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Cyanobacteria Ecotypes in the North Pacific

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Thesis Advisor

Anne Thompson, PhD

Portland State University

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Abstract

Phytoplankton are an essential part of nutrient cycling in the marine environment. Of particular interest

are *Synechococcus* and *Prochlorococcus*, two closely-related groups of cyanobacteria that are among the

most abundant photosynthetic cells on the planet. However, the environmental factors that drive

evolution of these bacteria into distinct ecotypes remains poorly understood. Here, we examine

cyanobacterial diversity along an understudied transect of the North Pacific Ocean. Nine surface-

seawater samples were analyzed using PCR of the *Prochlorococcus* ITS region and high-throughput DNA

sequencing. We observed an abundance of HL-II *Prochlorococcus* in subtropical regions, an abundance

of HL-I *Prochlorococcus* in temperate regions, and an abundance of *Synechococcus* in cooler, coastal

regions. When superimposed beside the temperature gradient observed along the transect, a clear

pattern emerges that suggests an important relationship between sea-surface temperature and the

Prochlorococcus ecotype community structure in the North Pacific Ocean.

Introduction

Prochlorococcus accounts for an estimated 5% of global photosynthetic activity. Due to their abundance and widespread distribution, small ecological changes in populations of *Prochlorococcus* could have large-scale implications for the global energy web. In order to better understand the nature of these changes, many studies have sought to examine *Prochlorococcus* diversity. In particular, much work has been devoted to the way *Prochlorococcus* lineages have partitioned into specialized high and low light "ecotypes" in the oligotrophic ocean (Bouman et al. 2006, Campbell et al. 1994, Farrant et al. 2016, Huang et al. 2012, Larkin et al. 2016, Malmstrom et al. 2010, Zwirglmaier et al. 2008). This has been accomplished through circumnavigational surface sampling (Bouman et al. 2006), repeated sampling at one location over time (Campbell et al. 1994), analyzing the bacteria's *petB* gene (Farrant et al. 2016), sampling across depth gradients (Huang et al. 2012), comparing the abundance of *Pro*chlorococcus in environments with different seasonal fluctuation patterns (Malmstrom et al. 2010), and comparing ecotypes in tropical and temperate zones (Zwirglmaier et al. 2008). Together, these studies have shown that incredible diversity exists in *Prochlorococcus*, even at the individual cell level, that ecologically distinct ecotypes coexist, and that environmental factors driving community structure and diversity remain poorly understood.

The North Pacific Ocean has an abundance of cyanobacteria. It also has diverse oceanographic conditions, nutrient availability, and other environmental factors that could influence cyanobacteria diversification. This makes it an ideal environment for studying cyanobacteria ecotype patterns with respect to oceanographic gradients.

Here, we examine how cyanobacterial diversity changes from the open ocean to a coastal environment in the North Pacific. We examined *Prochlorococcus* and *Synechococcus* ecotypes by comparing diversity found at the 16S/23S ITS level. Our work offers interesting insights into and

temperature-based shifts of *Prochlorococcus* ecotypes in the North Pacific, leading to an improved

understanding of diversity in this abundant bacterium.

Materials and Methods

Oceanographic Sampling

Filtered seawater from nine North Pacific Ocean sites was collected in March-April of 2017 aboard the

R/V Sikuliaq Cruise #SKQ201703S (Figure 1, Table 1). The latitude sampled ranges from 26.1 degrees

North to 42.3 degrees North, beginning at the warm and nutrient-poor open ocean near Station ALOHA

and ending at the relatively cold and nutrient-rich coastal region near Newport, OR. The samples were

collected from the ship's uncontaminated flow-through seawater system, while temperature and salinity

information were simultaneously collected by the Sea-Bird Scientific SBE 45 microTSG. Satellite data

were acquired from the MUR SST database maintained by the California Institute of Technology.

