frustulia	WAT		
Eukaryota	Dinoflagellata	Dinophyceae	Gymnodiniphycidae	Gymnodinium clade	Spiniferodinium	WAT		
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS4 marine group	WAT		
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS5 marine group	WAT		
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Polaribacter	WAT		
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	JL ETNP Y6	Unknown 1000942	WAT		
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86 clade	Unknown 1000950	WAT		
Eukaryota	Unknown 1002968	Trebouxiophyceae	Chlorellales	Unknown 1003011	Nannochloris	WAT		
Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	Robiginitomaculum	SED		
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Thalassobius	SED		
Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Flammeovirgaceae	uncultured 1059	SED		
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Desulfotalea	SED		
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Sandaracinaceae	uncultured 11491	SED		
Eukaryota	Ochrophyta	Diatomea	Coscinodiscophytina	Fragilariales	Licmophora	SED		
Eukaryota	Ochrophyta	Diatomea	Bacillariophytina	Bacillariophyceae	Nitzschia	SED		
Eukaryota	Dinoflagellata	Dinophyceae	Gymnodiniphycidae	Gymnodinium clade	Spiniferodinium	SED		
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Aquibacter	SED		
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Lutibacter	SED		
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Maribacter	SED		
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Muriicola	SED		
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Robiginitalea	SED		
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Ulvibacter	SED		
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Zeaxanthinibacter	SED		
Bacteria	Proteobacteria	Gammaproteobacteria	BD7 8 marine group	Unknown 1002603	Unknown 1000892	SED		
Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Ectothiorhodospiraceae	Unknown 1000902	SED		
Bacteria	Proteobacteria	Gammaproteobacteria	E01 9C 26 marine group	Unknown 1002611	Unknown 1000908	SED		
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	JTB255 marine benthic group	Unknown 1000984	SED		
Bacteria	Chlorobi	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	Ignavibacterium	SED		
Eukaryota	Arthropoda	Insecta	Orthoptera	Unknown 1003417	Unknown 1001292	SED		
Eukaryota	Ciliophora	Intramacronucleata	Spirotrichea	Hypotrichia	Holosticha	SED		
Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Saprospiraceae	Phaeodactylibacter	SED		
Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Saprospiraceae	uncultured 1272	SED		
Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	S15 21	Unknown 1000338	SED		
Eukaryota	Unknown 1003810	Unknown 1003811	Unknown 1003812	Unknown 1003813	Unknown 1001715	SED		
Eukaryota	Ochrophyta	Diatomea	Coscinodiscophytina	Fragilariales	Licmophora	SED	SH	
Eukaryota	Ochrophyta	Diatomea	Bacillariophytina	Bacillariophyceae	Nitzschia	SED	SH,	MJ
Eukaryota	Unknown 1002968	Ulvophyceae	Unknown 1003017	Unknown 1003018	Unknown 1001105	SEA	SH,	MJ

Table 2.1 Shared (core) and unique core taxa for environment type and location.

Core taxa found across all samples, sediment (SED) or seawater (SEA) environment types, and Sheltered (SH), Minor Outlet (MN), or Major Outlet (MJ) site locations based on richness. Core phyla and classes highlighted in yellow are specific to that environment type. Core genera highlighted in yellow indicated unique Operational Taxonomic Unit (OTU) numbers for repetitive names.

2.5 Discussion

Microbial communities in the coastal Baja California region are understudied relative to Western coastal regions, and community dynamics among multiple domains within Baja California were unknown prior to this study. We characterized sample diversity within (alpha diversity) and between (beta diversity) coastal microbial communities by examining bacteria, archaea and microbial eukaryotes in both the sediment and seawater of Puerto Nuevo, Baja California. Our findings support the hypotheses that: (1) the variation in diversity is greater in coastal sediment microbial communities than seawater microbial communities along a 0.45 km range and (2) prokaryotic and eukaryotic microbial communities exhibit similar composition patterns in coastal sediment but different composition patterns in seawater. Our findings that coastal communities differ among sample sites and between environment type (sediment, seawater) are consistent with global patterns of microbial biodiversity; for example, studies on the Baltic Sea coastline and the coral reef systems of Indonesia find similar patterns as our study (Langenheder and Ragnarsson 2007; Hao et al. 2016). Furthermore, our observed differences for bacterial, archaeal, and eukaryal microorganisms between sites within a small 0.45 km range illustrate the necessity for future studies to expand geographical and temporal sampling in this region to better understand the microbial ecology and biodiversity patterns of Puerto Nuevo, Baja California.

The finding that the sediment environment type exhibits higher bacterial richness when compared to seawater is consistent with previous literature investigating bacterial diversity in and along the Pacific (Daly et al. 2014; Walsh et al. 2015; Cleary et al. 2017), with less being known in this regard for archaeal and eukaryal microorganisms. These results could be explained by the physical nature of the sediment environment type, allowing for an increase in the formation of microbial mats and biofilms by providing a surface for microorganisms to attach. In addition, the sediment is composed of minerals, and as such it supports the electric coupling of complex microbial redox reactions, which may serve important roles in biogeochemical cycling and the maintenance of ecological homeostasis (Nielsen and Risgaard-Petersen 2015). In general, sediment is a stratified solid gradient that provides niche stability to microorganisms, whereas seawater is a dynamic liquid that is constantly in flux. These two environment types, however, are not mutually exclusive; the seawater environment type is a necessary contributor to refreshing the microbial populations within coastal environments (Won et al. 2017), including the sediment. We note that we used different extraction kits for seawater and sediment. While we did include a blank as a control for the seawater extraction kit and observed little to no detectable DNA in the blank sample, there is always the possibility of different levels of bias from the sequencing results of the DNA samples extracted using different extraction methods. Nonetheless, we observed some overlap of core taxa between seawater and sediment across sites (Table 1), which suggests an interaction between these communities. Additionally, we observed consistency in microbial community composition between sites, which is particularly interesting for sediment samples, since sediment samples often display microspatial heterogeneity (Downing and Rath 1988; Stoyan et al. 2000). Overall, our study provides the framework for

future studies to examine the microbial composition and taxa preferences between and among multiple environment types at a particular location site, and is a starting point for understanding the underlying functional implications that these preferences may play within specific ecosystems.

We observed distinct core taxa present for coastal Puerto Nuevo with three eukaryal genera specific to the sediment core taxa of one or more sampling sites (Table 1). Interestingly, *Nitzschia* and Unknown 1001105 were core genera (found in the sediment) that distinguish the Sheltered (SH) and Major Outlet (MJ) sites from the Minor Outlet (MN) site. *Nitzschia* has been found in regions with observed elevated nitrogen levels (Machado et al. 2016), and is a known toxin-producing diatom in marine and freshwater environments. *Licmophora* is another diatom which, unlike *Nitzschia*, is negatively impacted by human nitrogen pollutants ([CSL STYLE ERROR: reference with no printed form.]) and could be in competition with *Nitzschia*. Interestingly, *Licmophora* is found in both the sediment and seawater whereas *Nitzschia* is only observed in the sediment (Table 1). Both *Nitzschia* and *Licmophora* were the only genera that showed up multiple times as distinguished core taxa for Puerto Nuevo microbial communities, be it sediment or seawater specific communities, or sampling site specific communities. Further investigation into the metabolomic profiles of these genera in relation to detailed biogeochemistry in the environments they are found may reveal novel information into the significance of these taxa in Puerto Nuevo and other coastal microbial communities.

We observed that different patterns of microbial taxa primarily depend on the environment type rather than the sampling site (Figures 3-4, Table 1). Akin to sediment hosting greater microbial biodiversity than seawater, we found a common pattern with previous literature in that beta diversity appears to be more important than alpha diversity in determining microbial community composition across environment types (Prober et al. 2015; Li et al. 2016). Many soil microbial ecology studies agree that drivers of microbial beta diversity vary across space. In specific reference to coastal and marine microbial communities, Barberán and Casamayor (2010) found that the significance of beta diversity and its drivers vary by phylum when specifically investigating bacterial Actinobacteria, Alphaproteobacteria, Bacteroidetes, Betaproteobacteria, Cyanobacteria, and Gammaproteobacteria (Barberán and Casamayor 2010). This seems to be a common observation, affirmed by current studies in vastly different coastal microbial communities (Campbell et al. 2015; Richa et al. 2017). Puerto Nuevo sediment microbial communities within a 0.45 km range do not significantly differ between sites or domains (16S and 18S), whereas seaweater eukaryal microbial communities do demonstrate heterogeneity for all sites, and bacterial and archaeal microbial communities specifically differ for the Major Outlet site (Figure 2.4). Explanation for these results may be rooted in the physical dynamics of coastal seawater compared to sediment. Indeed, several studies demonstrate how the microbial community composition of aquatic and marine environments depend on scale (Green and Bohannan 2006; Lozupone and Knight 2007; Langenheder and Ragnarsson 2007), and while we did not explicitly test for scale, we observed statistically significant community composition variation to exist even for small 0.45 km ranges. Moreover, our study is consistent with previous studies observing the mixing of marine and terrestrial communities, where coasts are unique interfaces for comparing the two interacting environments. While more studies comparing coastal seawater and sediment are needed, especially for microbial eukaryotes, a recent study (Chen et al. 2017) on a coastal environment of Southern China found similar patterns as we have found in this study for Puerto Nuevo in that the environment type and geographic location

impacted the community composition, a finding that is analogous to previous studies focused exclusively on bacterial communities (Hill et al. 2003; Lozupone and Knight 2007; Langenheder and Ragnarsson 2007; Martiny et al. 2011; Campbell et al. 2015; O'Brien et al. 2016). Another recent study in China's coastal waters reported on the biogeography of microbial eukaryotes (Zhang et al. 2018), further adding to our knowledge of microbial community composition studies.

Overall, our study is consistent with other studies, while providing new information on microbial diversity for Puerto Nuevo. For example, studies in other locations (Camanocha and Dewhirst 2014, [CSL STYLE ERROR: reference with no printed form.]; Wasmund et al. 2016) found that Chlorobi, a photosynthesizing bacterial phylum that is known to contribute to sulfur cycling, is generally present in the sediment. Our results also indicated that Chlorobi are present in the sediment of Puerto Nuevo. Also consistent with other studies in other locations, the photosynthesizing Cyanobacteria have been observed to exist preferentially in seawater (Gao Yonghui et al. 2014; Makhalanyane et al. 2015; Paerl 2017), and we find this to be the case in Puerto Nuevo as well. In addition, Alphaproteobacteria and Gammaproteobacteria, which have been observed to be common phyla across multiple environment types in other regions (Barberán and Casamayor 2010; Sun et al. 2013; Franco et al. 2017; Richa et al. 2017), were also found in the sediment and seawater of Puerto Nuevo. While we do see archaea representative of abundant or rich taxa (Figure 3), we did not find any archaeal groups in the core taxa of Puerto Nuevo (Table 1). The lack of archaea in the core taxa of Puerto Nuevo is a novel finding in terms of marine microbial composition, and suggests that future studies should incorporate the inclusion of microbial eukaryotes in microbial community composition studies, as our results indicate that there is stronger co-occurrence between bacteria and microbial eukaryotes than between archaea and other domains.

2.6 Conclusions

In this investigation, we have expanded our understanding of microbial diversity and community composition in a near-shore marine environment of Baja California – a coastal region that has been generally understudied. Our analysis of coastal microbial communities just North of Puerto Nuevo, Baja California, which combined 16S and 18S rRNA gene sequencing approaches of coastal seawater and sediment, identified strong relationships between sampling sites and environment types consistent with previous studies. Our findings also highlight the differences of small scale (0.45 km) beta diversity, and demonstrate the significance of integrating multi-domain, environment type, and sampling sites into microbial composition studies to provide ecological context to microbial biodiversity potentially impacted by human-induced climate change and development.

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The effect of fertilizer on the growth rate of the diatom Licmophora abbreviata | The Expedition

Chapter 3

The venom microbiome of marine neogastropod *Californiconus californicus* is distinct from the surrounding environment and is compartment-specific

3.1 Abstract

Over 100,000 species of animals have convergently evolved venom glands, yet surprisingly little is known about venom microbiomes. We analyzed the microbiome amplicon data of adult *Californiconus californicus* venom glands collected at five sampling locations along the California-Baja coastline of North America. Using 16S and 18S rRNA gene sequencing, we show that the *C. californicus* venom microbiome is distinct from that of the surrounding seawater environment ($p < 0.001$). We observed conservation of archaeal and bacterial (16S) communities across the five locations and over the time points collected. Microbial eukaryote communities (18S), however, significantly differed ($p < 0.05$) by location and time, with a greater abundance of parasitic protists (eugregarinoids) in the venom glands during June than December. Additionally, we explored microbial variation along discrete sections of the venom gland. We found that the distal venom duct, where conotoxin activity is known to occur, harbors a unique microbial community distinct from other venom gland compartments. Our results add to the growing consensus that the venom gland microbiome is a specialized, complex microenvironment, with multiple intra-specific host niches.

3.2 Introduction

More than two decades after the sequencing of the first microbial genome (Fleischmann et al. 1995, Loman and Pallen 2015), the impact of Next Generation Sequencing (NGS) on microbiology has profoundly influenced our understanding of microbial evolution and ecology (Allen and Banfield 2005, Hugenholtz and Tyson 2008, Hug et al. 2016, Zaremba-Niedzwiedzka et al. 2017). Sequencing technologies have vastly expanded our awareness of the interactions between microbial and macrofaunal life (Gilbert and Tauber 2016, Brunham 2018, Gilbert et al. 2018, Noguiera et al. 2018, Rees et al. 2018). Metagenomic analyses of the human gut microbiome (Gill et al. 2006, Blaut 2018), for example, unveiled a plethora of unculturable microorganisms with the potential to impact nutrition and health (Hattori and Taylor 2009, Barka et al. 2016, Shapira 2016, Heintz-Buschart and Wilmes 2018). With more than 8.7 million animal species estimated on Earth (Mora et al. 2011), animal microbiome research is broadening beyond the study of traditional model systems and organs (Turnbaugh et al. 2007, Beck et al. 2012, Hickey et al. 2012, Hacquard et al. 2015, Barhndorff et al. 2016, Fitstevens et al. 2016, Taroncher-Oldenburg et al. 2018, National Academy of Sciences 2018). Microbiome research has recently extended to the study of venomous animals, which have toxin-producing sacs or glands evolved to capture prey and defend against predators (Gopalakrishnakone 2017). These specialized venom organs span across terrestrial and marine vertebrates (reptiles, fish, mammals) and invertebrates (cnidarians, arthropods, molluscs) (Mebs 2001, Gopalakrishnakone 2017). The few sequencing studies to date that have sampled from venom and characterized their microbiomes (Goldstein et al. 2013, Torres et al. 2017, Esmaeilishirazifard et al. 2018) highlight that venomous animals are an understudied source of microbial biodiversity.

Approximately 80% of habitable Earth is underwater and difficult to access, and thus acts as a largely untapped area in terms of our understanding of microbial biodiversity and evolution (Briggs 1994, Heidelberg and Gilbert 2010). For example, from sponges, considered to be among the oldest phyla (Porifera) of animal life over evolutionary time, it is known that microbial symbionts can be transmitted both vertically and horizontally, and that there are many medically-relevant compounds produced between microbes and their hosts (Montalvo et al. 2005, Taylor et al. 2007, Bull and Stach 2011, Webster and Taylor 2011, Hentschel et al. 2012, Moitinho-Silva et al. 2017). In another example, from corals, it is known that variant clades of microeukaryote dinoflagellates of the endosymbiotic mutualist *Symbiodinium* play roles in maintaining reef health (Bourne et al. 2016, Peixoto and Rosado et al. 2017, Putnam et al. 2017, Webster and Reusch 2017). Thus far, Conidae is the only marine clade of venomous animals to be investigated for symbionts within its venom gland, including Actinobacteria (*Gordonia*, *Nocardiopsis, Streptomyces*) and Proteobacteria (*Stenotrphomonas*), that are thought to contribute to venom chemistry (Peraud et al. 2009, Lin et al. 2010, Lin et al. 2013a, Lin et al. 2013b, Lin et al. 2014, Quezada et al. 2016, Quezada et al. 2017, Torres et al. 2017). Little is known, however, about the microbial community composition of the highly specialized venom gland within a host species across ecological space and geological time (Ul-Hasan et al. ToxiconX *in press*).

