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ARTICLE

Bacterial assemblages associated with coral species of the Mexican Central Pacific

Ensamblajes bacterianos asociados a especies de coral del Pacífico central mexicano

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Resumen.- El papel funcional de las bacterias asociadas a corales y su contribución a la salud del coral es aún desconocido en gran medida. Es necesario que primero se caracterice el ensamblaje microbiano del coral y sus cambios en la diversidad a través de las especies de coral, el espacio y tiempo. Los corales ramificados (*e.g.*, género *Pocillopora*) son los principales constructores arrecifales a nivel mundial. Este estudio evaluó la estructura bacteriana asociada al mucus y tejidos de *Pocillopora damicornis* y *Pocillopora verrucosa*, así como del agua de mar y sedimentos circundantes en 6 sitios del Pacífico central mexicano. Se emplearon las técnicas DGGE y RFLP del ADNr 16S para generar los perfiles de bandeo o evaluar la diversidad. Además, se evaluó la relación del ensamblaje bacteriano-coral con variables ambientales y espaciales del entorno arrecifal (de cada sitio), utilizando análisis multivariados. Se obtuvieron 20 Unidades Taxonómicas Operacionales (OTU) diferentes, siendo los sedimentos los que presentaron mayor número. Se encontró una especificidad de grupos bacterianos para cada especie de coral, así como entre el tejido y mucus de cada especie. Los resultados mostraron que los grupos de bacterias dominantes variaron entre sustratos y entre sitios, encontrando, sólo una variación espacial significativa. Las variables ambientales que explican la variación de los grupos bacterianos dominantes en corales y agua de mar fueron las coberturas de macroalgas carnosas, coral vivo y esponja. En cambio, la variación en los sedimentos fue explicada por las coberturas de arena, escombro y roca.

Palabras clave: Ensamblajes bacterianos, corales *Pocillopora*, variación espacio-temporal, DGGE, Pacífico mexicano

Abstract.-The functional role of coral-associated bacteria and their contribution to coral health is still largely unknown. The first necessary step to address this gap in the knowledge is based on characterization of the microbial assemblage of the coral and the species-specific, temporal and spatial variation in its diversity. Branched corals (*e.g.*, genus *Pocillopora*), are the main builders of coral reefs worldwide. This study evaluated the bacteria associated with the mucus and tissues of *Pocillopora damicornis* and *Pocillopora verrucosa*, as well as that of the seawater and surrounding sediments, in 6 sites of the Mexican Central Pacific during summer and winter seasons. The molecular techniques DGGE and RFLP were used with the 16S rDNA to assess the most abundant bacterial OTUs. The relationships between the bacterial-coral assemblage and environmental and spatial variables of the reef surroundings were also evaluated, using the multivariate analyses. Twenty different Operational Taxonomic Units (OTU) were obtained, with the highest number presented by the sediments. Specificity of bacterial groups was found for each coral species, as well as between the tissue and mucus of each species. The results showed that the bacterial dominant groups were similar between seasons, but these showed significant spatial variations among substrates within sites, as well as per substrate across all sites. The environmental variables that explained the variation of the dominant bacterial groups in corals and sea water were the coverages of fleshy macroalgae, live coral and sponge. In contrast, variation in the sediments was explained by the coverages of sand, rubble and rock.

Key words: Bacterial assemblages, *Pocillopora* corals, spatial-temporal variation, DGGE, Mexican Pacific

Introduction

Coral reefs face degradation worldwide, mainly as a result of environmental stress factors of anthropogenic origin. These stresses include increased sea surface temperatures, coastal degradation, pollution, diseases and the synergistic effects of multiple stress factors (Ban *et al*. 2014). These changes damage the equilibrium between the coral and its associated microbiota (*i.e.*, symbiotic dinoflagellates, endolithic algae, fungi, bacteria, archaea and viruses) (Ceh *et al*. 2011). It is recognized that this microbial biota plays a functional role in the daily metabolism, health, resistance, recruitment and resilience of the corals (Bourne & Webster 2013).

Bacterial assemblages are diverse and active in coral ecosystems (Krediet *et al*. 2013). They are dynamic and occupy different niches: i) In the corals, within the mucus (surface mucopolysaccharide layer) (Morrow *et al*. 2012) and tissues (Sweet *et al*. 2011); ii) In the sea water (Bourne & Munn 2005) and surrounding marine sediments (Carlos *et al*. 2013). However, variation in their diversity among coral species and the functional role of these bacteria remain poorly understood. Mutualistic benefits have been reported between the bacteria and corals, including fixation of nitrogen and carbon (Bourne & Webster 2013) and exchange of secondary metabolites (Littman *et al*. 2009), among others.

Recent research shows that the bacterial assemblage associated with the coral changes as a function of environmental conditions (Lee *et al*. 2012, Li *et al*. 2014). The corals modify their bacterial microbiota as a mechanism of acclimatization to environmental changes (Reshef *et al*. 2006). In order to study bacteria-coral interactions, it is necessary to determine the resident microbiota and evaluate its spatio-temporal stability (Mouchka *et al*. 2010). Certain studies report that each coral species presents specific bacterial assemblages, regardless of geographic distance (Bourne & Webster 2013, Krediet *et al*. 2013).

Culture-independent techniques, such as denaturing gradient gel electrophoresis (DGGE), restriction fragment length polymorphism (RFLP), clone libraries and sequencing of 16SrDNA, have identified a wide range of bacterial groups associated with the corals (Littman *et al*. 2009). Recently, massive sequencing has provided greater knowledge of the bacteria associated with corals and is used for studies of microbial ecology (Li *et al*. 2014). Moreover, DGGE (Muyzer *et al*. 1993) is widely used to estimate the bacterial structure and diversity associated with marine invertebrates (Rodríguez-Lanetty *et al*. 2013). This technique is relatively straight forward, highly reproducible, rapid and reliable and thus represents an attractive alternative for the analysis of the bacterial assemblages in environmental samples.

This study represents the first comparative analysis of bacterial assemblages associated with coral ecosystems and uses two coral species of wide distribution in the Mexican Central Pacific (MCP) as a case study. This region is characterized by the presence of an important richness and live coverage of corals, where those of the genus *Pocillopora* constitute the main reef builders (Reyes-Bonilla *et al*. 2013). Furthermore, it is an area with excellent representation of the coral ecosystems of the northern sector of the Eastern Tropical Pacific. The objective of this study was to analyze spatio-temporal variation in the assemblage of dominant bacterial groups associated with the mucus and tissue of *Pocillopora damicornis* and *Pocillopora verrucosa*, sea water and sediments in the MCP, as well as to correlate the variation in the diversity with environmental variables of the ecosystem.

Materials and methods

Study area and sampling

The MCP includes the coast of the states of Nayarit, Jalisco and Colima in Mexico (Fig. 1A). It is characterized by the presence of relatively productive waters in a transition zone of 3 oceanic currents: i) the Costa Rica Coastal Current (CRCC), which brings warm water from the south; ii) the California Current (CC), which carries cold water rich in nutrients in a north-south direction; and iii) the Gulf of California Current. This allows the occurrence of 3 seasons per year (Wyrtki 1966). Samples were taken in August 2013 and January 2014 in 6 sites along the MCP: i) Costa Fragata Somero (CFS) in Isla Isabel, which is located to the north and in the mouth of the Gulf of California (Fig. 1B); ii) Zona de Restauración (ZR) in the Islas Marietas, located in the northeast of Bahía de Banderas (Fig. 1C); iii) Pelícanos (P) in Bahía Chamela, located on the central coast of Jalisco (Fig. 1D); iv) Cuastecomatito (CU) in Bahía Cuastecomates-Punta Melaque, located on the southern coast of Jalisco (Fig. 1E); v) Carrizales (CRZ) in Bahía Ceníceros; and vi) Punto B (PB) in Bahía Santiago, both found in Colima (Fig. 1F).

Samples were taken in 3 apparently healthy colonies of *P. damicornis* and *P. verrucosa* from between 1 and 6 m in depth at each site (Fig. 1). From each colony, a fragment of length 2-3 cm was collected and placed in a sterile plastic bag with sea water for transportation to land. Mucus was collected from each fragment with a sterile cotton bud and stored in a 2 ml cryovial with sterile sea water (Guppy & Bythell 2006). Tissue was obtained by removal with pressurized air at ambient temperature (Bourne & Munn 2005). Samples were preserved in liquid nitrogen until subsequent processing. In addition, triplicate

samples of the sea water were taken at each site using KIMAX sterile glass bottles at a distance of ~10 cm from each coral colony. The sea water was filtered with Sterivex nitrocellulose membranes of 0.22 mm (Millipore, Billerica). Finally, triplicate samples of the sediment were taken at each site, using 50 ml conical polypropylene tubes at ~10 cm from the colonies. The filters with the microorganisms retained from the sea water and the sediment samples were preserved in liquid nitrogen for transportation to the laboratory.

