ORGANIC CARBON SOURCES AND THEIR TRANSFER IN A GULF OF MEXICO CORAL REEF ECOSYSTEM

By

© Laura Carreón-Palau (B.Sc., M.Sc.)

A thesis submitted to the School of Graduate Studies
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in the Faculty of Science

Department of Biology

Memorial University of Newfoundland

June, 2015

St. John's Newfoundland, Canada

ABSTRACT

Coral reefs face unprecedented threats throughout most of their range. Poorly planned coastal development has contributed increased nutrients and sewage contamination to coastal waters, smothering some corals and contributing to overgrowth by macroalgae. My approach to assessing the degree to which coral reef ecosystems have been influenced by terrestrial and anthropogenic organic carbon inputs is through the use of carbon (C) and nitrogen (N) stable isotopes and lipid biomarkers in a marine protected area, the Coral Reef System of Veracruz: Parque Nacional Sistema Arrecifal Veracruzano (PNSAV) in the southwest Gulf of Mexico. Firstly, I used a C and N stable isotope mixing model and a calculated fatty acid (FA) retention factor to reveal the primary producer sources that fuel the coral reef food web. Secondly, I used lipid classes, FA and sterol biomarkers to determine production of terrestrial and marine biogenic material of nutritional quality to pelagic and benthic organisms. Finally, I used coprostanol to determine pollutant loading from sewage in the suspended particulate matter. Results indicate that phytoplankton is the major source of essential metabolite FA for marine fish and that dietary energy from terrestrial sources such as mangroves are transferred to juvenile fish, while seagrass non-essential FA are transferred to the entire food web mainly in the rainy season. Sea urchins may be the main consumers of brown macroalgae, especially in the dry season, while surgeon fish prefer red algae in both dry and rainy seasons. C and N isotopic values and the ratio C:N suggest that fertilizer is the principal source of nitrogen to macroalgae. Thus nitrogen supply also favored phytoplankton and seagrass growth leading to a better nutritional condition and high retention of organic

carbon in the food web members during the rainy season when river influence increases. However, the great star coral *Montastrea cavernosa* nutritional condition decreased significantly in the rainy season. The nearest river to the PNSAV was polluted in the dry season; however, a dilution effect was detected in the rainy season, when some coral reefs were contaminated. In 2013, a new treatment plant started working in the area. I would suggest monitoring δ^{15}N and the C: N ratio in macroalgae as indicators of the nitrogen input and coprostanol as an indicator of human feces pollution in order to verify the efficiency of the new treatment plant as part of the management program of the PNSAV.

ACKNOWLEDGEMENTS

I am grateful to my supervisor, Dr. Christopher C. Parrish, for accepting to help me and for sharing his knowledge of tracing organic carbon in cold waters and showing me how to apply it to coral reefs; thanks for his patience, support, good advice, and generosity. I am thankful to my supervisory committee for their support, to Horacio Pérez España for inviting me to participate in the project 'Fuentes orgánicas de carbono y nitrógeno y su función sobre la estructura trófica en el Sistema Arrecifal Veracruzano Fomix-Veracruz 37567 ' [Organic sources of carbon and nitrogen and their function on the trophic structure of the Coral Reef System of Veracruz], his support in the field work and good advice. Thanks go to Dr. Annie Mercier for her conscientious revisions and good advice. Also I want to thank the other members of the Project, especially Jorge Arturo Del Angel-Rodriguez and Sergio Aguiñiga-García for their support in the first chapter. To all the bachelor students that worked in the project from Instituto Tecnológico de Boca del Río, Veracruz, their help in the collection and labelling of samples was fundamental.

The Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico, and the Veracruz state government co-financed the field and lab work. Analytical work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC). Thanks go to the Ph.D. fellowships from CONACYT (grant number 117304) and from the School of Graduate Studies, Memorial University of Newfoundland

I appreciate the support of my work place the Centro de Investigaciones Biologicas del Noroeste in La Paz, Baja California Sur, Mexico. Dr. Sergio Hernández-Vázquez, Dr. Illie Racotta-Dimitrov, Dr. Bertha Olivia Arredondo-Vega, Dr. Pedro Cruz-Hernández

and Dr. Daniel Lluch-Cota provided access to all the administrative facilities to complete my thesis. Also Dr. Arredondo-Vega generously provided me the lab facilities to be more efficient in the biochemical analysis.

The Departments of Biology and Ocean Sciences at Memorial University were great places to work, thanks to Jeanette Wells and Linda Windsor, I really appreciated their expertise. Thanks to the friendship of Wally Martin, Zhao Sun, Stefanie Hixson, Giseli Swerts-Rocha, Jean Tucker, Lindsey Hemphill, Khalil Eslamloo, Neus Campanya and all the shuttle community. The people from the Society of Spanish at MUN made me enjoy this thesis with the Latino dancing breaks. The warm people from St. John's, Elizabeth and Caroline Watton, Cameron and Holly Dunn, Ted and Margarita Miller, and Dr. Parrish's family: Leslie, Nicholas, and Kathleen all shared their generosity and friendship with us and made my son happy. I can´t express how much I appreciate that.

To my friends Lourdes Castillo, Oscar Meruvia, Soledad Porta and Alejandro Buren for all the talks and fun that we had together. Thanks go to my mother Angela Palau Betancourt for her visits to St. John's and my sisters Martha, Silvia and Alicia and my brothers Carlos and Miguel, they gave me the love and confidence to face any challenge.

I dedicate this thesis to the memory of my father Miguel Carreón-Lopez and our first land lord in St. John's Maxwell Watton; they taught me to face the dark side of life with love, hope, and humor. Finally, I feel deeply thankful to my husband Jorge A. Del Angel-Rodríguez and my son Aarón Sinhué Del Angel-Carreón, my partners in this adventure for their love and enthusiasm to learn and discover.

Table of Contents

List of Tables

List of Figures

List of Symbols, Nomenclature or Abbreviations

Acetone Mobile Polar Lipids (AMPL): A mixture of pigments and chloroplast-associated glycolipids which strongly indicates photosynthetic organisms.

Alcohols (ALC): Organic compounds in which a hydroxyl functional group (-OH) is bound to a carbon atom, usually connected to other carbon or hydrogen atoms. A high alcohol level suggests degradation of zooplankton-derived wax esters.

Branched fatty acids (BFA): Fatty acids synthesized by many microorganisms (most often with an *iso*- or an *anteiso*-methyl branch) and synthesized to a limited extent in higher organisms. They can also enter animal tissues via the diet, especially those of ruminants.

Bioactive metabolites (BM): Biologically active lipid mediators (C_{20} fatty acids and their metabolites), including prostaglandins, thromboxanes, leukotrienes and other oxygenated derivatives, which exert their effects at very low concentrations.

Essential fatty acids (EFA): Both of the parent fatty acids linoleic (LA) 18:2ω6 and α-linoleic (ALA) 18:3ω3 can be synthesised in plants, but not in animal tissues, and they are therefore essential dietary components.

Fatty acids (FA): Compounds synthesized in nature via condensation of malonyl coenzyme A units by a fatty acid synthase complex. They usually contain even numbers of carbon atoms in straight chains (commonly C_{14} to C_{24}), and may be saturated or unsaturated, and can contain a variety of substituent groups.

Fatty acid nomenclature: The number before the colon specifies the number of carbon atoms, and that after the colon, the number of double bonds. The position of the terminal

double bond can be denoted in the form (*n*-x), where *n* is the chain-length of the fatty acid and x is the number of carbon atoms from the last double bond, assuming that all the other double bonds are methylene-interrupted. Thus linoleate and α-linolenate are 18:2(*n*-6) and 18:3(*n*-3), respectively or 18:2*ω*6 and 18:3*ω*3.

Free fatty acids (FFA): The uncombined fatty acids or nonesterified fatty acids found in an organism which usually come from the breakdown of a triglyceride (triacylglycerol).

Hopanoids (HP): Natural pentacyclic compounds (containing five rings) based on the chemical structure of hopane. Their primary function is to improve plasma membrane strength and rigidity in bacteria. In eukaryotes (including humans) sterols serve a similar function.

Hydrocarbons (HC): Organic compounds which contain only carbon and hydrogen. There are four classes of hydrocarbons: Alkanes, alkenes, alkynes and aromatics.

Isoprenoids (IS): Class of organic compounds made up of two or more structural units derived from isoprene. Isoprene is a five-carbon hydrocarbon with a branched-chain structure. In isoprenoids, isoprene units are linked into larger molecules having from two to thousands of five-carbon units that can take the form of both linear structures and rings. Many of these molecules play a wide variety of roles in plant and animal physiological processes and as intermediates in the biological synthesis of other important molecules. Some examples include: Monoterpenes e.g. citral from lemongrass oil; diterpenes e.g. phytol, a precursor of chlorophyll; Triterpenes e.g. squalene, the precursor of cholesterol and other steroids; and tetraterpenes e.g. carotene, the pigment in carrots and a precursor of vitamin A.

Ketones (KET): Class of organic compounds containing a carbonyl group (-C=O) bonded to two carbon atoms. Ketones can be produced by lipid metabolism, usually because of starvation.

Lipids: Hydrophobic compounds related closely to fatty acid derivatives through biosynthetic pathways (e.g. prostanoids, aliphatic ethers or alcohols) or by their biochemical or functional properties (e.g. cholesterol and plant sterols).

Lipid classes: Hydrophobic or amphipathic small molecules present in lipid extracts which include hydrocarbons (HC), steryl esters (SE), waxes (WE), ketones (KET), triacylglycerol (TAG), free fatty acids (FFA), alcohols (ALC), sterols (ST), acetone mobile polar lipids (AMPL), and phospholipids (PL).

Monounsaturated fatty acids (MUFA): Straight- or normal-chain (even-numbered), monoenoic components, i.e. with one double bond, which make up a high proportion of the total fatty acids in most natural lipids. Normally the double bond is of the *cis*- or *Z*-configuration, although some fatty acids with *trans*- or *E*-double bonds are known.

Odd-chain fatty acids (OFA): fatty acids with odd numbers of carbons biosynthesized from propionyl-CoA and acetyl-CoA, rather than to two acetyl-CoA at the final step. The propionyl-CoA is not a substrate for the TCA cycle or other simple pathways.

Pentacyclic triterpenoids (PT): The pentacyclic triterpenes or saponins are common secondary plant metabolites and are synthesized *via* the isoprenoid pathway to produce a hydrophobic triterpenoid structure (aglycone) containing a hydrophilic sugar chain (glycone). Saponins have antimicrobial activities and protect against attempted pathogen infections.

