Title

Alterations of the human skin microbiome after ocean water exposure

Authors

Nielsen, Marisa Chattman

Jiang, Sunny C

Publication Date

2019-08-01

DOI

10.1016/j.marpolbul.2019.06.047

HHS Public Access

Author manuscript

Mar Pollut Bull. Author manuscript; available in PMC 2021 April 22.

Published in final edited form as:

Mar Pollut Bull. 2019 August ; 145: 595–603. doi:10.1016/j.marpolbul.2019.06.047.

Alterations in the human skin microbiome after ocean water exposure

Marisa Chattman Nielsen[1], **Sunny C. Jiang**[1,2,*]

[1]Environmental Health Sciences, University of California, Irvine.

[2]Civil and Environmental Engineering, University of California, Irvine.

Abstract

Skin is the body's first line of defense against invading microorganisms. The skin microbiome has been shown to provide immunity against exogenous bacterial colonization. Recreational water exposures may alter the skin microbiome and potentially induce skin infections. This study explored the link between ocean water exposures and the human skin microbiome. Skin microbiome samples were collected, using swabs, from human participants' calves before and after they swam in the ocean, and at 6 hours and 24 hours post-swim. Genomic analysis showed that skin microbiomes were different among individuals before swimming. But after swimming, microbial communities were no longer different, which was demonstrated by a decrease in inter-sample diversity. Taxonomic analysis showed that ocean bacteria, including potential pathogens, replaced the native skin bacteria and remained on the skin for at least 24 hours post-swim. This research provides insight into the relationship between the human skin microbiome and the environment.

Keywords

microbial community; skin infection; vibrio; next-generation sequencing; skin microbiome; recreational water

Introduction:

It is estimated that 41% of the U.S. population swim in oceans, lakes, rivers or streams each year (*National Survey on Recreation and the Environment (NSRE) 2000–2002.*). Even though exercise and recreational activities have numerous health benefits such as improved aerobic fitness and cardiovascular health (Oja et al., 2015), poor water quality and reports of

*Corresponding author: 844 Engineering Tower, University of California, Irvine, CA 92697. sjiang@uci.edu; Tel: 949-824-5527.

Authors' contributions: Marisa Chattman Nielsen: Conception, Data Curation, Formal Analysis, Visualization, Investigation, Writing-Original Draft Preparation. Sunny Jiang: Conception, Writing-Reviewing and Editing, Investigation, Supervision. All authors read and approve the manuscript.

Declarations:

Ethics approval and consent to participate: This study was approved by the University of California, Irvine Institutional Review Board (IRB #2017–3751). Participants gave verbal consent to enroll in this study.

Availability of data and material: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare no competing interests.

recreational water related illness (RWRI) can significantly impact the value of beaches. Exposure to these waters can cause negative health effects including: gastrointestinal and respiratory illness, ear infections, and skin rashes (Collier et al., 2015). In fact, 16.3% of all ocean beachgoers reported a new health issue after going to the beach (Collier et al., 2015). Similarly, windsurfers were 2.9 times more likely to get one or more of the following symptoms after windsurfing in contaminated water: gastroenteritis, conjunctivitis, otitis and skin infection. The relative risk of the symptoms increased with reported numbers of times the windsurfers fell into the water (Dewailly et al., 1986). Arnold et al., (2017) reported that surfers in San Diego, CA were three times more likely to get a skin infection during dry weather months and nearly five times more likely during wet weather months than those with no water exposure. Among various RWRI, skin irritation or infections are frequently reported by those that engage in recreational water activities, but are less studied than gastrointestinal illnesses.

Recreational beach waters are often contaminated by wastewater and storm-water runoff (Arnold et al., 2017). The presence of a variety of pathogens, such as: *Salmonella spp.*, *Shigella spp., Campylobacter spp.*, *Vibrio spp.*, *Staphylococcus aureus*, intestinal parasites, viruses and other organisms in sewage and storm-water runoff can cause illness in humans that contact the water. The 2009–2010 Waterborne Disease and Outbreak Surveillance System (the most recent report) reports 24 disease outbreaks associated with natural (untreated water including rivers, lakes, streams, oceans) recreational waters (Hlavsa et al., 2014). Southern California coastal regions are among the most urbanized in the world (Arnold et al., 2017) and most of the rainfall occurs during the wet weather season (November to May) (Cao et al., 2017). Human fecal contamination, which includes potentially pathogenic bacteria, of ocean water is significantly higher in the wet weather months due to the increase of storm-water run-off during rainfall events (Cao et al., 2017).

Wastewater and storm-water runoff are not the only sources of potential pathogens in ocean water. Naturally occurring bacteria, such as *Vibrio* species and *Mycobacterium* species, are found in marine environments all over the world and can cause human disease (Sridhar and Deo, 2017; Yu, 2018). Environmental parameters (e.g. temperature, turbidity, salinity, sea level height and climate change) can contribute to the virulence and abundance of *Vibrio* species (Yu, 2018). Several *Vibrio* species such as *Vibrio cholerae* and *Vibrio parahemolyticus* are well known human pathogens. And *Vibrio vulnificus* is considered one of the most dangerous waterborne pathogens, causing severe wound infections and septicemia (Bisharat et al., 2005). While predominately found in warm waters such as the U.S. Gulf Coast, these pathogenic *Vibrio* species have all been detected in the coastal waters of Southern California (Dickinson et al., 2013), and have been implicated in non-foodborne infections in all coastal regions of the U.S. and other parts of the world (Dechet et al., 2008). Furthermore, the extensive use of antibiotics has affected environmental bacteria, including *Vibrio* species, rendering them more resistant to antibiotics which makes treating these skin infections especially difficult (Han et al., 2007). Naturally occurring atypical *Mycobacterium species*, such as *Mycobacterium marinum* and *Mycobacterium scrofulaceum*, have been associated with skin infections directly related to aquatic exposure (Griffith et al., 2007; Sridhar and Deo, 2017). These organisms can cause self-limiting, slowly-healing ulcers as well as more invasive health effects (e.g. joint and bone infections)

in up to 29% of the cases (Gonzalez-Santiago and Drage, 2015). As the climate changes and ocean temperatures rise, *Vibrio vulnificus* and other organisms that prefer warmer temperatures may increase in abundance in locations that are not currently suitable (Kaffenberger et al., 2017). This could result in increased water contamination and more frequent infections.

Skin is the body's first line of defense, both physically and immunologically, during exposure to contaminated water. Recent studies have shown that the human skin microbiome plays an important role in immune system function against localized and systemic diseases, and infection (Hannigan and Grice, 2013). The human skin microbiome refers to the microorganisms that inhabit human skin. The microbial composition differs among individuals and skin sites but individuals are more similar to themselves than they are to others (Grice et al., 2009)(Schommer and Gallo, 2013). The topographical difference in microbial composition is associated with the skin types. For example, sebaceous sites (i.e. face, back), moist sites (i.e. axilla, groin, toe webs) and dry sites (i.e. forearm, buttocks, calf) all have different microbial communities, even in the same individual (Hannigan and Grice, 2013). Even though there is much variability among individuals and body sites, there is no significant temporal variation among individuals. Most bacteria detected on normal human skin belong to the following phyla: Actinobacteria (51.8%), Firmicutes (24.4%), Proteobacteria (16.5%), and Bacteroidetes (6.3%) (Grice et al., 2009). The dominant genera are also quite stable and include: *Staphylococcus, Corynebacterium, Propionibacterium, Lactobacillus and Streptococcus* (Grice et al., 2009; Pereira et al., 2017).