Of the three microbiome studies that sampled directly from the venom of terrestrial komodo dragons (Goldstein et al. 2013), marine cone snails (Torres et al. 2017), and terrestrial snakes and scorpions (Esmaeilishirazifard et al. 2018), we argue that cone snails are perhaps the

strongest model system contenders for advancing the study of the venom microenvironment. There are over 800 species of cone snails identified globally (Peters et al. 2013), each with its own unique array of between 50 to 350 conotoxins (small peptide toxins distinctive to Conidae) in a venom cocktail, and over 8000 conotoxins identified to date (Gao et al. 2017). Amongst the commonly found cone snail species (Peters et al. 2013), several can be maintained and reared relatively easily in the laboratory environment, and thus there is much potential for establishing gnotobiotic cone snail populations for use in *in vitro* experiments (Page 2012). In addition, cone snail anatomy is comparatively simple, making it straightforward to sample their venom glands in quantities sufficient for microbiome (Torres et al. 2017), transcriptomic (Ducancel et al. 2014), proteomic (Prashanth et al. 2014, Gorson and Holford 2016, Robinson et al. 2017), and metabolomic (Neves et al. 2015) characterization. Prevailing gaps in the venom microbiome literature include a lack of microbial beta diversity in the venom across space and time as it occurs in the natural environment, an absence of 18S rRNA gene amplicon sequencing of the venom, and little focus on distinct compartments of the venom gland(s) in parallel to their toxin activity gradient. Based on these knowledge gaps, along with the ample amount of known information on the biochemistry and evolution underlying host conotoxins (Robinson and Norton 2014), we believe that the detailed study of cone snail venom glands may serve as an ideal example of venom-microbe symbiosis, setting the foundation for the exploration of less feasible venomous species.

The cone snail species *Californiconus californicus* (Reeve 1844), known as the California cone snail, represents a good choice for a comprehensive venom-microbial ecology study for several reasons. First, *C. californicus* is an abundant venomous marine gastropod found by the hundreds along the intertidal to subtidal Baja-California coastline (Peters et al. 2013) and is at a low risk of endangerment from sample collections relative to other cone snail species (Chivian et al. 2003, Duterte and Lewis 2011, Peters et al. 2013). Second, *C. californicus* is relatively harmless to humans (Whysner and Saunders 1963, Whysner and Saunders 1966, Elliott and Rafferty 1979, Gilly et al. 2011) and thus easily collectible in the wild and uncomplicated to handle in the laboratory. Third, *C. californicus* is the only cone snail species within its population range (Peters et al. 2013), and the only extant species of its genus within the Conidae family (Puillandre et al. 2015, Puillandre et al. 2017), presenting a dynamic venom cocktail both unique and generalized in comparison to other conids (Biggs et al. 2010, Elliger et al. 2011, Gilly et al. 2011). Finally, *C. californicus* venom gland anatomy in relation to contoxin production and how the activity gradient can contribute to its uniquely generalist predatory behavior is well described (Saunders and Wolfson 1961, Kohn 1966, Whysner and Saunders 1966, Marshall et al. 2002, Duda and Palumbi 2004, Stewart and Gilly 2005, Biggs et al. 2010, Elliger et al. 2011, Gilly et al. 2011), and is one of few cone snails species with its whole venom gland transcriptome sequenced (Lavergne et al. 2015, Phuong et al. 2016). For these reasons, we chose to study the community composition of adult *C. californicus* venom across space and time, and along discrete sections of its venom gland.

In this study, we analyzed the microbial community composition of bacteria, archaea, and microbial eukarya residing in the adult *Californiconus californicus* venom gland by sequencing community 16S rRNA and 18S rRNA genes from snails collected across several locations (Monterey, San Diego, and Puerto Nuevo) and over several times points (in Puerto Nuevo). We also analyzed the community composition within the distinct compartments of the venom gland. We characterized the *C. californicus* core microbiome using amplicon sequencing to determine

(1) if there are differences in the microbial community of the venom gland compared to the surrounding seawater environment, (2) if variation occurs in the venom gland microbial community across the spatially separated sites and across time, and (3) if the alpha diversity within the venom gland identifies unique community patterns across microbial domains. Our findings support the overarching hypothesis that the *C. californicus* venom microbiome is specialized and constant across space and time, with compartment-specific microbial biodiversity.

3.3 Materials and Methods

3.3.1 Sample Sites and Collection

Adult *C. californicus* animals were collected along their natural ranges within the Northern California and Southern California Bight marine ecoregions of the California-Baja coastline (Peters et al. 2013, GitHub repository tables). The three major locations sampled from North to South are (a) Del Monte Beach in Monterey, California 36.608 N, -121.882 E, (b) Mission Bay in San Diego, California 32.761 N, -117.247 E, and (ci-ciii) three nested sites in Puerto Nuevo, Baja California along a 0.45 km range between 32.248 N, -116.948 E and 32.246 N, -116.944 E (Figure 3.1, Table 3.1, Supplemental Figure 3.1). All sites are frequently exposed to human recreational activity and rich in *Zostera* eelgrass beds. Specimens at site location (a) could only be collected by diving a minimum of 6 m, whereas specimens at sites (b-ciii) were collected by snorkel at low tide (~1 m in depth each). Two to three replicates of seawater samples adjacent to collected specimens were collected at each site as previously described (Quast et al. 2013, Ul-Hasan et al. 2019).

Seawater samples (200 mL) were filtered on-site using sterile 60 mL syringes with 25 mm hydrophilic polyethersulfone 0.1-micron membrane filters (Supor-200 PES; Pall Laboratories) at an approximate rate of 15 mL/min. Filters were then transferred into individual, sterile 2 mL Eppendorf tubes, immediately frozen on dry ice, and stored at -80 °C until further processing. All samples were handled with sterile nitrile gloves both on- and off-site.

C. californicus specimens of similar size (15 – 35 mm, depending on population) were collected and immediately frozen on dry ice on-site, then stored at -80 °C until further processing. Specimens were thawed at room temperature in filtered, sterile seawater to maintain tissue integrity. The venom gland was then dissected out of the animal host, washed three times with sterile seawater as previously described (Peraud et al. 2009, Torres et al. 2017), cut in half or sectioned, then flash frozen again for DNA extraction for amplicon sequencing. Whole venom glands were categorized as wildtype from site locations for the summer of 2017, different time points for the Puerto Nuevo Minor Outlet site location, and sectioned by venom gland compartment from a subset of the San Diego population (Figure 3.1, Table 3.1). Animals used for venom compartment section comparison were kept in captivity under ambient conditions and with exposure to wildtype seawater for calibration.
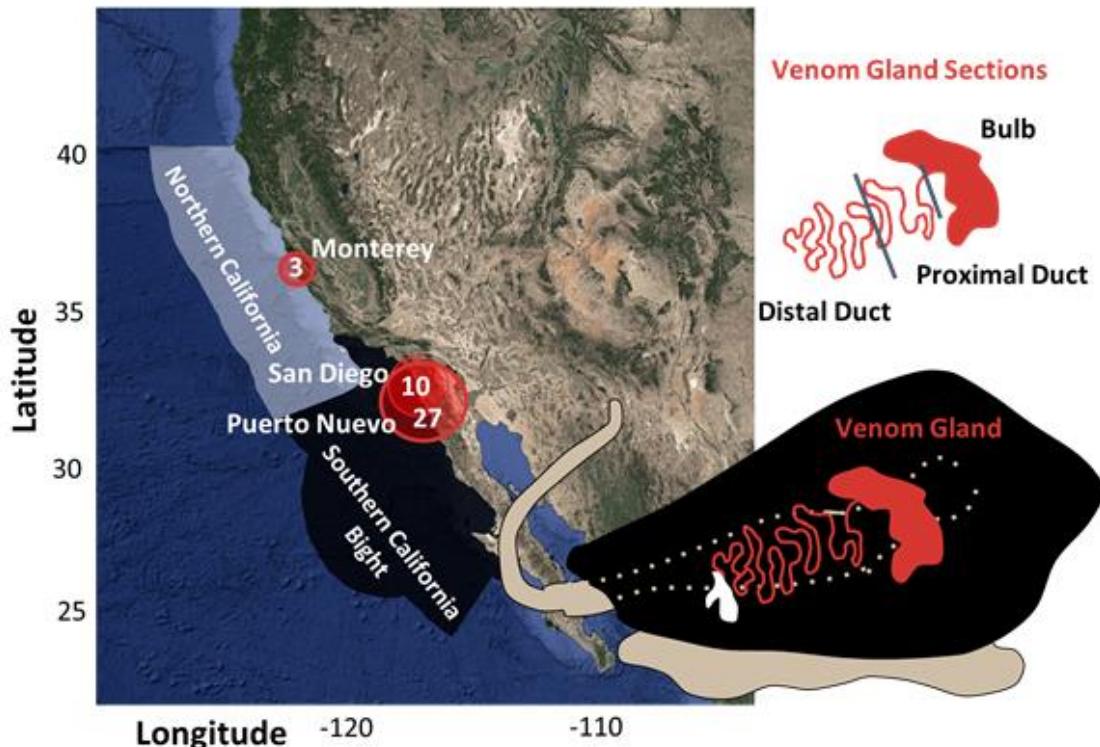
Figure 3.1. Map of samples analyzed.
Wild venom glands were sampled from Monterey (Del Monte), San Diego (Mission Point), and Puerto Nuevo (3 nested sites: Sheltered or SH, Minor Outlet or MN, and Major Outlet or MJ). Spatial and longitudinal (June 2016, 2 consecutive days in December 2016, and June 2017 for the MN site nested within Puerto Nuevo) variation of the adult *Californiconus californicus* venom gland microbiome was assessed. The San Diego population was kept in captivity for several weeks, with exposure to wild seawater, for venom gland sectioning (whole gland, distal duct, proximal duct, and bulb) and microbiome assessment. Snail venom gland anatomy is shown with the radula sac (white) and pharynx (dotted) as points of reference.

Table 3.1. Breakdown of samples analyzed in this study across space, time, and gland section for 16S and 18S rRNA gene regions.
Seawater and venom samples collected in summer 2017 are represented for Monterey (MB), San Diego (SD), and Puerto Nuevo (PN) as three nested sites (Sheltered as SH, Minor Outlet as MN, and Major Outlet as MJ). Compartment comparisons were additionally sampled from the San Diego population after being kept in captivity for several weeks as the whole venom gland control (Whole), distal duct (DD), proximal duct (PD), and bulb. Temporal samples were taken from the Minor Outlet site for Summer 2016 and Winter 2016 (Days 1 and 2).

	MB	SD	PN		
			SH	MN	MJ
SEA	2	2 (+ 2 control seawater)	2	2	3

	Compartments					Time			
	Whole	DD	PD	Bulb		Sum 16	Win 16 Day 1	Win 16 Day 2	
VEN 3	5				4	5			4
SEA	2					3	3	3	
VEN	5	4	5	5		4	5	5	

3.3.2 DNA Extraction, PCR Amplification for Validation, and Illumina Amplicon Sequencing

DNA from 200 mL of sterile, RNase free water used to wash and collect venom from dissected venom glands, and 200 mL filtered environmental seawater samples was extracted using the QIAGEN DNeasy Blood & Tissue Kit (Qiagen™, Valencia, CA, United States) according to the manufacturer's protocol. Filters were cut in 2 mm strips using sterilized scissors and the microbial film on the filter was then homogenized using the Omni Bead Ruptor (Omni International™, Kennesaw, GA, United States) using 0.1, 0.5, and 1.4 micron beads. All extracted DNA from seawater samples were each diluted to a final concentration of 5 ng per mL.

Ribosomal RNA gene amplification was performed for all samples and sequenced over 3 runs. The 16S gene was amplified to identify bacteria and archaea, targeting either the V4 (FW 515 F 5'- GTGYCAGCMGCCGCGGTAA-3', RV 805R 5'- CCGYCAATTYMTTTRAGTTT-3') or V4-V5 (FW 515 F 5'- GTGYCAGCMGCCGCGGTAA-3', RV 926R 5'- CCGYCAATTYMTTTRAGTTT-3') region (Tremblay et al. 2015, Parada et al. 2016). For eukaryotes, the V4 region of the 18S rRNA gene (FW 5'- CCAGCASCYGCGGTAATTCC-3', RV 5'- ACTTTCGTTCTTGATYRA-3') was targeted (Stoeck et al. 2010). PCRs were performed in 25ul reactions containing a final concentration of 1x AccuStart II PCR SuperMix (Quantabio™, Beverly, MA, United States), 10 ng of template DNA, 500nM of each primer, and 10 ug/ul bovine serum (ThermoFisher Scientific™, Waltham, MA, United States) for amplification validation checks, conducted prior to amplicon sequencing. Both 16S rRNA gene regions were amplified by denaturation at 94 °C/3 min, followed by 30 cycles of denaturation at 94 °C/30 sec, annealing at 50 °C/30 sec, elongation at 72 °C/1 min, with a final elongation of 72 °C/10 min. The 18S rRNA gene V4 region was amplified by denaturation at 94 °C/3 min, followed by 30 cycles of denaturation 94 °C/30 sec, annealing at 60 °C/30 sec, elongation at 72 °C/1.5 min, and a final elongation of 72 °C/10 in. After visual validation by gel electrophoresis, 250 ng in 50 uL of each amplicon was sent to the Joint Genome Institute for paired-end amplicon sequencing on the Illumina MiSeq platform (Illumina™, San Diego, CA, United States). Samples were sequenced with a unique 12 bp barcode tag sequence within each run.

Raw sequences, publicly accessible upon free registration at the Joint Genome Institute Genome Portal: IDs 1191512 and 1191514, were preprocessed for analysis through the QIIME2

implementations of a number of programs (Boleyn et al. 2019). Full code for this work is available at https://github.com/sabahzero/Ccalifornicus_Venom-Microbiome. Samples were denoised into amplicon sequence variants (ASVs) using the DADA2 method (Callahan et al. 2016). Taxonomy was assigned using the Silva database v132 (Quast et al. 2013). All libraries were subset to a common lowest read count for each primer sets (1397 ASVs for 16S rRNA libraries and 1023 for 18S rRNA libraries), then converted to proportions (relative abundances). The resultant datasets include 174 of the 179 original 16S rRNA amplifications, and 166 of the 173 18S rRNA amplifications.

3.3.3 Post-Processing Data Analyses and Statistics

The number of specimens used in 16S rRNA gene and 18S rRNA gene microbial community analyses, after data clean-up, are indicated in the table (Metadata_Clean.csv for samples used in analysis and qiime_dada2_mapfile.txt for total sample pre-processing, available via https://github.com/sabahzero/Ccalifornicus_Venom-Microbiome). All samples can be found in the raw ASV files folder on the Github repository. Sampling buffers were sequenced as controls and are removed from final analysis. For the community analysis, singletons and doubleton ASVs were removed, as well as Caenogastropoda and human host reads. Reads were proportioned for each sample according to the lowest sum total read count of a given sample over 1000 for either 16S rRNA gene or 18S rRNA gene. Specimens were included in analyses if both 16S rRNA gene and 18S rRNA gene amplicon libraries passed criteria. A total of 75 samples from venom glands were usable for this microbial community analyses. Of the 179 specimens sampled, 140 passed filter criteria. This study focuses on 77 of these samples (Figure 3.1, Table 3.1). We include additional sampled specimens in the online dataset to address ongoing investigations beyond the scope of this study, such as venom microbial community adaptation to pressure or in response to a broad-spanning antibiotic frequently used in aquaculture (tetracycline). The biodiversity of each site (alpha diversity) and among sites (beta diversity) were determined for the 77 samples. For alpha diversity, we utilized Shannon's and Simpson's indices based on read abundance. For differential abundance, we compared relativized ASV reads of taxa by log-fold change. For core taxa as indicators of the community, we took a DESeq2 approach and compared taxa richness of each ribosomal marker gene across all venom glands for the three different sites and two seasons.

   Biological replicates for the venom gland were sampled with a minimum threshold of n=3, and for environmental seawater controls with a minimum threshold of n=2 (Table 3.1, Metadata_Clean.csv). The MN site for June 2016 is represented by n=4 venom glands, and n=3 seawater. December 2016 Day 1 is represented by n=5 venom glands and n=3 seawater samples. December 2016 Day 2 is represented by n=5 venom glands and n=3 seawater samples. December 2016 Day 3 is represented by n=3 seawater samples. No venom gland sequence outputs for both 16S and 18S rRNA were sufficient in quality for this day. June 2017 is represented by n=5 venom glands and n=2 seawater samples.