Phospholipids (PL): Class of lipids containing a diacylglycerol, a phosphate group, and a simple organic molecule such as choline. They are essential components of membranes where they share a structural function with sterols, and can be used to indicate freshly biosynthesized material

Polyunsaturated fatty acids (PUFA): Fatty acids with methylene-interrupted double bonds, i.e. with two or more double bonds of the *cis*-configuration separated by a single methylene group. In higher plants, the number of double bonds in fatty acids only rarely exceeds three, but in algae and animals there can be up to six. Two principal families of polyunsaturated fatty acids occur in nature that are derived biosynthetically from linoleic (9-*cis*,12-*cis*-octadecadienoic) and α-linolenic (9-*cis*,12-*cis,*15-*cis*-octadecatrienoic) acids, those families are named omega-6 and omega-3 respectively.

Saturated fatty acids (SFA): Straight- or normal-chain, saturated components (even-numbered). The most abundant saturated fatty acids in animal and plant tissues are straight-chain compounds with 14, 16 and 18 carbon atoms, but all the possible odd- and even-numbered homologues with 2 to 36 carbon atoms have been found in nature in esterified form. They are named systematically from the saturated hydrocarbon with the same number of carbon atoms, the final 'e' being changed to 'oic'.

Sterols (ST) and **Steryl esters (SE):** Group of steroids with a tetracyclic ring system usually with a double bond in one of the rings and one free hydroxyl group. Sterols are found both in the free state (sterols e.g. cholesterol), where they have an essential role in maintaining membrane fluidity, and in esterified form (steryl ester e.g. cholesterol esters). Steryl esters are stored in intracellular lipid droplets from which they are mobilized upon

demand and hydrolyzed to yield free sterols and fatty acids. In plants, cholesterol is rarely present in other than small amounts, but phytosterols such as sitosterol, stigmasterol, avenasterol, campesterol and brassicasterol, and their fatty acid esters are usually found, and they perform a similar function.

Triacylglycerols (TAG): Consists of a glycerol moiety with each hydroxyl group esterified to a fatty acid. In nature, they are synthesised by enzyme systems, which determine that a centre of asymmetry is created about carbon-2 of the glycerol backbone, so they exist in enantiomeric forms, i.e. with different fatty acids in each position. Their primary biological function is to serve as a store of energy.

Wax esters (WE): In their most common form, wax esters consist of fatty acids esterified to long-chain alcohols with similar chain-lengths. The latter tend to be saturated or have one double bond only. Such compounds are found in animal and plant tissues, and in microbes. They have a variety of functions, such as acting as energy stores, waterproofing and lubrication.

List of Appendices

I General Introduction and Overview

I.1 Importance of marine protected areas for coral reefs

Coral reefs are one of the pinnacles of life on earth, unmatched in diversity and beauty for us to admire, understand, cherish, and to conserve for generations to come. Corals are members of the phylum Cnidaria and class Anthozoa, comprising over 6,000 known species. Anthozoans also include sea fans, sea pansies, and sea anemones. Stony corals belong to the order scleractinia the largest order of anthozoans, and are the group primarily responsible for laying the foundations of, and building up, reef structures by calcium aragonite deposition (Veron 1995). Most scleractinians are colonial animals composed of hundreds to thousands of individuals, called polyps; however, solitary forms exist. In tropical reefs they have a symbiotic relationship with dinoflagellates (zooxanthellae). Approximately 800 species of reef-building scleractinians are described in shallow waters, yet fewer than 10 without zooxanthellae are known to make substantial cold deep-water reef frameworks (Freiwald et al. 2004).

Metazoan taxa that are involved in key ecological interactions on reefs belong to eight phyla; Porifera, Cnidaria, Annelida, Sipuncula, Arthropoda, Mollusca, Echinodermata, and Chordata (Glynn and Enochs 2011). These phyla occupy different habitats in the reef: encrusting (scleractinians and poriferans), attached (bivalves and gorgonians), boring (annelids and sipunculans), burrowing (annelids), vagile (crustaceans and echinoderms), and nektonic or planktonic, such as, brachyurans, carideans, mysids, and cephalopods (Ginsburg 1983). Some suspension and filter feeders, such as sponges, bryozoans,

bivalves, barnacles, and ascidians live hidden in crevices, drill, or etch their own holes within coral skeletons (so-called bioeroders). Also, fish play an essential role in the ecology of coral reefs as herbivores, predators and bioeroders.

Abundance and diversity of many taxa are related to the structural complexity of the reef, most commonly measured as the rugosity index (Luckhurst and Luckhurst 1978). The diversity of tropical corals is far greater in the Indo-Pacific, particularly around Indonesia, the Philippines, and Papua New Guinea. Many other groups of marine fauna show similar patterns. The Caribbean Sea possesses a smaller number of species, with few common species between the two regions and less biodiversity (Longhurst and Pauly 1987).

Coral reefs are located along the coastlines of over 100 countries and provide a variety of ecosystem goods and services (Burke, et al. 2011). Reefs serve as a major food source for many developing nations, they provide barriers to high wave action that buffer coastlines and beaches from erosion, and they supply an important revenue base for local economies through fishing and recreational activities. Coral reefs are threatened by the increase in green house gases and coastal pollution. The increase in green house gases - methane, tropospheric ozone, and atmospheric CO_2 -, has two effects, the increase of sea surface temperature; global warming, and the uptake of CO_2 by the ocean; ocean acidification.

The uptake of anthropogenic carbon since 1750 has led to ocean acidification with an average decrease in pH of 0.1 units (IPCC, 2007). Predictions based on experimental and field observations indicate that the combined effects of rising temperatures, and ocean acidification could reduce coral calcification and increase the frequency of bleaching

events (Hoegh-Guldberg et al. 2007, Anthony et al. 2008). Coral bleaching occurs when the mutualistic relationship between the coral host and symbiotic dinoflagellates is destabilized, and symbionts are lost from the coral polyps. This has occurred over vast areas of the world's oceans, in response to periods with warmer-than-normal sea surface temperature. When temperatures exceed summer maxima by 1° to 2°C for 3 to 4 weeks, this obligatory endosymbiosis disintegrates with ejection of the symbionts and coral bleaching (Hoegh-Guldberg, 1999). Corals may survive and recover their dinoflagellate symbionts after mild thermal stress, but typically show reduced growth, calcification, and fecundity (Hoegh-Guldberg et al. 2007).

Environmental and biological factors affecting scleractinian corals, the principal habitat providers in a coral reef, also affect species composition and ecological function of reef communities. We can summarize these factors as follows: 1) the increase of vulnerability to diseases and mortality, as a result of thermal stress, 2) the potential reduction of calcification rates if ocean acidification increases, 3) the reduction of the capacity of recovery by recruitment and growth due to competition with macroalgae, which is favoured by polluted coastal water with extra nutrients and overfishing of herbivores. Those factors are related to anthropogenic activities, therefore the most effective, and immediate, strategy to undertake is to reduce local effects such as industrial and agricultural pollution, eutrophication, and overfishing.

Marine protected areas (MPA) are currently the best management tool for conserving coral reefs (Mumby and Harborne 2010). However, no-take areas (NTA) provide the most effective protection for extractive activities such as fishing, affording a spatial refuge for a portion of the stock from which larvae and adults can disperse to adjoining

exploited areas (Palumbi et al. 2003). Ecological modeling studies indicate that at least 30% of the world's coral reefs should be NTA, to ensure long-term protection and maximum sustainable yield of exploited stocks (Hastings and Botsford, 2003). According to the World Resources Institute, 20% of the coral reef area is in partially or effective MPAs (Burke, et al. 2011). The challenge for the future is to increase the effectiveness of the MPAs, based on the following criteria: 1) Selection of reefs with higher coral and fish fecundities, 2) evaluation of currents and connectivity, 3) identification of sites with lower sedimentation rates to deploy artificial reefs, 4) control of overfishing at least to protect herbivore biomass, 5) conservation of nursery mangrove areas and 6) watershed management.

The coral reef system of Veracruz (Parque Nacional Sistema Arrecifal Veracruzano: PNSAV) is the largest in the southern Gulf of Mexico and functions as a bridge for various species dispersed between the Caribbean and Florida reefs (Jordán-Dahlgren 2002). In the PNSAV, there are several fisheries of commercial importance, extraction of shells for souvenir products, harvesting of ornamental aquarium fish for export, and for-profit recreational activities that include island tourism and SCUBA diving (Arceo and Granados-Barba 2010). Untreated wastewater discharges (both domestic and industrial) from adjacent cities are considered the major environmental threat (Ortiz-Lozano et al. 2005). The coral reef system is located on a terrigenous platform receiving an effluent of 2.65×10^9 m^3 $year^{-1}$ directly from the Jamapa River and 44.66×10^9 m^3 $year^{-1}$ indirectly from the Papaloapan River (CNA, 2011), the eighth and the second largest Mexican rivers flowing into the Gulf of Mexico, respectively (CNA, 2011).

During the rainy season, turbidity plumes are measured on order of 10 km offshore (Ricono 1999). Sedimentation rates as high as 2 kg m^{-2} day^{-1} have been recorded in the reef area (Pérez-España et al. 2012). Input of sediments and nutrients are usually deleterious for coral reefs, which prefer oligotrophic conditions and clear water (Veron 2000). Authorities and managers are concerned about the effects of wastewaters; this discharge is around of 34 $x10^6$ m^3 y^{-1} (Sistema de Agua y Saneamiento Metropolitano de Veracruz 2005).

My approach to assessing the degree to which the PNSAV has been influenced by terrestrial and anthropogenic inputs of organic carbon is through the use of carbon (C) and nitrogen (N) stable isotopes and lipid biomarkers. C and N stable isotopes reveal trophic levels and origin of carbon from primary producers, $\delta^{15}N$ from macroalgae reveal information on nitrogen sources, C:N ratios reveal enriched dissolved inorganic nitrogen sources that promote growth of primary producers. Lipid classes and fatty acids can be used to determine production of terrestrial and marine biogenic material of dietary (nutritional) value to pelagic and benthic organisms. Meanwhile, sterols are structural biomarkers related to taxonomic origin and are well conserved in invertebrates as filter feeders and primary consumers. Other sterols such as the 5β-stanol coprostanol can be used to determine pollutant loading, from sewage (Parrish et al. 2000).

I.2 The organic carbon cycle

The organic carbon (OC) cycle operates on multiple time scales with a small fraction of the global reservoir actively exchanged. For the marine system, the sources are principally recently synthesized material from autotrophic production, which annually

contribute 44–50 Pg C y^{-1} of new organic carbon (1 Pg=1x10^{15} g: Harvey 2006). This is supplemented by terrestrial carbon arriving from rivers ranging from 0.25–0.36 Pg C y^{-1} for dissolved OC and less for particles (Schlünz and Schneider 2000). Atmospheric input is a quantitatively minor fraction from the perspective of total organic input, but has indirect importance for transport of essential trace metals needed for phytoplankton growth (Siefer et al. 1999, Fung et al. 2000). The sources of organic matter are dependent upon the intensity of the autochthonous signal and the proximity and magnitude of inputs from coastal higher plants, and sediments carried by rivers. Primary production by microalgae is the largest of these sources to the marine system, but terrestrial material eroded from rivers has received heightened interest in recent years as a recorder of changing coastal systems and increased sea level (Harvey 2006).