DNA extraction and purification

The DNA was extracted from the sea water and sediment samples using the kit UltraCleanSoil DNA (MoBio, Carlsbad, CA). The DNA of the mucus and tissue was obtained using the modified protocol of Ausubel (2002). Purification of the DNA was performed with the kit Wizard DNA purification (Promega, Madison, WI). The quality and quantity of the DNA was determined with an Epoch nanodrop (260/280) and visualization was performed on 1% agarose gels. In total, DNA was extracted from 216 samples, corresponding to 36 tissues and 36 mucus samples from each coral species, as well as 36 sea water and 36 sediment samples.

PCR amplification of 16S rDNA

Amplification of the 16S rDNA was performed with universal primers 27f (52-GAGTTTGATCCTGGCTCAG-32) and 1525r (52-AGAAAGGAGGTGATCCAGCC-32) for bacteria (Warneke *et al*. 2007). The final volume per PCR reaction was 50 ml and consisted of: 0.1 mM of each primer, 9 mL of 10X concentrated PCR buffer, 50 mM/L of Tris-HCl at pH 8.2, 18 mM/L of $MgCl_2$, 500 mM/L KCl, 2 ml of DNTPs (10 mM/L), 1 mL of DNA at 5 mM, 1.25 U of GoTaqFlexi DNA polymerase (Promega, Madison WI) and double distilled water. Amplification was performed in a Apollo DNA Cycler® thermocycler (NyxTechnik Inc.) under the following conditions: 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 2 min, after which a final elongation step at 72°C for 10 min was performed. The PCR products were purified with the Wizard DNA purification kit (Promega, Madison, WI).

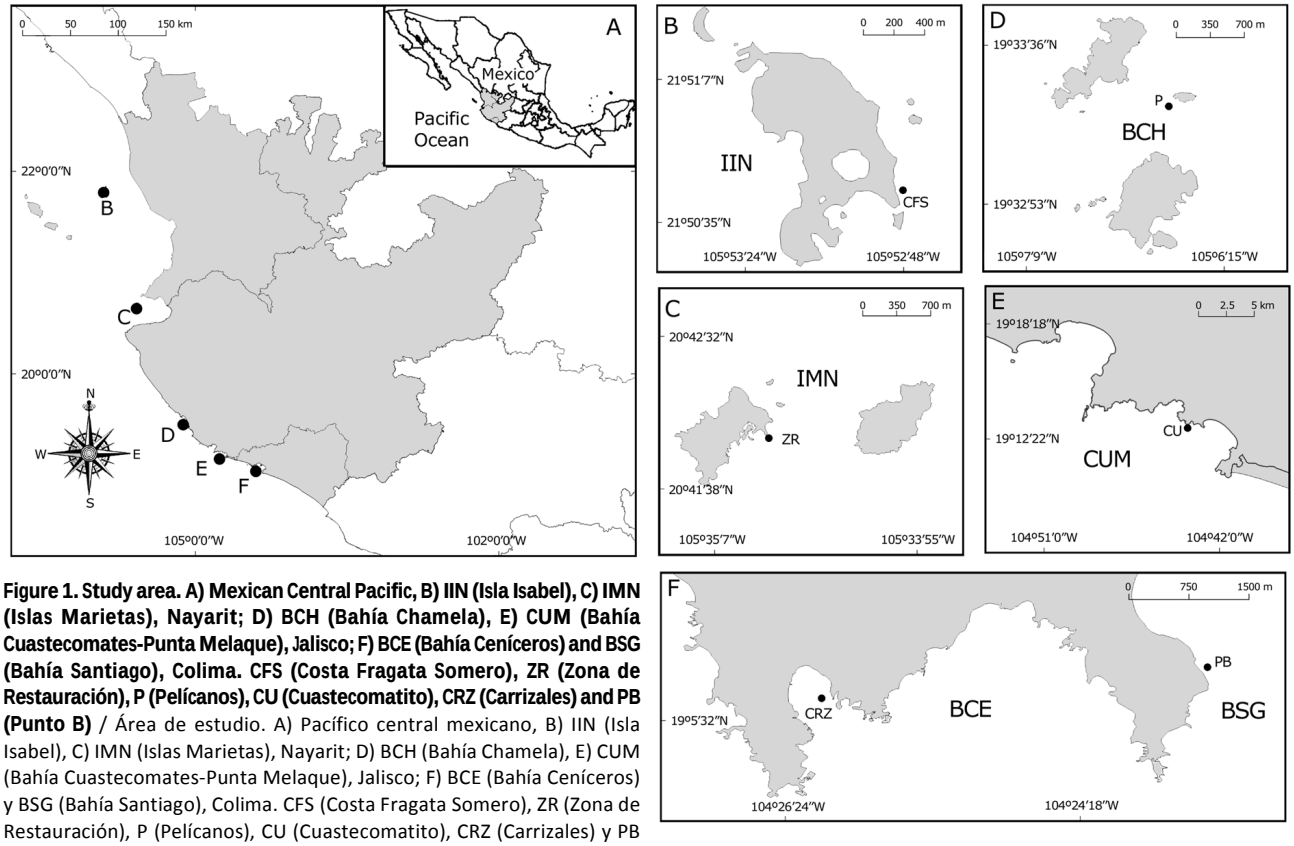

Figure 1. Study area. A) Mexican Central Pacific, B) IIN (Isla Isabel), C) IMN (Islas Marietas), Nayarit; D) BCH (Bahía Chamela), E) CUM (Bahía Cuastecomates-Punta Melaque), Jalisco; F) BCE (Bahía Ceníceros) and BSG (Bahía Santiago), Colima. CFS (Costa Fragata Somero), ZR (Zona de Restauración), P (Pelícanos), CU (Cuastecomatito), CRZ (Carrizales) and PB (Punto B) / Área de estudio. A) Pacífico central mexicano, B) IIN (Isla Isabel), C) IMN (Islas Marietas), Nayarit; D) BCH (Bahía Chamela), E) CUM (Bahía Cuastecomates-Punta Melaque), Jalisco; F) BCE (Bahía Ceníceros) y BSG (Bahía Santiago), Colima. CFS (Costa Fragata Somero), ZR (Zona de Restauración), P (Pelícanos), CU (Cuastecomatito), CRZ (Carrizales) y PB (Punto B)

DGGE analysis

The V3 region was amplified by PCR with the primers 341f-GC (52-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGCACGGGGGCCCTACGGGAGGCAGCAG-3´) and 907r (52-CCGTCAATTCMTTTGAGTTT-3´) (Muyzer *et al*. 1993). The reaction mixture was prepared under the same conditions as described above. Amplifications were conducted with a touchdown protocol (Ferris *et al*. 1996): 94°C for 2 min, followed by 10 cycles of 94°C for 30 s, 30 cycles of 65°C (decreasing by 1°C in each cycle) and 72°C for 45 s, followed by 20 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 45 s 2 min, after which a final elongation step at 72°C for 5 min.

The PCR products were analyzed in a DGGE TTGEK-2401-220 (CBS Scientific Company) system. The PCR products were run on 8% acrylamide gels with a 30-70% linear gradient of urea-formamide, using 1X TAE buffer. Electrophoresis was conducted at 60°C and 70 volt for 16 h. Gels were removed and stained for 10 min with an SYBR Gold nucleic acid stain (Molecular Probes Inc., Eugene, OR) in 1XTAE buffer. Gels were distained by rinsing with 1X TAE buffer and subsequently photographed using a UV transilluminator.

RFLP analysis

The PCR amplifications products of 16S rDNA were digested with the restriction enzymes Alu I (Promega) and Hae III (Promega). The restriction reactions for both enzymes were prepared with NEB Buffer 2 (Promega), 10U of the enzyme and 15 ml of PCR product. The samples were incubated at 37°C for 6 h and analyzed in 1.5% agarose gels in 1X TAE buffer at 85 volt for 40 min (Grimont & Grimont 1986).

Environmental variables

Measurements were taken in triplicate of sea surface temperature (SST), dissolved oxygen and salinity with the probes YSI-55 and YSI-30. In order to estimate the concentration of ammonium, nitrate + nitrite, phosphate and silicate, three sea water samples were taken (in 30 ml Sarstedt polypropylene tubes) per site. These samples were filtered with 2.5 cm fiberglass discs of pore size 0.2 mm (GF/F Whatman), which had been previously oven-treated for 2 to 3 h at 450°C. These were then placed in sterile 30 ml polypropylene jars and stored at -25°C until subsequent processing in a Skalar Flowanalyzer CFA SAN plus nutrient analyzer. Granulometry (determination of percentage of gravel, sand, clays and silt) was performed in the sediments following the method of Buoyocoz (1928).

The benthic structure was recorded in the sampling sites using 3 linear transects, each of 20 m in length. Along each transect, 5 quadrats of 1 m^2 were placed equidistantly (~5 m) to record the coverage of hermatypic corals, soft corals, hydrocorals, articulated and encrusting calcareous algae, fleshy macroalgae, rubble, rock, sand, dead coral and algal turfs.

Statistical analysis

One-way analyses of similarity (ANOSIM), based on Sorensen similarity matrices, were conducted to evaluate variation in the number of Operational Taxonomic Units (OTU) among *Seasons* (SE, 2 levels: Summer and Winter), *Sites* (SI, 6 levels: Costa Fragata Somero, Zona de Restauración, Pelícanos, Cuastecomatito, Carrizales and Punto B) and *Substrates* (SU, 6 levels: mucus and tissue of *P. damicornis* and *P. verrucosa*, sea water and sediments). Likewise, other ANOSIM were calculated to contrast the variation in the OTUs among sites per substrate type, as well as among the substrates within each site. Statistical significance was tested with 9.999 permutations in Primer V6.1+PERMANOVA (Clarke & Gorley 2006).