   All statistical tests and visualizations were conducted in R (Core R Team 2011) using the phyloseq (McMurdie and Holmes 2013), vegan (Oksanen et al. 2011), and DESeq2 (Love et al. 2014) packages with all code and package information available at the Github repository. For statistical tests, we evaluated significance at the $\alpha = 0.05$ level. Changes in microbial community structure among sites were analyzed using non-parametric multivariate analysis of variance

(PERMANOVA, Anderson 2001) with Bray-Curtis distances (Bray and Curtis 1957) for the abundance datasets and Jaccard indices (Jaccard 1912) for richness datasets. A Bonferroni p-value correction (Bonferroni 1936) was used to determine pairwise differences between sites. Beta diversity differences in community structure and associated statistics were visualized using proportion of variance for principal coordinate analysis (PCoA) along two axes. For all univariate data, we used analysis of variance (ANOVA) to determine significant differences. We used q-q plots and scale-location plots to inspect normality and homoscedasticity, respectively. Where significant differences were detected, Tukey's Test of Honest Significant Differences was used to determine the range of differences among the sites and interactions.

3.4 Results

3.4.1 Post-Processing Amplicon Sequence Data Results

After filtering, a total of 24279 unique ASVs (12995 for 16S rRNA gene and 11284 for 18S rRNA gene) were observed from all raw sequence data. A total of 9054 unique ASVs corresponding to 7473020 reads (4800 ASVs from 2522054 reads 16S rRNA gene 2522054 reads, and 4254 ASVs from 4950965 reads 18S rRNA gene reads) were observed among the 77 selected samples (Figure 3.1, Table 3.1). Reads of 1397 or more was the minimum sample sum for the 16S rRNA gene and 1023 for the 18S rRNA gene, with median read lengths at ~380 bp.

3.4.2 The Venom Microbiome Across Space and Time

For both the archaeal/bacterial and fungal communities, the wild venom microbiome was significantly distinct from the surrounding seawater environment ($p < 0.001$, Figure 3.2). This result is consistent when expanded to additional exploratory experiments between the host and surrounding environment (Supplemental Figures 2-3). Overall, the top named bacterial and archaeal phyla found in wild venom are, in order of abundance, Proteobacteria, Bacteroidetes, *Cand.* Patescibacteria, Actinobacteria, Cyanobacteria, Verrucomicrobia, Firmicutes, Acidobacteria, Nanoarchaeaeota, and Planctomycetes. The top named microbial eukaryote phyla, in order of abundance, belong to the Ochrophyta, Cercozoa, Apicomplexa, Arthropoda, Annelida, Ciliophora, Dinoflagellata, Archaeplastida, Metamonada, and Chlorophyta groups (Supplemental-Results.rmd). With regards to the uniqueness of the venom microbiome, the bacterial community was enriched in the phyla Bacteroidetes and *Cand.* Patescibacteria, and Protobacteria class Alphaproteobacteria. In the eukaryotic domain, the venom gland was enriched in Ochrophyta plastids, as compared to the external environment. Overall, the relative abundance of Nanoarchaeota, Dinoflagellata, Cercozoa, Ciliophora, and Apicomplexa was much lower than that seen in environmental seawater samples (Figure 3.2).

Archaeal and bacterial venom microbial communities were consistent across space ($p = 0.056$) and time ($p = 0.302$), while venom microbial eukaryotes were dependent on space ($p = 0.019$) and time ($p = 0.038$) (Figure 3.3). Multiple comparisons among locations for variation in eukaryotic microbial communities were not significant ($p < 0.05$). Differential abundance analysis revealed that *Gregarinasina eugregarinorida* (a group of parasites within the phylum, Opisthokonta) was significantly enriched ($p = 0.001$) during the summer (Supplemental Figure

3.1). *G. eugregarinorida* was also enriched in the San Diego population while *Parvamoeba rugata* was observed in only in Puerto Nuevo populations (Supplemental Figure 3.2).
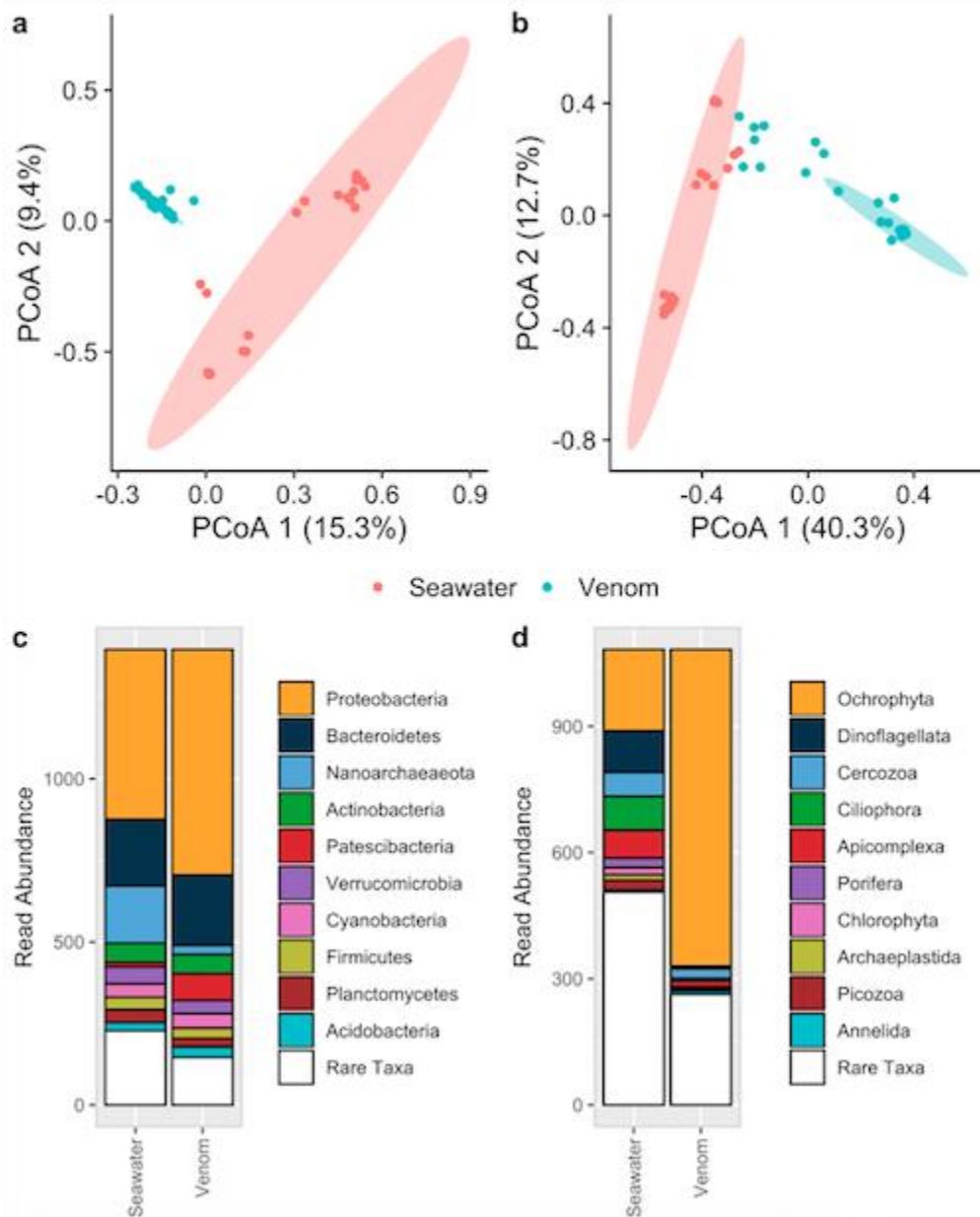
Figure 3.2. PCoAs of microbial community composition and barcharts of top phyla for venom versus seawater in the natural environment.
Comparison of ordination plots (PCoAs) with 95% confidence ellipses for microbial communities of (a) 16S rRNA gene representing archaea and bacteria and (b) 18S rRNA gene representing microbial eukaryotes by media type (whole venom gland and seawater) indicating that the venom microbiome is significantly different from the seawater the environment (p < 0.001 under PERMANOVA using Bray-Curtis). Bar chart breakdowns of top phyla for © both 16S gene and (d) 18S microbial communities reveal an abundance of Proteobacteria,

Bacteroidetes, Patescibacteria, and Ochrophyta in the venom versus the seawater. Rare taxa are displayed as currently undescribed taxa with an abundance less than the mean read count for a given sample, which are overall higher in count (less taxa described, comparatively) for 18S than 16S microbial communities.
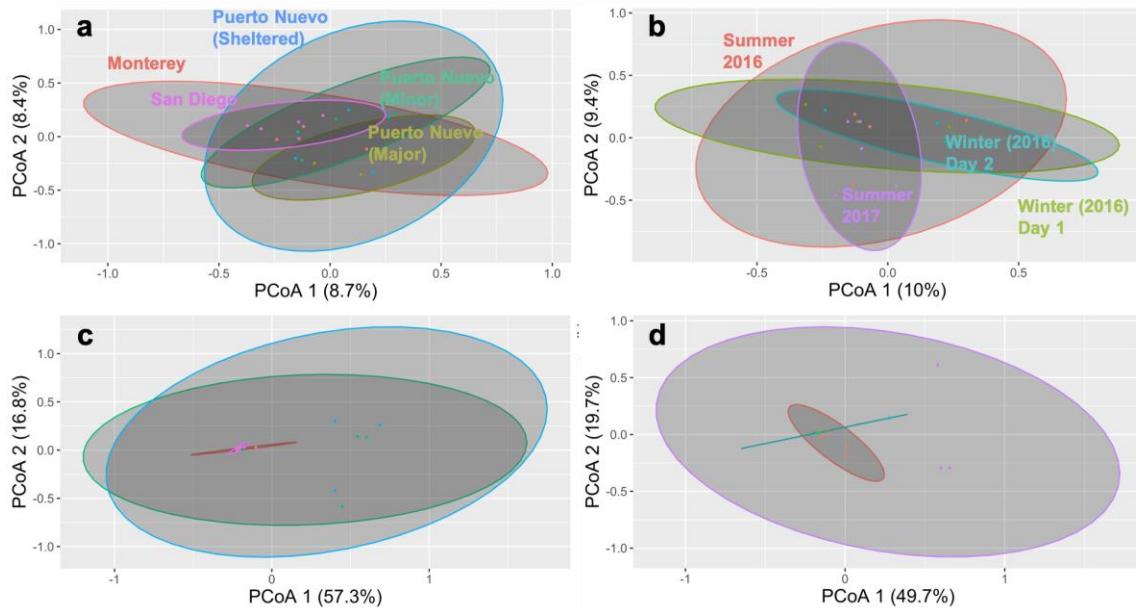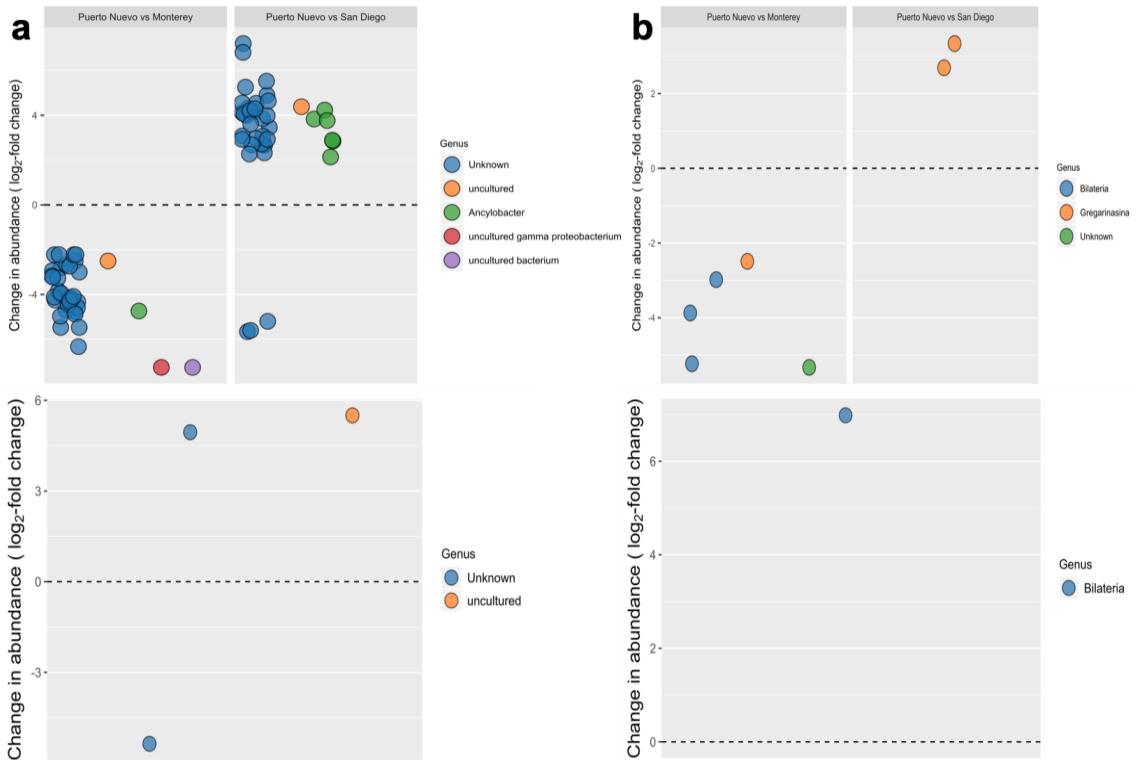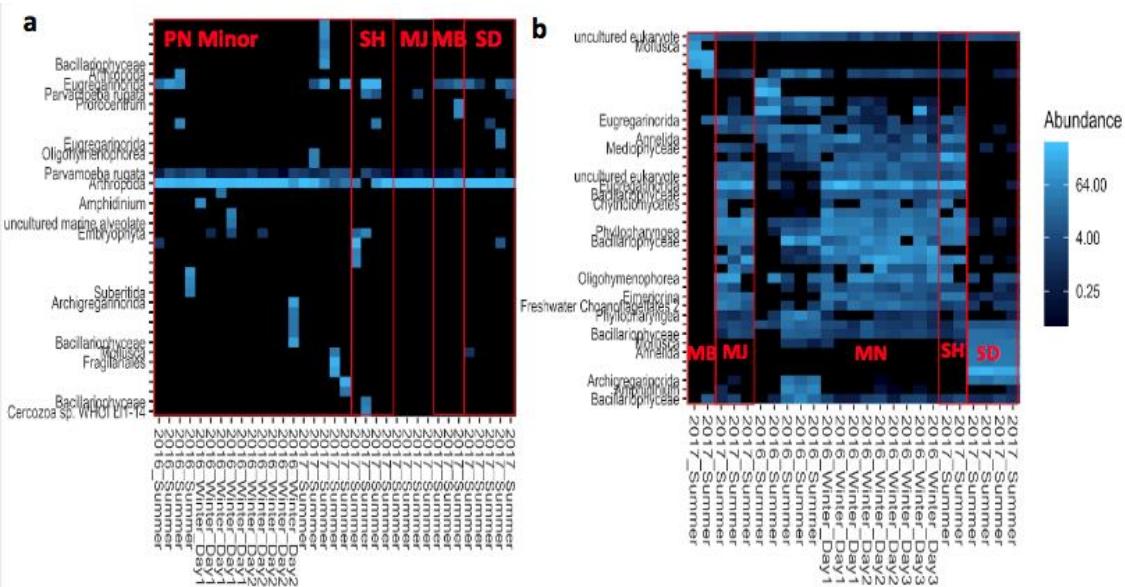
Figure 3.3. Venom microbial community variation across space and time.
Principal coordinate analyses of microbial communities for 16S rRNA gene across (a) locations and (b) time points within the Puerto Nuevo Minor Outlet site for 18S rRNA gene (c-d) reveal domain to be an important factor when evaluating the venom microbiome. For 18S rRNA gene, venom microbial communities for the Puerto Nuevo Sheltered and Minor sites show a broader composition than that of other locations. Sites were controlled for time. 18S rRNA gene microbial communities vary more by year than by season (summer versus winter) or day.

Supplemental Figure 3.1. Top 10 venom microbiome genera by location and time.
Comparison of locations for collection during Summer 2017, with Puerto Nuevo as the reference
for 16S rRNA gene (a) and 18S rRNA gene (b) indicate that bacteria decrease in log-fold
abundance for Monterey and increase for San Diego. For time across the Puerto Nuevo Minor
Outlet site, categorized by season (summer or winter, with winter as the season of reference),
few top genera show differential abundance for either (c) 16S rRNA gene or (d) 18S rRNA gene.