Stable isotopes are ideally suited to increase our understanding of element cycles in ecosystems. The δ values are measures of the amounts of heavy and light isotopes in a sample. Increases in these values denote increases in the amount of the heavy isotope components. Stable isotopes record two kinds of information. Where physical and chemical reactions fractionate stable isotopes, the resulting isotopic distributions reflect reaction conditions (process information). Stable isotope distributions also record information about the origins of samples (source information). The source sets an isotopic baseline that can subsequently be shifted by isotopic fractionation. A well-studied example is C isotope fractionation in photosynthesis. A 1974 study showed that terrestrial C_3 plants average -27.8‰. This was about 20‰ more negative than the source of carbon for plants, CO_2 in air (δ ^{13}C CO_2 = -7.4‰ in air). The overall plant isotopic composition

thus reflected both source (-7.4‰) and fractionation (-20.4‰) information: -27.8 (Peterson and Fry, 1987 and references therein).

On the other hand lipids are primarily dietary in pelagic zooplankton and other consumers. Matthews and Mazumder (2005) demonstrated that stable isotope analysts should carefully consider the consequences of dietary lipids in the interpretation of consumer $\delta^{13}C$. Stable isotope of C and N analyses and isotope mixing models have been used to estimate the relative contribution of the different potential food sources to the biomass of fish (Kaussoroplis et al. 2001). This model is based on Bayesian inference offering a quantification of which solutions are most likely, incorporating many more sources of variability within the model, while allowing for multiple dietary sources and then generating potential dietary solutions as true probability distributions (Parnel et al. 2010). The contribution of the present study is the use of the mixing model to quantify the fatty acids trophic transfer from primary producers instead of food items.

Detailed evaluation of the sources and reactivity of organic matter in coastal sediments have also been made using biomarker compounds covering a range of reactivity, including lignin oxidation products, lipids and carbohydrates (Hedges et al. 1997). Their lipid fraction comprises saturated and aromatic hydrocarbons, fatty acids, alcohols and sterols (Venkatesan et al. 1987) most of which are not sinthesized *de novo*, and some analyses include compound-specific isotope ratios (Canuel et al. 1997, Copeman et al. 2009). Approaches that have used multiple techniques have been most successful in identitying the sources of organic matter in complex nearshore sediments. The analysis of the isotopic composition of individual compounds has shown that trends in the isotopic

composition of lipids were consistent with $\delta^{13}C$ from the total organic carbon (Canuel et al. 1997). Land-derived OC that flows to the oceans in rivers includes recently biosynthesized plant debris, detritus, dissolved humic substances accompanied by older soil humus, and recycled fossil OC eroded from sedimentary rocks. However, particulate organic matter of terrestrial origin suffers rapid and remarkably extensive remineralization at sea (Hedges et al., 1997). In contrast, urban sewage discharges are OC sources that drive alterations in the habitat, the decline of biodiversity, and the loss of the resources and services provided by coastal ecosystems (Costanza et al. 1997, Daily et al. 1997).

I.3 Use of biomarkers to trace organic carbon sources through the ecosystem

I.3.1 Primary producers

Living organisms biosynthesize a very small subset of the billions of bio-molecules that can be assembled in theory from carbon, hydrogen, oxygen, nitrogen, sulphur, and phosphate, and these molecules can be regarded as biomarkers. Their presence in an environment reflects their synthesis by the primary producer organisms. Some molecules are produced only by a certain species or classes of organisms. The key criteria to select a biomarker are: the information content**,** robustness of the molecule**,** and feasibility of detection and analysis. Lipids form 10-20% of OC in most organisms. They are an extensively studied class of compounds which are analytically accessible, and some lipids particularly sterols, are chemically stable and structurally diverse (Parrish et al. 2000, Volkman 2005). For such applications, it is vital that we understand how biochemical pathways have evolved over time, in parallel with the evolution of species on earth as

codified in the three kingdoms of life Archaea, Bacteria and Eukaryota (Volkman 2005). There are three scales of processes analyzed with biomarkers: First, geological processes in ancient sediments where the presence of a biomarker may only provide information about the existence of a biosynthetic pathway rather than the presence of a particular group of organisms. Second, geochemical processes in younger sediments (5 years old) where the source specificity and biosynthesis of cyclic isoprenoids such as hopanoids, sterols and pentacyclic triterpenoids, are commonly used to assign sources of organic matter (Volkman 2005), and third those studies of bound lipids and macromolecular organic matter in organisms (food web tracers) and sediments that reveal ecological processes, where the most common biomarkers are sterols and fatty acids (Parrish et al. 2000 and references therein).

Sterols are isoprenoid membrane lipids related to the primary producer: β-sitosterol (higher plants), brassicasterol (diatoms), dinosterol (dinoflagellates) and fucosterol (macroalgae) (Parrish et al. 2000 and references therein). Fatty acids (FA) are important components of all living cells; they are a source of metabolic energy, essential constituents of cell membrane lipids, and precursors of bioactive metabolites (Tocher and Sargent 1990). Microalgae differ in FA composition depending on their taxonomic position (Parrish et al. 2000, Brocks and Pearson 2005, Berge and Barnathan 2005). At the next trophic level, zooplankton forms an essential link between primary producers and higher-order consumers. The fatty-acid trophic biomarkers concept is based on the observation that marine primary producers preserve certain FA patterns that may be conservatively transferred throughout the pelagic marine environment (Berge and Barnathan 2005) and in aquatic food webs in general.

I.3.2 Urban sewage

The water quality of coastal or river waters can be affected by sewage discharges and the results are variable pathogenic bacteria concentrations with negative effects on recreational uses, public safety or shellfish sanitary status (Feldhusen 2000, Dorfman and Sinclair-Rosselot 2008), and severe oxygen deficiencies that can induce the asphyxia of the biota of estuaries and coastal areas (Ortiz-Lozano et al. 2005). Some sterols or their ratios have been proposed as biomarkers, coprostanol (5β-cholestan-3β-ol) is a 27 carbon stanol formed in the gut of higher mammals by the stereospecific reduction of the double bond of cholesterol. Coprostanol constitutes about 60% of the total sterols in human faeces (Leeming et al. 1996). Other mammals such as pigs, sheep, cows and cats have coprostanol in their faeces, but total concentrations and, most importantly, amounts relative to other sterols are very much less (Leeming et al. 1996). This compound has been used as a biomarker for the presence of human faecal matter in the marine environment (Bethell et al. 1994, Chaler et al. 2001). In contrast to bacteria coprostanol can remain hundreds of years in sediments (D'Anjou et al. 2012). In addition, it provide information of the degree of treatment efficiency, for instance, from an average of six secondary and tertiary treatment plants, coprostanol, cholesterol, campesterol, and stigmastanol had percent removal rates of greater than 95% (Furtula et al. 2012).

I.4 Organic carbon trophic transfer

Local ecosystems are never totally isolated in space because they receive and lose considerable amounts of matter and organisms in many forms and from various processes (Polis et al. 1997, 2004). These flows could be either direct, when the nutrient is moved

between locations in its inorganic form, or indirect, when the nutrient is moved between locations sequestered in the biomass (live or dead) of organisms (Gravel et al. 2010). Transfers from terrestrial to aquatic ecosystems (Graham et al. 2006) and in the opposite direction (Gratton et al. 2008) are common. These transfers influence both ecosystem productivity and structure (Christie and Reimchen 2008). There are also important biological transportations in oceans, varying in strength and direction on tidal or seasonal basis (e.g., Varpe et al. 2005). The primary producers deplete the limiting nutrient in the ecosystem in which it is found. The nutrient sequestered in the biomass of primary producers is either consumed by the herbivore or returned to the detritus compartment following natural death. The dead biomass of herbivores is also converted to detritus and/or transferred to upper trophic levels in reef ecosystems (Horn 1989).

The rates at which primary production is retained by herbivores and higher trophic levels are important parameters in understanding organic carbon transfer dynamics in these systems. The nutritional basis of the interaction between organism and environment are named nutritional ecology (Raubenheimer, et al., 2009). For instance, the relative percentage of lipid classes helps to characterize the nutritional condition of an organism. Triacylglycerols (TAG) are short-term energy storage lipids, whereas wax esters (WE) are long-term energy storage lipids. During starvation stress, TAG are utilized before WE (Lee and Patton 1989). Acetone mobile polar lipids (AMPL) are a mixture of pigments and chloroplast-associated glycolipids which strongly indicates photosynthetic organisms.

Phospholipids (PL) and sterols (ST) are primarily membrane structural components, and dominance of these lipid classes in the lipid profile indicates a constant food source. Cholesterol is the main ST in most animals, and the presence of other ST may provide

good trophic indicators (Drazen et al. 2008). For example, phytosterols are synthesized by algae and plants. Their main function is to regulate membrane fluidity (Rozner and Garti 2006). Sterol content is a major means by which eukaryotic cells modulate and refine membrane fluidity, permeability, and the function of various membrane proteins (Martin-Creuzburg and von Elert , 2009 and references therein). It has been shown that the ordering capacity provided by cholesterol is of significantly greater magnitude than that of any of cholesterol's metabolic precursors (Dahl et al. 1980). Likewise, the ordering effect provided by phytosterols differs from that of cholesterol, which may limit direct substitution of cholesterol by phytosterols in animal membranes (Haines 2001). Even phytosterols are structurally similar to cholesterol, although they have an extra hydrophobic carbon chain at the C-24 position (Trautwein et al. 2003). Herbivorous insects and also the crustaceans examined to date use dietary sterols to synthesize cholesterol. Therefore, most species studied are capable of dealkylating and reducing common C-24-alkyl phytosterols, such as sitosterol or stigmasterol, to cholesterol (Grieneisen 1994; Behmer and Nes 2003). However, more than 200 different types of phytosterols have been reported in plant material (Moreau et al. 2002), and not all of them are suitable as cholesterol precursors allowing us to consider them as biomarkers.

The nutritional condition of the consumer is the result of food intake, absorption, assimilation, biosynthesis, energy metabolism catabolism and excretion. Some indicators of nutritional quality of primary producers and nutritional condition of consumers includes the stoichiometric carbon to nitrogen ratio (Elser et al. 2007, El-Sabaawi et al. 2012), proportion of energy storage molecules such as TAG (Frasser, 1989), and their relation to structural molecules such as ST (Hooper and Parrish, 2009).

Lipid classes, sterols and fatty acids have been used to understand food web relationships, in some marine ecosystems, for instance abyssal polychaetes, crustaceans, and a cnidarian had carnivory as the predominant mode of foraging (Drazen et al. 2008), also lipid classes were used in deep-sea coral reefs for insights into coral physiology and information on species general health (Hamoutene et al. 2008). On the other hand coprostanol and other human derived contaminants like pharmaceuticals, steroids and hormones in surface waters have raised awareness of the role played by the release of treated or untreated sewage on water quality along sensitive coastal ecosystems such as wetlands and coral reefs which are in close proximity to large metropolitan cities (Singh et al. 2009).