A healthy microbiome protects the host from colonization and infection by opportunistic and pathogenic microbes (Hannigan and Grice, 2013). Recent research has demonstrated that changes in the microbiome can leave the host susceptible to infection and influence disease states (Naik et al., 2015)(Nakatsuji et al., 2017). For example, Naik et al. (2015) showed that *Staphylococcus epidermidis*, a normal human commensal, activated skin-resident dendritic cells and specific T cells that helped protect the skin from invading pathogens (Naik et al., 2015). Nakatsuji et al. (2017) demonstrated that human skin commensal bacteria produced antimicrobials to prevent *S. aureus* infection. They also showed that patients with cutaneous disorders were deficient in these protective organisms (Nakatsuji et al., 2017).

The skin microbiome not only protects hosts from pathogen colonization but also may modulate the pathogenesis of a variety of cutaneous disorders (Meisel et al., 2016). Alterations in the microbial communities on the skin have been linked to psoriasis, atopic dermatitis, acne and chronic wound infections (Gardiner et al., 2017; Rocha and Bagatin, 2018; Zeeuwen et al., 2013). Environmental factors that alter microbiome diversity (Price et al., 2009) are often associated with disease conditions (Gardiner et al., 2017). For example, Chang et al. showed a healthy microbiome differed significantly from the microbiome associated with psoriasis. Normal skin bacterial species, such as *Staphylococcus epidermidis* and *Propionibacterium acnes,* were less abundant but opportunistic pathogens like *Staphylococcus aureus* were more abundant in psoriatic patients than in healthy patients (Chang et al., 2018). This was attributed to a decrease in immunoregulatory bacteria such a *S. epidermidis* and *P. acnes,* that subsequently led to increased colonization by *S. aureus* (Chang et al., 2018). Similar characteristics have been observed in patients with atopic

dermatitis. Skin microbiome dysbiosis in the affected individuals results in decreased normal commensal bacteria and increased colonization by *S. aureus* (Williams and Gallo, 2017).

Much of the current research on the skin microbiome parallels that on the gut microbiome and human health. Alterations in the gut microbiome are not only physically associated with the gastrointestinal illnesses, like irritable bowel syndrome, inflammatory bowel disease and colon cancer, but also other diseases such as rheumatoid arthritis, obesity and Parkinson's disease. This past research demonstrates that the effects of change in the normal microbiome may have a greater and more far reaching impact than previously thought (Gilbert et al., 2016). While direct exposure to pathogens can cause infection, the role of the human microbiome in immunity and infectious disease development has become increasingly recognized. Characterizing the changes in the resident skin microbiota associated with recreational water exposure may provide insight into the complex and fragile balance between healthy skin and skin infection.

High throughput sequencing technologies, like next-generation sequencing (NGS), have revolutionized microbiome research. NGS utilizes sequencing parallelization that results in millions of reads originating from specific amplified DNA sequences. The 16S rRNA gene is highly conserved among bacteria, but also has numerous variable regions that facilitate bacterial identification. The taxonomic composition of the microbiome has proven to be an important feature for distinguishing healthy individuals from those with disease states in numerous studies (Gilbert et al., 2016).

Understanding the changes of the skin microbiome during recreational water exposure and the role of the human microbiome against pathogen invasion and infection can offer new strategies in protecting humans against RWRI. This research provides the foundation for the investigation of the potential link between alterations in the human skin microbiome and increased risk of infections. This research may aid in the revision of safety guidelines for exposure and the development of diagnostic and therapeutic tools that can help correct alterations in the skin microbiome for treatment or prevention of infections.

Methods:

Sample collection

This study was approved by the University of California, Irvine Institutional Review Board (IRB #2017–3751). Sample collection occurred in April, 2018 at Huntington Dog Beach in Huntington Beach, CA. A large poster summarizing the study and asking for volunteers was displayed at the collection site. Interested participants inquired and were given a detailed study description if they met the participant criteria. Only those who were 18 years of age or older, could speak and read English, and could swim were allowed to enroll. Participants gave verbal consent to enroll in this study. We obtained skin microbiome samples from nine participants including three males and six females, age ranges from 24–39, with no sunscreen application, infrequent exposure to the ocean and beach (once per month or less), no shower/bath in the past 12 hours, no antibiotic usage in the past 6 months, and no active infections. No identifying information was collected from the participants; samples were assigned a number (1 through 9). Samples were collected from an 8cm x 8cm section of skin

on the back of the participants' calves using rayon-tipped swabs moistened in sterile saline. The calf was selected as the body site of interest because it has a large flat surface area and sustains constant water exposure while wading/swimming without requiring the participant to be completely submerged in the water. Samples were collected before the individuals swam in the ocean. They were then instructed to swim or wade in the ocean for 10 minutes and the second set of samples was collected after they completely air-dried, which took approximately 20–30 minutes. The before and after samples were collected from the same calf but on different sections of the skin to ensure the sample collection taken before swimming did not remove bacteria from the section of skin swabbed after swimming. The before swimming samples were collected from the right side of the right calf and the after swimming samples were collected from the left side of the right calf after the area had air-dried.

The participants were then instructed not to shower or to wash the leg area for 24 hours and were trained using the above-mentioned swabbing method to collect their own samples for the 6 hour and 24 hour post-swim collections. The 6 hour sample was collected from the right side of the left calf and the 24 hour sample was collected from the left side of the left calf. Participants were instructed to keep the samples on ice after collection and investigators met with participants to retrieve the samples; no samples were shipped to the laboratory. Ice packs and coolers were provided, if requested. All samples were received on ice and processed within 24 hours of collection. An ocean water sample (75ml) from the swim site, at the time of sample collection, was collected and analyzed in the same manner as the experimental samples.

DNA extraction, PCR amplification and 16s rRNA gene sequencing

All samples were kept on ice until centrifuged to concentrate bacteria. Cell pellets were frozen at −80°C within 24 hours of collection. DNA was extracted from the cell pellets, and a single-step 30 cycle PCR was performed for the 16S rRNA gene V4 variable region using PCR primers 515F/806R (515F: 5′-GTGCCAGCMGCCGCGGTAA-3′; and 806R: 5′-GGACTACVSGGGTATCTAAT-3′). PCR conditions were: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes. All samples underwent DNA extraction, PCR and analysis by NGS using the 16S rRNA gene V4 variable region on an Ion Torrent PGM at MR DNA Laboratory (Shallowater, TX.).

Analysis and interpretation of sequencing results

Sequence data were analyzed using QIIME (Caporaso et al., 2010) at The University of California, Irvine. After importing the raw sequencing data, sequences were demultiplexed; primers, barcodes, short sequences, sequences with ambiguous base calls, and sequences with homopolymers exceeding 6 bp were removed using QIIME default settings. After initial quality control, each sample had between 6,320 and 167,432 DNA sequences, with an average of 97,048 sequences per sample and a total of 3,299,632 sequences in the data set. Chloroplast sequences were removed from the data. Sequences were filtered using a cut-off quality score of 25, clustered (using Uclust at 97% sequence similarity) into an open

reference operational taxonomic unit (OTU) table and taxonomically classified (using Uclust consensus taxonomy assigner).