Supplementary Figure 3.2. Heatmaps of top 18S rRNA region taxa comparing venom to seawater by location and time.
A focus on top 18S taxa found for (a) venom and (b) seawater by location and time show Arthropods to dominate the venom spatially and temporally. Labels not shown are taxa currently undescribed.

3.4.3 The Venom Microbiome is Localized Across the Gland, With Variation by Treatment

Amplicon data analysis revealed that the microbial community of the distal venom duct is significantly ($p < 0.05$) different from the proximal venom duct and the venom bulb across domains (Figure 3.4). The distal duct, the site of conotoxin production, also has the most restricted alpha diversity of the three venom gland regions analyzed (Figure 4, Supplemental-Results.rmd). Verrucomicrobia, Acidobacteria, Planctomyetes, Annelida, and Dinoflagellata are comparatively more abundant whereas Bacteroides, Cyanobacteria, and Apicomplexa are less abundant phyla than in other gland sections (Figure 3.4). Additionally, the captive animal venom microbiome (Venom_WT) microbial biodiversity is more varied compared to wild counterparts. Further breakdown of microbial abundance by top taxa (Supplemental Figure 3.3) are consistent in showing Eugregarinorids abundant across the venom gland, as well as and Arthropods.
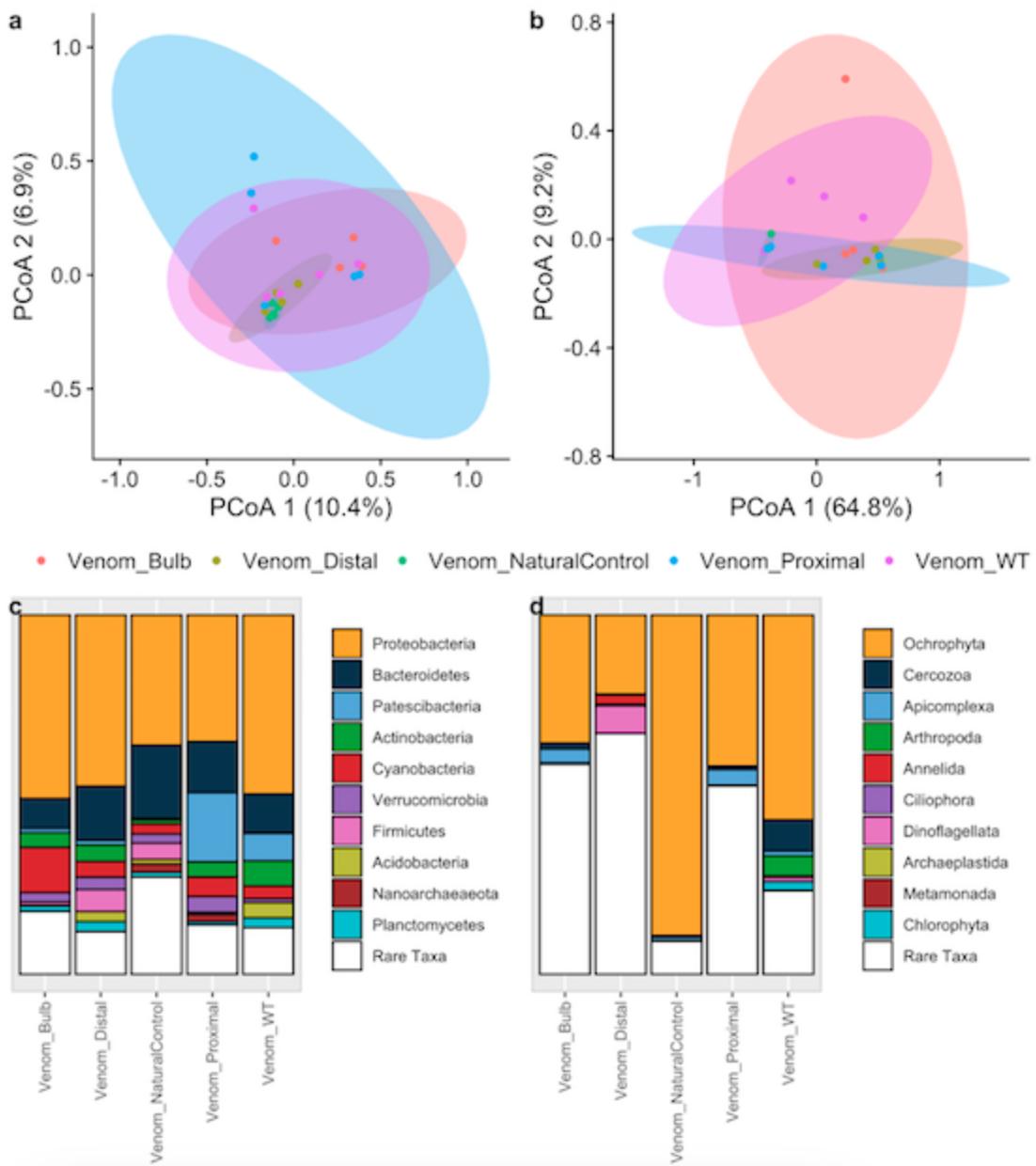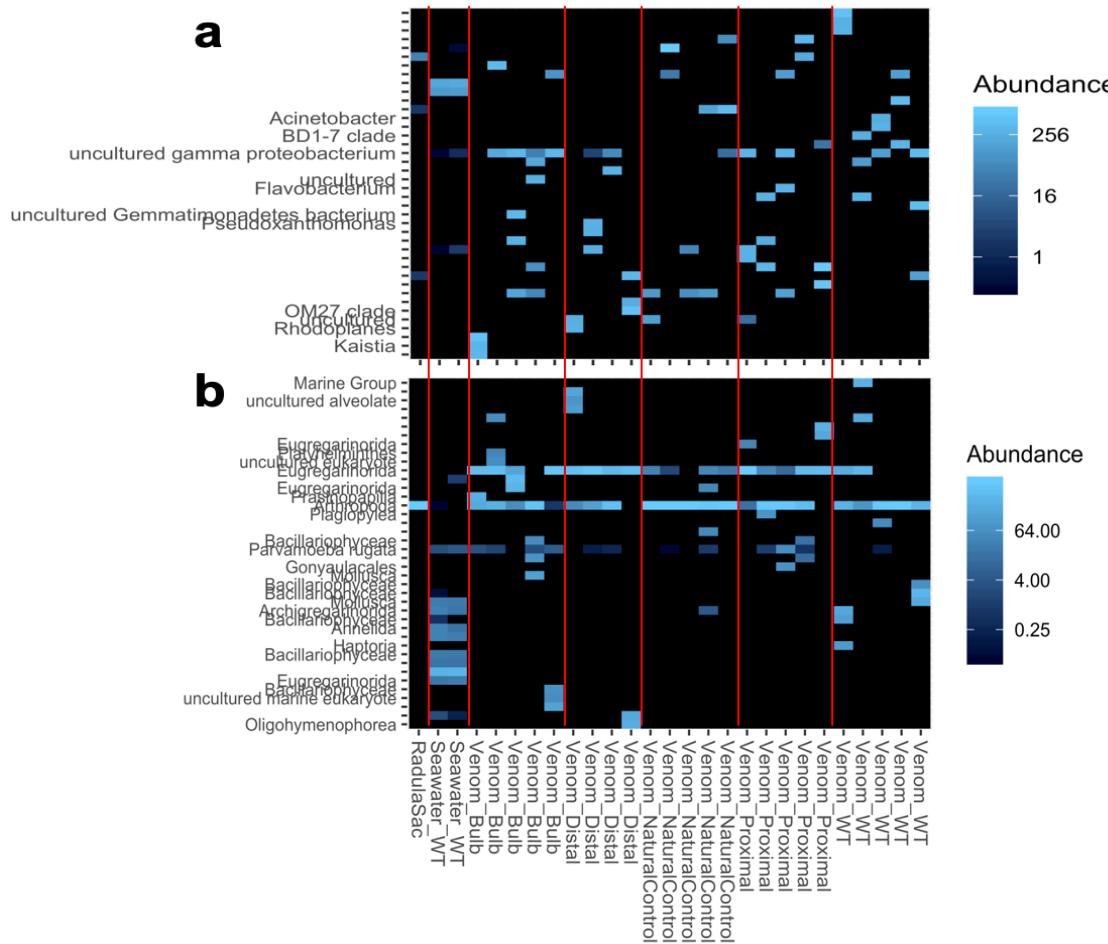
Figure 3.4. PCoAs of microbial community composition alpha diversity of top phyla for venom by gland compartment.
Comparison of ordination plots (PCoAs) with 95% confidence ellipses for microbial communities of (a) 16S rRNA gene representing archaea and bacteria and (b) 18S rRNA gene representing microbial eukaryotes by gland compartment, including whole gland (Venom WT) and whole gland as found in the natural wild environment (Venom_NaturalControl). The venom microbiome was significantly distinct for the distal duct compared to other gland sections for 16S and 18S microbial communities ($p < 0.05$ under PERMANOVA using Bray-Curtis). Top phyla from results found in the wild venom for (c) 16S gene and (d) 18S show an abundance of Verrucomicrobia, Acidobacteria, Planctomyetes, Annelida, and Dinoflagellata in the distal duct compared to the bulb and proximal duct venom gland sections. Rare taxa are displayed as

currently undescribed taxa with an abundance less than the mean read count for a given sample, which are overall higher in count (less taxa described, comparatively) for 18S than 16S microbial communities for these media.

Supplemental Figure 3.3. Heatmaps of microbial abundance across venom gland sections for top 40 taxa
Heatmaps of top 40 families for (a) 16S rRNA gene and top 40 phyla for (b) 18S rRNA gene across venom gland sections, including seawater and radula tissue as controls as well as the wild venom gland (Venom_NaturalControl) show many unknown taxa (displayed as unlabeled). An unculturable gammaproteobacterium appears in multiple samples of the venom bulb, whereas Eugregarinorids and Arthropods are found across the venom gland.

3.5 Discussion

3.5.1 Venom as a Microenvironment

While several venom microbiomes have been described in recent years (Goldstein et al. 2013, Torres et al. 2017, Esmaeilishirazifard et al. 2018), the venom gland had largely been viewed as

a sterile environment, with most studies emphasizing the antimicrobial properties of venom (Ul-Hasan et al Toxicon X, *in press*). This study contributes to the growing body of work confirming that venom possesses a specialized microbiome distinct from the surrounding environment by comparing the microbial community present in host venom for species *C. californicus* (Figures 2-4).

While we did observed Actinobacteria (Peraud et al. 2009, Lin et al. 2010, Lin et al. 2013a, Lin et al. 2013b, Lin et al. 2014, Quezada et al. 2016, Quezada et al. 2017) and *Stenotrophomonas* (Torres et al. 2017) ASVs in our *C. californicus* venom amplicon data, they did not exhibit high abundance the venom as compared to the seawater environment (Figure 3.2, Supplemental Figure 3.2). We did observe Actinobacteria to be a constant phylum across gland sections, but at low abundances (Figure 3.4). Of these studies, Torres et al. 2017 is the only study to use culture-independent methods (16S rRNA gene sequencing) to compare 11 species (hepatopancreas gut, body, and venom duct microbial communities) of Conidae from around the world. One explanation for the differences we observed versus those of Torres et al. 2017 could be due to the different populations sampled. Adult *C. californicus* in the Torres et al. 2017 study were sampled from Catalina Island, whereas we sampled from other areas and observed differences in the microbial community by location (Supplemental Figure 3.2). Another potential difference between these two studies is that we extracted venom from the whole venom gland, whereas Torres et al. 2017 extracted venom and host tissue from the venom duct (proximal and distal). Taken together, these results highlight the importance of sampling from both host animal tissue and the environment for identifying taxa specific to the gland.

Stenotrophomonas is a Gammaproteobacterium, and we consistently observed unique Gammaproteobacteria taxa to be more abundant in the venom than the seawater by approximately 2-fold (Figure 3.2, Supplemental Figure 3.3). Torres et al. 2017 used a Fluorescence in situ hybridization (FISH) label to identify *Stenotrophomonas* in the venom duct and lumen of host *Conus virgo*, and found that *Stenotrophomonas* was preferentially localized in the lumen versus the exterior venom duct. We observed that the distal duct, closest in proximity to the opening of the pharynx and where conotoxin activity occurs, had discrete microbial biodiversity compared to other gland compartments (Figure 3.4, Supplemental Figure 3.3). An additionally interesting observation is that archaea are less abundant in the venom than the surrounding seawater, though Nanoarchaeota remains as one of the top phyla identified in wild venom (Figure 3.2, Figure 3.4, Supplemental-Results.rmd). In the literature, identification of archaea in microbiomes infers complexity (Moissl-Eichinger et al. 2018) and Nanoarchaeota are described as a marine phyla that exhibits ectosymbiotic characteristics (Thurber et al. 2009, Horz 2015, Merrifield and Rodiles 2015, John et al. 2019). Both our results and those of Torres et al. 2017 support the idea that there is selective pressure from either the host, microbes, or both in determining the microbes that remain and the microbes that are excluded from the venom gland.

3.5.2 Microbial Eukaryote Diversity in the Venom Gland

By including 18S rRNA gene sequencing in our amplicon study, we observed a high abundance of parasitic eukaryotes in the venom (Figures 2 and 4, Supplemental Figures 1-3). The San Diego site in particular appears to demonstrate a greater abundance of venom parasites than either Monterey or Puerto Nuevo (Supplemental Figure 3.2). Given that parasites in cone snails are largely unexplored and that cone snails are top predators in the gastropoda realm (Duterte et al.

2014), this may be an area of interest for future studies. Furthermore, the complex relationships between parasites and host fitness (Jakubowski et al. 2005, Davis et al. 2009, Duterte et al. 2010, Abdel-Rahman et al. 2011, Duterte et al. 2014) may be important in understanding venom transcriptomics as they vary inter- or intra- specifically. Since identifying key venom symbionts is a primary interest in venom-microbiome research, exploring the microbiome as it relates to fitness through prey capture may be another important and understudied area of future interest.

3.5.3 Marine Invertebrate Host Microbiomes Across Space and Time

Many microbiome studies have observed clear patterns in microbial community distribution by time and geography (Revellaid et al. 2014, Bik et al. 2016, Chu and Vollmer 2016, Thomas et al. 2016, Fahimipour et al. 2017, Dunphy et al. 2019). Interestingly, we observed that the adult *C. californicus* microbiome is fairly conserved over time, however the venom microbial community corresponding to the 18S rRNA gene significantly differed over the three time points sampled (Figures 3-4, Supplemental Figure 3.3). Gammaproteobacteria and Proteobacteria dominate the venom microbiome (Figure 3.2, Supplemental-Results.rmd), which is similar to what has been observed for other marine host-microbe studies (Revellaid et al. 2014, Cleary et al. 2017, Fahimipour et al. 2017).

3.6 Conclusions

Using adult *C. californicus* as the host system, we found that venom microbial communities are distinct from those of the environment and that the venom microbiome is consistent across space and time. We also observed several unknown venom microbe taxa, expanding our knowledge of microbial biodiversity in the venom gland. Overall, our results contribute to our understanding of how the venom microbiome may vary by (a) host species within a clade, (b) intraspecifically in relation to environmental factors such as space and time, and (c) within different sections and media types of the gland.

3.7 References

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Chapter 4

Applications of symbioses from the natural world to science culture and practice

4.1 Science Communication - The Biota Project: A Case Study of a Multimedia, Grassroots Approach to Scientific Communication for Engaging Diverse Audiences

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4.1.1 Abstract

The Biota Project communicates science to populations historically ignored by the scientific community. The Biota Project is comprised of a team of young professionals from a myriad of backgrounds and locations with interests in promoting science accessibility and equity. We do this by highlighting research conducted by scientists from underrepresented groups in relatable yet underrated locations with the intention of increasing the participation of underrepresented populations in science. The Biota Project centers on the scientific definition of symbiosis as a tool for both educating and learning from its followers. We deliver stories on the environments of our own backyards by merging art and science and distributing these publicly available stories widely online through short films, media clips, drawings, paintings, blogs, and e-newsletters. This project demonstrates a fresh, transferable perspective on strengthening science communication in a way that conjoins scientific discovery with social justice through the promotion of critical thinking by its target audience. Likewise, contributors learn how to better support local communities with each new story and environment. The Biota Project thus sets a symbiotic tone for re-calibrating the balance between academics, researchers, and local communities. When science is made relevant through understanding, its quality and significance are enhanced, and public recognition of its value is increased.