Table 1. Locations of surface-seawater collection sites

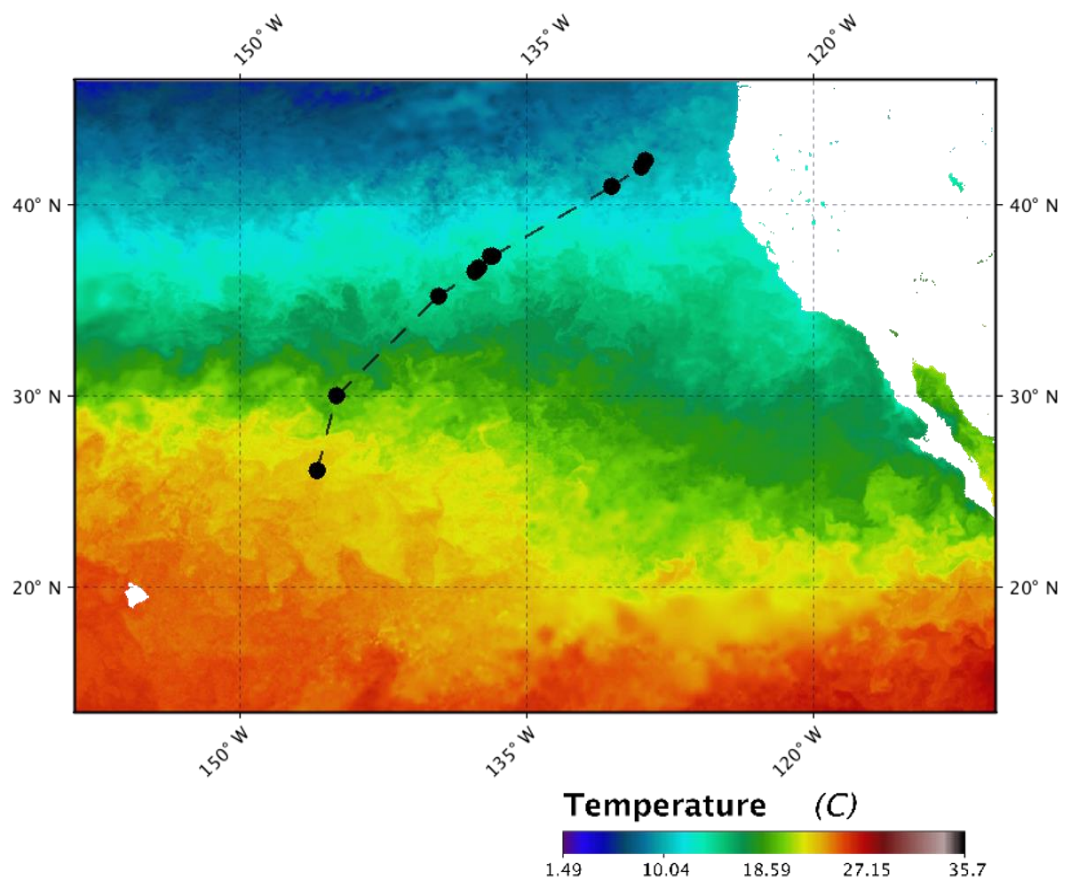| Sample	Date Collected	Sampling Location
A	3/9/2017	26.0749 N, -146.0189 W
B	3/17/2017	30.2650 N, -145.6328 W
C	3/30/2017	35.2482 N, -139.6439 W
D	3/31/2017	36.7022 N, -137.5114 W
E	3/31/2017	37.2987 N, -136.9502 W
F	4/2/2017	41.2684 N, -130.5722 W
G	4/2/2017	41.2953 N, -130.5758 W
H	4/2/2017	41.9689 N, -129.0283 W
I	4/2/2017	42.3078 N, -128.8542 W

Figure 1. Sample Stations for DNA extractions depicted in context of sea surface temperatures detected by satellite in March 2017.

DNA Extraction

200 mL samples of surface seawater concentrated on Sterivex filters were stored at stored at -80° C until processing. DNA was extracted using a previously described phenol:chloroform procedure (Wright et al.2009), and quantified via QuBit high sensitivity fluorometer for dsDNA (Qiagen). Extracted DNA was stored at -20° C.

PCR of Intergenic Transcribed Spacer Region

To amplify cyanobacterial DNA, PCR was performed on the ITS region of the samples ranging in concentration from 2µM – 20 µM and using Illumina-adapted and barcoded primers described by Nathan Ahlgren (N. Ahlgren, personal communication, May 2018) (Table 2). The PCR was performed using the following program: initial denaturation at 94°C for 2 minutes, followed by 30 cycles of 94° C for 20 seconds, 55° C for 20 seconds, 65° C for 1 min, and a final extension at 65° C for two minutes. Bands of approximately 550 bp in length were excised from agarose gel, and purified using the Invitogen PureLink Quick Gel Extraction kit. Amplicons with the barcodes were pooled before sequencing.

Table 2. Cyanobacterial-specific 16S/23S ITS Primers used in this study (N. Ahlgren, personal communication, May 2018).