Presence/absence matrices were constructed with the DGGE and RFLP banding profiles to perform non-metric multidimensional scaling (NMDS) analysis, based on Sorensen similarities (Clarke & Gorley 2006). The DGGE matrix was used to determine the similarity of the bacterial assemblages for each of the substrates among all of the sampling sites, as well as to estimate the similarity of the assemblages of all the substrates for each sampling site (local level). The RFLP matrix served to corroborate the findings of the NMDS analysis of the DGGE matrix.

The relationship between the bacterial assemblage and the environmental-spatial variables was evaluated with canonical additive partitions, based on canonical correspondence analysis (CCA), assuming a unimodal relationship among them (Legendre & Legendre 1998). The biological variables corresponded to the *Y* matrices constructed with the number of OTUs present. The *X* matrices were constructed with all of the aforementioned environmental variables. With the spatial variables, a *W* matrix was constructed using a superficial trend analysis based on geographic coordinates in UTM represented as a third order polynomial (Legendre & Legendre 1998). The CCA identified which *X* and *W* variables best explained the variation of *Y*. The models were conducted at the level of sites based on the results of the ANOSIM. The Trace statistic was used to analyze the fit of the model, since it represents the variation of *Y* explained by all of the canonical axes. In order to reduce multicollinearity among predictive variables, Pearson correlations (r) were used, eliminating those with $r \geq 0.90$.

Likewise, a variance inflation factor (VIF) ≤ 10 was used. Statistical significance was tested with 9.999 permutations under a reduced model in CANOCO v4.5 (Ter Braak & Smilauer 2002).

Results

DGGE patterns

The DGGE banding pattern showed 7 to 13 bands per sample with a total of 20 bands. It was assumed that each band observed corresponded to one OTU. The highest number of bands (13) was found in the sediments of Costa Fragata Somero and Zona de Restauración in summer and winter, and in Pelícanos in winter. In the tissue of *P. damicornis* of Costa Fragata Somero, Zona de Restauración, Pelícanos and Cuastecomatito, 12 bands were observed. The lowest number was found in the sea water of Costa Fragata Somero and Zona de Restauración (7), Pelícanos and Cuastecomatito (8) (Appendix A, Table A1). The banding profiles (DGGE) of the bacterial assemblage in the tissues and mucus of *P. damicornis* and *P. verrucosa*, sea water and sediment differed among some sites, while the banding pattern among the replicates of the substrates was identical (Appendix A, Fig. A1). However, none of the sites presented the total number of bands found (20 OTUs). Some were exclusive; 17 bands in sea water and sediments, 18, 19 and 20 in sediments. In contrast, 2, 7, 10 and 14 bands were present in most of the substrates and sites, with the exception of sea water (Appendix A, Table A1).

Variation in the bacterial assemblages

The ANOSIM showed significant spatial variations in the composition of bacterial OTUs among sites and substrates, but did not show significant temporal variation (Appendix B, Table B1). The pairwise comparisons of the factor *Substrate* showed significant differences. In contrast, in the factor *Site*, it was observed that most of the sites have a particular bacterial assemblage, except between Costa Fragata Somero and Zona de Restauración. In terms of OTU, the most similar sites were Carrizales and Punto B. These sites were also those most dissimilar to the rest of the sites (Appendix B, Table B1).

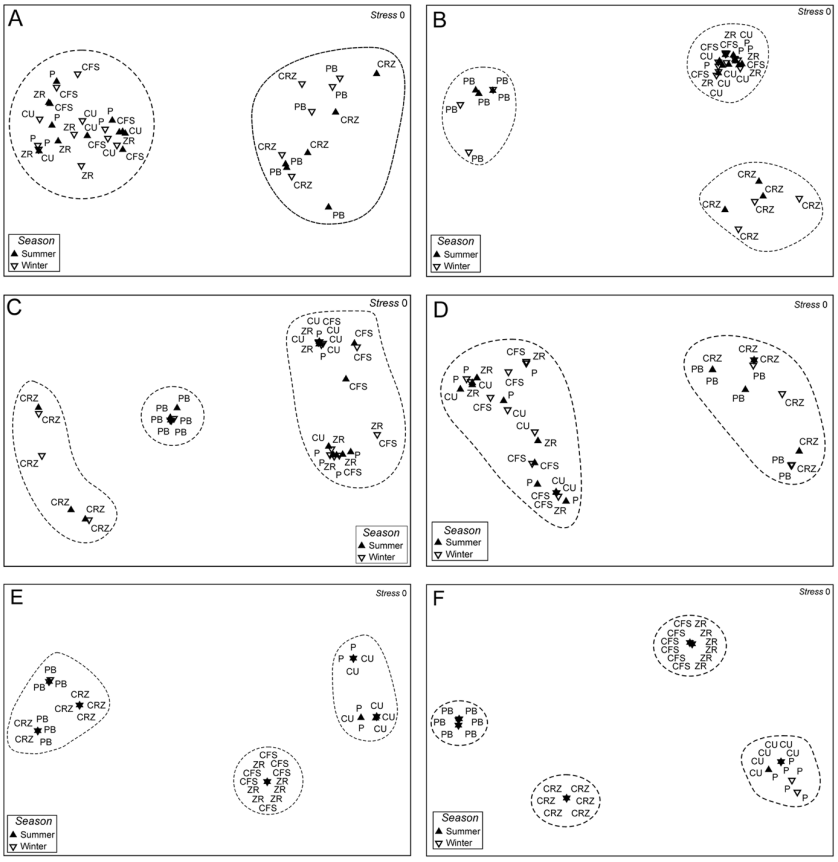

Figure 2. Nonmetric multidimensional scaling (NMDS) of the substrates among the sites from Mexican Central Pacific. A) *P. damicornis* tissue; B) *P. verrucosa* tissue; C) *P. damicornis* mucus; D) *P. verrucosa* mucus; E) Sea water and F) Sediments. Codes: CFS (Costa Fragata Somero), ZR (Zona de Restauración), P (Pelícanos), CU (Cuastecomatito), CRZ (Carrizales) and PB (Punto B) / Ordenamiento multidimensional no métrico (NMDS) de los sustratos entre los sitios del Pacífico central mexicano. A) Tejido de *P. damicornis*; B) Tejido de *P. verrucosa;* C) Mucus de *P. damicornis*; D) Mucus de *P. verrucosa*; E) Agua de mar y F) Sedimentos. Códigos: CFS (Costa Fragata Somero), ZR (Zona de Restauración), P (Pelícanos), CU (Cuastecomatito), CRZ (Carrizales) y PB (Punto B)

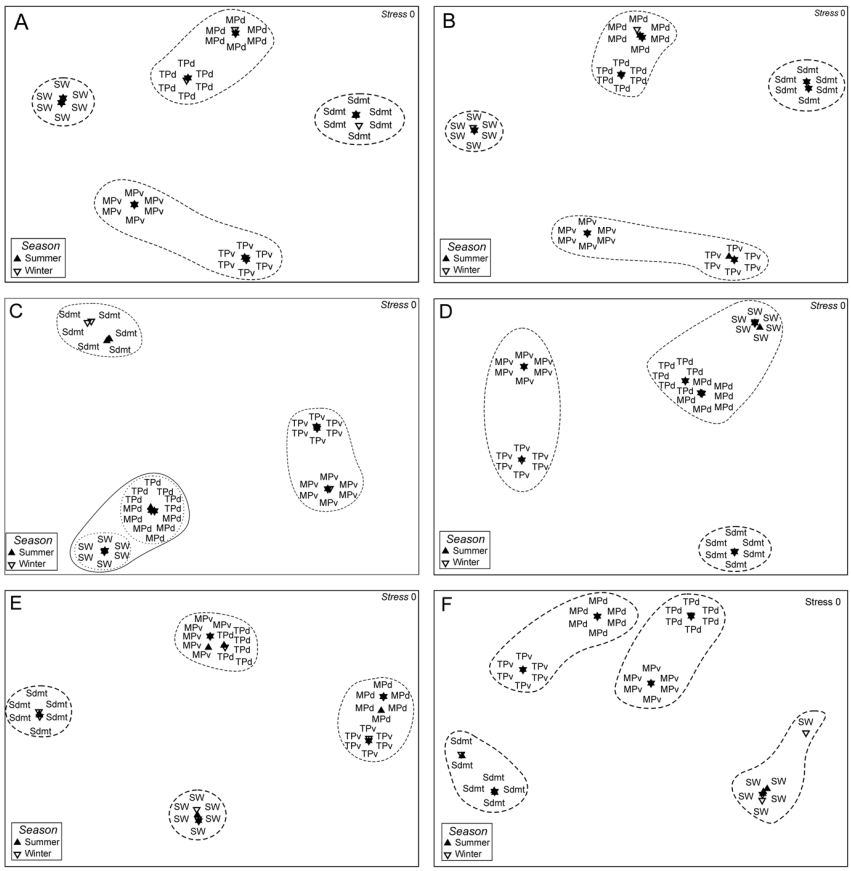

Figure 3.Non-metric multidimensional scaling (NMDS) of the substrates within the sites from Mexican Central Pacific. A) Costa Fragata Somero, B) Zona de Restauración, C) Pelícanos, D) Cuastecomatito, E) Carrizales and F) Punto B. Code: TPd (*P. damicornis* tissue), TPv (*P. verrucosa* tissue), MPd (*P. damicornis* mucus), MPv (*P. verrucosa* mucus), SW (Sea water) and Sdmt (Sediments) / Ordenamiento multidimensional no métrico (NMDS) de los sustratos dentro de los sitios del Pacífico central mexicano. A) Costa Fragata Somero, B) Zona de Restauración, C) Pelícanos, D) Cuastecomatito, E) Carrizales y F) Punto B. Códigos: TPd (Tejido de *P. damicornis*), TPv (Tejido de *P. verrucosa*), MPd (Mucus de *P. damicornis*), MPv (Mucus de *P. verrucosa*), SW (Agua de mar) y Sdmt (Sedimentos)