4.1.2 Purpose and rationale

The importance of scientific communication cannot be overstated. It is integral for developing new ideas and research questions among scientists, affecting policy and educating the public about the nature of the universe around us (Cash et al. 2003; Fischhoff 2013). Women and ethnic and racial minorities continue to be overlooked or

marginalized in this process (Blake 1993; Nisbet and Scheufele 2009). Whereas there are many initiatives to promote underrepresented parties in the sciences in the United States, reports by the National Center for Science and Engineering Statistics at the National Science Foundation (2013, 2018) reveal that the increase of underrepresented minority enrollment in undergraduate studies has yet to be reflected in the overall Science, Technology, Engineering and Mathematics (STEM) workforce. This has resulted in an underrepresentation of these identities in the sciences (Summers and Hrabowski 2006; Jackson et al. 2014). Hence, the lack of diversity in the sciences remains an important international topic for addressing how science can serve as a critical bridge between informing the public on current research and increasing innovation within its own community through inclusion. Connecting science to everyday life is a major tenet of scientific communication by raising scientific understanding (Nisbet and Scheufele 2009). The effectiveness of science in decision-making on a societal level and participation on a personal level depends on one's ability to communicate science, especially to diverse audiences. Many science communication projects fail to connect with diverse audiences due to the inability to maintain relevance via appropriate current media outlets or falling outside of an audience's interests and/or traditions (Lee 2013). These failures are affirmed by examples such as expert scientific panels and plenary speakers on television and at national scientific conferences in the United States continuing to be overwhelmingly white and male in contrast to the overall population demography (Casadevall and Handelsman 2014; Casadevall 2015). In addition, science communicators themselves often do not reflect the diversity of people they mean to serve and, in turn, do not reach these groups adequately as observed by Science Magazine with 45 of the top 50 science stars of Twitter being males (You 2014). Underrepresented groups are not only underserved but are also underrepresented in targeted science communication. Key science issues such as climate change are not communicated well and sometimes barely reach minority groups (Lee 2013), raising concerns about the adaptive capacities of underserved communities in times of natural disasters and post-disaster recovery (Archer 2003; Schlosberg and Collins 2014). Ineffective science communication can construct silos of dominant groups, creating marginalized conversations and unintentionally acting exclusively in practice (Dawson 2018). Without first understanding the communities and the history behind these communities, those who try to communicate with underrepresented groups can unknowingly create distrust, feelings of inaccessibility to science, and a perception of lack of care for inclusion in the sciences (Treise and Weigold 2002; Nisbet and Scheufele 2009; Grant 2016; Dawson 2018). A proposed solution for this communication gap comes from studies demonstrating how audience engagement and participation lead to better learning outcomes (Dickinson et al. 2012). In this article, we describe a grassroots approach that (1) focuses on symbiotic relationships in nature, which can then be translated into relationships within a community, (2) celebrates diversity by highlighting research from underrepresented scientists, (3) integrates multimedia art that is appealing to diverse audiences, and (4) provides a call to action, empowering individuals to address socioenvironmental issues in their communities. Entitled "The Biota Project" (www.thebiotaproject.org), our overall aims are to increase parity in science education, bolster inclusion in the sciences, and expand layperson interest in and understanding of the natural world.

4.1.3 Origin and approach

The Biota Project was conceptualized in 2013 by two individuals of differing expertise and interests. While one is interested in film production and the other interested in science, both are first-generation American, queer women of color from an underserved, low-income community in Salt Lake City, Utah (USA). In each of their respective fields, they encountered both implicit and explicit bias as well as marginalization. As a means of proactively addressing these issues, the duo then developed a team comprised of budding artists and scientists to initiate a science education film series geared toward an ethnically diverse target audience from both technical and non-technical backgrounds. Communications additionally targeted members of the millennial generation, as they sought to instigate change by centering on a generational demographic entering the workforce and becoming involved in decision-making processes. In 2016, The Biota Project adapted its mission to incorporate multiple projects as intersections to their short film theme. Such projects include operations and organization, film production, and social media and communications. Individuals in each of the project teams learned from and worked with each other throughout the process, building a "mutualistic symbiosis" that functioned to push the projects forward while working toward personal development. For example, one science writer in the Social Media and Communicators project team was unfamiliar with developing an e-newsletter. A fellow team member on the project had familiarity with tools (e.g., MailChimp, a commonly used email marketing service) to implement a regular subscription e-newsletter. The two members worked collaboratively to create an e-newsletter, with each newsletter release describing symbiotic relationships relating to a unified theme. Captured in the January 2017 newsletter, the theme of the newsletter discussed symbiotic relationships occurring within colder environments and how the people observing changes in these relationships interact with their environments in the wake of climate change. While taking on and testing out numerous media platforms through multiple projects had been overwhelming at times, such an approach was proven successful for The Biota Project in best identifying and then catering to its target audiences. Today, The Biota Project team continues to meet bi-weekly with updates on project progress. Project teams meet on a need-by-need basis; some project teams maintain regularly scheduled meetings due to the nature of their project whereas other project teams have few but intensive workshop meetings lasting several hours. These meetings are specialized to the needs of the project teams and their members' schedules, accepting of fluidity and fostering inclusion. The nature of The Biota Project and team members' locations (see section "Challenges, Lessons Learned, and Next Steps") require regular modern communications through telephone, instant messaging, and e-mails. When a product is ready for release, all members review it and coordinate with project teams to promote the product accordingly. This interactive "on-call" approach is appreciated by our audience and humanizes us as a team, building a community in and of itself where people are interested in the sciences while also feeling a sense of belonging and ownership.
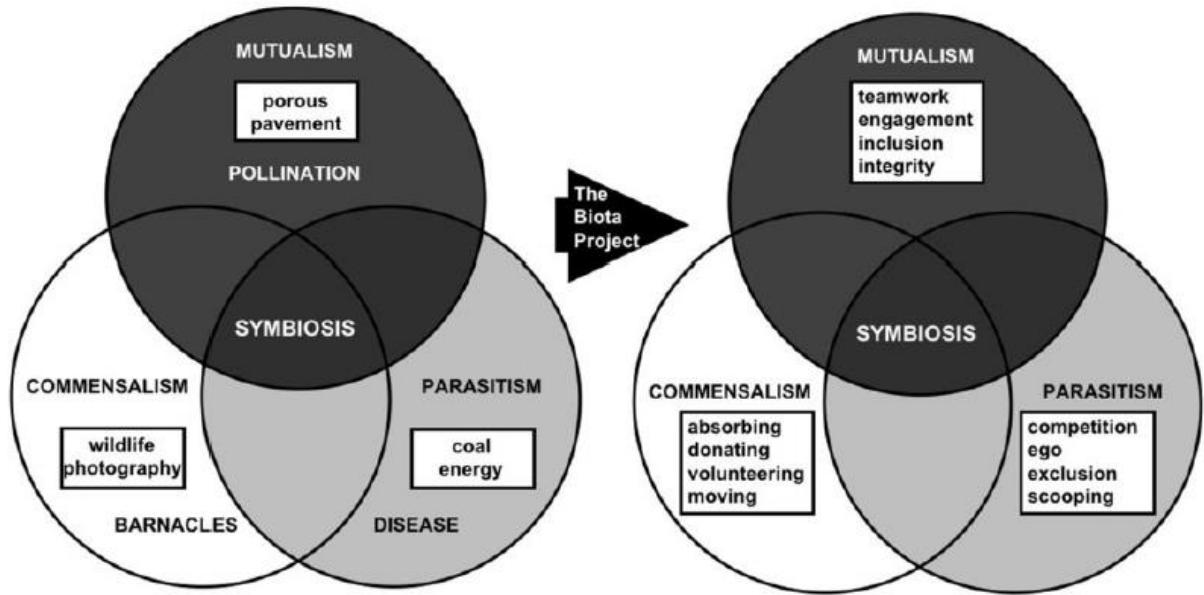
Fig. 4.1.1 The Biota Project highlights examples of the three major types of symbioses in nature to draw parallels of how humans interact with their environments and each other (boxed). Modes of symbiosis are practiced within The Biota Project to promote a healthy and evolutionarily advantageous working environment (mutualisms of teamwork, engagement, inclusion, and integrity) with awareness of other overall neutral (commensal) or negative (parasitic) working environment habits.

4.1.4 Symbiotic relationships in nature and human society

Symbiosis is defined as the interaction between two species living in close proximity and can be divided into three broad types: (1) parasitism, where one species benefits from the interaction while the other species is harmed; 2) commensalism, where one species benefits from the interaction while the other species is unaffected; and 3) mutualism, where both species benefit from the interaction (Moran 2007). We distribute information about and increase the understanding of this concept by highlighting these relationships within nature and draw parallels between nature and human society's ability to interact with it (Fig. 1). In The Biota Project's first film, we focused on symbiotic relationships in the vernal pools ecosystem of California's Central Valley (https://www.thebiotaproject. org/projects/). We highlighted the commensal dynamic between the California ground squirrel (Otospermophilus beecheyi) and the tiger salamander (Ambystoma tigrinum). In this ecosystem, ground squirrels dig extensive burrows that create habitat for tiger salamanders and other vernal pool species after burrows are vacated (Fitch 1948). We used this example of commensalism to relay how humans can act symbiotically with their surrounding ecosystems. For instance, cattle grazing in the vernal pools ecosystem has been shown to reduce invasive non-native plant species (Marty 2005). Thus, using symbiotic relationships as a metaphor for interactions between humans and the environment can spread a message that environmental conservation and economic development are not mutually exclusive.

Furthermore, practicing symbiosis through diverse perspectives within our science communication team and with our audience creates effective and productive spaces described in the next sections.

4.1.5 Celebrating diversity by practice

The Biota Project directly tackles challenges of diversity and inclusion by ensuring consistent representation in leadership team composition, a minimal hierarchical structure, and a membership reflective of the audiences it aims to engage. The past and current members of The Biota Project represent a range of expertise and backgrounds (Table 1). Research confirms teams comprised of people who are more different from each other than similar results in greater preparation for working through conflicts and facilitating trains of thoughts (Watson et al. 1993; Diaz- Garcia et al. 2013). In turn, this brings different perspectives into a project, potentially leading to new insights and knowledge. This is particularly true for innovation in the sciences (Watson et al. 1993; Nathan and Lee 2013). We believe diversity in our group and approach to science communication is a strength and has enabled the success The Biota Project as this draws a keen interest from audiences by taking nontraditional communication approaches. The following is a quote from one of our followers about what their interests were in The Biota Project: [The Biota Project] is a space for communities to come together and learn about each other through commonalities. I believe community strength comes through connections and encounters - the more we break down community walls, the more we can focus on issues that truly matter. (A. E. Jolin, Pers. interview, May 30, 2016). The following is a quote from a contributor of The Biota Project who produced original music for films and documentaries. The contributor spoke about reasons for joining the group: I've been playing mostly live music since I was a teenager and have never really composed something. For me this is a new type of symbiosis; I know how to be a part of a live band that is made up of parts but sounds like a unit. Now, instead of being one part of a band I am one part of The Biota Project experience. It's a great challenge and new way of growing. (B. Usami, Pers. interview, January 13, 2016). We have found that creating a discussion with, versus lecturing to, our audience results in an organic and amplified interest. In our December 2016 newsletter entitled "Winter, Bison, and Parasitic Mistletoe," we discussed a common winter tradition not so familiar to the general public. Known as the "Winter Count," this tradition of record-keeping and story-telling is practiced by the Lakota and Dakota Peoples of the Great Plains. We shared the tradition's history, how the tradition is celebrated, the communities celebrating the holiday, and how the tradition overall encompasses themes of ecological and cultural symbioses. We translated and shared information about the mutualistic relationship between bison (Bison bison) and prairie dogs (Cynomys spp.) (Fahnestock and Detling 2002), the bison's role in shaping the prairie vegetation (Knapp et al. 1999), and personal narratives of the Native Peoples' strong ties to the American Plains bison (Bison bison). We intentionally consulted perspectives of The Biota Project team members and subscribers who practice this winter tradition in this process to appropriately and respectfully incorporate their input on the feature. The outcome was a lasting impression of how communicating stories through diverse perspectives enables us to create valuable scientific content in reaching broad audiences.

2013	2014	2015	2016	2017	2018
Film & Art	Film & Art	Film & Art	Film & Art	Film & Art	Film & Art
Science	Film & Art	Film & Art	Film & Art	Film & Art	Film & Art
	Film & Art	Film & Art	Film & Art	Film & Art	Science
	Film & Art	Film & Art	Film & Art	Film & Art	Science
	Science	Film & Art	Film & Art	Film & Art	Science
	Science	Film & Art	Film & Art	Film & Art	
	Science	Science	Film & Art	Science	
	Science	Science	Film & Art	Science	
	Science	Science	Film & Art	Science	
	Science	Science	Science	Science	
	Science	Science	Science	Science	
	Linguistics	Science	Science	Science	
		Linguistics	Science	Business	
		Linguistics	Science		
		Business	Science		
			Science		
			Science		
			Science		
			Linguistics		
			Business		
Inception	Themes	Expand	Expand	Expand	Focus
	Recruit	Build	Fund	Fund	Re-Brand
	Audience	Edit	Awards	Outreach	Non-Profit
	Film	Fund	Polish	Focus	Expand

Table. 4.1.1 A breakdown of The Biota Project's active team member composition from inception to present day Science backgrounds range from ecology to technology to engineering. Film and art backgrounds range from film production to music synthesis to visuals. Many team members held overlapping skillsets and interest with dominating topics representing a given member. Education levels vary from a high school diploma through professorship, and multiple categories of underrepresentation organically present throughout. The Biota Project consistently demonstrates themes of symbiosis between science and art, with specialties intermixed within subteams. Each year is summarized with decided aims and resulting outcomes as a science communication group for that given time frame. Now transitioning from a grassroots to a more formal organization, we recommend science communication groups of any sort test multiple platforms, size ranges, and dynamics over different sets of time before concluding what their deliverable product is as a widely accessible entity.

4.1.6 Art as a symbiotic tool for connecting viewers

Art is often viewed as the antithesis of science. However, these two disciplines are more similar to each than different. Both disciplines require critical thinking and observations, promoting further investigation of the world and its processes. In addition, studies have found that the perception of mathematical equations excites the same regions of the brain linked with the perception of fine art (Zeki et al. 2014). Art has long been a device for communicating science. For example, Leonardo da Vinci is best known for his Renaissance paintings and yet many of his works were informed by observation and scientific investigation in physiology and anatomy,