This is the first study that combines the interaction of nutrients (C:N ratio) and other substances (e.g., TAG, essential FA, HUFA, and ST) in primary producers with the nutritional condition of several components of a coral reef food web which is influenced by terrestrial and anthropogenic inputs including contamination from untreated sewage.

I.5 Thesis overview

To identify the effect of river influence on adjacent coral reef ecosystems we need to understand the complex mixture of organic carbon present in coastal waters. Organic carbon is a product of biological synthesis; its sources are particles, the dissolved fraction, and biomass due to primary production.

In Chapter 1, I used carbon and nitrogen stable isotopes and fatty acids to reveal the organic carbon sources fueling a coral reef food web. Subsequently, a stable isotope

mixing model was used in order to estimate the relative contribution of terrestrial and marine primary producers to the bulk carbon and nitrogen of upper trophic food web members. Then the FA concentration per dry biomass unit in consumers was corrected for the relative contribution of each primary producer according to the mixing model, and the primary producer's FA transfer was evaluated in higher trophic levels using the trophic retention factor. Tracking the organic carbon sources with the mixing model it was possible to differentiate fatty acids from primary producers and their trophic transfer across trophic levels.

In Chapter 2, I used organic carbon to nitrogen molecular-ratios and lipids to discriminate food sources and seasonal shifts in nutritional condition in a Southwest Gulf of Mexico coral reef food web. The non-metric multidimensional scaling analysis of lipid class, fatty acid and sterol profiles suggested a better nutritional condition and high retention of organic carbon in the food web during the rainy season when river influence increases. The only exception was the great star coral *Montastrea cavernosa* with significantly lower nutritional condition.

In Chapter 3, I evaluated the quality of suspended particulate matter (SPM) using lipid classes, fatty acids and sterols and the degree of human fecal pollution in the SPM of the PNSAV. SPM was a good source of energy from triacylglycerols, saturated and monounsaturated FA, but had poor quality in terms of essential and highly unsaturated FA. The station located upstream of the Jamapa River was contaminated according to all indicators in the dry season. In contrast, the stations in the river mouth, Sacrificios reef and offshore of Anton Lizardo town reached values of coprostanol higher than the regulation limit for tropical marine coastal waters in the rainy season. There were three

sites clearly uncontaminated in both seasons, Enmedio and Cabezo reefs, as well as the river offshore with no presence of coprostanol.

Finally, I summarize the results of this work and assess implications for coral reef trophic ecology and the utility of the stable isotope mixing model, fatty acid retention factors and sterol biomarkers to trace terrestrial *vs* marine organic carbon.

I.6 Thesis objectives

The specific thesis objectives were:

1) To reveal key primary producer sources of organic carbon that fuel a coral reef food web using a carbon and nitrogen stable isotope mixing model and a calculated fatty acid trophic retention factor.

2) To evaluate the nutritional quality of primary producers, mangrove, seagrass, green, red and brown macroalgae, phytoplankton and zooxanthellae to pelagic and benthic consumers using C:N ratios, lipid class, fatty acid and the sterol profiles.

3) To evaluate the seasonal input of organic carbon from urban sewage in the suspended particulate matter using sterols (coprostanol) as biomarkers of human feces pollution.

I.7 Bibliography

Anthony, K. R. N., D. I. Kline, G. Diaz-Pulido, S. Dove, and O. Hoegh-Guldberg. 2008. Ocean acidification causes bleaching and productivity loss in coral reef builders. PNAS 105 (45): 17442-17446.

Arceo, P. and A. Granados-Barba. 2010. Evaluating sustainability criteria for a marine protected area in Veracruz, Mexico. Ocean Coast. Manag. 53: 535-543.

Berge, J. P. and G. Barnathan. 2005. Fatty acids from lipids of marine organisms: Molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. Adv. Biochem. Eng. Biotechnol. 96: 49–25.

Behmer , S.T., D. O. Elias and E. A. Bernays. 1999. Post-ingestive feedbacks and associative learning regulate the intake of unsuitable sterols in a generalist grasshopper. J. Exp. Biol. 202: 739–748 .

Bethell, P. H., L. J. Goad, R. P. Evershed, and J. Ottaway. 1994. The study of molecular markers of human activity: the use of coprostanol in the soil as an indicator of human faecal material. J. Archaeol. Sci. 21: 619-632

Brocks, J. J., and A. Pearson. 2005. Building the biomarker tree of life. Rev. Mineral. Geochem. 59:233-258.

Burke, L., K. Reystar, M. Spalding, and A. Perry. 2011. Reefs at risk revisited. World Resource Institute, Washington, D. C. United States of America Pp. 114.

Chaler, R., B. R. T. Simoneit, and J. O. Grimalt. 2001. Bile acids and sterols in urban sewage treatment plants. J. Chromat. A, 927: 155-160.

Christie, K. S., and T. E. Reimchen. 2008. Presence of salmon increases passerine density on Pacific Northwest streams. Auk 125:51–59.

Cleveland, A. and W. L. Montgomery. 2003. Gut characteristics and assimilation efficiencies in two species of herbivorous damselfish (Pomacentridae: *Stegastes dorsopunicans* and *S. planifrons*). Mar. Biol. 142:35-44

CNA, 2011. Atlas del Agua en México 2011. Comisión Nacional del Agua, Gobierno Federal. México, D.F. 133 pp. (available at http://www.conagua.gob.mx/CONAGUA07/Publicaciones/Publicaciones/SGP-18-11.pdf). (consulted on December 5, 2012).

Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B.,Limburg, K., Naeem, S., O'Neill, R.V., Paruelo, J., Raskin, R.G., Sutton, P., van den Belt, M., 1997. The value of the world's ecosystem services and natural capital. Nature 387, 253–260.

Dahl , C. E. , J. S. Dahl, and K. Bloch. 1980 . Effect of alkyl-substituted precursors of cholesterol on artificial and natural membranes and on the viability of *Mycoplasma capricolum*. Biochem. 19 : 1462 – 1467.

D'Anjou R. M. D., R. S. Bradley, N. L. Balascio, and D. B. Finkelstein. 2012. Climate impacts on human settlement and agricultural activities in northern Norway revealed through sediment biogeochemistry. PNAS 109(50): 20332–20337.

Dorfman, M. and K. Sinclair Rosselot. 2008. Testing the Waters. Quality at vacations beaches. Natural Resources Defense Council 18th Edition. Anthony Clark Editor. 447 pp

Drazen, J. C., C. F. Phleger, M. A. Guest, and P. D. Nichols. 2008. Lipid, sterols and fatty acid composition of abyssal holothurians and ophiuroids from the North-East Pacific Ocean: food web implications. Comp. Biochem. and Physiol., Part B 151: 79–87

El-Sabaawi, R. W., , T. J. Kohler, E. Zandona, J. Travis, M. C. Marshall, S. A. Thomas, D. N. Reznick, M. Walsh, J. F. Gilliam, C. Pringle, A. S. Flecker. 2012. Environmental and organismal predictors of intraspecific variation in the stoichiometry of a neotropical freshwater fish. PLoS ONE 7(3): e32713. doi:10.1371/journal.pone.0032713

Feldhusen, F. 2000. The role of seafood in bacterial foodborne diseases. Microbe Infect. 2:1651-1660.

Freiwald, A., J. H. Fossa°, A. Grehan, T. Koslow, J. M. Roberts. 2004. Cold-water coral reefs (United Nations Environment Programme–World Conservation Monitoring Centre, Cambridge).

Furtula V., J. Liu, P. Chambers, H. Osachoff, C. Kennedy and Joanne Harkness. 2012. Sewage treatment plants efficiencies in removal of sterols and sterol ratios as indicators of fecal contamination sources. Water Air Soil Pollut., 223:1017–1031 DOI 10.1007/s11270-011-0920-8

Ginsburg, R. N. 1983. Geological and biological roles of cavities in coral reefs. *In:* Barnes DJ (ed) Perspectives on coral reefs. Clouston, Australia, pp 148–153

Glynn P. W., and I. C. Enochs. 2011. Invertebrates and their roles in coral reef ecosystems. *In:* Coral Reefs: An Ecosystem in Transition Zvy Dubinsky and Noga Stambler Editors. Springer Science+Business Media B.V. pp. 521.

Graham, M. D., R. D. Vinebrooke, and M. Turner. 2006. Coupling of boreal forests and lakes: effects of conifer pollen on littoral communities. Limnol. Oceanog. 51:1524–1529.

Gratton, C., J. Donaldson, and M. J. Vander Zanden. 2008. Ecosystem linkages between lakes and the surrounding terrestrial landscape in northeast Iceland. Ecosystems 11:764–774.

Gravel, D., F. Guichard, M. Loreau, and N. Mouquet. 2010. Source and sink dynamics in meta ecosystems. Ecology 91(7): 2172-2184.

Grieneisen , M. L. 1994. Recent advances in our knowledge of ecdysteroid biosynthesis in insects and crustaceans. Insect Biochem. Mol. Biol. 24 : 115–132.

Haines , T.H. 2001 . Do sterols reduce proton and sodium leaks through lipid bilayers? Prog. Lipid Res. 40 : 299–324.

Hamoutene D., T. Puestow, and J. Miller-Banoub. 2008. Main lipid classes in some species of deep-sea corals in the Newfoundland and Labrador region (Northwest Atlantic Ocean). Coral Reefs 27: 237–246 DOI 10.1007/s00338-007-0318-7

Hastings, A., and L. W. Botsford. 2003. Comparing designs of marine reserves for fisheries and for biodiversity. Ecolog. Applic. **13**:S65–S70.

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. Mar. Freshwat. Res. 50: 839-866.

Hoegh-Guldberg, O., P. J. Mumby, A. J. Hooten, R. S. Steneck, P. Greenfield, E. Gomez, C. D. Harvell, P. F. Sale, A. J. Edwards, K. Caldeira, N. Knowlton, C. M. Eakin, R. Iglesias-Prieto, N. Muthiga, R. H. Bradbury, A. Dubi, and M. E. Hatziolos. 2007. Coral reefs under rapid climate change and ocean acidification. Science 318:1737-1742.

Hedges J. I., R. G. Keil and R. Benner. 1997. What happens to terrestrial organic matter in the ocean?. Organic Geochem. 27(5-6): 195-212.

Horn M. H. 1989. Biology of marine herbivorous fishes. Oceanog. Mar. Biol. Ann. Rev. 27: 167-272.

IPPC, 2007. Intergovernmental Panel on Climate Change. Climate Change 2007: Synthesis Report. Valencia, Spain, pp 26–73.

Jordán-Dahlgren, E. 2002. Gorgonian distribution patterns in coral reef environments of the Gulf of Mexico: Evidence of Sporadic Ecological Connectivity? Coral Reefs 21: 205-215.