Alpha and beta diversity analyses were created from the resulting OTU table with taxonomic assignments. The OTU alignment failures were removed. Simpson and Shannon indices were calculated in QIIME and the table outputs were uploaded into R Studio using R version 3.5.0 “Joy in Playing” (R Studio Inc., Boston, MA) for boxplot generation and further statistical analyses. P values were calculated using the Wilcoxon Rank Sum Test in R Studio.

Results:

Skin microbiome diversity

Alpha diversity: To investigate whether the skin microbiome changes after ocean water exposure, we first examined the alpha diversity (intra-sample diversity) metrics of the samples collected before swimming, after swimming, 6 hours post-swim and 24 hours post-swim. Results from all participants were pooled together by sample collection time. Community richness (chao1-abundance-based richness estimator and observed OTUs), evenness (Simpson index) and overall diversity (Shannon index) are shown in Figure 1a-1d and Table S1. Overall, we observed statistically significant differences in microbial diversity before and after the subjects swam in the ocean (Figure 1c-1d). Microbial diversity was the highest immediately after swimming, followed by 6 hours post-swim and before swimming. Samples collected 24 hour post-swim had the lowest diversity. Over time, the bacterial communities decreased in diversity as they trended towards baseline (before swimming). These results indicate that skin microbiome is altered by exposure to ocean water and changes are evident for at least 24 hours post-swim.

Beta diversity: We further explored the effects of ocean water exposure on the skin microbiome using beta diversity metrics (inter-sample diversity). We generated a Weighted Unifrac Distance Matrix, which is a qualitative representation of the difference in communities using phylogenetic branches that are weighted by the relative abundances of sequences. Using this matrix, Principle Coordinate Analysis (PCoA) plots were created (Figure 2). Principle Coordinate 1 (PC1) represents 54.52% variation, Principle Coordinate 2 (PC2) 8.22% and Principle Coordinate 3 (PC3) 6.84%. After ocean water exposure, we observed a distinct cluster indicating that even though the alpha diversity (intra-sample diversity) was the highest, the samples from individual subjects were not distinctly different from one another. Each point on Figure 2 represents a sample from a human subject identified by the first number and followed by either B (before swimming), A (after swimming), 6 (6 hours post-swim) or 24 (24 hours post-swim). As time passed, the beta diversity measurements trended toward baseline (before swim) for each individual subject. As illustrated by the dash-lines in Figure 2a, the skin microbiome of subjects 1 and 2 slowly returned to the microbiome signature of the skin before swimming and at different rates. Similar patterns were also observed for other subjects but the dash-lines were not included on the graph to avoid overcrowding of the lines. It should be noted, participant 3 and 4 did not have 6 hour or 24 hour samples collected, participant 8 and 9 did not have 6 hour

samples collected and participant 6's before sample was below the sampling depth of 25,000 sequences for the analyses and was not included.

Taxonomy changes in the skin microbiome after exposure

The predominating phyla (Figure 3a) on the skin changed after swimming when compared to before swimming. *Actinobacteria* decreased from 34% to 6.7%, *Firmicutes* decreased from 32.3% to 9.4%, *Proteobacteria* slightly decreased from 24.8% to 24% and *Bacteroidetes* increased from 7% to 41.8%. As time passed, the bacterial community composition trended towards baseline. In comparison, the ocean water sample was comprised of 63% *Proteobacteria*, 17.2% *Bacteroidetes*, 8.4% *Cyanobacteria*, 7.4% *Actinobacteria*, 1.7% *Verrucomicrobia* and 0.7% *Firmicutes*.

A similar difference was seen at the familiae level (Figure 3b). Before swimming, the predominating familiae on the skin were *Micrococcaceae* (23.4%), *Staphylococcaceae* (17.7%), *Corynebacteriaceae* (6.7%), *Streptococcaceae* (5.4%) and *Lactobacillaceae* (3.4%). After swimming, the predominating familiae were *Flavobacteriaceae* (29.6%), *Puniceicoccaceae* (6.7%), *Cryomorphaceae* (5.9%), *Rhodobacteraceae* (4.2%) and *Corynebacteriaceae* (4%). At 24 hours post-swim, *Staphylococcacea*e began to establish dominance at 13.5% and *Corynebacteriaceae* increased to 9.6%. These changes were even more obvious at the genus level (Figure 3c). Before swimming, the skin is inhabited by indigenous bacteria such as *Staphylococcus, Streptococcus*, and *Corynebacteria,* as expected (Grice et al., 2009). After swimming, however, those organisms were significantly reduced and ocean-borne bacteria predominated. Even though a significant amount of normal skin flora was washed off and subsequently replaced by marine bacteria, the data demonstrate that as time passed, indigenous flora began to re-establish.

Changes in the skin microbiome of individual participants

Taxa alterations were also evident in individual subjects as time passed (Figure 4). The phyla and genus relative abundance was shown side by side for individuals 2, 5 and 7 to demonstrate that the individual participant results were similar to those seen in the pooled data shown in Figure 3. At the phyla level (Figure 4a), *Firmicutes* and *Actinobacteria* initially decreased in relative abundance after exposure to ocean water and slowly increased back to baseline levels. *Bacteroidetes* increased after swimming and slowly decreased as time passed. The ocean water appeared to simultaneously wash off resident skin bacteria and deposit ocean-borne bacteria onto the skin. This change may be dependent on the relative abundance of *Proteobacteria* (62.94%), *Bacteroidetes* (17.18%), *Actinobacteria* (7.45%) and *Firmicutes* (0.70%) present in the ocean water. These data are summarized for each individual participant (Table S2). All of the participants acquired bacteria from the genus *Vibrio* after swimming (Figure 4b). This genus includes potential pathogens, although specific pathogenic species were not identified because organisms were only reported to the genus level. While this genus made up a very small percentage of total OTUs (0.37%) on the participants' skin, it still demonstrated that *Vibrio spp.* were present on the skin after swimming in the ocean. In some participants, these organisms persisted for 6 hours, and in one participant (7), for 24 hours. It is also worth mentioning that the fraction of *Vibrio spp.* detected on human skin was more than 10 times greater than the fraction of *Vibrio spp.* in

the ocean water sample (only 0.032%), suggesting it has a specific affinity for attachment to human skin. Even though the human skin microbiome differed greatly between individuals, the effects of ocean water exposure on the skin microbiome were similar among individuals.

Approximately 17.2% of all bacteria detected on human skin after swimming were likely ocean bacteria because they were found in the ocean water but were not detected on the participants before they entered the water (Figure 4c). These ocean bacteria persisted on the skin for at least 24 hours and decreased in concentration over time. The top 3 most abundant ocean bacterial genera that were detected in the after swimming samples are shown in Figure 4c and Table S3. The most abundant of these were members of the family *Pelagibacteraceae* (unknown genus), *Sediminicola spp.*, and *Pseudoalteromonas spp*. Again, Figure 4 data demonstrated that exogenous bacteria found on the skin after swimming, originated from the ocean, and persisted on the skin of individual human subjects for at least 24 hours.

Discussion:

Linking skin microbiome change with skin health

Skin is the primary barrier protecting us from the external environment. The microbiome is currently believed to be an integral part of our immune system because of the association between host and microbial factors and the downstream effects on immune cells (Belkaid and Tamoutounour, 2016; Zitvogel et al., 2018). A healthy microbiome, which is largely stable over time (Oh et al., 2016), has been shown to defend our bodies from invading pathogens and protect us from disease. Alterations in the skin microbiome have been associated with skin diseases (Furue et al., 2018). Our data demonstrate for the first time that ocean water exposure can alter the diversity and composition of human skin microbiota. A portion of the native skin microbiota was replaced by ocean bacteria, which was reflected in the increase in diversity and detection of ocean-borne bacteria in post-swim skin microbiome samples. This conclusion was further confirmed by the observation that although most individuals had different microbiomes when compared to one another pre-swim, they all had similar compositions after swimming, as shown in the beta-diversity analyses.