seen in his strong interest in nature (Fig. 2a). There is a growing trend in the United States to integrate art with the sciences. In 2010, the United States acknowledged the importance of STEM education for youth to promote discovery and innovation (Dejarnette 2012). Maeda (2013) made the argument that STEM alone would not lead to discovery, and rather that these disciplines require the addition of art to critically think about dense concepts and new ideas. Aptly, STEM is transitioning into STEAM (Science, Technology, Engineering, Arts, and Mathematics) and is being adopted by many K-12 institutions (Robelen 2011; Bequette and Bequette 2012; Watson and Watson 2013). Additionally, the universal language of art engages with all audiences regardless of one's skill set or demographic (de Oliveira 2017). The mission of The Biota Project is part of the growing national and international phenomena that melds the sciences and art to distribute science education. The Biota Project uses various forms of artistic media, such as drawings, cartoons, music, and film, to explain scientific principles and transform cultural perceptions. Our organization's team is comprised of both trained scientists and artists of various disciplines to engage in community dialogue. One strategy we have taken is incorporating artwork from audience members directly into our science writing, recruiting artists to our team to newly depict original art pieces such as the diversity of animals with gastroliths (Fig. 2b). The following is a quote from the artist of Fig. 2b. The artist describes their experience being a part of The Biota Project: I really like art and science. It's kind of the first thing that drew me to The Biota Project. I like that their goal [which] is to make science more interesting and also expressing that more towards millennials. I think it's a great way to get people involved and aware of our environment, and what better way to do that than through art and make it interesting for everybody. . . It is a great experience to be a part of this growing organization. (L. Hagerman, Pers. interview, December 3, 2016). In addition to visual artists, The Biota Project has members who are animators, graphic designers, sculptors, and musicians. Our films and documentary contents are original work. The overall aesthetic of our work holds strong appeal to millennial audiences as both a reflection of the members' interests, cultures, and styles and maintaining societal relevancy. Quotes gathered from interviews with our subscribers and followers revealed that the process of using science and art to connect ecosystems with the community is what drew their interest to The Biota Project. The co-founder of The Biota Project says this: I'm compelled to contribute to The Biota Project because art has the power to create and reinforce our culture. Animation, television, music; all of these forms of media are time capsules of an idea that has the ability—long after the creators themselves have died—to change the way that people think. The Biota Project has the potential to change the minds of people, and will empower them to develop supportive relationships within and with our world." (J. Abubo, Pers. interview, January 19, 2016).
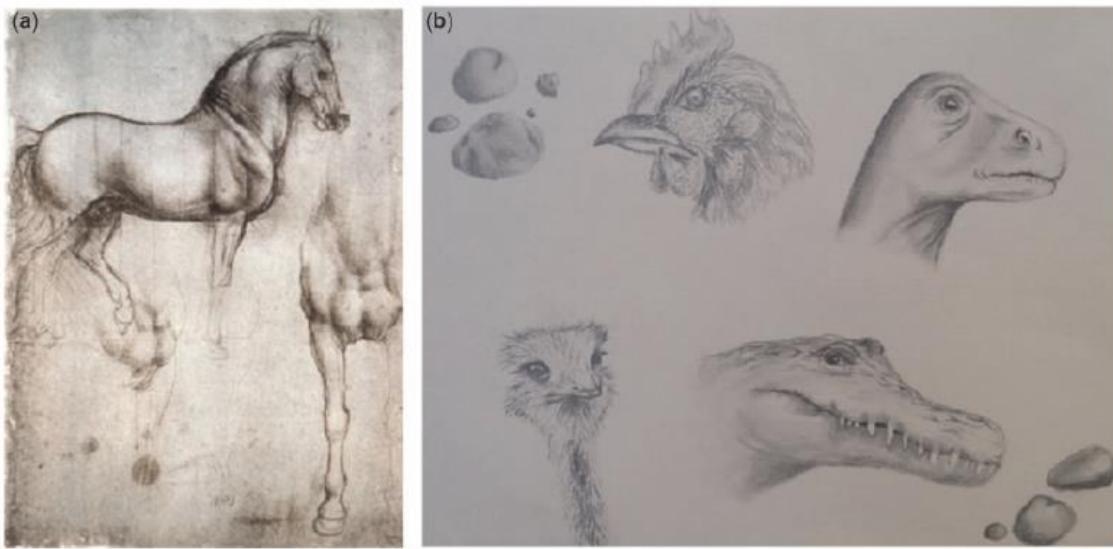
Fig. 4.1.2 Drawings depicting the physiology and anatomy of animal species. (a) "Study of Horses" by Leonardo da Vinci (1490) Retrieved from (https://www.leonardodavinci.net/) and (b) "Predators of Rock" by Leesa Hagerman @art_by_lah (2016), former member and artist of The Biota Project. Gastroliths are stones swallowed and either held in the muscular gizzard or passed through the digestive system along with food by animals that lack grinding teeth (Wings 2007), similar in conception to that of the da Vinci illustrations.

4.1.7 Call to action: Empowering and engaging audiences as stewards

A key component of The Biota Project is recognition of its audience's interests and strengths. We aim to shift the paradigm of top-down communication and allow for scientific information to be a two-way dialogue. We execute this approach by asking our audience what they want to learn about with us, delivering the information to them without reducing the quality of the science. For example, the first excerpt of our short film project on the vernal pools was to take a tour through downtown Merced, California. The town is adjacent to the "University of California, Merced Vernal Pools and Grassland Reserve." During this tour, members of the local community were asked what they knew about the vernal pools and what they would like to know. Locals knew little about the area or research activities therein but were deeply curious. One individual was particularly interested in the endangered fairy shrimp (Order Anostraca) found in the vernal pools. Questions from the local community then drove the narrative of the episode. The end result was a film that provided information and resources based on the curiosities of its audience, continuing to exist as an ongoing and engaged conversation with the local community. Social media has been another helpful tool in understanding our audiences. For instance, we create polls to inquire what subscribers want to see as our science communication organization continues to develop and expand. We highlight our viewers as integral contributors to our content by spotlighting their interests in The Biota Project alongside their general personal and professional interests. We interview our subscribers in The Biota Project, asking them questions about their interests in symbiosis and what drew them to The Biota Project. We find

this community-integrated approach contributes to an additional level of inclusivity, democracy, and a sense of belonging. Moreover, these interactions keep us current as a nexus of science, media, and social justice. Our products are constantly changing and evolving to match the needs of our audience. The empowerment of these communities through accessible means has proven critical for moving away from stagnancy and moving toward progress in the sciences. Our mission is to not only expose and engage our audience to scientific content but also ignite interests in ways previously absent. We have done this by distributing our short film on the vernal pool ecosystems as an educational conversation piece through the "University of California, Merced Vernal Pools and Grasslands Reserve," ValleyPBS, and the Center for Information Technology Research in the Interest of Society. The film highlights research on the reserve done by people from different educational levels and backgrounds, and connects the science with community members, further involving the larger public in scientific research and understanding. Engaging with our audience, even after completion of our projects, has had lasting effects. For instance, the vernal pools film continues to be shown to students annually in a yearly naturalist class at the "University of California, Merced Vernal Pools and Grassland Reserve." In addition, members have expressed pride in the film's music soundtrack being locally sourced and credited. These introductory exposures to a location culturally viewed as "undesirable" have transformed the conversation and sparked new interest and stewardship through the lens of different and fresh, yet locally rooted perspectives.

4.1.8 Challenges, lessons learned, and next steps

Since its inception, The Biota Project has encountered challenges common to many new organizations, including team coordination, recruitment and retention of skilled and knowledgeable partners, and refinement of its outreach approach (Table 1). In this section, we share some of the obstacles encountered, describe the associated lessons learned, and summarize our next steps as a resource for others to build upon in their own initiatives. The first obstacle we faced was how to logistically coordinate across multiple time zones while balancing our careers and education. Our past and present members reside in different cities across the United States and around the world. We all hold full-time jobs and/or commitments while working on The Biota Project. Thus, our initial planning and coordination across time zones presented itself as a challenge. Addressing this challenge, we created a system heavily based on the use of online communication. Most of our communication is through email, instant messaging, and social media, keeping each other accountable to our deadlines through default checks and balances. When external commitments arise for a team member, others work to compensate to maintain stability within The Biota Project. This system gives us the flexibility to balance our different schedules while collaborating on multiple projects worldwide. We coordinate group meetings as needed by using Doodle or When2Meet for building a consensus on meeting times and meet collectively via video-conferencing (such as Google Hangouts). Additionally, because our products are digital and are stored in a shareable folder via the 'Cloud' (Google Drive), all members can access and exchange files and documents as well as distribute and advertise products remotely. For these reasons, The Biota Project serves as an example for successful coordination among members of an organization cognizant of physical location and socioeconomic means. It is an important pillar in the mission of our group that we practice the inclusion we preach in our organizational methods. With so many different schedules and

lifestyles, recruiting and retaining members able to contribute beyond a year is another challenge. The Biota Project has ranged from as few as 3 to as many as 20 fully active members at a given time as these roles are voluntary and unpaid while members juggle external commitments. Our team addressed this hurdle by developing a sustainable model that welcomes short-term contributors while also establishing a core group of organizers. We outline internships for those interested in short-term projects while simultaneously setting up the process of becoming a legal entity, which requests long-term commitment from interested parties. The process of becoming a 501(c)(3) educational nonprofit is extensive and, once established, will recognize our team to be an official organization for developing paid positions with the hope of accruing increased funding for expanding inclusion. This status will assist us with another barrier we have encountered throughout the organization's growth, which is how to finance The Biota Project. Quality film content is critical to the strategy of The Biota Project, with early funds sought for production and editing. Our attempt to do this through formal, academic means was at first received with skepticism and unsuccessful in gaining support for our approach. As an alternative, we launched a Kickstarter crowd-sourced funding campaign in December 2015 (https://www.kickstarter.com/projects/ 1622253977/biota), surpassing our modest goal of $2000. We believe the campaign was successful because of (1) belief in our product from the Department of Energy Joint Genome Institute and associated animation support donated by their partners at Illumina Studios and (2) buy-in and advertising from those who know us and our work ethic on a personal level. Together, these sources of support helped us demonstrate that we were serious about the quality of our vision to our target audience. The original financial support allowed us to create a promotional video with high-quality animations about the Great Salt Lake as an overlooked ecosystem that can serve as an asset to Salt Lake City. We showed that there are microbial extremophiles that can be used as a potential biofuel to mitigate air pollution from oil refinement. This jumpstarted our initiative in a fundamental way and has since led to multiple grants, awards, and opportunities. We anticipate continuously learning and growing as young professionals through our involvement in this organization. The Biota Project, therefore, acts as a living body that develops over time, providing inspiration to both scientists and non-scientists by truly interacting with diverse audiences to address the needs of science communication and science overall.

4.1.9 Conclusions

The Biota Project is a young organization (conceptualized in 2013, launched in 2016) having made tremendous strides in a comparatively short amount of time. Through trial and error, the organization has polished its processes to direct current projects and develop ongoing endeavors. (1) By creating a "symbiotic relationship" within ourselves, different disciplines work together to achieve the goal of highlighting nature and societal relationships to nature. (2) Diverse expertise and backgrounds bring about innovative modes of communicating science and reach new audiences that have been historically excluded from conversations in the Western sciences. (3) Our interests in different artistic media and tools allow us to learn how to apply our scientific work in order to fulfill the mission of The Biota Project valued by ourselves and, most importantly, our audiences. (4) Finally, mutually egalitarian discussions between us and our audiences engage interest not only toward natural ecosystems but also towards strengthening

communities as an evolutionarily advantageous mechanism for long-term survival in theory and practice.

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4.2 Science Education - BiotaQ: A STEM outreach program for Merced County K 9-12 facilitated by UC Merced graduate and undergraduate students (Memorandum of Understanding)

Proponents: Kimber Moreland, Mario Banuelos, Noelle Anderson, Sabah Ul-Hasan

4.2.1 Summary

BiotaQ is a collaboration between graduate and undergraduate students at the University of California, Merced (UC Merced) whose mission is to provide outreach to our local community, specifically those that are currently underserved, to influence students in engineering and science studies. The program provides graduate students hands-on opportunities to build on their STEM (science, technology, engineering, and mathematics) expertise by developing related learning modules with the assistance of enthusiastic undergraduates. Both undergraduate and graduate students deliver this newly developed material to junior and senior public high school students in Merced County. With the oversight and approval of the graduate students' respective advisors, these dynamic modules are then taught to the students in an after-school setting. Following NGSS (Next Generation Science Standards), graduate and undergraduate students transform into tutors and mentors, and engage high schoolers in module activities.

Legislative order has enacted state law for the purpose of developing scientifically and technologically literate citizens (Education Code 60605.85, SB 300 Chapter 624). The BiotaQ project has been conceived at a critical time in the State of California's history, where Merced County's position on education is actively changing. Teaching STEM through BiotaQ is an active component of the community's continued effort to maintain and promote academic activity in Merced's youth population. Merced County schools have recently reported increased test scores in Math and English (Austin 2016). The program additionally strengthens bridges between students of the underrepresented groups at UC Merced and that of the Merced community.

The positive change in Merced County's education demonstrates the timeliness of BiotaQ's fruition with a strong, lasting impression on the heels of California's legislative edict. Therefore, BiotaQ proposes a continued implementation of 4, 120-minute after-school workshops (excluding transportation) once per week for 4 consecutive weeks every Spring. In chronological order, workshops will address the four a major pillars of science (biology, chemistry, mathematics, and physics) through interdisciplinary activities directly related to graduate student research. These workshops are bookended by an introductory field trip to the UC Merced campus and a panel showcasing the diversity of careers in science. Pre- and post-surveys data from participants are taken in order to make effective curriculum and to determine the project outcomes. The known total budget of this program for a group of ~40 junior and senior high school students is $41,160. This budget supports transportation and material costs, refreshments, care packages for visiting students, and the services of 7 to 10 UC Merced and Merced College graduate (3-4) and undergraduates (4-6) associated with each respective workshop. If there is

continued success, gauged by surveys distributed to the high school students at the end of each module, the BiotaQ program will expand in module topic, number, and high school students served, per each academic year and will be updated accordingly. BiotaQ is an initiative financially supported by the Merced Union High School District (MUHSD) and logistically supported by UC Merced.

Key contributions of BiotaQ:
- Merced County high school students gain exposure to STEM, research, and higher education
- UC Merced strengthens its mission of being an official Community Engagement campus
- UC Merced graduate students develop NGSS lectures directly related to their research
- UC Merced undergraduate students gain teaching experience and graduate student mentors
- UC Merced strengthens its partnerships with MUHSD and CSU Fresno
- MUHSD strengthens its access to current research in alignment with NGSS course material

4.2.2 Program Overview

During the Spring semester of each academic year, BiotaQ will provide junior and senior high school students, with priority given to Yosemite High School students, with modules on and off the UC Merced campus that grant them exposure to STEM career possibilities and related skill sets for the workforce. The first event is an on-site campus visit. The 2nd- 4th events are interactive 120-minute workshops led by 2-3 UC Merced graduate students and assisted by up to a total of 10 graduate and undergraduate students. The final event is a career panel discussion, open to the students and their families. After-school workshops will be held at a local Merced high schools and the final career panel will be held off campus at a public location to be determined. Some workshops will involve field trips as funding and resources permit, such as the UC Merced campus, Vernal Pools & Grasslands Reserve.

Intellectual Merit
The proposed outreach disseminates research and introductory technical skills to underserved students in Merced County high schools. It also provides both undergraduate and graduate students opportunities to communicate their research to a non-technical audience. Moreover, this program provides pedagogical, active-learning training for both undergraduate and graduate students who are considering a career with a teaching focus.

Module	Expected Attendees	Expected Assistants
Campus Visit	Ideal: 70, Min: 20, and Max: 80	Grads: 6, UGs: 8
Career Panel	Ideal: 150, Min: 50, Max: 250	Grads: 3, UGs: 6
Agriculture and Soils	Ideal: 40, Min: 20, Max: 70	Grads: 3, UGs: 6

Microbes and Microscopes	Ideal: 40, Min: 20, Max: 70	Grads: 3, UGs: 6
Population Dynamics and Bioinformatics	Ideal: 40, Min: 20, Max: 70	Grads: 3, UGs: 6

Table. 4.2.1 Personnel Requirements
These numbers are expected to double with increased school collaborations and module types. We also hope to include CANRA training to assist the graduate students going into teaching careers.

Date	# Students	Workshop activity
Week 1-March	70	UC Merced Campus Visit
Week 2-March	40	Agriculture and Soils (Yosemite High School)
Week 3-March	40	Microbes and Microscopes (Yosemite High School)
Week 4-March	40	Population Dynamics and Bioinformatics (Yosemite High School)
Week 5- April	40	Career Panel (Downtown location TBD)

Table. 4.2.2 Workshop Schedule
To increase student involvement and continue working to make BiotaQ a sustainable and lasting program, BiotaQ's 2019 event will be a 2-hour program with demos of the modules, a short module presenting potential careers in STEM, and will focus on promoting enthusiasm for STEM education events.

4.2.3 Expected Outcomes and Impact

Outcomes
1. To develop and compile STEM modules that fully incorporate the NGSS.
2. To teach science education to local, underserved public high school students.
3. To develop assessments of students and teachers and use outcome data to determine impact on local community and further improve program efficacy.

Impact

BiotaQ is a collaborative venture that is positioned to make a positive impact in an underserved community, not only in relation to economic conditions, but also in science and technology education (STEM). This project has deployed effective educational modules that meet such a need. As relevant tracks of these modules are completed over an iterative public school year basis, outcome developments will begin from the collected data received via completion surveys. The abstract goal of BiotaQ is to serve as proof-of-concept for a sustainable program in the space of youth teaching and learning in the Central Valley region and beyond.

Benefits

Overall, BiotaQ has proven to be a successful STEM outreach program that benefits high school students, UCM graduate and undergraduate students, faculty, and the Central Valley community. It teaches advanced leadership skills and engages with the local community in a way that is unique to this area.

High school students: access to science in a relatable and applicable way, to see people whom the students can relate to in STEM careers, interaction with college students, exposure to STEM concepts relevant to their everyday lives, learn something meaningful.

Undergraduate Students: leadership skills, engagement with community, connection with graduate students and UC Merced research, communication skills (social and interpersonal)

Graduate Students: leadership skills, engagement with community, improving mentoring skills, curriculum development, skill development for job market

Faculty: engagement with community, improving mentoring skills

UC Merced: engagement with community, recruitment opportunity, aligns with UCM mission (public service, undergraduate and graduate engagement, and cross-disciplinary)

Community: connection with the university, access to UC Merced's resources

Measure of Success

Anonymous surveys will be administered to teachers and students after the program and to students before and after the module to assess program success. Surveys for teachers will provide us with an idea of how we can best use our modules to complement the current curricula and to identify material which the teachers may want students to be exposed to but have not been able to implement. Specific pre-module surveys for students assist us in catering to the student's current knowledge, interests, and expectations for the program. Post-module surveys will allow us to identify how effective modules were and how they can be improved for future runs of the program. The results of all of these surveys will be used when developing future modules and expanding these modules to be done in other schools. Summarized results of these surveys will be publically available on our website.