Koussoroplis A. M., B. Alexandre, M. E. Perga, E. Koutrakis, G. Bourdier, and C. Desvilettes. 2011. Fatty acid transfer in the food web of a coastal Mediterranean lagoon: Evidence for high arachidonic acid retention in fish. Estuar. Coast. Shelf Sci. **91:** 450–461.

Lee R. F. and J. S. Patton 1989. Alcohol and waxes. In: Ackman RG (ed) Marine Biogenic Lipids, Fats and Oils. CRC Press, Boca Raton, FL, p 73–102

Leeming R., A. Ball, N. Ashbolt, and P. Nichols. 1996. Using fecal sterols from humans and animals to distinguish faecal pollution in receiving waters. Waters Res. 30(12):2893-2900.

Longhurst, A. R., and D. Pauly. 1987. Ecology of tropical oceans. Academic Press INC. United states of America pp. 407.

Luckhurst, B. E., and Luckhurst, K. 1978. Analysis of the influence of substrate variables on coral reef fish communities. Mar. Biol. 49:317-323.

Matthews, B., and A. Mazumder. 2005. Temporal variation in body composition (C : N) helpsexplain seasonal patterns of zooplankton d^{13}C. Freshwater Biology 50: 502–515, doi: 10.1111/j.1365-2427.2005.01336.x

Martin-Creuzburg D. and E. von Elert. 2009. Ecological significance of sterols in aquatic food webs. M.T. Arts et al. (eds.), Lipids in Aquatic Ecosystems, 43

DOI: 10.1007/978-0-387-89366-2_3, © Springer Science + Business Media

Mumby, P. J., and A. R. Harborne. 2010. Marine reserves enhance the recovery of corals on caribbean reefs. PLoS ONE 5(1): e8657. doi:10.1371/journal.pone.0008657

Ortiz-Lozano, L., A. Granados-Barba, V. Solis-Weiss, and M.A. Garcıa-Salgado. 2005. Environmental evaluation and development problems of the Mexican Coastal Zone. Ocean Coast. Manag. 48:161–17

Palumbi, S. R, S. D. Gaines, H. Leslie, and R W. Robert. 2003. New Wave: high-tech tools to help marine reserve research. Front. Ecol. Environ. 1(2):73–79

Peterson B. J. and B. Fry. 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 18:293-320.

Parnell, A. C., R. Inger, S. Bearhop, and A. L. Jackson. 2010. Source partitioning using stable isotopes: Coping with too much variation. Plos One 5: 9672, doi:10.1371

Parrish, C. C., T. A. Abrajano, S. M. Budge, R. J. Helleur, E. D. Hudson, K. Pulchan Y C. Ramos. 2000. Lipid and phenolic biomarkers in marine ecosystems: Analysis and applications. in: P. Wangersky (Ed.) The handbook of environmental chemistry. Vol. 5, Part D. Springer-Verlag, Berling. 193-223 Pp.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Ann. Rev. Ecol. System. 28: 289–316.

Polis, G. A., M. Power, and G. R. Huxel, editors. 2004. Food webs at the landscape level. University of Chicago Press, Chicago, Illinois, USA.

Raubenheimer, D., S. J. Simpson and D. Mayntz. 2009. Nutrition, ecology and nutritional ecology: toward an integrated framework. Func. Ecol. 23: 4–16

Ricono, N. A. 1999. Seasonal water quality impacts of riverine and coastal waters on coral reefs of Veracruz, México. M. Sc. Thesis. Department of physical and life sciences, Corpus Christi, Texas A & M University. Texas, USA.

Rozner S. and N. Garti. 2006. The Activity and absorption relation-ship of cholesterol and phytosterols. Coll. Surf. A 282-283: 435–456

Trautwein E. A., G. S. M. J. E. Duchateau, Y. Lin, S. M. Mel'nikov, H. O. F. Molhuizen, F. Y. Ntanios. 2003. Proposed mechanisms of cholesterol-lowering action of plant sterols. Eur. J. Lipid Sci. Technol. 105:171–185.

Singh, S. P., A. Azua, A. Chaudhary, S. Khan, K. L. Willett, P. R. Gardinali. 2010. Occurrence and distribution of steroids, hormones and selected pharmaceuticals in South Florida coastal environments. Ecotoxicology, 19:338–350

Tocher, D. R. and J. R. Sargent. 1990. Effect of Temperature on the Incorporation into phospholipid classes and metabolism via desaturation and elongation of n-3 and n-6 polyunsaturated fatty acids in fish cells in culture. Lipids 25(8):435-442.

Varpe, O., O. Fiksen, and A. Slotte. 2005. Meta-ecosystems and biological energy transport from ocean to coast: the ecological importance of herring migration. Oecologia 146:443–451.

Veron, J. E. N. 1995. Corals in Space and Time: The biogeography and evolution of the scleractinia. UNSW Press, Sydney, Australia pp. 321.

Veron, J. 2000. Corals of the world. Australian Institute of Marine Science, Melbourne, Australia. 463 Pp.

Veron, J. E. N**.** 2008. A Reef in Time. The great barrier reef from beginning to end.The Belknap Press of Harvard University Press. London England pp. 289.

Volkman J. K., S. M. Barrett, S. I. Blackburn, M. P. Mamsouri, E. L. Sikes and F. Gelin. 1998. Microalgal biomarkers: A review of recent research developments. *Organic Geochemistry. 29:(5-7) 1163-1179.*

Volkman, J. K. 2005. Sterols and other triterpenoids: source specificity and evolution of biosynthetic pathways. Organic Geochemistry 36:139–159

II Co-authorship statements

I am the first and corresponding author on all of the manuscripts produced from this thesis. I identified the research questions and designed and performed the entire field sampling protocols and biochemical analysis. I completed all statistical data analysis and manuscript preparations.

Dr. Chris Parrish is a co-author on all the manuscripts that were produced from all chapters. Dr. Parrish helped with identification of research ideas, sampling protocols, manuscript preparation, and lab financial support.

Dr. Horacio Perez España is a co-author on all the manuscripts that were produced from all chapters. Dr. Perez España helped with identification of research ideas, sampling protocols, manuscript preparation, and field and lab financial support.

Jorge Del Angel MSc is co-author on the manuscript that was produced from Chapter 1. He helped in the sampling protocol design, collection and analysis of samples, and the use of the stable isotope mixing model.

Dr. Sergio Aguiñiga Garcia is co-author on the manuscripts that was produced from Chapter 1 and Chapter 2. He helped in the organic carbon and nitrogen stable isotope sample preparation and use of the stable isotope mixing model.

The publications produced from this thesis include:

Chapter 1: Carreón-Palau, L. C. C. Parrish, J. A. del Ángel-Rodríguez, H. Pérez-España, and S. Aguiñiga-García. 2013. Revealing organic carbon sources fueling a coral reef food web in the Gulf of Mexico using stable isotopes and fatty acids. Limnology and Oceanography, 58(2): 593–612

Chapter 2:

Carreón-Palau, L., C. C. Parrish, H. Pérez-España, and Sergio Aguiñiga García. Seasonal Shifts in C: N atios, Lipid Classes, Fatty Acids and Sterols in a Gulf of Mexico Coral Reef Food Web under River Influence. Target journal is Progress in Oceanography.

Chapter 3:

Carreón-Palau, L., C. C. Parrish, H. and Pérez-España. Organic Carbon from Urban Sewage in the Suspended Particulate Matter of a Gulf of Mexico Coral Reef under River Influence. Target journal is Marine Pollution Bulletin

III Research Chapters

Chapter 1 Revealing Organic Carbon Sources Fueling a Coral Reef Food Web in the Gulf of Mexico Using Stable Isotopes and Fatty Acids

A version of this chapter was published in Limnology and Oceanography 2013. 58(2): 593–612

1.1 Abstract

C and N stable isotopes and FA revealed primary producer organic carbon sources that fuel a coral reef food web with river influence. A stable isotope mixing model was used to assess the relative contribution of six different primary producers to nine of the most ubiquitous invertebrate and fish consumer's bulk carbon. Mangrove and phytoplankton were two sources difficult to differentiate in some consumers; likely solutions involved one or the other but not both at the same time. FA concentration in upper trophic levels was corrected for the primary producer's relative contribution according to the mixing model, and FA retention was evaluated using a calculated trophic retention factor (TRF). The C_{18} FAs, 18:2ω6 or linoleic acid (LIN) and 18:3ω3 or α-linolenic acid (ALA) were plentiful in mangrove, sea grass, and green algae, but decreased across trophic levels with a TRF$\leq$ 1, probably due to decomposition of drifting leaves and then consumer metabolism. In contrast, macroalgae and phytoplankton FAs, 24:1ω9, and highly unsaturated fatty acids (HUFAs), arachidonic acid (ARA) 20:4ω6, docosapentaenoic acid (DPA) 22:5ω3, and docosahexaenoic acid (DHA) 22:6ω3, showed trophic accumulation (TRF > 1), while eicosapentaenoic acid (EPA) 20:5ω3 had similar concentrations across trophic levels (TRF ~ 1), suggesting the following degrees of

HUFA retention: DHA > ARA > EPA. This study indicates that phytoplankton are the major source of essential dietary nutrients for all fish, and that dietary energy from mangroves is transferred to juvenile fish *Caranx hippos*, while sea grass nonessential FAs are transferred to the entire food web. Moreover, among the species studied, the sea urchin *Echinometra lucunter* is the major consumer of brown and green algae, while red algae were consumed by the surgeon fish *Acanthurus chirurgus*.

1.2 Introduccion

Coral reefs are located along the coastlines of over 100 countries and provide a variety of ecosystem goods and services. Reefs serve as a major food source for many developing nations, they provide barriers to high wave action that buffer coastlines and beaches from erosion, and they supply an important revenue base for local economies through fishing and recreational activities. This is the case of the Veracruz reef system National Park (Parque Nacional Sistema Arrecifal Veracruzano: PNSAV) located in the southwest Gulf of Mexico. The PNSAV was declared a marine protected area in 1992 because of its high biodiversity. However, the PNSAV is under considerable pressure from fisheries, tourism, and from intense traffic of large ships to Veracruz harbor. Also, it receives high rates of sedimentation ranging between 0.006 and 2 kg m^2 d^{-1}, turbidity, and organic matter related to the three rivers flowing into the PNSAV: La Antigua in the north, Jamapa in the middle and Papaloapan in the south (Perez-España et al. 2008). The myriad sources of organic matter in the oceans depend on the intensity of the autochthonous signal and the proximity and magnitude of inputs from coastal higher plants and transport by rivers. Primary production by microalgae is the largest

of the organic carbon sources to the marine systems, but terrestrial material eroded from rivers has received heightened interest in recent years as a recorder of changing coastal systems and increased floods and sea level (Harvey, 2006).