Primer Type	Sequence (5' to 3')
Forward	CGTACTACAATGCTACGG
Reverse	GGACCTCACCCTTATCAGGG

Sequencing and Bioinformatic Processing

The samples were sent for Illumina MiSeq high throughput DNA sequencing at the OSU Center for Genome Research and Biocomputing, using 2x250 bp reads. Samples were demultiplexed by barcodes on reverse reads at the sequencing facility. The QIIME pipeline was used to quality filter the raw

sequence data (Q > 30), demultiplex the reads with the forward barcode, and call operational taxonomic units (OTUs) (for description of QIIME scripts, see supplementary information). A representative sequence for each OTU was selected and identified using BLAST (Altschul et al. 1997).

Results

Diversity and Distribution

Site 'A' exhibited the greatest diversity of the samples studied, with 'minority-type' OTUs (OTUs besides the three most abundant OTUs, see Figure 3) contributing to approximately 46.0% of OTU representation. In contrast, sites C, D, and E exhibited the least diversity, with minority OTUs providing only 8.0-9.3% of overall OTU coverage.

The top 3 OTUs identities (Figure 3) were determined to most closely resemble the organisms *Synechococcus* sp. CC9902 (BLAST accession number CP000097.1), *Prochlorococcus marinus* subsp. pastoris str. CCMP1986 (BLAST accession number BX548174.1), and *Prochlorococcus* sp. RS04 (BLAST accession number CP018346.1). *Prochlorococcus marinus* subsp. pastoris str. CCMP1986 is a HL-I ecotype of *Prochlorococcus*, and *Prochlorococcus* sp. RS04 is a HL-II ecotype of *Prochlorococcus*.
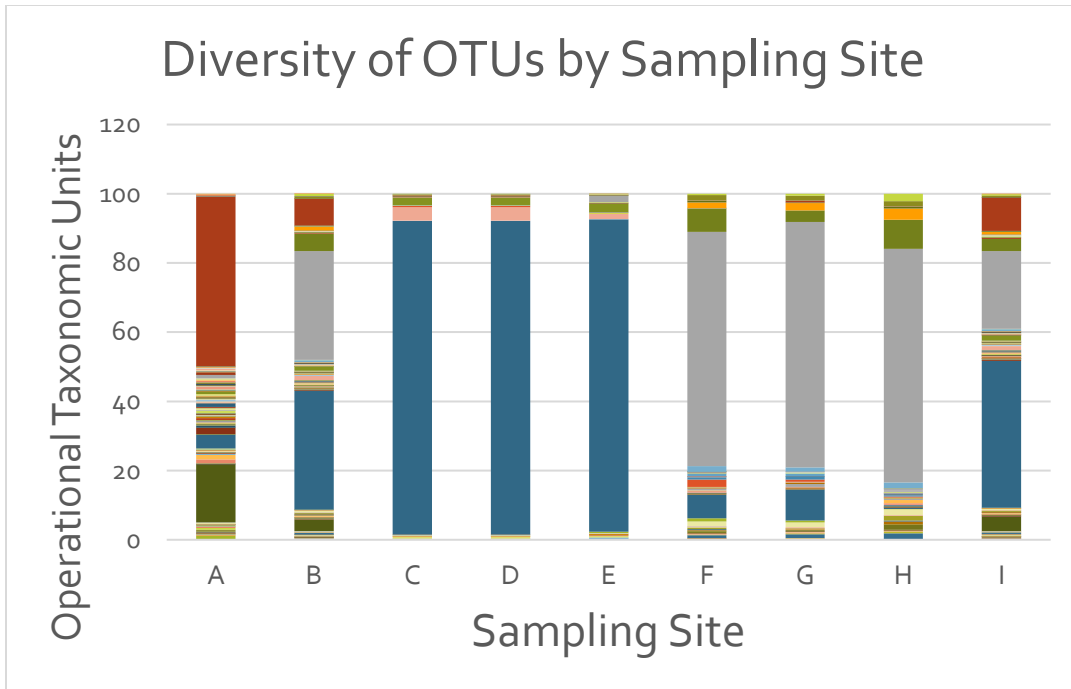
Figure 2. Site 'A' exhibits the greatest diversity of all the samples studied. Sites 'C', 'D', and 'E' exhibit the least diversity.
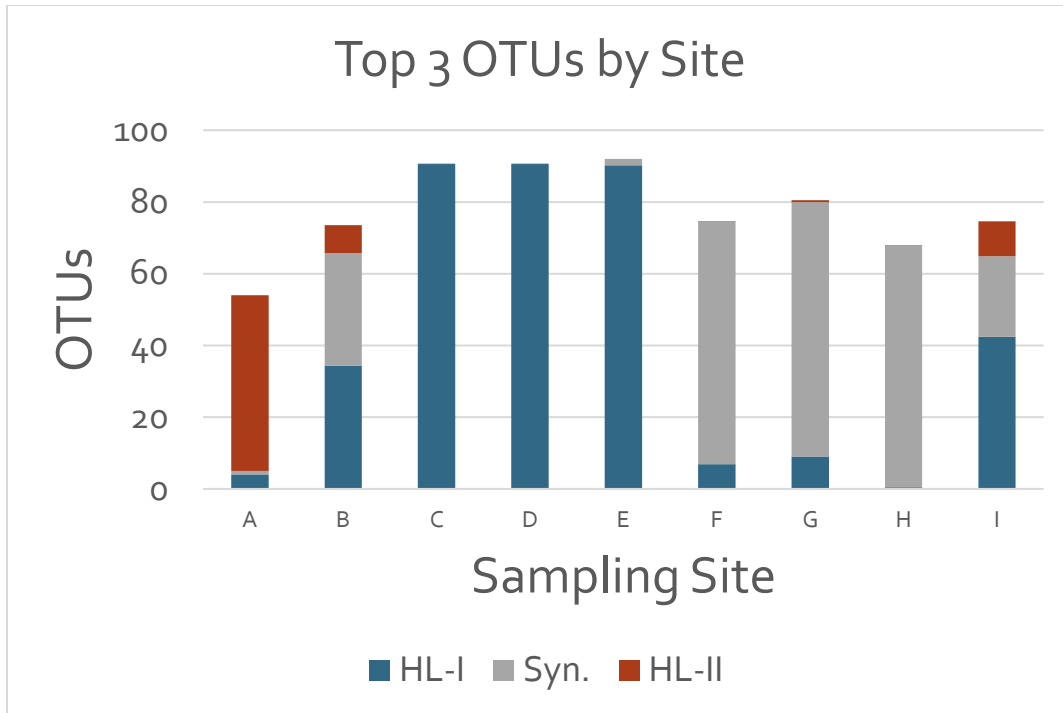