Analysis of each substrate at regional level showed significant differences among sites (Appendix B, Tables B2). The pairwise comparisons of the OTUs of *P. damicornis* tissues and *P. verrucosa* mucus showed that Costa Fragata Somero, Zona de Restauración, Pelícanos and Cuastecomatito are similar in composition of bacterial OTUs, but differ in this regard to Carrizales and Punto B in Colima. The bacterial assemblage of these latter sites did not present variation. In the *P. verrucosa* tissues and *P. damicornis* mucus, the composition of the microbiota of the sites in Nayarit and Jalisco were similar to each other and different to those of Colima, which also presented differences among themselves. The pairwise comparisons on the sea water samples showed that the bacterial assemblages of the sites belonging to the same state did not present significant differences. In the sediments, significant differences were found in the composition of OTUs in most of the sites, except for Costa Fragata Somero *vs* .Zona de Restauración in Nayarit (Appendix B, Table B2).

The NMDS ordination of the tissue samples of *P. damicornis* showed that Punto B and Carrizales were more similar in term of OTU, but also dissimilar to the rest of the sites (Fig. 2A). In contrast, the tissue samples of *P. verrucosa* showed that Carrizales and Punto B were dissimilar to each other and to the rest of the sites (Fig. 2B). The composition of OTUs of the tissue samples of both coral species was similar for the sites that corresponded to the states of Jalisco and Nayarit, but different to those of Colima (Fig. 2A-B). In the mucus of *P. damicornis*, greater similarity of OTU was observed among the sites from Jalisco and Nayarit, but these differed to those of Carrizales and Punto B, with the latter sites also found to be different to each other (Fig. 2C). The mucus samples of *P.*

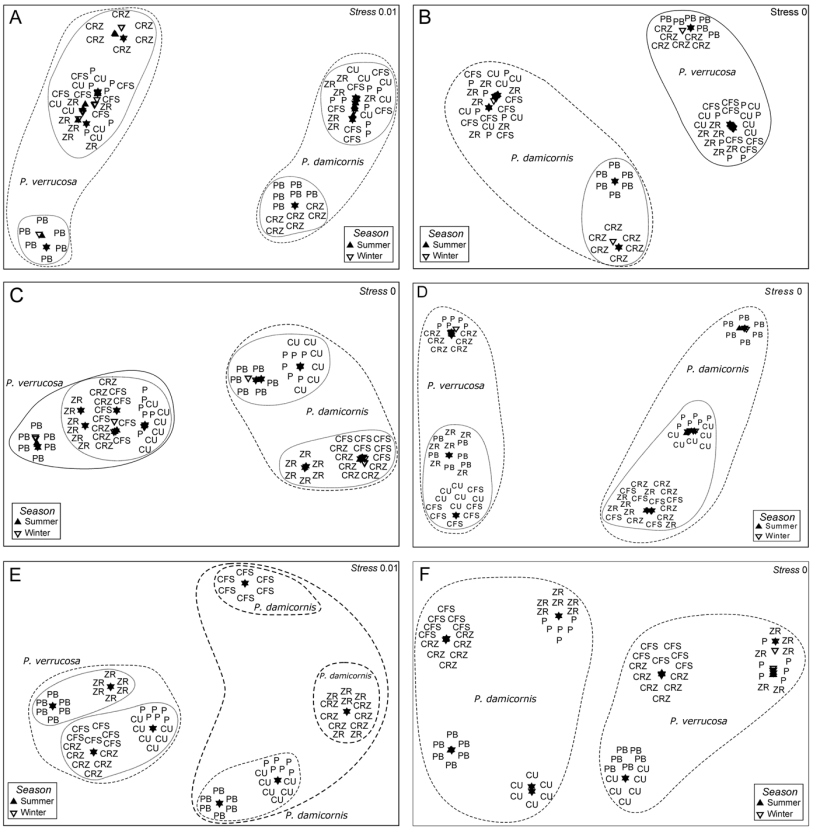

Figure 4. Non-metric multidimensional scaling (NMDS) of the operational taxonomic units (OTUs) in the tissue (A, C and E) and mucus (B, D and F) of *P. damicornis* and *P. verrucosa*. A-B) Denaturing Gradient Gel Electrophoresis (DGGE), C-D) Restriction Fragment Length Polymorphism (RFLP) with the enzyme *Alu I* and, E-F) RFLP with the enzyme *Hae III*. CFS (Costa Fragata Somero), ZR (Zona de Restauración), P (Pelícanos), CU (Cuastecomatito), CRZ (Carrizales) and PB (Punto B) / Ordenamiento multidimensional no métrico (NMDS) de las unidades taxonómicas operacionales (OTUs) en los tejidos (A, C y E) y mucus (B, D y F) de *P. damicornis* y *P. verrucosa*. A-B) Electroforesis en gel con gradiente desnaturalizante (DGGE), C-D) Polimorfismo de longitud de fragmentos de restricción (RFLP) con la enzima *Alu I* y, E-F) RFLP con la enzima *Hae III*. CFS (Costa Fragata Somero), ZR (Zona de Restauración), P (Pelícanos), CU (Cuastecomatito), CRZ (Carrizales) y PB (Punto B)

verrucosa did not show differences between Carrizales and Punto B, but both were different to the rest of the sites, which were similar to each other in terms of OTU (Jalisco and Nayarit) (Fig. 2D). In the sea water, 3 groupings were observed in the NMDS. The samples of the sites of each state were more similar to each other, but differences were evident in the composition of OTUs among sites of different states (Fig. 2E). In the sediment, a pattern similar to that of the sea water samples was found, except that the sites from Colima (Carrizales and Punto B) were different (Fig. 2F).

The results of the ANOSIM provided evidence that the composition of bacterial OTUs differed among the substrates of the sites. The pairwise comparisons showed a specificity of bacterial assemblage per substrate (Appendix B, Table B3). The NMDS of these analyses showed a similar pattern in all of the sites. It was found in Costa Fragata Somero, Zona de Restauración, Pelícanos and Cuastecomatito that the tissue and mucus samples grouped themselves according to each coral species (Fig. 3A-D). However, this pattern was not observed in Carrizales and Punto B, where tissue samples of *P. verrucosa* were similar to mucus samples of *P. damicornis* and, equally, mucus samples of *P. verrucosa* were similar to tissue samples of *P. damicornis* (Fig. 3E-F). The composition of OTUs in the sediments differed to that found in coral species and sea water in each sampling site. This was similar in the sea water, with the exception of Pelícanos and Cuastecomatito, where it was observed that the composition of OTUs was more similar between the mucus and tissue of *P. damicornis* (Fig. 3C-D).The NMDS ordinations of the pattern of variation of DGGE bands showed that the composition of OTUs of mucus and tissue was different in both coral species (Fig. 4A-B). This was corroborated through

the NMDS analysis of the RFLPs (enzymes Alu I and Hae III) of the DGGE banding pattern, showing separation between the species in terms of their OTU composition (Fig. 4C-F).

Canonical additive partitions

The bacterial structure found in the tissue and mucus of both coral species, as well as in the sea water and sediments was explained only by the pure spatial component [a], since the spatial variables (*W*) did not show a significant relationship to the bacterial OTUs. For this reason, the spatially structured [b] and purely spatial [c] environmental variation did not contribute in the analyses. The total explained variation [*a*+*b*+*c*] ranged from 78.06 to 95.37%, with statistical significance values of between $0.0001 < P < 0.0344$ (Table 1). In the mucus of *P. damicornis* and tissue of *P. verrucosa*, the variables that explained the variation of the composition of the bacteria were coverage of live coral, sponges and fleshy macroalgae (Table 1). These variables also explained the variation in the composition of the bacterial OTUs of the tissue of *P. damicornis*, mucus of *P. verrucosa* and sea water, but with the inclusion of salinity. In contrast, the variation in the sediments was explained by the sandy texture and coverage of sponges and live coral (Table 1). The contributions of the predictive variables of this variation are presented in Appendix B, Table B4.