The dramatic changes in the skin microbiome from a normal indigenous microbiome signature to a completely different diversity signature after ocean water exposure signifies the importance of understanding the relationship between ocean exposure and skin health. Washing off native skin bacteria could weaken immunity against exogenous bacteria in the ocean. Ocean bacteria, including *Vibrio spp.,* are clearly evident on the skin after exposure although *Vibrio* is not the predominant bacterial genus in the ocean water. This result implies that skin has the ability to attract exogenous bacteria from recreational water. *Vibrio vulnificus*, a naturally occurring pathogen in marine water, can cause necrotizing wound infections that can result in sepsis and death (Oliver, 2015). Although it is not the intention of this study to expose participants to water of poor quality, the results of the study imply that microbial pathogens would attach to human skin if they were present in the water.

Attachment and persistence of pathogens on the skin not only has implications for increased risk of skin infections, but harmful organisms found in recreational water of poor quality

may infect humans through the fecal-oral route. It is possible that as pathogens persist on the skin, there is a chance for accidental transfer from the skin to the mouth, or even transfer to another individual. This could cause gastrointestinal illness not only at the beach, but in the hours or days post-exposure. This is especially dangerous for children and immunocompromised individuals whom are more susceptible to infection.

Study Limitations

There were several limitations in this study. The 'before swimming samples' were collected after most of the individuals had entered the beach area (with the exception of participant 1 and 2) but before they entered the water. In some cases, participants were walking and standing in the sand for 2 hours or more before samples were collected. Participants 1 and 2 did not have ocean bacteria detected on the skin before they entered the water, but all the other participants did. We believe that aerosols produced by the waves and skin contact with the sand may deposit a small amount of ocean-borne bacteria onto the skin. Ideally, all 'before swimming samples' should have been collected before participants entered the beach area but the phenomenon that ocean bacteria may be acquired on the skin without entering the water should be investigated.

The participant size in this study was small and conclusions from the study population may not apply to the general population. However, the microbiome sample size is sufficient for the study because even though only 9 participants were analyzed, 2–4 different time point samples were collected on each individual for a total of 34 samples. The samples averaged 97,048 bacterial sequences per sample. This sample size is similar to numerous highly influential human skin microbiome studies published in recent years. For example, Grice et al. investigated the human skin microbiome in healthy individuals and used samples from only 10 human participants (Grice et al., 2009). Another study assessing the temporal stability of the human skin microbiome used samples from 12 healthy human participants for genomic analysis (Oh et al., 2016). Studies on the association between certain disease states and the skin microbiome have also utilized small participant numbers. A 2018 study investigating the difference in the skin microbiome of healthy individuals and those with atopic dermatitis was based on 10 diseased individuals compared to 8 healthy individuals (Furue et al., 2018). We had the opportunity to include additional participants but instead chose to adhere to strict study criteria designed to minimize confounding factors and additional variables.

Medical history was not collected from the participants and sex differences were not assessed due to the small number of participants. Chronic skin disorders and other characteristics may influence how the human skin microbiome responds to environmental exposures. In order to account for these variables, each person served as their own control to assess the effects of ocean water exposure and minimize confounding factors. Additional studies are necessary to understand how unique skin characteristics influence the effects of ocean water exposure on the human skin microbiome.

Another potential limitation was the use of 16S rRNA gene hypervariable region 4 sequencing for our study. V4 region was selected for the greatest coverage of bacteria with different niches due to the large number of environmental and human commensal bacteria

present in our samples. However, V4 has limitations in detection of some human skin commensals, particularly *Propionibacteria spp.* 16S rRNA gene sequencing of the V4 region is likely to identify as many bacteria as possible and to elucidate the effects of ocean water on the skin microbiome with the expectation that metagenomic analysis would be a useful tool for future research.

Lastly, this study only analyzed the skin microbiome on the calf. This body site was chosen because of its large, flat surface area and sustained water contact while wading. Different body sites are known to have different microbial community compositions. We anticipate these effects would be similar on other body sites and may even be accentuated in areas that have a lower abundance of commensals, have a higher abundance of more fastidious commensals, have the ability to trap water (inside the ears and nasal cavity), and/or maintain contact with the ocean sediment (toes).

Future research

There has been little research devoted to investigating the effects of environmental exposures on the skin microbiome. The microbiome can be altered in response to external substances, such as antibiotics and toxic chemicals, however, ocean water is unique in that it removes resident bacteria and simultaneously deposits foreign bacteria on the skin. A large portion of the population is exposed to ocean water, therefore, a better understanding of ocean exposure and skin microbiome may protect public health during water recreational activities. This is especially of concern with increasing water temperatures and pollutant runoffs and a higher concentration of pathogens in natural waters (Kaffenberger et al., 2017). Future work to connect changes in the skin microbiome with a prospective epidemiological study in poor quality water (i.e. post-storm condition) may offer a direct link between changes in the microbiome and skin infections.

Some participants encountered more drastic changes in the skin microbiome that persisted for a longer time as compared to the other participants. The physical characteristics of an individual's skin, such as: skin type, hydration level, skin product usage, sun exposure, hygiene, etc., may affect the changes seen. Some participants have a less diverse skin microbiome before swimming (as measured by species richness) and would therefore appear to have a larger increase in diversity after swimming. Research by Wang et al. (2016) has shown that differences in the human skin microbiome may be governed by differences in available carbon sources on the skin. They demonstrated that increasing sucrose on the skin promoted the fermentative capabilities of *S. epidermidis*, but not *P. acnes*. When *P. acnes* and *S. epidermidis* were co-cultured in the presence of sucrose, *P. acnes* growth was diminished (Wang et al., 2016). Individuals have different levels of available sugars which support the growth of different indigenous bacteria (Wang et al., 2016). Such differences may also support the attachment and persistence of exogenous bacteria on the skin. The skin microbiome and its responses to environmental exposure may also differ by sex. There is an unexplained observation reported in the literature that males are more likely to acquire *Vibrio vulnificus* (Tacket et al., 1984) and *Aeromonas spp*. infections (Baddour, 1992) after water exposure. Future research in this area may shed new light on wound and other

necrotizing infections and the differences in the skin microbiome of males and females after exposure.

Wastewater, storm-water, discharges from animal agriculture, aquaculture and hospitals all contribute to the release of antibiotics and antibiotic resistant bacteria into the environment. Antibiotic resistant organisms have been found in ocean water (Dang et al., 2008; Di Cesare et al., 2012; Leonard et al., 2015), which present additional risk to recreational bathers for acquiring antibiotic resistant infections (Di Cesare et al., 2012; Morroni et al., 2016). Future metagenomic research is needed to elucidate the connection between recreational water exposure and acquisition of antibiotic resistance organisms on human skin.