The main contributor of organic carbon from mangrove forests that border the river margins near the PNSAV is probably *Rizhophora mangle* as it is in direct contact with salt water during high tides in the river zone (Contreras-Espinosa and Warner 2004). The other higher plant growing in the PNSAV, *Thalassia testudinum*, is the most frequent and spacially-dominant sea grass species in the PNSAV lagoon reefs (Terrados et al. 2008). River discharge seems to increase availability of phosphorus to seagrass *T. testudinum* from the water column, increasing the content of orthophosphate 3 to 4 times in leaves and improving their growth when the salinity decreases from 36‰ to 31‰ (Terrados et al. 2008). The benthic primary producers: sea grasses and macroalgae make an important contribution to the productivity of coral reefs, principally for fish communities associated with the reef lagoons (Ayukai 1995).

Naturally occurring stable isotopes of nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) have been used as source tracers, and to determine relative trophic levels of organisms in marine food webs. This approach is based on the principle that stable isotope ratios of consumer tissue can be related to that of diet (DeNiro and Epstein 1981). Between trophic levels enrichment of 3 – 4‰ ^{15}N is generally observed (Michener and Shell 1994) because metabolic pathways select ^{14}N over their isotope ^{15}N and ^{12}C over their isotope ^{13}C. Carbon shows little (1‰) or no change in the relative abundance of ^{13}C between primary producers and first level primary consumers (Hobson et al. 1995) and is therefore

an indicator of C sources of primary producers in systems with isotopically distinct sources. Differences in primary producers values of $\delta^{13}C$ are explained by the inorganic C source (CO_2) incorporated during photosynthesis, for instance the high $\delta^{13}C/^{12}C$ values of seagrass is related to using bicarbonate as an inorganic carbon source (Beer et al. 2002), bicarbonate has a less negative $\delta^{13}C$ than CO_2, 0‰ and -9‰, respectively, and its incorporation by the seagrass may lead to a relatively high $\delta^{13}C$ compared with terrestrial plants like mangrove (Lapointe et al. 2004). The stable isotope approach is an important ecological tool; the majority of work has been in the area of animal foraging and resource partitioning. Much progress has been facilitated by isotopic mixing models, which allow the proportional contribution of sources (dietary items) within a mixture (consumer tissue) to be estimated, and thereby diet composition to be inferred (Parnell et al. 2010). In this study the mixing model was used to determine each of the primary producers' contributions instead of the dietary items to discriminate the origin of carbon and nitrogen in upper food web members. Primary producer contributions were used to quantify the trophic transfer of specific source FA.

Plants, algae, and cyanobacteria biosynthesize different polyunsaturated FA (PUFA: Bell and Tocher, 2009) that can be accumulated in consumers and traced as specific food web sources. Saturated and monounsaturated FA can be stored in fat tissue and can later be mobilized to provide fuel for short or long-term energy demands (Kainz and Fisk 2009). Some authors describe highly unsaturated fatty acids (HUFA) of 20 and 22 carbons in plankton as markers to assess microalgal-derived FA, and odd-chain saturated (OFA) and branched-chain FA (BFA) as bacterial biomarkers (Napolitano 1999). *De novo* biosynthesis of PUFA including HUFA is restricted to organisms at the base of the

aquatic food chain, and fish cannot biosynthesize HUFA *de novo*, but bioconvert PUFA precursors, to various degrees to target HUFA in freshwater fish (Tocher 2003). In contrast, studies in marine fish have consistently shown very little desaturation of PUFA precursors occurs with no production of HUFA. Marine species studied have at least some activity for both the C_{18} to C_{20} fatty acid elongase and the Δ5 desaturase. This is consistent with the genes determining both enzymes being present in marine species. However, the Δ5 fatty acid desaturase gene but not the C_{18}-C_{20} elongase gene is well expressed in freshwater fish cells. In contrast, the C_{18}-C_{20} and C_{20}-C_{22} elongase gene(s) but not the Δ5 desaturase gene is well expressed in marine fish cells. Thus, it can be deduced that the apparent inability of marine fish to convert 18:3ω3 to 20:5ω3 and 22:6ω3 is due not to the complete absence of the required genes in a particular species, but to one or more of the required genes not being sufficiently well expressed (reviewed by Bell and Tocher, 2009). Thus, the trophic transfer of PUFA and HUFA from primary producers to upper trophic levels is critical for the health of higher trophic level marine fish. Changes to food web structure and components may alter this important process (Kainz and Fisk 2009). In coral reefs, there is a growing concern that Scleractinian coral-dominated systems are being supplanted at an increasing rate by macroalgae dominated systems, the latter being favored by eutrophication and reduction of herbivores as a result of disease and over-fishing (Fabricius 2011). The PNSAV is a coral reef system in which the most distant reefs are located between 25 and 50 km from the three rivers.

The contribution of primary producer organic carbon sources that fuel the PNSAV coral reef food web was used to indicate macroalgae consumer species that should be protected. A carbon and nitrogen stable isotope mixing model was used to estimate the relative

contribution of different primary producer sources to the bulk carbon of upper trophic food web members. Then the FA concentration per mg dry weight in upper trophic levels was corrected by the relative contribution of each primary producer according to the mixing model. Finally, retention of primary producer FA was evaluated across trophic levels using a calculated trophic retention factor (TRF).

1.3 Material and methods

1.3.1 Study area

The Veracruz Reef System is located adjacent to the cities of Veracruz and Boca del Rio, and Anton Lizardo village in the Southwest Gulf of Mexico (Fig. 1-1), between 19°01'35'' and 19°16'10''N and between 96° 13'00'' and 95°45'40''W. The PNSAV reef communities are mainly affected by two seasonal atmospheric and oceanographic conditions. During winter they are affected by frontal incursions of northerly systems locally known as 'nortes' which produce winds of 120 km h^{-1}. This dry season has monthly precipitation of 34±15 mm (November to May), and the marine current runs southward. During summer, the atmospheric conditions are dominated by tropical storms from the south which produce high monthly precipitation rates of 265±99 mm (June to October), and the marine current runs northward (Zavala-Hidalgo et al. 2003). Sea temperature differed among months with highest temperatures between May and October 2007, ranging from 26°C to 30°C. The lowest temperatures were recorded from November 2007 to March 2008 at 20 – 22°C. Also, salinity differed among months; the lowest values were recorded in September 2007 with a value of 15 practical salinity units (PSU) at the mouth of the Jamapa River and 25 PSU in the Cabezo reef. The highest

salinity values of 38 PSU were recorded in April and August 2007 and in January and April 2008 (Okolodkov et al. 2011).

1.3.2 Selection of food web members

Primary producers were selected based on the broader seasonal occurrence of the majority of the dominant macroalgae and sea grasses according to Okolodkov et. al. (2007). For upper food web member selection, we used criteria based on biomass, frequency, abundance and presence in both dry and rainy seasons from a previous study in the PNSAV (Perez-España, et al. 2008). Around 71 groups were put into a mass balanced isotope calibrated model Ecopath with Ecosim 6.2 (EwE, Christensen et al. 2005; H. Perez-España unpublished), and we selected 15 groups to be analyzed. Functional groups in Table 1-1 were determined from literature and gut contents studies in the PNSAV species (Perez-España unpubled; Santander-Monsalvo 2010). The basic estimates from the parameterization section of Ecopath allowed the estimation of the trophic level (TL). TL of 1 is assigned to primary producers, and for consumers the TL is estimated as 1+[the weighted average of prey trophic level]. So, if for example, a consumer eats 40% plants (TL = 1) and 60% herbivores (TL = 2), its trophic level will be 1+[(1x0.4)+(2x0.6)]= 2.6.

1.3.3 Sampling methods

Primary producers were collected at the end of the dry season in April 2007 and 2008. The specimens of green algae *Halimeda opuntia*, brown algae *Dyctiota* sp. and red algae *Galaxaura* sp. as well as the most abundant seagrass *T. testudinum* were taken by

divers in the lagoon af the Cabezo reef. Mangrove leaves were collected by hand (with nitrile gloves) from trees located in Arroyo Moreno in the Jamapa river margin. Suspended particulate matter (SPM) was collected using plankton sampling protocols (Okolodkov et al. 2011), at nine stations through the Veracruz Reef System National Park: the mouth of the Jamapa River, the Enmedio, Cabezo, Anegada de Afuera, Anegada de Adentro, Verde, and Sacrificios reefs, one offshore station between Anegada de Adentro and Anegada de Afuera, and one offshore from Anton Lizardo village (Fig. 1-1). Collection was done by net tows: with 30 and 120 μm meshes to collect phytoplankton and zooplankton, respectively. Those samples were taken from a boat, towing at 2 km h^{-1} for 5 min, and an aliquot was identified (Okolodkov et al. 2011) as part of the same project. Plankton and sea grass samples were also collected at the end of the rainy season in October 2008, in order to investigate seasonal variation in their FA. The upper food web members were collected at the end of the rainy season in September – October 2007 and at the end of the dry season in April – May 2008. Samples were collected in the Cabezo, Rizo, and Salmedinita reefs in the south and in the Sacrificios and Anegada de Adentro reefs in the north. Divers went to the deepest part of the reefs and followed a transect to the shallowest part of the reefs, collecting big fishes with a harpoon and small fishes with an anesthetic solution of eugenol (Santander-Monsalvo 2010). Plankton samples were centrifuged to concentrate the biomass and macroalgae were cleaned of epiphytes. Sea urchins (*Echinometra lucunter*), and small fish (*Coryphopterus personatus* and *Haelichoeres burekae*) were collected for subsequent analysis of whole soft tissue. For big fish, homogenation was difficult to do, therefore subsamples of muscle tissue were taken from the surgeon fish *Achanturus chirurgus*, the hogfish *Bodianus rufus*, the

yellowtail snapper *Ocyurus chrysurus*, and the jack *Caranx hippos* for stable isotope and fatty acid analysis. Lipid classes and sterol analyses were also performed on these samples and the data were analyzed in Chapter 2. Jack adults (88±1 cm and 7750±848 g) were collected in open water while juveniles (24±2 cm and 176±31 g) were collected at the Jamapa river mouth.

1.3.4 Stable isotopes

The samples were freeze dried in a Lyophilizer Virtis 5L and ground to a homogeneous fine powder. Lipids were not extracted in order to include this major dietary source in the isotopic signature (Matthews and Mazumder 2005). Subsamples of about 2 mg were weighed with an analytical balance (Heraeus maximum load of 60 g), and analyzed for $\delta^{15}N$, and $\delta^{13}C$ at the University of California in Santa Cruz. The analyses were made on an isotope-ratio mass spectrometer (OPTIMA) interfaced with continuous flow to a Carlo Erba NA 1500 elemental analyzer. These results were presented with respect to the international standards of atmospheric nitrogen (AIR, N_2) and Vienna Pee Dee belemnite (V-PDB) for carbon. The average instrumental precision for nitrogen was 0.14‰ and for carbon was 0.08‰. The stable nitrogen and carbon isotope ratios are expressed as:

$\delta^{15}N$ or $\delta^{13}C = [(R_{sample} : R_{standard})-1] \times 1000$, where R is $^{15}N:^{14}N$ for $\delta^{15}N$ and $^{13}C:^{12}C$ for $\delta^{13}C$

The means and standard deviation of C and N stable isotopes were plotted to detect relative position among primary producers and upper trophic levels.