Discussion

In this study, a total of 20 OTUs were observed, where each represented a bacterial species (Muyzer *et al*. 1993). It was found that *P. damicornis* presented a higher diversity of bacterial groups than *P. verrucosa*. Differences in the bacterial diversity among coral species have been documented in Caribbean species (Morrow *et al*. 2012), as well as those of the Australian Great Barrier Reef (GBR) (Kvennefors *et al*. 2010). However, each coral species maintains a characteristic bacterial microbiota, since they form species-specific associations with certain bacterial groups (Littman *et al*. 2009, McKew *et al*. 2012), modifying these associations in relation to stress events, such as bleaching (Bourne *et al*. 2008). Similarly, in the GBR, bacterial group specificity was identified in *Acropora millepora* (Littman *et al*. 2009), *A. hyacinthus* and *Stylophora pistillata* (Kvennefors *et al*. 2010) and, in the Mexican Caribbean, in *Porites astreoides* and *A. palmata* (McKew *et al*. 2012). The results of this study provide evidence that the tissue and mucus of *P. damicornis* and *P. verrucosa* have a specificity of bacterial groups that are dominant in the Mexican Central Pacific, demonstrating a species-specific coral-microbial relationship.

Table 1. Canonical additive partition of the variation of bacterial operational taxonomic units in the different substrates in the coral reef ecosystem in the Mexican Central Pacific / Partición aditiva de la variación de las unidades taxonómicas operacionales bacterianas en los diferentes sustratos en los ecosistemas de coral del Pacífico central mexicano

	[*a*]	[*b*]	[*c*]	[*d*]	Total. Exp [*a*+*b*+*c*]	Env. Var. [*a*+*b*]	Spat. Var. [*b*+*c*]
Tissue of *Pocillopora damicornis*	95.12	0.0	0.0	4.88	95.12	95.12	0.0
					$P = 0.0224$	$P = 0.0224$	*n.s*
	Environmental variables: RSC, FMA, LCC and SAL						
Tissue of *Pocillopora verrucosa*	78.06	0.0	0.0	21.94	78.06	78.06	0.0
					$P = 0.0107$	$P = 0.0107$	*n.s*
	Environmental variables: RSC, FMA and LCC						
Mucus of *Pocillopora damicornis*	85.06	0.0	0.0	14.94	85.06	85.06	0.0
					$P = 0.0343$	$P = 0.0343$	*n.s*
	Environmental variables: RSC, FMA and LCC						
Mucus of *Pocillopora verrucosa*	86.78	0.0	0.0	13.22	86.78	86.78	0.0
					$P = 0.0262$	$P = 0.0262$	*n.s*
	Environmental variables: RSC, FMA, LCC and SAL						
Sea water	95.37	0.0	0.0	4.63	95.37	95.37	0.0
					$P = 0.0002$	$P = 0.0002$	*n.s*
	Environmental variables: RSC, FMA, LCC and SAL						
Sediments	83.71	0.0	0.0	16.29	83.71	83.71	0.0
					$P = 0.0235$	$P = 0.0235$	*n.s*
	Environmental variables: % TA, RSC and LCC						

Notes: Environmental variables were included in X matrices, (Env. Var) with the codes: LCC live coral cover, RSC sponge, FMA fleshy macroalgae, SAL salinity and %TA percentage of sandy texture, n.s. not statistically significant ($P > 0.05$)

The [a + b+ c] fraction= Y vs. X and W; [a + b]= Y vs. X; [b + c]= Y vs. W; [a] = pure environmental variation; [b]= spatially-structured environmental variation; [c]= pure spatial variation; and [d]= unexplained variation. Total Exp. Total explained variation [a + b + c]

This study found that the bacterial assemblage at local level was different between the mucus and tissue of the corals studied, as well as between the samples of sediment and sea water. This supports the theory that the corals have a different microbiota between their compartments and the surrounding environment (Kvennefors *et al*. 2010, Krediet *et al*. 2013). It was shown that the composition of dominant bacteria in both coral species differed to that of the sea water, which had previously been observed in *P. damicornis* (Bourne & Munn 2005). It is thought that some members of the bacterial assemblage of the sea water act as seeds of the coral microbiota, such that these may acquire certain specific bacteria from the environment without vertical transmission (Sunagawa *et al*. 2010). In this way, the bacteria of the coral mucus could be acquired from the surrounding sea water (Guppy & Bythell 2006), or indeed by re-suspension of benthic sediments (Sweet *et al*. 2011). It has also been suggested that the coral mucus is constituted by transitory bacteria that originate from other environmental sources, causing the mucus to present similar ribotypes to those of the sea water and sediment (Bourne & Webster 2013).

No significant similarity was found between the bacteria of the coral and of the sediments, although the bacteria present in the sediments could colonize the coral surfaces (Schöttner *et al*. 2013). The sediments could serve as a reservoir of opportunistic pathogenic agents that can generate diseases and mortality in the corals. For this reason, study of the bacterial assemblages associated with the sediments in coral reefs contributes to the understanding of the synergy between the bacteria of the sediment and those of the corals (Carlos *et al*. 2013), particularly when changes are evaluated in bacterial composition in the corals (Guppy & Bythell 2006). It has been documented that the resident bacteria of the coral compete for nutrients and ecological niches with other invasive microbial in the mucus and tissue (Littman *et al*. 2009). It is considered that specific invertebrate-microbial associations play an important role in the maintenance of healthy coral and protect it from invasion by pathogenic microbes (Li *et al*. 2014). For this reason, changes in bacterial consortia can predict the appearance of signs of disease and can be used as indicators of coral reef health (Bourne & Webster 2013).

Other studies have shown that the bacterial assemblages associated with the corals present spatio-temporal variations (Lee *et al*. 2012, Krediet *et al*. 2013). However, the spatial analysis indicated that the sites of Nayarit and Jalisco did not differ in terms of bacterial composition and presented specificity despite the geographic distance. In the sites of Colima, the dominant groups of bacteria differed from those of the other sites. Furthermore, the bacterial composition of the sea water and sediments differentiates in a north-south latitudinal gradient (Nayarit, Jalisco and Colima). This coincides with the changes observed in the bacterial assemblages of *Orbicella* (formerly *Montastraea*) and *P. astreoides* in sites in the Caribbean (Morrow *et al*. 2012). In *Acropora* and *Porites* of the Mexican Caribbean and Indonesia, McKew *et al*. (2012) demonstrated an important spatial variation in the assemblages of geographically different sites, but did not find differences between the species and the surrounding sea water. Similarly, Littman *et al*. (2009) reported that the assemblages associated with *A. millepora* in the GBR were grouped according to the geographic location of the sites and were not associated with coral species. This indicates that the dominant bacteria differ among geographically distant corals. Furthermore, it was observed that the bacterial assemblage was stable in summer and winter, suggesting that this was due to fluctuations in temperature of <10°C. In contrast, the assemblage associated with colonies of *Oculina patagonica* in the Mediterranean Sea varied between summer and winter because of the larger changes in temperature that occur in this area (>20°C) (Koren & Rosenberg 2006). In this study, the NMDS and ANOSIM based on DGGE profiles suggest that there is no important temporal variation in the bacterial assemblages among the coral species and substrates studied. However, it is necessary to consider that the fluctuations of temperature between summer and winter in the study area were less than 5°C, which could explain such 'stability' in both seasons. Previous studies suggest that the bacterial assemblages of healthy coral soften respond to seasonal fluctuations (Koren & Rosenberg 2006).

Different studies have explored the effects of environmental parameters on the density, diversity and microbial composition of coral ecosystems (Lee *et al*. 2012, Kelly *et al*. 2014, Li *et al*. 2014). For example, Bourne *et al*. (2008) correlated the presence of *Vibrio* spp. with increased temperatures and decreased density of zooxantellae of *A. millepora* during a bleaching event on Magnetic Island (GBR). All of this supports the hypothesis that 'everything is everywhere, but the environment selects' (Baas-Becking 1934). Nevertheless, Guppy & Bythell (2006), using DGGE profiles, did not find a correlation between the structure of the bacterial assemblage associated with the mucus of *O. faveolata* and variables considered representative of sea water quality.

In the sediments, changes in bacterial composition were correlated with the coverage of macroalgae, sponges and live coral. The high coverages of fleshy macroalgae have a negative effect on the health of the coral reefs since they generate abrasion and produce a shade effect on the coral tissues (McCook *et al*. 2001). Likewise, they cause elevated levels of dissolved organic carbon (DOC) due to the excess of photosynthates they release in the water column. This acts to

alter the equilibrium between the corals and their associated microbiota and accelerates the growth of coral mucus bacteria (Ceh *et al*. 2011). Kelly *et al*. (2014) indicate that sites with higher coverages of fleshy macroalgae present a high abundance of *Gammaproteobacteria* (*Enterobacterial* and *Pseudomonadal*), while a greater abundance of *Alphaproteobacteria* is associated with sites with higher live coral cover. In this sense, evidence is provided by the fact that Carrizales and Punto B present the highest coverage of fleshy macroalgae and direct supply of nutrients through rainwater runoff compared to the rest of the sites (apart from Costa Fragata Somero) (Appendix B, Table B4). This could explain the difference between the bacterial assemblages of these sites and those of the other studied sites.