Our survey results from 2016 indicate that the modules were a success in a multitude of ways. The highlights are listed below:

- ~90% of students reported that they had a more positive view of UC Merced after the campus tour.
- In all three modules, no student attendees were less interested in the material post-module.

- After the Agriculture and Soils module, 50% of students were more interested in the subject than before and 25% of attendees were more likely to pursue soil science careers.
- In the Microbes and Microscopes module, nearly 70% of students were more interested in the subject after the module and approximately 50% of attendees were more likely to pursue microbiology or physics post-module.
- In the Population Dynamics and Bioinformatics module, 40% of students were more interested in the subject after the module. Approximately 80% of attendees were either previously unfamiliar or intimidated by computer programming before the module, and approximately 25% of attendees were more likely to pursue mathematics or computer science post-module.

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4.3 Science Policy – Bringing Parks Back to the People: Revisiting the Dual Mandate and Core Values of the National Park Service

*Cassidy Jones, *Nate Shipley and *Sabah Ul-Hasan
The George Wright Forum (34.1) 2017

4.3.1 Introduction

The National Park Service (NPS) is tasked with protecting natural and cultural resources while simultaneously providing opportunities for public use and enjoyment. This dichotomous mission, known as the "dual mandate," defines NPS's unique and complex purpose. In 2016, NPS's centennial year, many national parks saw record-breaking visitation (Repanshek 2016; Tabish 2016). The impacts associated with increased visitation garnered extensive scrutiny and focused attention on the challenges of managing for both resource integrity and social engagement.

Leading up to the centennial, NPS prioritized making the national parks relevant to all Americans (National Park Service 2011; National Park Service Stewardship Institute 2015). Though national park visitation is greater than ever (Flowers 2016), many Americans still appear to be unconnected to the parks (Peterson 2014). Enhancing relevancy and engagement while mitigating the ways in which people impact park resources presents yet another pair of disjointed challenges for NPS.

As we examine the core values of NPS, we review the historical treatment of the dual mandate and attend to the marginalization of the "public enjoyment" aspect of the NPS mission.

We then explore ways for NPS to embrace leisure and recreation in order to foster stewardship among an increasingly diverse and urbanized American citizenry. To secure relevancy and reinforce conservation, we ultimately recommend that NPS re-calibrate its internal priorities to encourage use of parks and engender a long-term connection to nature.

4.3.2 Where have we been?

The dual mandate stems from the NPS Organic Act of 1916, which states that the agency shall manage national parks for resource conservation and public enjoyment. Tension between the two edicts of the dual mandate developed quickly, and in 1925 NPS's first director, Stephen Mather, reasoned it would be impossible for the public to enjoy parks without maintaining intact resources (Martin 2005). The Redwood Act of 1978 (amending the General Authorities Act of 1970) supported Mather's position by stating protection should take precedence over use by the people whenever the two are in conflict (Dilsaver 1994). Current NPS management policies reaffirm resource protection as NPS's predominant duty (National Park Service 2006). While stringent resource protection policies have guided vital national park conservation decisions, we maintain that NPS should establish equally high standards for providing opportunities for public enjoyment. Alternatively, by minimizing its charge to provide public enjoyment, NPS further distances itself from the American people and from its duty to cultivate citizen stewardship.

The astonishing scenery and unique story of this country are assets shared by all Americans, and NPS must engage with the public as responsible owners and stewards of their communally owned parks. Yet, in current dialogue people are referred to in sterile terms, such as "carrying capacity" or "number of visitors," and the public enjoyment function of the dual mandate has taken a back seat in research discourse and management practice. Figure 1 illustrates how researchers have focused more on issues related to protection of the national parks from the people than on designing experiences for the people.

An imbalanced approach to researching and managing national parks may have contributed to the challenges NPS now faces. Within its overarching agency goal of achieving relevance, NPS addresses multiple issues connected to the public enjoyment edict. Cultural disconnect among young people, poor representation of diverse populations (both as park visitors and in the NPS workforce), and increasing incidents of visitor transgression in parks all are complex problems of public enjoyment (Peterson 2014). With this in mind, we consider the commendable work NPS is doing to address such issues, and we urge NPS to take further action by adopting an internal priority shift toward public enjoyment.

Fig 4.3.1(Above) A search through the Web of Science database for publication titles containing the phrases "dual mandate" or "national park service" yielded 297 articles dating back as far as 1922. After removing all prepositions from the titles and variations of "dual mandate," "national park service," and "United States of America," the resulting Wordl figure demonstrates that commonly used terms within these 297 titles are "historic," "manage," "land," "policy," and "area." (Below) Searching these same 297 titles for "relevant" or "inclusion" or "visitor" or "connect" yielded only 12 articles, the first being published in 1979. The resulting Wordl figure suggests a research bias for management, policy, and resources of parks over the treatment of people, enjoyment, and experiences.

4.3.3 Where are we going?

New park interpretation practices exhibit NPS's desire to focus more on visitor enjoyment and engagement. For example, park interpreters are beginning to use facilitated dialogue techniques to create interpretive programs that involve the lived experiences and perspectives of visitors (Stephen T. Mather Training Center 2013). Outside of park settings, a growing number of new programs and strategic plans invite people to explore and connect with NPS. Initiatives include: The Urban Agenda, a plan to connect NPS to people living in cities (National Park Service Stewardship Institute 2015); OneNPS, a strategic objective to activate the synergy of parks and NPS programs in communities (National Park Service Stewardship Institute 2015); and Every Kid in Park, a program to give all fourth graders in America access to federal lands and waters (US Department of the Interior 2017). In addition to new programs, recently designated national

monuments, such as César E. Chávez and Charles Young Buffalo Soldiers, contribute to a more complete narrative of this nation's heritage.

Furthermore, in conjunction with the designation of Stonewall National Monument in 2016, NPS announced a National Park Service Heritage Initiative to identify and interpret LGBTQ sites and stories, indicating the agency's commitment to important, underrepresented American stories (National Park Service n.d.). These park practices, programs, designations, and research initiatives show how NPS is actively seeking ways to make its work relevant to a modern American citizenry.

Despite work currently being done, there is still a need to promote a people-focused culture on-site and within park operations, management, and administration. When people visit their national parks, it is crucial for them to be treated as stewards and conservationists rather than as threats to resources. Furthermore, people need to feel emotionally connected to parks in order to develop a sense of ownership and an ethic of stewardship.

4.3.4 How do we bridge the past with the future?

Leisure is a direct motivation for the public to visit this country's national treasures (Snepenger et al. 2006). People who visit national parks do not do so to be instructed; rather, they visit to experience and be moved by the grandeur of iconic places (Figure 2). Emotion is a critical and fundamental motivation of human behavior (Dolan 2002; Phelps and LeDoux 2005). Thus, if people are emotionally connected to parks and feel as if they belong, they are more likely to support the parks and treat them respectfully. By focusing on leisure and recreation as mechanisms that foster emotional connection, NPS can help visitors develop an ethic of care and a willingness to safeguard parks for future generations.
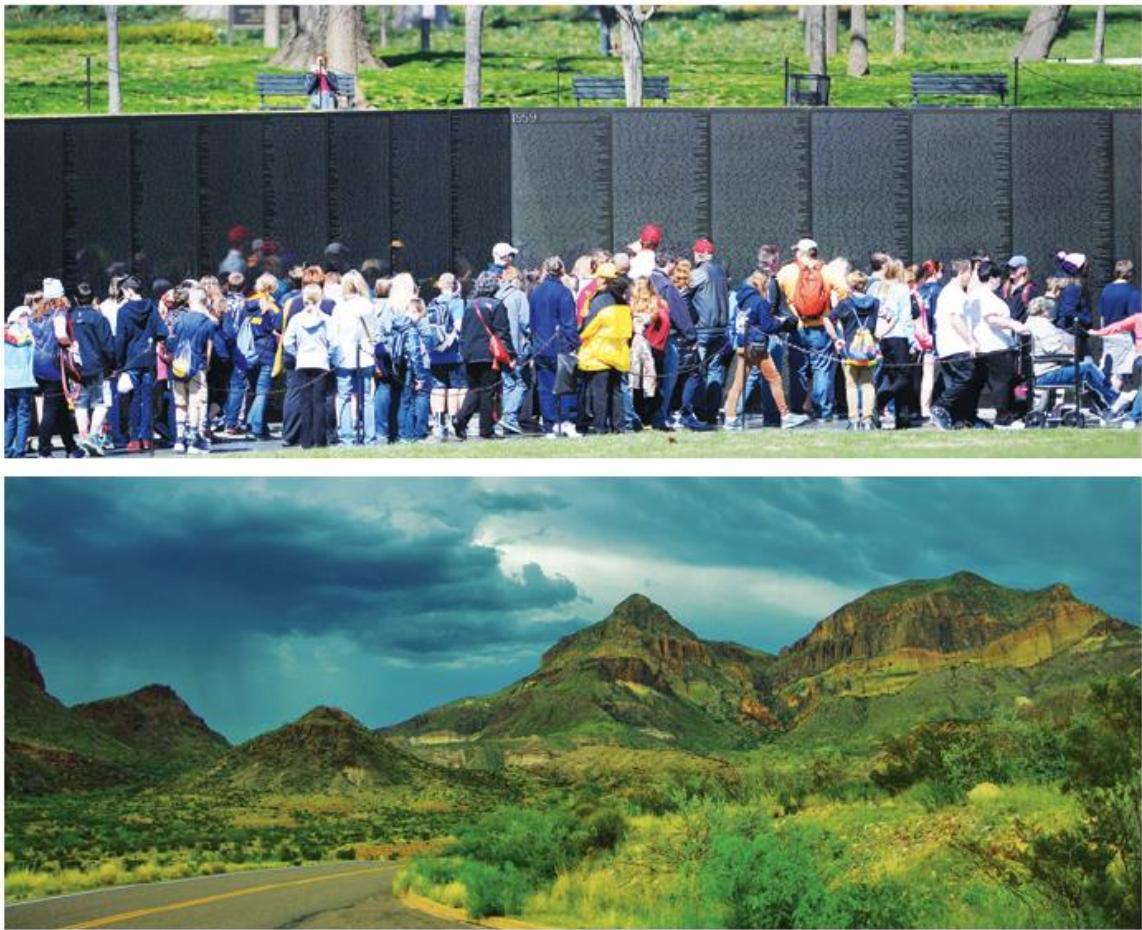
Fig 4.3.2 People don't come to national parks to learn lessons. They come to be emotionally moved by the experience of iconic places. (Above) Vietnam Veterans Memorial (photo courtesy of Marvin Lynchard/Department of Defense). (Below) Big Bend National Park (photo courtesy of Niagara66 via Wikimedia Commons).

While continued focus on providing leisure is one method for sustaining support for parks, further consideration should be given to the unique park characteristics that appeal to various visitor identities. One potential method for understanding how national parks appeal to people is examining the brand of NPS. Graves (2013) presents a relevant psychological rationale underlying consumer behavior: when people buy products, they may often do so largely based on the branding of the product as opposed to an overt rationalization of the purchase decision. Extensive marketing research has constructed an entire consumer psychology of brands (Schmitt 2012), providing vital concepts such as brand attachment and brands as identity signals. Applying psychological principles of branding, NPS can design a brand that people trust and value, much like they trust and value their favorite brand of car or computer. With this in mind, we are compelled to ask some difficult questions: Does the current brand of NPS reflect the duality of its mission? Does the NPS brand suggest positive emotional experiences for visitors, or does it instill a sense of restriction to the public?

If the NPS brand communicates how it sustains rather than restricts access to parks, the agency may appeal more broadly to people who are not already natural resource enthusiasts and avid outdoor recreationalists. NPS can better define and exemplify its brand by reconsidering the public image it portrays. For instance, NPS can emanate a sense of familiarity to visitors by presenting parks as special places and not just as protected areas. Similarly, a renewed focus on serving visitors may stimulate profound, lifelong connections to national parks that extend beyond one-time visits.

In order to manage a possible rebranding, NPS should consider restructuring its current ranks agency-wide. By involving more communicators, marketers, psychologists, sociologists, and other professionals from the social science disciplines, NPS would be better positioned to attend to both prongs of the dual mandate equally. By building a workforce that hosts specialists in human behavior and other social disciplines, NPS can better create a foundation that reflects both the resource and social aspects of stewarding the national parks. Lastly, NPS should cultivate stronger external relationships with state, regional, and local parks and nature centers (Figure 3). Research suggests that regularly occurring family leisure activities are better predictors of overall family cohesion than those that require greater investments in time, money, or effort (Zabriske and McCormick 2001). Similarly, environmental socialization research suggests the importance of recurring, expanding, and frequent interaction with nature in the developmental stages of many "natural-history-oriented young adults" (James, Bixler, and Vadala 2010).

Considered together, core family leisure and environmental socialization conceptually support the recommendation that NPS should consider strengthening relationships with local nature-based parks. While some natural resource professionals may reason a single visit to a national park provides a transformative experience, it is an unlikely outcome for most visitors. It is more likely that visitors develop lifelong interests in nature through repeated emotional experiences with nearby nature. By supporting public engagement with nearby parks and natural spaces, NPS can develop visitor interest in local natural and cultural heritage, which may evolve into a broader interest in protecting and enjoying national parks.

Fig 4.3.3 NPS should cultivate stronger external relationships with state, regional, and local parks and nature centers. Research suggests that regularly occurring family leisure activities are better predictors of overall family cohesion than those that require greater investments in time, money or effort. Among the groups Santa Monica Mountains National Recreation Area partners with are the City of Malibu Parks & Recreation Department, The Children's Nature Insititute, California State Parks, and Los Angeles County Recreation & Parks Department. Photo courtesy of the National Park Service.

4.3.5 Summary

The dual mandate enunciated in the National Park Service (NPS) Organic Act has guided administration and management of America's national parks since 1916, shaping an enduring and inspiring legacy. But as modern society evolves and new generations mature, NPS must direct increasing energy and attention to maintaining its cultural relevancy. While acknowledging the importance of preserving resource integrity, NPS would benefit immensely from making a commitment to care for its visitors in the same manner in which it cares for the resources under its purview.

NPS can strengthen its relationship with the American people by talking with visitors as opposed to talking to and about them (Figure 1a); after all, people come to the national parks to seek emotional and fulfilling leisure experiences, not to be lectured and managed (Snepenger et al. 2006). NPS can expand its workforce to include people with educational backgrounds in social disciplines to balance staff who specialize in science and conservation, a restructuring that honors the dual mandate. NPS can allocate resources to constructing new affiliations with state, regional, and local parks, nature centers, and cultural heritage sites to encourage more frequent and recurring experiences in parks and nature beyond the occasional visit to a national park.

To many Americans, NPS is the green and gray uniform, the arrowhead, the American bison, the giant sequoia, and purple mountains' majesty. However, if a modern public recognizes national parks as crucial bastions of the nation's cultural and natural history, NPS is more likely to endure as a relevant cultural concept for all Americans. By seeking ways to become not just relevant but indispensable, NPS encourages the American public to become invested in national parks. Though conservation work is both prudent and necessary, by providing opportunities for quality public enjoyment, NPS fosters key stakeholder support that will protect the national parks in perpetuity.