Figure 3. The top 3 most abundant OTUs were plotted with respect to distribution across the transect. The transect begins with an abundance of *Prochlorococcus marinus* subsp. pastoris str. CCMP1986 (HL-II) at site 'A', followed by a more diverse distribution at site 'B', an abundance of *Prochlorococcus* sp. RS04 (HL-I) at sites 'C'-'E', an abundance of *Synechococcus* sp. CC9902 (Syn.) at sites 'F' - 'H', and a final diverse distribution at site 'I'.
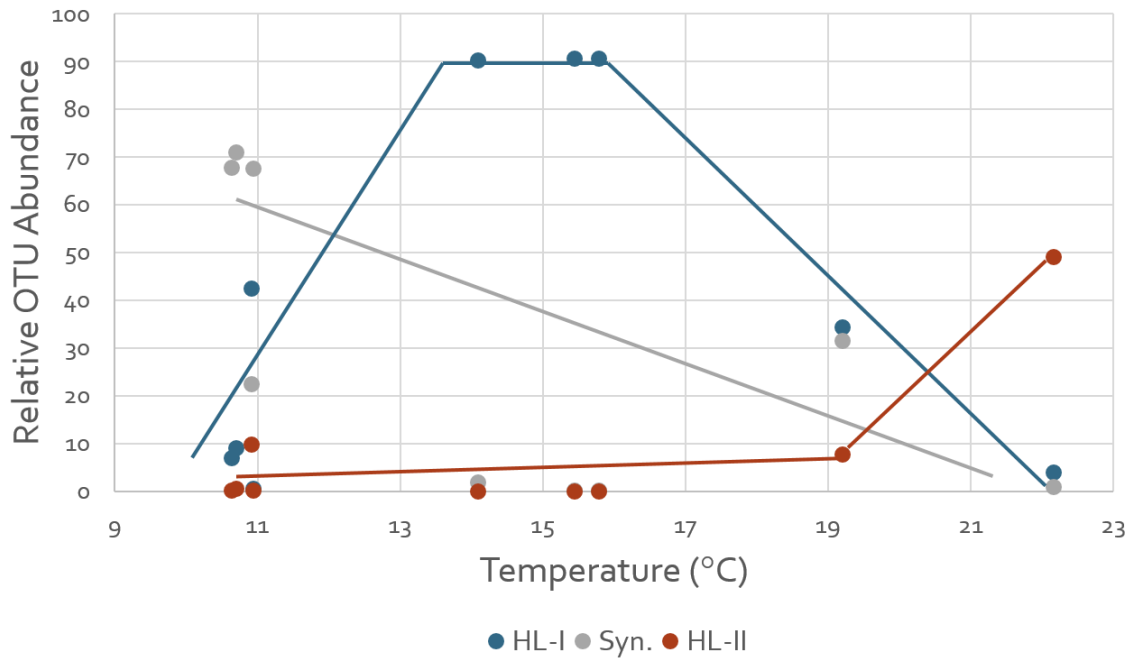
Figure 3. Three trends become apparent when inspecting OTU abundance with respect to temperature. 1) HL-II ecotypes along the transect are more abundant at higher temperatures. 2) Synechococcus abundance becomes more abundant at cooler temperatures along the transect. 3) HL-I ecotypes occupy an intermediate temperature range compared to the other two. (Trendlines drawn by eye.)
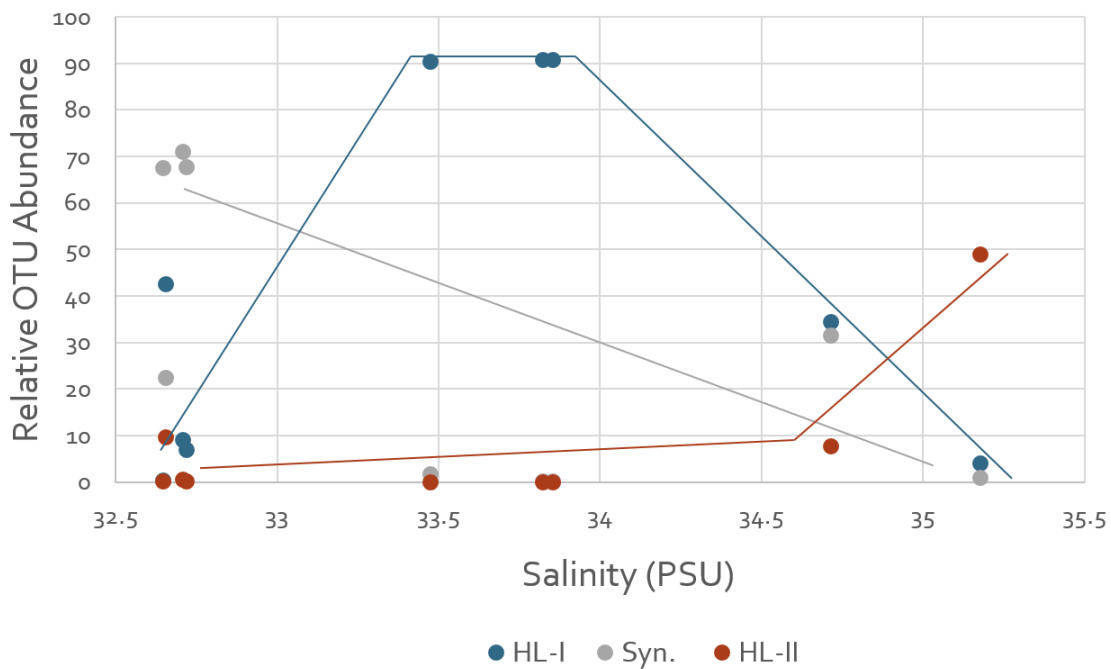
Figure 4. The trends for salinity closely mirror the trends for temperature (see Figure 4). (Trendlines drawn by eye)