The bacterial assemblage associated with the sponges is dynamic and, as with the corals, is interconnected with that of the sea water and sediments, suggesting that the bacterial microbiota of corals in sites with high coverage of sponges is possibly influenced by this condition (Webster & Taylor 2012). In this sense, and despite the fact that the sites Carrizales and Punto B in Colima differed from the other sites, the greatest coverage of sponges was recorded only in the site Punto B and it is probable that this can explain why the bacterial assemblage of Punto B differed among substrates and sites.

Li *et al*. (2014) observed that rainfall and dissolved oxygen were the environmental parameters that most influenced the variation of the bacterial assemblage of the mucus, tissue and skeleton of *P. lutea* in the Luhuitou reef, in northern China. Chen *et al*. (2011) found that rain was the factor most correlated with the bacterial assemblage of *Isopora palifera* in Tan-Tzei Bay southeast of Taiwan. The observed influence of rainfall supports the notion that some bacteria of the coral could be derived from terrestrial soils. However, in this study, no correlation was found between the quantity of dissolved oxygen (data not presented) and variation in bacterial groups. Moreover, the influence of rainfall and its effect on the runoff of continental water riverine influx were not evaluated.

This study represents the first effort made to understand the structure and spatio-temporal variation of the bacterial assemblages in the two most abundant coral species in the Mexican Central Pacific. It was determined that the corals *P. damicornis* and *P. verrucosa* have a specificity of dominant bacteria, which are in most cases maintained despite geographic distance and temporal variation. The bacteria vary in the compartments of the holobiont and environmental variables, such as coverage of live coral, macroalgae and sponges; play a significant role in the variation of the composition of dominant bacteria in corals, sea water and sediments. In order to further understand the magnitude of the change in structure and dynamics of the bacterial assemblages in the corals of the Mexican Central Pacific and their potential relationship with the health of the reef ecosystem, future studies could employ metagenomic techniques that allow estimation of the less abundant OTUs, the 'Rare Biosphere'.

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Appendix A

Table A1. Number of bacterial OTUs (bands) present in the tissue and mucus *P. damicornis* and *P. verrucosa*, sea water and sediment. Code: CFS: Costa Fragata Somero; ZR: Zona de Restauración; P: Pelícanos; CU: Cuastecomatito; CRZ: Carrizales and PB: Punto B / Número de OTUs bacterianos (bandas) presentes en el tejido y mucus *P. damicornis* y *P. verrucosa*, agua de mar y sedimento. Códigos: CFS: Costa Fragata Somero; ZR: Área de Restauración; P: Pelícanos; CU: Cuastecomatito; CRZ: Carrizales y PB: Punto B

Substrates	Sites	Season	Bands																			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total
Tissue	CFS	Summer	1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	0	0	0	0	0	12
P. damicornis	ZR		1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	0	0	0	0	0	12
	P		1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	0	0	0	0	0	12
	CU		1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	0	0	0	0	0	12
	CRZ		1	0	1	1	1	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0	10
	PB		1	0	1	1	1	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0	10
	CFS	Winter	1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	0	0	0	0	0	12
	ZR		1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	0	0	0	0	0	12
	P		1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	0	0	0	0	0	12
	CU		1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	0	0	0	0	0	12
	CRZ		1	0	1	1	1	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0	10
	PB		1	0	1	1	1	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0	10
Tissue	CFS	Summer	1	1	1	1	0	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	9
P. verrucosa	ZR		1	1	1	1	0	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	9
	P		1	1	1	1	0	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	9
	CU		1	1	1	1	0	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	9
	CRZ		1	1	1	1	0	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	9
	PB		1	1	0	0	0	0	1	1	0	1	1	1	1	1	0	0	0	0	0	0	9
	CFS	Winter	1	1	1	1	0	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	9
	ZR		1	1	1	1	0	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	9
	P		1	1	1	1	0	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	9
	CU		1	1	1	1	0	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	9
	CRZ		1	1	1	1	0	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	9
	PB		1	1	0	0	0	0	1	1	0	1	1	1	1	1	0	0	0	0	0	0	9
Mucus	CFS	Summer	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	10
P. damicornis	ZR		1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	10
	P		1	0	1	1	1	1	1	0	1	1	1	1	0	1	1	0	0	0	0	0	12
	CU		1	0	1	1	1	1	1	0	1	1	1	1	0	1	1	0	0	0	0	0	12
	CRZ		1	1	1	0	1	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	9
	PB		1	1	1	0	1	1	1	0	1	1	1	1	1	0	0	0	0	0	0	0	11
	CFS	Winter	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	10
	ZR		1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	10
	P		1	0	1	1	1	1	1	0	1	1	1	1	0	1	1	0	0	0	0	0	12
	CU		1	0	1	1	1	1	1	0	1	1	1	1	0	1	1	0	0	0	0	0	12
	CRZ		1	1	1	0	1	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	9
	PB		1	1	1	0	1	1	1	0	1	1	1	1	1	0	0	0	0	0	0	0	11

Table A1 continued. Number of bacterial OTUs (bands) present in the tissue and mucus *P. damicornis* and *P. verrucosa,* sea water and sediment. Code: CFS: Costa Fragata Somero; ZR: Zona de Restauración; P: Pelícanos; CU: Cuastecomatito; CRZ: Carrizales and PB: Punto B / Número de OTUs bacterianos (bandas) presentes en el tejido y mucus *P. damicornis* y *P. verrucosa,* agua de mar y sedimento. Códigos: CFS: Costa Fragata Somero; ZR: Área de Restauración; P: Pelícanos; CU: Cuastecomatito; CRZ: Carrizales y PB: Punto B

Substrates	Sites	Season	Bands																				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total
Mucus	CFS	Summer	1	1	1	0	1	1	0	1	0	1	1	0	1	0	0	0	0	0	0	0	9
P. verrucosa	ZR		1	1	1	0	1	1	0	1	0	1	1	0	1	0	0	0	0	0	0	0	9
	P		1	1	1	0	1	1	0	1	0	1	1	0	1	0	0	0	0	0	0	0	9
	CU		1	1	1	0	1	1	0	1	0	1	1	0	1	0	0	0	0	0	0	0	9
	CRZ		1	0	1	0	1	1	0	1	1	1	1	0	1	1	0	0	0	0	0	0	10
	PB		1	0	1	0	1	1	0	1	1	1	1	0	1	1	0	0	0	0	0	0	10
	CFS	Winter	1	1	1	0	1	1	0	1	0	1	1	0	1	0	0	0	0	0	0	0	9
	ZR		1	1	1	0	1	1	0	1	0	1	1	0	1	0	0	0	0	0	0	0	9
	P		1	1	1	0	1	1	0	1	0	1	1	0	1	0	0	0	0	0	0	0	9
	CU		1	1	1	0	1	1	0	1	0	1	1	0	1	0	0	0	0	0	0	0	9
	CRZ		1	0	1	0	1	1	0	1	1	1	1	0	1	1	0	0	0	0	0	0	10
	PB		1	0	1	0	1	1	0	1	1	1	1	0	1	1	0	0	0	0	0	0	10
Sea water	CFS	Summer	1	0	1	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	7
	ZR		1	0	1	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	7
	P		0	0	1	1	0	1	0	1	1	0	1	1	0	0	1	0	0	0	0	0	8
	CU		0	0	1	1	0	1	0	1	1	0	1	1	0	0	1	0	0	0	0	0	8
	CRZ		1	0	1	1	1	0	0	1	1	0	0	1	1	0	0	0	1	0	0	0	9
	PB		1	0	1	1	1	0	0	1	1	0	0	1	1	0	0	0	1	0	0	0	9
	CFS	Winter	1	0	1	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	7
	ZR		1	0	1	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	7
	P		0	0	1	1	0	1	0	1	1	0	1	1	0	0	1	0	0	0	0	0	8
	CU		0	0	1	1	0	1	0	1	1	0	1	1	0	0	1	0	0	0	0	0	8
	CRZ		1	0	1	1	1	0	0	1	1	0	0	1	1	0	0	0	1	0	0	0	9
	PB		1	0	1	1	1	0	0	1	1	0	0	1	1	0	0	0	1	0	0	0	9
Sediment	CFS	Summer	1	1	0	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	0	0	13
	ZR		1	1	0	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	0	0	13
	P		0	0	0	1	1	0	1	1	1	1	0	1	1	0	1	0	1	1	0	0	11
	CU		0	0	0	1	1	0	1	1	1	1	0	1	1	0	1	0	1	1	0	0	11
	CRZ		0	0	0	0	0	1	1	1	1	0	1	1	1	1	0	1	1	1	0	0	11
	PB		0	0	0	0	0	1	1	1	1	0	1	1	1	1	0	1	0	0	0	0	9
	CFS	Winter	1	1	0	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	0	0	13
	ZR		1	1	0	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	0	0	13
	P		0	0	0	1	1	0	1	1	1	1	0	1	1	0	1	0	1	1	1	1	13
	CU		0	0	0	1	1	0	1	1	1	1	0	1	1	0	1	0	1	1	0	0	11
	CRZ		0	0	0	0	0	1	1	1	1	0	1	1	1	1	0	1	1	1	0	0	11
	PB		0	0	0	0	0	1	1	1	1	0	1	1	1	1	0	1	0	0	0	0	9