1.3.5 Fatty acids

All samples were freeze dried on a Lyophilizer Virtis 5L, and weighed with an analytical balance (Heraeus maximum weight of 60 g). Total lipids were extracted with chloroform: methanol: water 2:1:0.8 according to Parrish (1999), and total lipid extracts were divided in two. The first was separated into lipid classes by Chromarod thin layer chromatography (Parrish 1987) to quantify the acyl lipids. The rest of the lipid extract was derivatized to obtain fatty acid methyl esters (FAME) with hydrochloric acid and methanol 5:95 heated at 85°C for 2.5 h (Sato and Murata 1988). FAMEs were extracted in pure hexane, and after 3 hexane washes, the hexane was evaporated under nitrogen and FAME were recovered in 0.5 mL of pure hexane. FAME were analyzed in a Varian 3800 gas chromatograph with a mass spectrometer detector (GCMS), on a 30 m × 0.25 mm × 0.25 μm Omegawax 250 column (Supelco). Chromatographic conditions were helium flow 0.9 ml min^{-1} and injector temperature 250°C. After injection, the temperature of the column was subjected to the following sequence: 110°C for 3 min, increased to 165°C at a rate of 30°C min^{-1}, maintained at 165°C for 2 min, increased to 209°C at a rate of 2.2°C min^{-1}, and maintained at 209°C for 18 min. The mass spectrometer detector was set at 260°C and ion source was set at 70 eV. Peaks were identified by retention time of standards and mass spectra interpretation (Appendix 1) Identification was based on McLafferty and Turecek (1993). Quantification was done by interpolation of peak areas with a calibration curve of 37 fatty acid standards (Supelco 47885-U). Areas were integrated with the Wsearch 32 software version 1.6 2005 (Australia). The FA individual concentration was computed considering resuspended volume of hexane. Total

concentration of identified FAME in the sample (μg mL^{-1}) was considered as 100%, an individual FAME was calculated as a proportion of the total identified FAME. This proportion was related to the acyl lipid fraction of dry biomass to obtain individual FA concentrations per unit of dry biomass (μg mg^{-1}). Lipids were separated into lipid classes, including hydrocarbons, SE/WE, ketones, TAG, free FA, alcohols, ST, AMPL and PL by Chromarod thin layer chromatography (Parrish, 1987a) acyl lipids included SE/WE, TAG, FFA, AMPL and PL. Concentrations of lipid classes were obtained by interpolation with a calibration curve constructed with five concentrations, ranging between 0.5 and 4.0 µg, of the following standards: nonadecane, cholesteryl sterate, 3-hexadecanone, triplamitin, palmitic acid, cetyl alcohol, cholesterol, monopalmitoyl, and phosphatidylcholine (Sigma-Aldrich).

1.3.6 Data analysis

The respective contribution of primary producer sources to the different consumers was assessed with a Bayesian isotopic mixing model available as an open source R package, Stable Isotope Analysis in R (SIAR). To perform the analysis, the R studio software (version 0.96.316 2009-2011) was used. The model allows the inclusion of isotopic signatures and fractionation together with the uncertainty (e.g., standard deviation) of these values within the model. The SIAR model is fitted via Markov Chain Monte Carlo (MCMC) methods producing simulations of plausible values of dietary proportions of sources consistent with the data using a Dirichlet prior distribution (Parnell et al. 2010). The resulting probability distributions of the dietary proportion solutions produced by SIAR allow direct identification of the most probable solution (i.e., the

median value) (Parnell et al. 2010). The average fractionation factors were set at 1.1±0.3‰ and 2.8±0.4‰ for C and N respectively because samples were not treated to remove lipids; according to McCutchan et al. (2003), $\delta^{13}C$ and $\delta^{15}N$ for muscle were lower but not significantly lower than in untreated samples. The use of the model was not constrained to the potential diet sources, all potential primary producer sources collected were considered for all the consumers. A matrix plot of source proportions showed Pearson's *r* values for the negatively correlated sources. This is a useful diagnostic tool as it identifies when the model is performing well, as indicated by low correlations between sources, or when the model is struggling to differentiate between sources, as indicated by significant correlations, if two sources are very close together, then likely solutions could involve one or other of the sources but not both at the same time (Parnell et al. 2010). The Minitab Statistics Software (version 15.1.1.0 2007) was used to test the significance of Pearson's *r* values. To obtain the *p*-value, I computed a *t*-test. The *t*-test is used to establish if the correlation coefficient is significantly different from zero, and hence, that there is evidence of an association between the two variables (primary producers).

Principal components analysis (PCA) was performed on major fatty acids from the sources. PCA was used to detect the correlated variables. A minimum explained variance above 60% for the 3 first components together was considered a good fit. A log ratio transformation was performed to normalize the percentage of total FAs prior to the multivariate statistical test for compositional data according to Aitchison (1986); the centered log ratio transformation consists of division by the geometric mean of the sample followed by a log transformation. A correlation matrix was used to detect the FA with higher contributions to primary producer separation. Source FAs with Pearson's

correlation ≥ 0.7 with components 1, 2 and 3 were further analyzed by Kruskall-Wallis non-parametric analysis using their concentrations expressed as μg mg^{-1}. Minitab Statistics Software (version 15.1.1.0 2007) was used for PCA and Kruskall-Wallis non-parametric analysis. The individual FA concentration per mg dry weight in upper trophic levels was corrected for the relative contribution from each primary producer according to the mixing model (Eq. 1),

$$[C_S] = [C_{cons}] \times (P_S/100) \quad (1)$$

Where C_S= Concentration of FA in the consumer corrected by the source proportion (μg mg^{-1}), C_{cons} = Concentration of FA in the consumer (μg mg^{-1}), P_s = Proportion of the source in the consumer (%).

The corrected FA retention from each primary producer was evaluated in upper trophic levels using the trophic retention factors (TRF). The TRF for fatty acids was calculated similarly to food web magnification factors (FWMF) of contaminants originally proposed by Fisk et al. (2001) and revised by Jardine et al. (2006). Trophic magnification factors provide information on the average change in contaminant concentration per relative trophic level (Müller et al. 2011). The extent to which fatty acids are retained depends on physiological requirements of the organisms. This may result in lower or higher FA concentrations per unit biomass and is regulated by the consumers, which is not the case for most contaminants. TRF for the HUFA 20:4ω6, 20:5ω3 and 22:6ω3 were evaluated also considering all sources together and without considering the SIAR data (i.e., not corrected by the source proportion), to compare the overall HUFA retention with that from each primary producer. Seasonal comparison was

done with analysis of covariance (ANCOVA with α=0.05), using Minitab software (version 15.1.1.0 2007).

In this study the whole body concentration was used from homogenized organisms for invertebrates and small finfish, while muscle was used in upper food web members for TRF calculation. Equation 2 calculates the change of FA per relative trophic level from each primary producer using the natural logarithm of the concentration dry weight ($C_{dry\ wt}$) of individual organisms *vs* their trophic level (TL):

$$\ln[C_{dry\ wt}/C_0]= \alpha+\beta \times TL \quad (2)$$

where C_0= A standard unit of concentration (1 μg mg^{-1}), α= intercept, β= slope, and TL = Trophic level with no units. TRF= e^{β}.

Minitab Statistics Software (version 15.1.1.0 2007) was used to test the significance of the regression by a basic analysis of variance: a probability value, p of < 0.05 means that the slope β is significantly different from zero or that the TRF= e^{β} is different from 1. Values of TRF= 1 denote no trophic change with respect to the source, TRF> 1 means trophic retention, and TRF< 1 means trophic reduction probably because of catabolism or chain elongation and/or desaturation.

Using the parameters calculated with Eq. 2, in those cases where the regression was significant, we can compute concentrations of each FA in each trophic level with Eq. 3:

$$C_{dry\ wt}/C_0=e^{\alpha}\times e^{\beta \cdot TL} \quad (3)$$

Isolating $C_{dry\ wt}$, we have the Eq. 4:

$C_{dry\ wt} = e^{\alpha} \times e^{\beta \cdot TL} \times C_0$

(4)

where $C_{dry\ wt}$=Concentration of FAs in a specific trophic level (μg mg^{-1} dry weight), e^{α}= initial concentration (unitless), e^{β}= Trophic Retention Factor (unitless), TL= Trophic level (unitless), and C_0 is a standard unit of concentration (1 μg mg^{-1}).

1.4 Results

1.4.1 Food web members

Trophic levels of the 15 groups collected for fatty acid and isotope analyses ranged from 1 – 3.7 (Table 1-1). The sample size was different for fatty acids (Table 1-1) and for stable isotopes (Table 1-2). Samples obtained with the 30 μm mesh plankton net were considered to have a minimum size of 30 μm but the maximum size was not measured. Organisms collected in those samples were identified at least to the phytoplankton group level. There were between 4 and 16 species of diatoms in the dry season compared with 30 – 36 species in the rainy season. Dinoflagellates showed similar numbers of species in both seasons, ranging from 3 – 10. The common phytoplankton species in the dry season was *Chaetoceros decipiens*, while in the rainy season it was *C. lacinius* and the cyanophyte *Trichodesdium* sp. Dry biomass was significantly higher in the dry season 39±14 mg m^3 (mean±confidence interval with α= 0.05), compared with 14±5 mg m^3 in the rainy season. Suspended particulate matter collected with a 120 μm plankton net was also considered to have a minimum size of 120 μm but the maximum size was not measured.

1.4.2 Stable isotopes of C and N

The contribution of primary producers to consumers is shown in Table 1-2. The mean $\delta^{13}C$ and $\delta^{15}N$ stable isotope values are shown at the bottom of Table 1-3 and in Figure 1-2. The isotopes were significantly different based on the confidence interval (α= 0.05) among primary producers, which were enough to trace them in the food web. Values of $\delta^{13}C$ for primary producers were clearly different, the most negative value coming from CO_2 was the mangrove *R. mangle* (-29.1±2.2‰), while for marine primary producers the source of C was dissolved bicarbonate and the values of $\delta^{13}C$ were less negative: phytoplankton (-19.2±2.1‰), seagrass *T. testudinum* (-15.4±1.7‰), green algae *H. opuntia* (-14.4±5.5‰) with high variability probably due to tissue carbonates, brown algae *Dyctiota* sp. (-14.0±2.7‰), and red algae *Galaxaura* sp. (-11.2±2.7‰).
N stable isotope data provided distinct end members (Table 1-3 and Fig. 1-2). Phytoplankton showed the highest level of $\delta^{15}N$ (5.1±0.6‰), while macroalgae had the lowest value of $\delta^{15}N$, red algae *Galaxaura* sp. (-2.0±1.5‰), green algae *H. opuntia* (-0.7±0.8‰) and brown algae *Dyctiota* sp. (-0.09±0.68‰). Such low values have been reported in macroalgae when the nitrogen source came from fertilizers (Umezawa et al. 2002), while in the seagrass *T. testudinum* (3.3±0.6‰) the source of N derives mainly from sediment pore water (especially ammonium) absorbed by roots and the water column (mostly NO_3^-) absorbed by leaves. The mangrove *R. mangle* had values of N from NO_3^- taken up via roots from the surrounding ground (2.0±0.2‰).