Providing evidence that ocean water exposures under certain circumstances (e.g., geographic, seasonal, exposure frequency) may increase health risks, will allow public health organizations to generate appropriate mitigation recommendations to help reduce the occurrence of RWRI. Potential strategies for reducing skin related health risks from exposure to ocean water could include protective recommendations (e.g., showering immediately post-swim, exposure time limits based on age, immune status and other characteristics) and therapeutic interventions targeted at re-population of normal skin commensals. Public and occupational health organizations will be better positioned to make recommendations that protect people while enabling them to continue to enjoy and work in marine environments.

Conclusions

Alterations of the human skin microbiome have been linked to skin diseases but the impact of recreational ocean water exposure on the human skin microbiome has not yet been studied. This study is the first step towards an understanding of the relationship between recreational water exposure, the skin microbiome, and potential skin infection. The following conclusions can be made from the results of this study:

- Ocean water exposure removed normal resident bacteria from human skin, which have been shown to modulate the immune system and provide protection against invading pathogens. Therefore, the removal of these symbiotic organisms could leave the host susceptible to infection.
- Ocean water exposure simultaneously deposited ocean-borne bacteria onto the skin, including potential pathogens that could cause infection.
- While the normal skin microflora re-established dominance as time elapses post-exposure, exogenous bacteria was present on the skin for at least 24 hours after swimming. This might present an opportunity for pathogenic bacteria to cause infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

Authors thank Dr. Scott Bartell, Dr. Ulrike Luderer, Dr. Debra Mauzy-Melitz, and Dr. Oladele Ogunseitan for helpful conversations relating to the design of the study and analysis of sequencing data.

Funding: Funding for this research was in part provided by NSF CBET 1806066, 1804166 and UCI Oceans Fellowship.

References:

Arnold BF, Schiff KC, Ercumen A, Benjamin-Chung J, Steele JA, Griffith JF, Steinberg SJ, Smith P, McGee CD, Wilson R, Nelsen C, Weisberg SB, Colford JM, 2017. Acute Illness Among Surfers After Exposure to Seawater in Dry- and Wet-Weather Conditions. Am. J. Epidemiol 186, 866–875. 10.1093/aje/kwx019 [PubMed: 28498895]

Baddour LM, 1992. Extraintestinal Aeromonas Infections—Looking for Mr. Sandbar. Mayo Clin. Proc 67, 496–498. 10.1016/S0025-6196(12)60402-9 [PubMed: 1405778]

Belkaid Y, Tamoutounour S, 2016. The influence of skin microorganisms on cutaneous immunity. Nat. Rev. Immunol 16, 353–366. 10.1038/nri.2016.48 [PubMed: 27231051]

Bisharat N, Cohen DI, Harding RM, Falush D, Crook DW, Peto T, Maiden MC, 2005. Hybrid Vibrio vulnificus. Emerg. Infect. Dis 11, 30–35. 10.3201/eid1101.040440 [PubMed: 15705319]

Cao Y, Raith M, Smith P, Griffith J, Weisberg S, Schriewer A, Sheldon A, Crompton C, Amenu G, Gregory J, Guzman J, Goodwin K, Othman L, Manasjan M, Choi S, Rapoport S, Steele S, Nguyen T, Yu X, Cao Y, Raith MR, Smith PD, Griffith JF, Weisberg SB, Schriewer A, Sheldon A, Crompton C, Amenu GG, Gregory J, Guzman J, Goodwin KD, Othman L, Manasjan M, Choi S, Rapoport S, Steele S, Nguyen T, Yu X, 2017. Regional Assessment of Human Fecal Contamination in Southern California Coastal Drainages. Int. J. Environ. Res. Public Health 14, 874. 10.3390/ijerph14080874

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R, 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–6. 10.1038/nmeth.f.303 [PubMed: 20383131]

Chang H-W, Yan D, Singh R, Liu J, Lu X, Ucmak D, Lee K, Afifi L, Fadrosh D, Leech J, Vasquez KS, Lowe MM, Rosenblum MD, Scharschmidt TC, Lynch SV, Liao W, 2018. Alteration of the cutaneous microbiome in psoriasis and potential role in Th17 polarization. Microbiome 6, 154. 10.1186/s40168-018-0533-1 [PubMed: 30185226]

Collier SA, Wade TJ, Sams EA, Hlavsa MC, Dufour AP, Beach MJ, 2015. Swimming in the USA: beachgoer characteristics and health outcomes at US marine and freshwater beaches. J. Water Health 13, 531–43. 10.2166/wh.2014.095 [PubMed: 26042984]

Dang H, Ren J, Song L, Sun S, An L, 2008. Dominant chloramphenicol-resistant bacteria and resistance genes in coastal marine waters of Jiaozhou Bay, China. World J. Microbiol. Biotechnol 24, 209–217. 10.1007/s11274-007-9458-8

Dechet AM, Yu PA, Koram N, Painter J, 2008. Nonfoodborne Vibrio Infections: An Important Cause of Morbidity and Mortality in the United States, 1997–2006. Clin. Infect. Dis 46, 970–976. 10.1086/529148 [PubMed: 18444811]

Dewailly E, Poirier C, Meyer FM, 1986. Health hazards associated with windsurfing on polluted water. Am. J. Public Health 76, 690–1. [PubMed: 3706597]

Di Cesare A, Vignaroli C, Luna GM, Pasquaroli S, Biavasco F, 2012. Antibiotic-Resistant Enterococci in Seawater and Sediments from a Coastal Fish Farm. Microb. Drug Resist 18, 502–509. 10.1089/mdr.2011.0204 [PubMed: 22546011]

Dickinson G, Lim K, Jiang SC, 2013. Quantitative Microbial Risk Assessment of Pathogenic Vibrios in Marine Recreational Waters of Southern California. Appl. Environ. Microbiol 79, 294–302. 10.1128/AEM.02674-12 [PubMed: 23104412]

Furue M, Iida K, Imaji M, Nakahara T, 2018. Microbiome analysis of forehead skin in patients with atopic dermatitis and healthy subjects: Implication of Staphylococcus and Corynebacterium. J. Dermatol 45, 876–877. 10.1111/1346-8138.14486 [PubMed: 29971837]

Gardiner M, Vicaretti M, Sparks J, Bansal S, Bush S, Liu M, Darling A, Harry E, Burke CM, 2017. A longitudinal study of the diabetic skin and wound microbiome. PeerJ 5, e3543. 10.7717/peerj.3543 [PubMed: 28740749]

Gilbert JA, Quinn RA, Debelius J, Xu ZZ, Morton J, Garg N, Jansson JK, Dorrestein PC, Knight R, 2016. Microbiome-wide association studies link dynamic microbial consortia to disease. Nature 535, 94–103. 10.1038/nature18850 [PubMed: 27383984]

Gonzalez-Santiago TM, Drage LA, 2015. Nontuberculous Mycobacteria. Dermatol. Clin 33, 563–577. 10.1016/j.det.2015.03.017 [PubMed: 26143432]

Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, NISC Comparative Sequencing Program, N.C.S., Bouffard GG, Blakesley RW, Murray PR, Green ED, Turner ML, Segre JA, 2009. Topographical and temporal diversity of the human skin microbiome. Science 324, 1190–2. 10.1126/science.1171700 [PubMed: 19478181]

Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn CF, Wallace RJ, Winthrop K, 2007. An Official ATS/IDSA Statement: Diagnosis, Treatment, and Prevention of Nontuberculous Mycobacterial Diseases. Am. J. Respir. Crit. Care Med 175, 367–416. 10.1164/rccm.200604-571ST [PubMed: 17277290]