Discussion

We explored the diversity of cyanobacteria types present along a transect of the North Pacific. Distinct patterns emerged with respect to temperature. For example, a majority abundance of *Prochlorococcus* was dramatically replaced with a majority abundance of *Synechococcus* as the sampling approached the Oregon coast. This transition occurred at the point in the transect where the surface temperature ranged from around 11-13° C (Figure 3). This is consistent with previous reports of Prochlorococcus thriving in oligotrophic conditions and declining in more nutrient-rich waters (Bouman et al. 2006). Most *Prochlorococcus* cyanobacteria are not known to use nitrate (Bouman et al. 2006), but most *Synechococcus* cyanobacteria are, suggesting that the availability of organic nitrate to organisms that graze on cyanobacterial might be increased in the colder, more nutrient-rich waters where *Synechococcus* dominate.

Zwirglmaier et al. found that HL-II ecotypes tend to be more abundant at subtropical and tropical zones, while HL-I ecotypes tend to be more abundant at temperate zones. This trend was also described in Johnson et al. 2006, where eMED4 (a HL-I ecotype) abundance was found to dominate cooler, high latitude waters. Similarly, our data showed an abundance of HL-II ecotypes present at 'Site A', corresponding to a subtropical latitude of 26.1° N (Table 1), and a clear abundance of HL-I ecotypes emerging at 'Site C', corresponding to a more temperate latitude of 35.2° N. The ecological impacts of an abundance of HL-I or HL-II ecotypes is being examined presently with respect to differences in carbon fixation and nutrient cycling. For example, it has been demonstrated that there are novel HL-I strain-specific genes that confer a stress response to phosphate starvation (Martiny et al. 2006), and other high-light ecotypes with increased iron-scavenging abilities (Malmstrom et. al 2013), already suggesting that different high-light ecotypes interact with trace nutrients in the ecosystem differently.

Our study is limited by an unequal distribution of reads across the transect. For example, we were able to get a tenfold increase in the number of reads at 'Site A' compared to the number of reads we were able to get at 'Site E'. Although all data presented was normalized with respect to the number of reads per site, the diversity trends at the sites with the least coverage (D E, J, and H) may not be as representative of the area sampled as the remaining sites that received a higher degree of coverage.

Because there were distinct trends with respect to the ecotypes and the gradients examined, temperature and salinity may help explain the ecotypical changes observed in the North Pacific. Future study of this transect should also examine the relationship of day length, silicate, oxygen, phosphate, and chlorophyll conditions with observed ecotypes, as these were demonstrated to be significant drivers of cyanobacterial community composition change (Larkin et al. 2016). It should also be noted that salinity was not found to be a significant driver of ecotype shifts in the Larkin et al. study, and was "merely correlated with large-scale oceanographic trends" (Larkin et al. 2016). As such, it is possible that our observed trends with respect to salinity are simply correlated to, and not a causative agent of cyanobacteria ecotype change. Additional future work might consist of conducting a phylogenetic analysis of the OTUs observed along the transect and comparing the resulting cladogram to similar analysis conducted in other studies, as in Larkin et al. 2016.

Overall, the data presented in our study suggest that temperature is an important ecological driver of cyanobacteria OTU diversification. We observed an abundance of HL-II *Prochlorococcus* in subtropical regions, an abundance of HL-I *Prochlorococcus* in temperate regions, and an abundance of *Synechococcus* in cooler, coastal regions. Other oceanic conditions might be explored along this transect to gain a better understand of the factors that drive the evolutionary partitioning of this globally important and very abundant organism.

Acknowledgements

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Supplementary Information

QIIME Scripts Work-Flow for Bioinformatic Analysis

#validate mapping files
validate_mapping_file.py -o vmf-map/ -m miseq122017_FWD_barcodes_R01.txt

Trim the forward reads to remove the variable '4 N' nucleotides.

get barcodes from the forward reads (R1) of all samples (that were already separated by RVS barcodes at sequencing facility)
extract_barcodes.py -f /path.fastq -o path -c barcode_single_end -l 5

split raw R2s based on fwd barcodes and quality filter (default errors in barcode)
split_libraries_fastq.py -o path -i path.fastq -b path/barcodes.fastq -m miSeq122017_FWD_barcodes_R01.txt
--barcode_type 5 --rev_comp_mapping_barcodes --rev_comp_barcode -q 30 -n 0

take fasta files from split library and check for "barcode on reverse reads", write only sequences that contain perfect match to RVS primer
files names will now have Unique Sample ID from split_libraries_fastq preceded by "RVSYes" from split_libraries...
split_libraries.py -m Rprimer_map.txt -f /path/seqs.fna -o /path/ --barcode_type 20 -e 0 --disable_primers

Remove "RVSYes" from fasta sequence names.

Concatenate all sequences and rename with .fna.

OTU picking
#pick OTUs denovo, best for when no reference database then summarize output
pick_de_novo_otus.py
biom convert
biom summarize-table

filter OTU table to remove all OTUs that do not contain at least 100 sequences then convert from biom format to summary and text file.
filter_otus_from_otu_table.py
biom convert
biom summarize-table