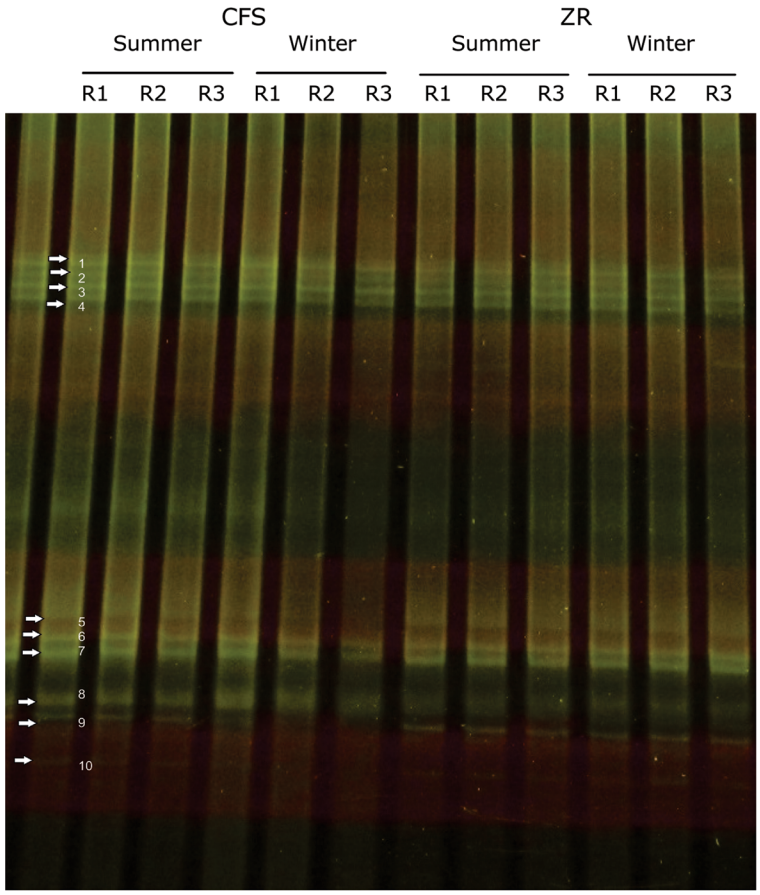

Figure A1. DGGE of PCR-amplified 16S rDNA fragments of *Pocillopora damicornis* mucus in summer and winter. Code: CFS: Costa Fragata Somero; ZR: Zona de Restauración; R1: Replica 1, R2: Replica 2 and R3: Replica 3. The arrows indicate the band number / Visualización de geles de DGGE a partir de fragmentos del ADNr 16S del mucus de *P. damicornis* colectadas en verano e invierno. Códigos: CFS: Costa Fragata Somero; ZR: Zona de Restauración; R1: Réplica 1, R2: Réplica 2 y R3: Réplica 3. Las fechas indican el número de banda

Appendix B

Table B1. One-way similarity analysis (ANOSIM), results from comparing the bacterial OTUs among *Season, Substrates* and *Sites* in the corals reef ecosystem on Mexican Central Pacific. Code: CFS (Costa Fragata Somero), ZR (Zona de Restauración), P (Pelicanos), CU (Cuastecomatito), CRZ (Carrizales) and PB (Punto B). TPd (Tissue of *P. damicornis*), TPv (Tissue of *P. verrucosa*), MPd (Mucus of *P. damicornis*), MPv (Mucus of *P. verrucosa*), SW (Sea water) and Sdmt (Sediment) / Resultados del análisis de similitudes (ANOSIM) para comparar los OTUs bacterianos entre la *Estación*, *Sustratos* y los *Sitios* en los ecosistemas de coral del Pacífico central mexicano. Códigos: CFS (Costa Fragata Somero), ZR (Zona de Restauración), P (Pelicanos), CU (Cuastecomatito), CRZ (Carrizales) y PB (Punto B). TPd (Tejido de *P. damicornis*), TPv (Tejido de *P. verrucosa*), MPd (Mucus de *P. damicornis*), MPv (Mucus de *P. verrucosa*), SW (Agua de mar) y Sdmt (Sedimentos)

	R statistic	Significance level
Global test		
Season	- 0.0009	0.9850
Substrates	0.7460	0.0001
Sites	0.6590	0.0001
Pairwise comparisons among *Substrates*		
TPd *vs* TPv	1	0.0001
TPd *vs* MPd	1	0.0001
TPd *vs* MPv	1	0.0001
TPd *vs* SW	1	0.0001
TPd *vs* Sdmt	1	0.0001
TPv *vs* MPd	1	0.0001
TPv *vs* MPv	1	0.0001
TPv *vs* SW	1	0.0001
TPv *vs* Sdmt	1	0.0001
MPd *vs* MPv	1	0.0001
MPd *vs* SW	1	0.0001
MPd *vs* Sdmt	1	0.0001
MPv *vs* SW	1	0.0001
MPv *vs* Sdmt	1	0.0001
SW *vs* Sdmt	1	0.0001
Pairwise comparisons among *Sites*		
CFS *vs* ZR	0	0.1
CFS *vs* P	0.333	0.0003
CFS *vs* CU	0.333	0.0001
CFS *vs* CRZ	1	0.0001
CFS *vs* PB	1	0.0001
ZR *vs* P	0.333	0.0001
ZR *vs* CU	0.333	0.0001
ZR *vs* CRZ	1	0.0001
ZR *vs* PB	1	0.0001
P *vs* CU	0.033	0.0185
P *vs* CRZ	1	0.0001
P *vs* PB	1	0.0001
CU *vs* CRZ	1	0.0001
CU *vs* PB	1	0.0001
CRZ *vs* PB	0.5	0.0001

Table B2. One-way similarity analysis (ANOSIM), results from comparing the bacterial OTUs among *Sites per Substrates* in the corals reef ecosystem on Mexican Central Pacific. Code: CFS (Costa Fragata Somero), ZR (Zona de Restauración), P (Pelícanos), CU (Cuastecomatito), CRZ (Carrizales) and PB (Punto B) / Resultados del análisis de similitudes (ANOSIM) para comparar los OTUs bacterianos entre los *Sitios por Sustratos* en los ecosistemas de coral del Pacífico central mexicano. Códigos: CFS (Costa Fragata Somero), ZR (Zona de Restauración), P (Pelícanos), CU (Cuastecomatito), CRZ (Carrizales) y PB (Punto B)

Test	R statistic	Significance level	Test	R statistic	Significance level
Global test			Global test		
Tissue of *P. damicornis*	0.533	0.0001	Mucus of *P. verrucosa*	0.533	0.0001
Pairwise comparisons tissue of *P. damicornis* among *Sites*			Pairwise comparisons mucus of *P. verrucosa* among *Sites*		
CFS *vs* ZR	0	0.100	CFS *vs* ZR	0	0.100
CFS *vs* P	0	0.100	CFS *vs* P	0	0.100
CFS *vs* CU	0	0.100	CFS *vs* CU	0	0.100
CFS *vs* CRZ	1	0.0002	CFS *vs* CRZ	1	0.0002
CFS *vs* PB	1	0.0002	CFS *vs* PB	1	0.0002
ZR *vs* P	0	0.100	ZR *vs* P	0	0.100
ZR *vs* CU	0	0.100	ZR *vs* CU	0	0.100
ZR *vs* CRZ	1	0.0002	ZR *vs* CRZ	1	0.0002
ZR *vs* PB	1	0.0002	ZR *vs* PB	1	0.0002
P *vs* CU	0	0.100	P *vs* CU	0	0.100
P *vs* CRZ	1	0.0002	P *vs* CRZ	1	0.0002
P *vs* PB	1	0.0002	P *vs* PB	1	0.0002
CU *vs* CRZ	1	0.0002	CU *vs* CRZ	1	0.0002
CU *vs* PB	1	0.0002	CU *vs* PB	1	0.0002
CRZ *vs* PB	0	0.100	CRZ *vs* PB	0	0.100
Global test			Global test		
Tissue of *P. verrucosa*	0.6	0.0001	Sea water	0.8	0.0001
Pairwise comparisons tissue of *P. verrucosa* among *Sites*			Pairwise comparisons sea water among *Sites*		
CFS *vs* ZR	0	0.100	CFS *vs* ZR	0	0.100
CFS *vs* P	0	0.100	CFS *vs* P	1	0.0002
CFS *vs* CU	0	0.100	CFS *vs* CU	1	0.0002
CFS *vs* CRZ	1	0.0002	CFS *vs* CRZ	1	0.0002
CFS *vs* PB	1	0.0002	CFS *vs* PB	1	0.0002
ZR *vs* P	0	0.100	ZR *vs* P	1	0.0002
ZR *vs* CU	0	0.100	ZR *vs* CU	1	0.0002
ZR *vs* CRZ	1	0.0002	ZR *vs* CRZ	1	0.0002
ZR *vs* PB	1	0.0002	ZR *vs* PB	1	0.0002
P *vs* CU	0	0.100	P *vs* CU	0	0.100
P *vs* CRZ	1	0.0002	P *vs* CRZ	1	0.0002
P *vs* PB	1	0.0002	P *vs* PB	1	0.0002
CU *vs* CRZ	1	0.0002	CU *vs* CRZ	1	0.0002
CU *vs* PB	1	0.0002	CU *vs* PB	1	0.0002
CRZ *vs* PB	1	0.0002	CRZ *vs* PB	0	0.100
Global test			Global test		
Mucus of *P. damicornis*	0.6	0.0001	Sediment	0.887	0.0001
Pairwise comparisons mucus of *P. damicornis* among *Sites*			Pairwise comparisons sediment among *Sites*		
CFS *vs* ZR	0	0.100	CFS *vs* ZR	0	0.100
CFS *vs* P	0	0.100	CFS *vs* P	1	0.0002
CFS *vs* CU	0	0.100	CFS *vs* CU	1	0.0002
CFS *vs* CRZ	1	0.0002	CFS *vs* CRZ	1	0.0002
CFS *vs* PB	1	0.0002	CFS *vs* PB	1	0.0002
ZR *vs* P	0	0.100	ZR *vs* P	1	0.0002
ZR *vs* CU	0	0.100	ZR *vs* CU	1	0.0002
ZR *vs* CRZ	1	0.0002	ZR *vs* CRZ	1	0.0002
ZR *vs* PB	1	0.0002	ZR *vs* PB	1	0.0002
P *vs* CU	0	0.100	P *vs* CU	0.2	0.0182
P *vs* CRZ	1	0.0002	P *vs* CRZ	1	0.0002
P *vs* PB	1	0.0002	P *vs* PB	1	0.0002
CU *vs* CRZ	1	0.0002	CU *vs* CRZ	1	0.0002
CU *vs* PB	1	0.0002	CU *vs* PB	1	0.0002
CRZ *vs* PB	1	0.0002	CRZ *vs* PB	1	0.0002