Han F, Walker RD, Janes ME, Prinyawiwatkul W, Ge B, 2007. Antimicrobial susceptibilities of Vibrio parahaemolyticus and Vibrio vulnificus isolates from Louisiana Gulf and retail raw oysters. Appl. Environ. Microbiol 73, 7096–8. 10.1128/AEM.01116-07 [PubMed: 17827331]

Hannigan GD, Grice EA, 2013. Microbial ecology of the skin in the era of metagenomics and molecular microbiology. Cold Spring Harb. Perspect. Med 3, a015362. 10.1101/cshperspect.a015362 [PubMed: 24296350]

Hlavsa MC, Roberts VA, Kahler AM, Hilborn ED, Wade TJ, Backer LC, Yoder JS, Centers for Disease Control and Prevention (CDC), 2014. Recreational water-associated disease outbreaks--United States, 2009–2010. MMWR. Morb. Mortal. Wkly. Rep 63, 6–10. [PubMed: 24402466]

Kaffenberger BH, Shetlar D, Norton SA, Rosenbach M, 2017. The effect of climate change on skin disease in North America. J. Am. Acad. Dermatol 76, 140–147. 10.1016/j.jaad.2016.08.014 [PubMed: 27742170]

Leonard AFC, Zhang L, Balfour AJ, Garside R, Gaze WH, 2015. Human recreational exposure to antibiotic resistant bacteria in coastal bathing waters. Environ. Int 82, 92–100. 10.1016/J.ENVINT.2015.02.013 [PubMed: 25832996]

Meisel JS, Hannigan GD, Tyldsley AS, SanMiguel AJ, Hodkinson BP, Zheng Q, Grice EA, 2016. Skin Microbiome Surveys Are Strongly Influenced by Experimental Design. J. Invest. Dermatol 136, 947–956. 10.1016/j.jid.2016.01.016 [PubMed: 26829039]

Morroni G, Di Cesare A, Di Sante L, Brenciani A, Vignaroli C, Pasquaroli S, Giovanetti E, Sabatino R, Rossi L, Magnani M, Biavasco F, 2016. Enterococcus faecium ST17 from Coastal Marine Sediment Carrying Transferable Multidrug Resistance Plasmids. Microb. Drug Resist 22, 523–530. 10.1089/mdr.2015.0222 [PubMed: 26982016]

MR DNA Laboratory. www.mrdnalab.com Shallowater, TX. [WWW Document], n.d. URL http://www.mrdnalab.com/ (accessed 12.6.18).

Naik S, Bouladoux N, Linehan JL, Han S-J, Harrison OJ, Wilhelm C, Conlan S, Himmelfarb S, Byrd AL, Deming C, Quinones M, Brenchley JM, Kong HH, Tussiwand R, Murphy KM, Merad M, Segre JA, Belkaid Y, 2015. Commensal–dendritic-cell interaction specifies a unique protective skin immune signature. Nature 520, 104–108. 10.1038/nature14052 [PubMed: 25539086]

Nakatsuji T, Chen TH, Narala S, Chun KA, Two AM, Yun T, Shafiq F, Kotol PF, Bouslimani A, Melnik AV, Latif H, Kim J-N, Lockhart A, Artis K, David G, Taylor P, Streib J, Dorrestein PC, Grier A, Gill SR, Zengler K, Hata TR, Leung DYM, Gallo RL, 2017. Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis. Sci. Transl. Med 9. 10.1126/scitranslmed.aah4680

National Survey on Recreation and the Environment (NSRE) 2000–2002. The Interagency National Survey Consortium Coordinated by the USDA Forest Service Recreation Wilderness and Demographics Trends Research Group and the University of Tennessee Human Dimens, 2004

Oh J, Byrd AL, Park M, Kong HH, Segre JA, Segre JA, 2016. Temporal Stability of the Human Skin Microbiome. Cell 165, 854–866. 10.1016/j.cell.2016.04.008 [PubMed: 27153496]

Oja P, Titze S, Kokko S, Kujala UM, Heinonen A, Kelly P, Koski P, Foster C, 2015. Health benefits of different sport disciplines for adults: systematic review of observational and intervention studies with meta-analysis. Br. J. Sports Med 49, 434–440. 10.1136/bjsports-2014-093885 [PubMed: 25568330]

Oliver JD, 2015. The Biology of Vibrio vulnificus. Microbiol. Spectr 3. 10.1128/microbiolspec.VE-0001-2014

Pereira SG, Moura J, Carvalho E, Empadinhas N, 2017. Microbiota of Chronic Diabetic Wounds: Ecology, Impact, and Potential for Innovative Treatment Strategies. Front. Microbiol 8, 1791. 10.3389/fmicb.2017.01791 [PubMed: 28983285]

Price LB, Liu CM, Melendez JH, Frankel YM, Engelthaler D, Aziz M, Bowers J, Rattray R, Ravel J, Kingsley C, Keim PS, Lazarus GS, Zenilman JM, 2009. Community Analysis of Chronic Wound Bacteria Using 16S rRNA Gene-Based Pyrosequencing: Impact of Diabetes and Antibiotics on Chronic Wound Microbiota. PLoS One 4, e6462. 10.1371/journal.pone.0006462 [PubMed: 19649281]

Rocha MA, Bagatin E, 2018. Skin barrier and microbiome in acne. Arch. Dermatol. Res 310, 181–185. 10.1007/s00403-017-1795-3 [PubMed: 29147769]

Schommer NN, Gallo RL, 2013. Structure and function of the human skin microbiome. Trends Microbiol 21, 660–8. 10.1016/j.tim.2013.10.001 [PubMed: 24238601]

Sridhar SCJ, Deo SCR, 2017. Marine and Other Aquatic Dermatoses. Indian J. Dermatol 62, 66–78. 10.4103/0019-5154.198052 [PubMed: 28216728]

Tacket CO, Brenner F, Blake PA, 1984. Clinical features and an epidemiological study of Vibrio vulnificus infections. J. Infect. Dis 149, 558–61. [PubMed: 6725989]

Wang Y, Kao M-S, Yu J, Huang S, Marito S, Gallo R, Huang C-M, 2016. A Precision Microbiome Approach Using Sucrose for Selective Augmentation of Staphylococcus epidermidis Fermentation against Propionibacterium acnes. Int. J. Mol. Sci 17, 1870. 10.3390/ijms17111870

Williams MR, Gallo RL, 2017. Evidence that Human Skin Microbiome Dysbiosis Promotes Atopic Dermatitis. J. Invest. Dermatol 137, 2460–2461. 10.1016/J.JID.2017.09.010 [PubMed: 29169458]

Yu S, 2018. Uncovering the geographical and host impacts on the classification of Vibrio vulnificus. Evol. Appl 11, 883–890. 10.1111/eva.12602 [PubMed: 29928297]

Zeeuwen PLJM, Kleerebezem M, Timmerman HM, Schalkwijk J, 2013. Microbiome and skin diseases. Curr. Opin. Allergy Clin. Immunol 13, 514–20. 10.1097/ACI.0b013e328364ebeb [PubMed: 23974680]

Zitvogel L, Ma Y, Raoult D, Kroemer G, Gajewski TF, 2018. The microbiome in cancer immunotherapy: Diagnostic tools and therapeutic strategies. Science (80-.) 359, 1366–1370. 10.1126/science.aar6918