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4.4 Proof of Concept – Exploring the venom ecosystem: The emerging field of venom-microbiomics and the Initiative for Venom Associated Microbes and Parasites (iVAMP)

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4.4.1 Abstract

Venom is a known source of novel antimicrobial natural products. The substantial, increasing number of these discoveries have unintentionally culminated in the misconception that venom and venom-producing glands are most likely sterile environments. Culture-dependent and -independent studies on the microbial communities in venom and venom-producing glands reveal the presence of archaea, algae, bacteria, endoparasites, fungi, protozoa, and/or viruses in these environments. Venom-centric microbiome studies are comparatively sparse to date, and the adaptive advantages that venom-associated microbes might offer to their hosts or that hosts might provide to venom-associated microbes remain unknown. In this article, we highlight the potential for the discovery of venom-microbiomes in the context of venom as an adaptive landscape. The sheer number of known venomous animals and the convergent evolution of the

venom gland, juxtaposed with the few studies that have identified microbial communities in venom, provides new possibilities for both biodiversity and therapeutic discoveries. We present an evidence-based argument for integrating microbiology as part of venomics and express a need for a meta-analysis of the literature to illustrate the overlap between these fields. We introduce iVAMP, the Initiative for Venom Associated Microbes and Parasites (http://sabahzero.github.io/ivamp/) as a growing consortium for interested parties to contribute and collaborate within this subdiscipline.
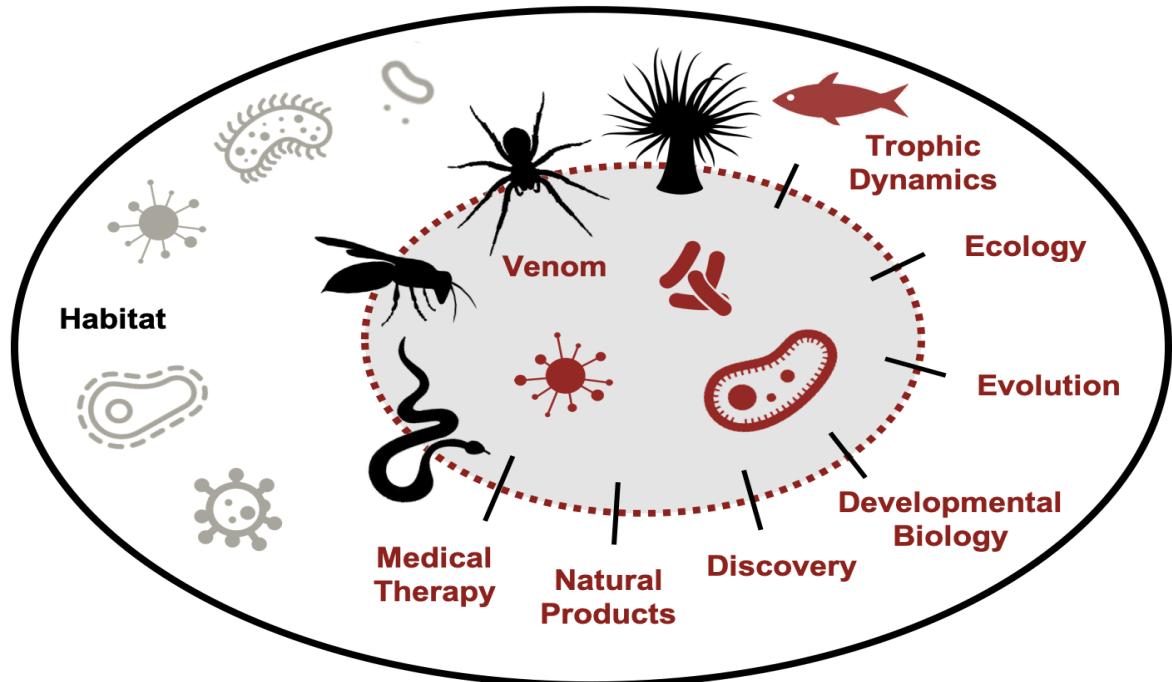

Fig 4.4.1 Graphical Abstract

4.4.2 Highlights

- Venom-microbiome studies as an integrative field of venomics and microbiology
- Introduction of a venom-microbiome research consortium iVAMP
- Argument for multi-omics-based discovery through an ecosystem framework

4.4.3 Text

While scientific interests in toxins and microbiology have persisted for centuries, less than 150 studies overlap between these two fields despite each significant advancing via next generation sequencing technology (Figure 1, Supplemental Table 1, Supplemental Code). The integration of genomics (Moran and Gurevitz 2006), transcriptomics (Pahari et al. 2007), and proteomics (Fry 2005) into venomics has contributed to new toxin discovery and associated biological activity (Oldrati et al., 2016; Calvete, 2017). Over the past 15 years, microbiome research has yielded

breakthroughs in our knowledge of unculturable microbial "dark matter" (Bernard et al. 2018), the origins of life (Spang et al. 2017), and human health (Clavel et al. 2016; Arnold et al. 2016). Providing ecological and evolutionary context has enhanced both microbiology (Boughner and Singh 2016; Hird 2017) and venomics (Prashanth et al., 2016; Sunagar et al., 2016; Calvete, 2017). We thus propose viewing the venom as an ecosystem key for investigating the dynamics of venom-microbe interactions, acting as a unique niche in which microbes may adapt.

(a) **(b)** **(c)**
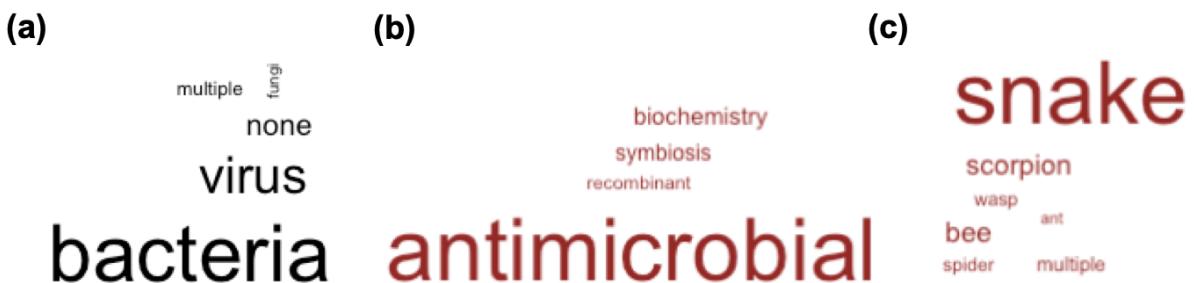
Fig 4.4.2. Word clouds representative of Supplemental Table 1 content.
A breakdown of 127 resultant articles from searching Web of Science for venom-microbe studies. (a) Most articles are either bacteria- or virus- specific, and a subset of 17 articles are not related to studies involving microbes. After removing these articles, investigation of the remaining 110 show (b) approximately 70% focus on venom toxins exhibiting antimicrobial properties and only about 10% looking at venom-microbe interactions. (c) Roughly 53% of studies focus on snake venom, the remaining from invertebrates.

Researchers in the fields of both venomics and microbiology share common interests in natural products (Katz and Baltz 2016; Robinson et al. 2017) and adaptive evolution (Phuong et al., 2016; Hird, 2017). With more information on the presence and diversity of venom-associated microbiomes (Table 1), future research efforts can focus on how microbes colonize and maintain themselves in venom glands as a starting point for integrating these fields (McFall-Ngai 2014; Nunes-Alves 2015). A complementary component of the venom ecosystem perspective could include the underlying biology of the host, where findings gained from microscopy (Schlafer and Meyer 2017) and biomechanics (Yevick and Martin 2018) can be translated to predictive models (Biggs et al. 2015) for identifying the underlying mechanisms of toxin and metabolite function (Sapp, 2016; Adnani et al., 2017). Determining which venom microenvironments are truly sterile, if any, will prove critical in our understanding of venom evolution (Conlin et al. 2014) and antimicrobial resistance (Adnani et al. 2017). Additionally, identifying microbial species that have adapted to these seemingly extreme environments (Rampelotto 2013) will open new avenues of research, and emphasizes the need for phylogenetically representative venom host species as emerging model systems to be bred axenically *in vivo* and allow researchers to test the functional roles of venom-associated microbes observed in the wild.

What naturally occurs in the venom microenvironment remains largely unknown; addressing this through directed microbiome sequencing experiments within a wildtype ecosystem framework strengthens our findings of animal associated microbes through multiple branches (McFall-Ngai et al. 2013). A variety of microbial studies have found that specific toxins are produced by certain bacteria (Hwang et al., 1989; Cheng et al., 1995; Pratheepa and

Vasconcelos, 2013; Stokes et al., 2014) and viruses namely those with RNA genomes show preference for residing in venom (Sanjuán et al., 2010; Debat, 2017) and can be abundant in venom glands (Sanjuán et al., 2010; Domingo et al., 2012; Debat 2017). These studies contrast the mainstream view of the venom microenvironment as sterile (Supplemental Table 1). However, (1) Compounds derived from or contained within venom demonstrating antimicrobial activity against clinical and/or reference strains (Almeida et al. 2018) may not reflect was occurs against wild-type strains that co-evolved within venom glands (Reis et al. 2018) and (2) cultured microbes (McCoy and Clapper, 1979; Peraud et al., 2009; Catalán et al., 2010;Quezada et al., 2017b, 2017a, 2017b; Silvestre et al., 2005; Yu et al., 2011) can produce compounds in a lab setting they may not produce in the wild (Simmons et al. 2008). Furthermore, most microbiome studies rely on captive individuals (Colston and Jackson 2016) and the call for microbiome studies to utilize wild-collected samples (Colston & Jackson, 2016; Hird, 2017) contributes to evidence suggesting that the captive environment may influence microbial composition of the oral/venom microbiome (Hyde et al. 2016), with implications that captivity already affects the host venom profile (Willemse et al., 1979; Freitas-de-Sousa et al., 2015). Studying the venom microbiome and considering the adaptive traits of microbes under selection in an ecological context as it occurs in the wild over in captivity clarifies the evolutionary pressures for these anti-microbial compounds found in venom (Figure 2). Complementing *in vitro*, *in vivo*, and natural venom microbiome experiments through culture -dependent and -independent techniques holistically contribute to our understanding of mutual symbioses, with room for predictive modeling to identify novel niches for microbial adaptation and competition (Bull et al. 2010).
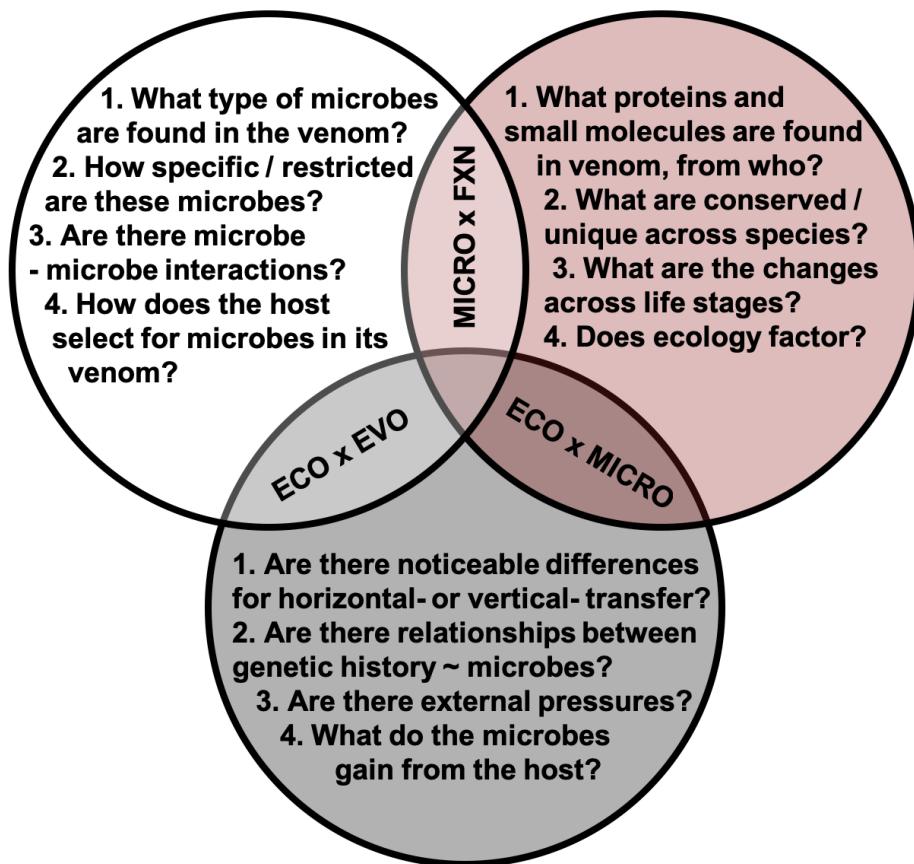
Fig 4.4.3. Proposed questions for venom microbiome exploration.
A venn diagram displaying the intersects of microbiology and venomics through an ecosystem focus, and some questions worthy of investigation therein.

A brief search shows that for the past 5 years, approximately one hundred papers per year have consistently been published on venom antimicrobial peptides (PubMed search term - antimicrobial AND peptide AND venom 14th Mar 2019). The few venom-microbiome studies to date (Table 1) demonstrate advantages of venom-microbiome research as a subdiscipline in what has become an international, collaborative cohort of researchers referred to as the Initiative for Venom Associated Microbes and Parasites (or iVAMP, http://sabahzero.github.io/ivamp/). In addition to interests in investigating the questions listed above, the iVAMP consortium serves as a tangible mode for pushing venomics further to the surface of current conversations in the scientific community with special interests in elevating historically overlooked voices (Cheng et al. 2018). This inclusive subdiscipline supports working with and for communities from which we sample rather than taking from them by emphasizing diverse representation and practice. For example, the Nagoya Protocol on Access and Benefit (Buck and Hamilton 2011) dictates that all governments must reach mutual agreement before genetic resources are accessed for product research. Involving scientists across the globe through initiatives such as iVAMP extends beyond the requirements of legislation, such as that of the Nagoya Protocol, to ensure science is accessible and contributed to by everyone. The approach of this initiative conversely expands the potential for scientific discovery.

STUDY	ORGANISM	TISSUE	WILD / CAPTIVE	APPROACH
WEBB B.A., SUMMERS M.D. 1990	Wasp	Venom gland	Captive	Culture, Sanger Sequencing
PERAUD ET AL 2009	Cone-snail (3 species)	Body, Hepatopancreas, Venom Duct	Wild	Culture, FISH, Sanger Sequencing
GOLDSTEIN ET AL 2013	Monitor Lizard	Saliva, Gingiva	Captive	Culture, Sanger Sequencing, 16S
DEBAT H.J. 2017	Spiders	Transcriptomes	Wild	Data-mining (NGS)
TORRES ET AL 2017	Cone-snail (8 species)	Venom Duct, Muscle, External Duct	Wild	16S, 454
ESMAEILI-SHIRAZIFARD ET AL 2018	Snakes (5 species) Spiders (2 species)	Venom, Oral Cavity	Wild, Captive	Culture, 16S, WGS
IVAMP IN PROGRESS	Snakes (multiple)	Venom, Venom Glands, Venom Ducts,	Wild, Captive	16S, RNAseq transcriptomics

		Oral Cavity, Muscle, Stomach and GIT		, Proteomics
IVAMP IN PROGRESS	Snake: *Crotalus scutulatus*	Venom, Venom Glands, Venom Ducts, Oral Cavity, Muscle, Stomach and GIT	Wild, Captive	16S, RNAseq transcriptomics , Proteomics
IVAMP IN PROGRESS	Spiders: *Stegodyphus*	venom glands, venom	Wild, Captive	16S, RNAseq transcriptomics , Proteomics
IVAMP IN PROGRESS	Cone-snail: *Californiconus californicus*	Venom, Venom Duct, Hepatopancreas, Shell, Egg	Wild, Captive	16S and 18S, Proteomics, Metabolomics

Table 4.4.1. Explicit Sequencing and Next-Generation venom microbiome studies
Next-Generation venom microbiome studies are comparatively recent, and few in number. Even so, the diversity of these studies by host and microbial community examined spotlight the potential benefits of integrating microbiology and venomics (Webb and Summers, 1990; Peraud et al., 2009; Goldstein et al., 2013; Debat, 2017; Torres et al., 2017; Esmaeilishirazifard et al., 2018).

iVAMP exists as a consortium able to acknowledge the politicization of science for the benefit of science accessibility, funding, reproducibility, and longevity. Science represents a significant value to society and, as such, it cannot be separated from the political realm (Choi et al. 2005). Scientists are often viewed as objective arbiters of facts who struggle to communicate to researchers in other scientific disciplines, the public, and to the stakeholders with whom the research impacts most significantly (Weber and Schell Word 2001). In an age of rapid communication and inexhaustible access to information, the incompetence of communication and transparency in science cannot be tolerated as public participation in science is vital (Dietz 2013). iVAMP supports effective communication in science by existing as a publicly available, interdisciplinary research organization and database. In doing so, we aim to have these open-source practices prevent counterproductive competition and instead lean towards interdisciplinary, collaborative scientific research. Bolstering public trust by creating research that is reproducible, integrative, and open-access will lay the groundwork for science persisting as useful well into the future. Likewise, expansion of data available in the description of microbes living in the many diverse venom host ecosystems contributes to currently absent aspects of holobiont and coevolutionary theory (Faure Denis et al. 2018).

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