Table B3. One-way similarity analysis (ANOSIM), results from comparing the bacterial OTUs among *Substrates* within of each *Sites* in the corals reef ecosystem on Mexican Central Pacific. Code: CFS (Costa Fragata Somero), ZR (Zona de Restauración), P (Pelícanos), TPd (Tissue of *P. damicornis*), TPv (Tissue of *P. verrucosa*), MPd (Mucus of *P. damicornis*), MPv (Mucus of *P. verrucosa*), SW (Sea water) and Sdmt (Sediment) / Resultados del análisis de similitudes (ANOSIM), resultados para comparar los OTUs bacterianos entre *Sustratos* dentro de cada *Sitio* en los ecosistemas de coral del Pacífico central mexicano. Códigos: CFS (Costa Fragata Somero), ZR (Zona de Restauración), P (Pelícanos), TPd (Tejido de *P. damicornis*), TPv (Tejido de *P. verrucosa*), MPd (Mucus de *P. damicornis*), MPv (Mucus de *P. verrucosa*), SW (Agua de mar) y Sdmt (Sedimentos)

Test	R statistic	Significance level	Test	R statistic	Significance level
Global test			Global test		
CFS	1	0.0001	CU	1	0.0001
Pairwise comparisons among *Substrates* within CFS			Pairwise comparisons among *Substrates* within CU		
TPd *vs* TPv	1	0.0002	TPd *vs* TPv	1	0.0002
TPd *vs* MPd	1	0.0002	TPd *vs* MPd	1	0.0002
TPd *vs* MPv	1	0.0002	TPd *vs* MPv	1	0.0002
TPd *vs* SW	1	0.0002	TPd *vs* SW	1	0.0002
TPd *vs* Sdmt	1	0.0002	TPd *vs* Sdmt	1	0.0002
TPv *vs* MPd	1	0.0002	TPv *vs* MPd	1	0.0002
TPv *vs* MPv	1	0.0002	TPv *vs* MPv	1	0.0002
TPv *vs* SW	1	0.0002	TPv *vs* SW	1	0.0002
TPv *vs* Sdmt	1	0.0002	TPv *vs* Sdmt	1	0.0002
MPd *vs* MPv	1	0.0002	MPd *vs* MPv	1	0.0002
MPd *vs* SW	1	0.0002	MPd *vs* SW	1	0.0002
MPd *vs* Sdmt	1	0.0002	MPd *vs* Sdmt	1	0.0002
MPv *vs* SW	1	0.0002	MPv *vs* SW	1	0.0002
MPv *vs* Sdmt	1	0.0002	MPv *vs* Sdmt	1	0.0002
SW *vs* Sdmt	1	0.0002	SW *vs* Sdmt	1	0.0002
Global test			Global test		
ZR	1	0.0001	CRZ	1	0.0001
Pairwise comparisons among *Substrates* within ZR			Pairwise comparisons among *Substrates* within CRZ		
TPd *vs* TPv	1	0.0002	TPd *vs* TPv	1	0.0002
TPd *vs* MPd	1	0.0002	TPd *vs* MPd	1	0.0002
TPd *vs* MPv	1	0.0002	TPd *vs* MPv	1	0.0002
TPd *vs* SW	1	0.0002	TPd *vs* SW	1	0.0002
TPd *vs* Sdmt	1	0.0002	TPd *vs* Sdmt	1	0.0002
TPv *vs* MPd	1	0.0002	TPv *vs* MPd	1	0.0002
TPv *vs* MPv	1	0.0002	TPv *vs* MPv	1	0.0002
TPv *vs* SW	1	0.0002	TPv *vs* SW	1	0.0002
TPv *vs* Sdmt	1	0.0002	TPv *vs* Sdmt	1	0.0002
MPd *vs* MPv	1	0.0002	MPd *vs* MPv	1	0.0002
MPd *vs* SW	1	0.0002	MPd *vs* SW	1	0.0002
MPd *vs* Sdmt	1	0.0002	MPd *vs* Sdmt	1	0.0002
MPv *vs* SW	1	0.0002	MPv *vs* SW	1	0.0002
MPv *vs* Sdmt	1	0.0002	MPv *vs* Sdmt	1	0.0002
SW *vs* Sdmt	1	0.0002	SW *vs* Sdmt	1	0.0002
Global test			Global test		
P	0.993	0.0001	PB	1	0.0001
Pairwise comparisons among *Substrates* within P			Pairwise comparisons among *Substrates* within PB		
TPd *vs* TPv	1	0.0002	TPd *vs* TPv	1	0.0002
TPd *vs* MPd	1	0.0002	TPd *vs* MPd	1	0.0002
TPd *vs* MPv	1	0.0002	TPd *vs* MPv	1	0.0002
TPd *vs* SW	1	0.0002	TPd *vs* SW	1	0.0002
TPd *vs* Sdmt	1	0.0002	TPd *vs* Sdmt	1	0.0002
TPv *vs* MPd	1	0.0002	TPv *vs* MPd	1	0.0002
TPv *vs* MPv	1	0.0002	TPv *vs* MPv	1	0.0002
TPv *vs* SW	1	0.0002	TPv *vs* SW	1	0.0002
TPv *vs* Sdmt	1	0.0002	TPv *vs* Sdmt	1	0.0002
MPd *vs* MPv	1	0.0002	MPd *vs* MPv	1	0.0002
MPd *vs* SW	1	0.0002	MPd *vs* SW	1	0.0002
MPd *vs* Sdmt	1	0.0002	MPd *vs* Sdmt	1	0.0002
MPv *vs* SW	1	0.0002	MPv *vs* SW	1	0.0002
MPv *vs* Sdmt	1	0.0002	MPv *vs* Sdmt	1	0.0002
SW *vs* Sdmt	1	0.0002	SW *vs* Sdmt	1	0.0002

Table B4. Variables that explain the variation of the bacterial OTUs in the corals reef ecosystem on Mexican Central Pacific. Code: CFS (Costa Fragata Somero), ZR (Zona de Restauración), P (Pelícanos), CU (Cuastecomatito), CRZ (Carrizales) and PB (Punto B). LCC (live coral cover), RSC (sponge), FMA (fleshy macroalgae), SAL (salinity) and %TA (percentage of sandy texture) / Variables que contribuyeron a la variación de los OTUs bacterianos en los arrecifes de coral del Pacífico central mexicano. Códigos: CFS (Costa Fragata Somero), ZR (Zona de Restauración), P (Pelícanos), CU (Cuastecomatito), CRZ (Carrizales) y PB (Punto B). LCC (coral vivo), RSC (esponjas), FMA (macroalgas carnosas), SAL (salinidad) y %TA (porcentaje de textura arenosa)

Variables	Season	Sites					
		CFS	ZR	P	CU	CRZ	PB
LCC	Summer	31.8	26.3	24.2	47.7	53	11.6
	Winter	31.8	26.3	24.2	47.7	53	11.6
RSC	Summer	--	--	--	0.3	--	4.2
	Winter	--	--	--	0.3	--	4.2
FMA	Summer	10.4	17.96	0.8	7.4	25.3	14.3
	Winter	10.4	17.96	0.8	7.4	25.3	14.3
SAL	Summer	36.5	36.3	35.9	36	35.7	35.6
	Winter	36.5	36.3	35.9	36	35.7	35.6
%TA	Summer	74.8	63.8	64.4	67.1	81.4	73.5
	Winter	76.3	62.1	67.8	71.9	79.1	77.1