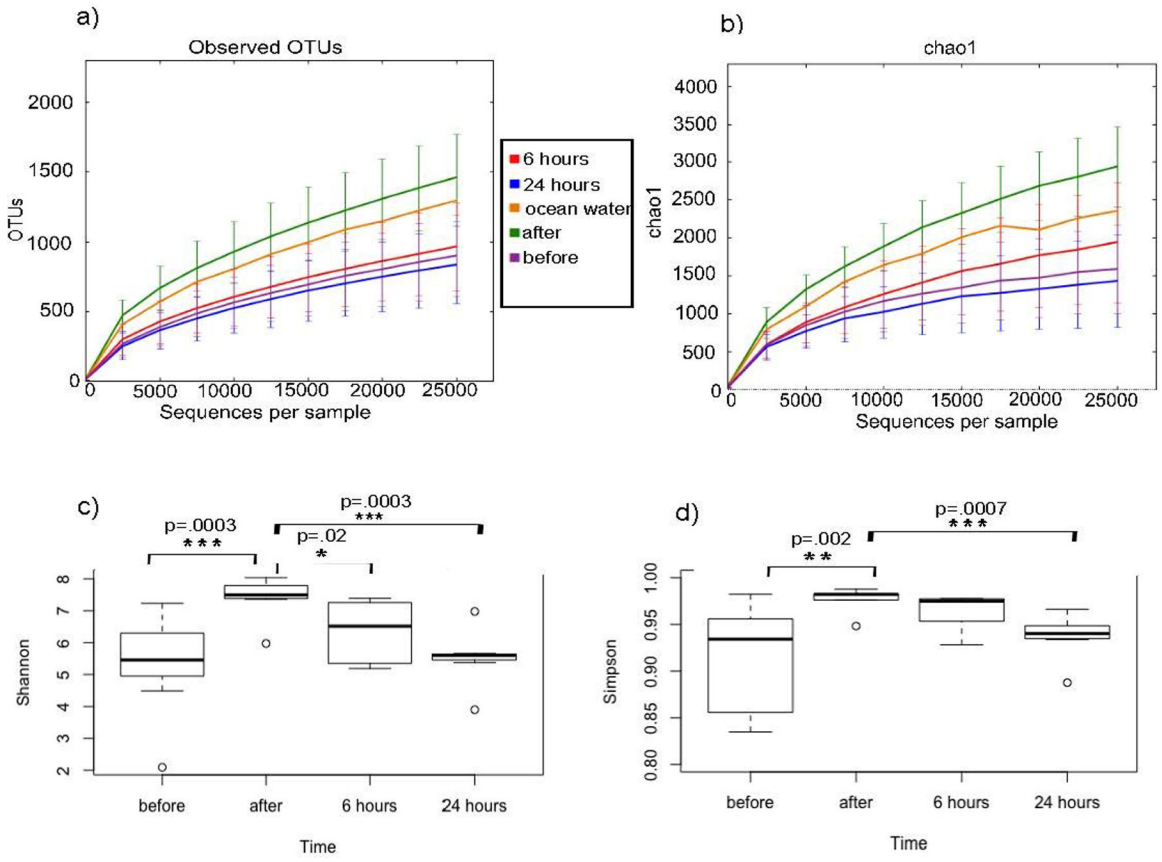

Figure 1.

The bacterial community alpha diversity of skin microbiomes before and after ocean swimming according to a) observed OTUs, b) Choa1 index c) Shannon index and d) Simpson index.

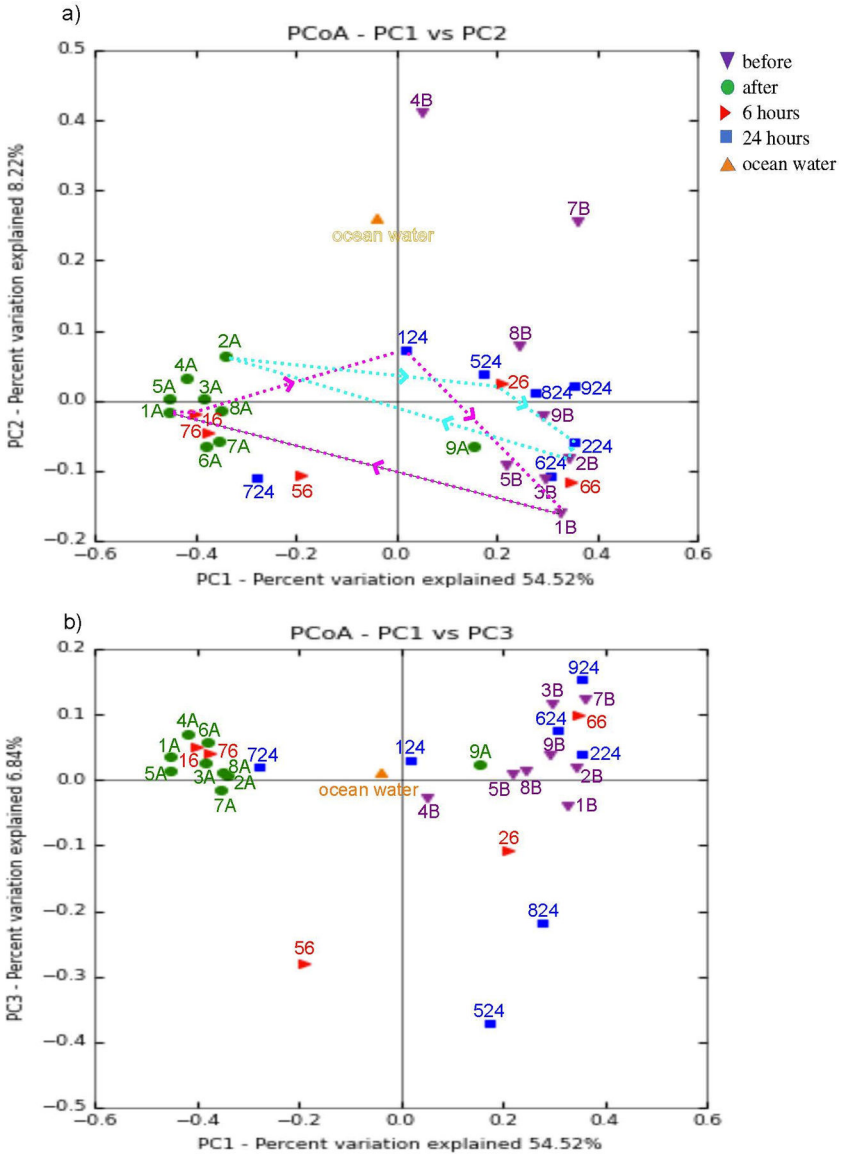

Figure 2.

The bacterial community beta diversity of skin microbiome before and after ocean swimming. Each point on the PCoA plot represents a skin microbiome sample where the first number indicates the human subject number. Beta diversity analysis was performed by weighted Unifrac PcoA where the 3 primary axes are shown a) PC1 vs PC2 and b) PC1 vs PC3. These coordinates represent 69.58% variation (PC1=54.52%, PC2=8.22%, and PC3=6.84%).

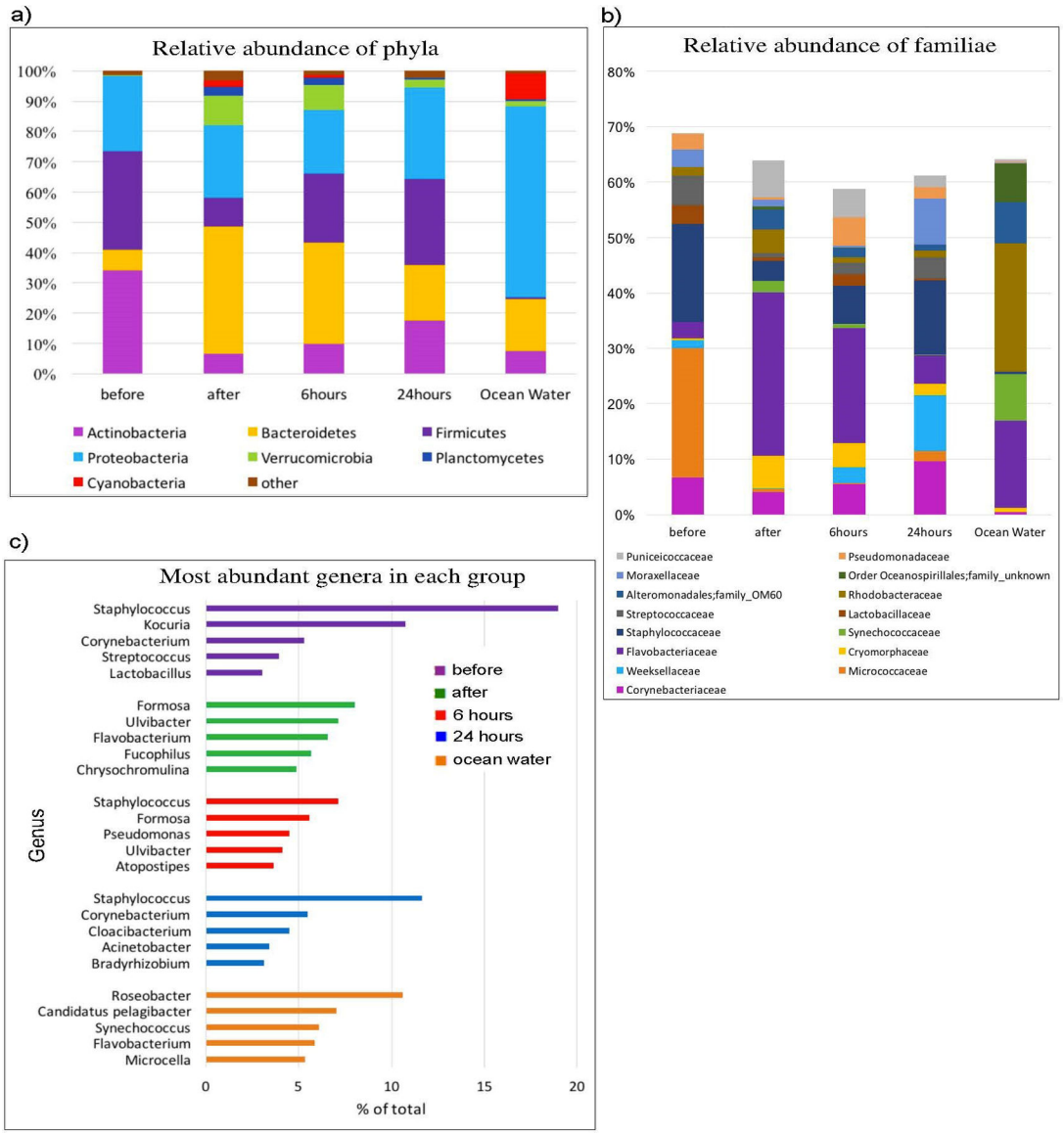

Figure 3.

Microbial community composition of the skin microbiome by a) most abundant phyla, and b) most abundant familiae in each category collected before swimmig (before), after swimming (after), 6 hours post-swim (6 hours) and 24 hours postswim (24 hours). C) Most abundant genera in each category.

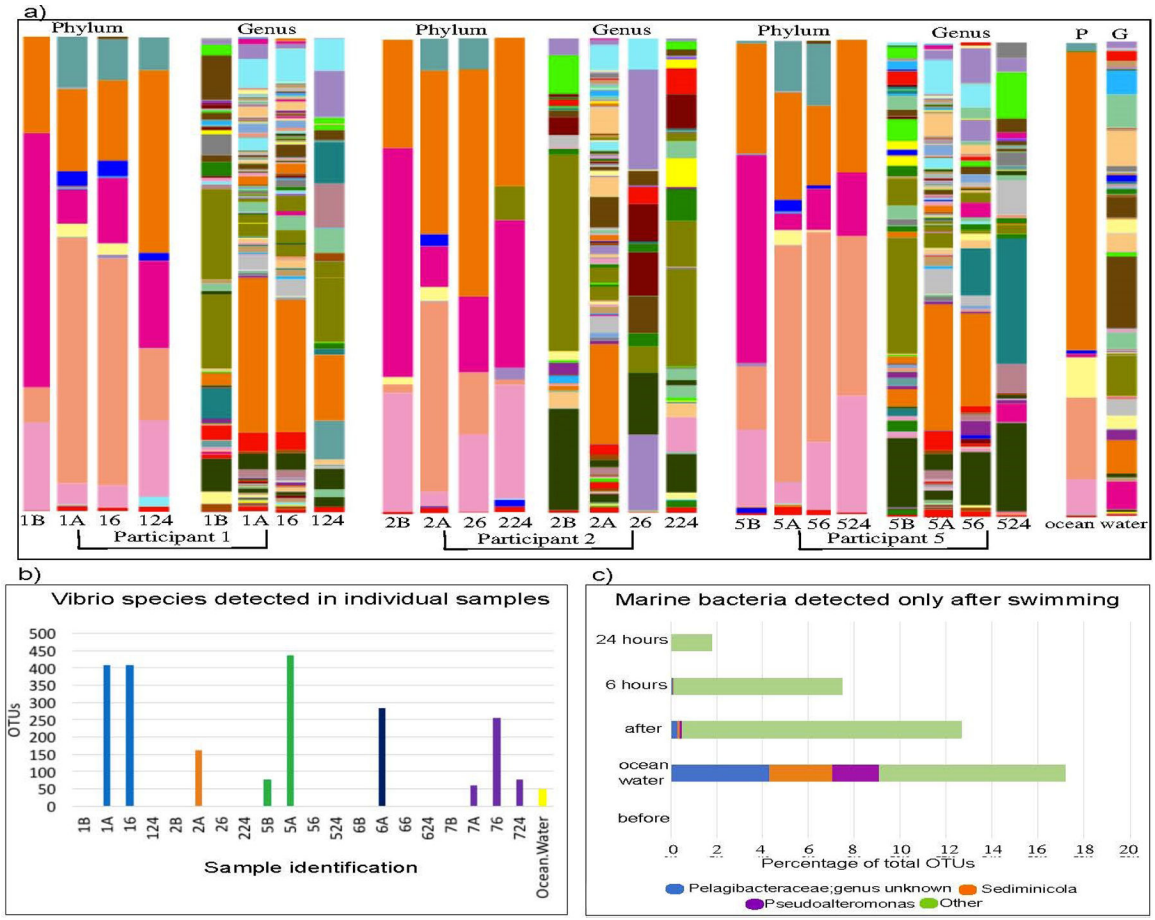

Figure 4.

Changes in microbial composition before and after ocean exposure on individual human subjects. a) Changes at the phylum and genus levels. b) OTU counts representing members of the members of the Vibrio genus detected in the samples. c) Bacteria that were detected on the skin only after swimming.