The zooplankto value $\delta^{15}N$ (4.9±1.6‰) was similar to phytoplankton (5.1±0.4‰). The sea urchin *E. lucunter* was a macroalgae consumer with a low $\delta^{15}N$ value of 3.3±0.6‰

which is 3‰ higher than macroalgae. The surgeon fish *A. chirurgus* was clearly not just a herbivore as the δ^{15}N values were 8±0.6‰, 8‰ higher than macroalgae and 5‰ higher than sea grass *T. testudinum,* and 3‰ higher than phytoplankton, suggesting that surgeon fish was omnivorous or detritivorous with a dietary contribution from macroalgae and phytoplankton (Fig. 1-2). The fish *C. personatus* and *H. burekae* had similar values of δ^{15}N (8.4±0.5 and 8.2±1.0, respectively), also values of δ^{13}C were similar between both species clearly related to phytoplankton (-17.7±0.4‰ and -17.5±1.8‰, respectively); while values of δ^{13}C for *A. chirurgus* were related to macroalgae and seagrass (-14.4±0.5‰). The hogfish *B. rufus* and the yellowtail snapper *O. crhysurus* had similar values of δ^{15}N (9.3±1.1‰ and 9.3±0.3‰, respectively); however, values of δ^{13}C for *B. rufus* was related to macroalgae and/or sea grass (-14.4±0.5‰) while the yellowtail snapper *O. chrysurus* was the finfish with a clear seasonal change in organic carbon source (see bottom of Tables 1-4 and 1-5). The latter showed a clear relationship with macroalgae in the dry season δ^{13}C of -13.1±0.6‰, compared with the rainy season, when δ^{13}C was -16.6±0.7 ‰ from phytoplankton. Finally the jack cravalle fish *C. hippos* with the higher trophic level of 3.7 (Fig. 1-2) showed differences between life stages. Juveniles had higher values for δ^{15}N (12.3±0.3‰) than adults (10.7±0.5‰) and in terms of δ^{13}C (-20.3±1.2‰) compared with adults (-14.8±0.5‰).

The isotope mixing model indicated that mangrove and phytoplankton were two sources that were difficult to differentiate, so likely solutions involve one or other of the sources but not both at the same time (Table 1-2). The mean relative contribution of phytoplankton in upper consumers ranged from 3 – 68%; ranges were red algae 2 – 52%, mangrove *R. mangle* 1 – 33%, seagrass *T. testudinum* 7 – 30%, brown algae *Dictyota* sp.

1 – 26%, and green algae *H. opuntia* 1 – 13%. Higher plant mangrove *R. mangle* showed high proportions in zooplankton samples of 33% in the dry season and 29% in the rainy season, but it was negatively correlated with phytoplankton which had 22% in both seasons. For the planktivorous small finfish *C. personatus* and *H. burekae* the mangrove contribution was 5 – 18%; for piscivorous fish *O. chrysurus,* the mangrove's contribution increased from 1% in the dry season to 12% in the rainy season, and for juveniles of *C. hippos*, it was 29% in the dry season and 29% in the rainy season (Table 1-2). In contrast, the lowest mangrove contribution was recorded in the sea urchin *E. lucunter* and surgeon fish *A. chirurgus*, 1– 3% in both seasons, and it was similar to the sea urchin consumer, the hogfish *B. rufus* which showed a low mangrove contribution from 2 – 4% in both seasons, similar to the adult's *C. hippos* of 6% in the dry season. The other higher plant growing in the PNSAV, the seagrass *T. testudinum* had a lower contribution to the zooplankton in the dry season of 7% compared with 15% in the rainy season. Meanwhile, seagrass contributions to the sea urchin were similar in both seasons with 11% and 9%, respectively. The surgeon fish had a higher seagrass contribution of 30% and 25% in dry and rainy seasons, respectively, similar to the rest of the consumers. The exception was juveniles of *C. hippos*, which showed an increase from 11% in the dry season to 17% in the rainy season (Table 1-2).

The contributions of green, red and brown macroalgae to the coral reef food web were different: the green algae *H. opuntia* showed the lowest contribution, 1– 4%, except in *E. lucunter, B. rufus* and adults of *C. hippos* with 24%, 13% and 16%, respectively. Red algae *Galaxaura* sp. showed the highest contribution ranging between 2 and 52%. Low contributions of 2 – 5% were observed in zooplankton, small fish *C. personatus* and *H.*

burekae, and juveniles of *C. hippos.* In contrast, the sea urchin *E. lucunter* showed the highest contribution from red algae of 52% in the dry season. The surgeon fish *A. chirurgus*, hogfish *B. rufus* and yellowtail snapper *O. chrysurus* showed red algae contributions ranging between 15% and 28%. Finally, brown algae *Dyctiota* sp. ranged between 1 and 26%. The lowest contribution was observed in the zooplankton, small fish *H. burekae,* and in juveniles of *C. hippos* at 1 – 8%. In contrast, the highest brown algae contribution was observed in *E. lucunter* of 26% and in the sea urchin consumer the hogfish *B. rufus*, it was 10% in the rainy season. The other consumers showed seasonal differences with higher brown algae contributions in the dry season: small finfish *C. personatus* 15%, yellow tail snapper *O. chrysurus* 15%, *and* adults of *C. hippos* 22% (Table 1-2).

Phytoplankton carbon was incorporated into all upper consumers analyzed, except for the sea urchin *E. lucunter* (3 – 4%) in which macroalgae and seagrass were the most important primary producer sources. Among the consumers, zooplankton showed a relatively lower contribution of 22% compared with the surgeon fish which is usually considered to be an herbivore and which had 29%. The other members showed organic carbon contributions ranging from 21 to 27%. The highest contribution from phytoplankton was observed in the small fish *C. personatus* with 68% in the rainy season (Table 1-2).

1.4.3 Fatty acids

Principal components analysis (PCA) was done using primary producer sources in the Veracruz coral reef ecosystem (TL=1) with six sample types: 1) mangrove *R. mangle*, 2)

seagrass *T. testudinum*, 3) green algae *H. opuntia*, 4) red algae *Galaxaura* sp., 5) brown algae *Dyctiota* sp., and 6) phytoplankton. Thirty-four variables were identified and quantified with proportions greater than 0.7% including the FA presented in Table 1-3. The response variables were the log ratio of the percent of fatty acid methyl ester (FAME). There is a significant difference based on the confidence interval ($\alpha = 0.05$) in the FA composition among some sources (Table 1-3): mangrove *R. mangle* and seagrass *T. testudinum* had similar percentage of 18:3ω3, 33±9% and 27±8%, respectively; significantly higher than green algae *H. opuntia* with 9±2%, which was significantly higher than the rest of the algae and phytoplankton with 0.6 - 2% of this FA ($p < 0.05$). A similar trend was observed with 18:2ω6, with similar proportions of 21±4% and 18±5% in mangrove *R. mangle* and seagrass *T. testudinum*, respectively and significantly higher than green algae *H. opuntia* with 7±1%, which was significantly higher than the rest of the algae and phytoplankton with 1.7 – 3.1% of this FA. In contrast, highly unsaturated FAs (HUFAs) 20:4ω6, 20:4ω3, 20:5ω3 and 22:5ω3 were absent in higher plants and present in green, red and brown algae, as well as phytoplankton. Essential metabolite FA (EMFA for marine fish *sensu* Parrish, 2009) composition showed different trends: while arachidonic acid (20:4ω6) had a significantly higher percentage in brown algae *Dictyota* sp. of 9±4%, eicosapentaenoic acid (20:5ω3) did not show differences among green, red and brown macroalgae, and phytoplankton, ranging from 4 to 9%, docosahexaenoic acid (22:6ω3) had significantly higher percentages in green algae *H. opuntia* and phytoplankton ranging from 5 to 8%, compared with red algae *Galaxaura* sp. with 0.7±0.6% ($p<0.05$), and it was not detected in the brown algae *Dictyota* sp. (Table 1-3).

The PCA showed underlying variables using a correlation matrix (Fig. 1-3). The first principal component, PC1, explains as much as 52.5% of the total variance and separates terrestrial from marine sources. Brown and red algae as well as phytoplankton fall to the right side of PC1 with higher contents of 14:0, 16:1ω7, 16:2ω4, 20:5ω3 and to a lesser extent 15:0, branched FA, and 22:6ω3, whereas seagrass and mangrove fall on the left side of PC1 with higher contents of 18:2ω6 and 18:3ω3. The green algae are near to the mean of PC1 sharing marine and higher plant FAs. The second principal component (PC2) explains 15.7% of the total variance and separates the red algae due to 16:0, 22:5ω3 and 24:1ω9, and green algae with 18:1ω7 and 20:3ω3 fall in the lower half and below brown algae and phytoplankton which fall above (Fig. 1-3A, B). PC3 explains 14.8% of the total variance and separates the brown algae well, with higher contents of 18:1ω5, 18:1ω9, 18:4ω3, 20:4ω6, and 22:4 ω3, from phytoplankton with higher content of 14:0, 16:1ω7, 16:2ω4, 22:6ω3, and branched FA (Fig. 1-3C, D). The first three underlying principal components accounted for 83.0% of the total explained variance and therefore were considered a good fit of the data to the model.

Fatty acid composition of upper food web members at the end of the dry season (Table 1-4) and at the end of the rainy season (Table 1-5) were considered to compare the PCA selected FAs. The major phytoplankton FAs were 14:0, 16:1ω7, 16:2ω4, 20:5ω3, and 22:6ω3. Those FAs were incorporated into all upper consumers analyzed, except the 22:6ω3 in the sea urchin *E. lucunter* (Table 1-4 and 1-5). The EFA 20:4ω6 showed the highest percentage in the brown algae *Dyctiota* sp. compared with the rest of the primary producers analyzed. This FA was detected in the sea urchin *E. lucunter* and the surgeon fish *A. chirurgus* with the highest proportion of 12±1% and 9±2%, respectively in both

seasons, transferring this FA proportion to the hogfish *B. rufus* during both seasons and to the yellowtail snapper *O. crhysurus* in the dry season (Tables 1-4 and 1-5). According to the PC analysis the FAs 18:1ω7 and 20:3ω3 were partially related to green algae *H. opuntia;* however, their percentages were similar among sea grass, red algae and phytoplankton.

The concentration of PCA selected FAs was compared among primary producers (Table 1-6). Among the FA 14:0, 16:1ω7, 16:2ω4, 20:5ω3, and 22:6ω3, which fall to the right (marine) side of PC 1 (Fig 1-3A, B), the only FA that showed significantly higher concentration in phytoplankton was 16:1ω7 with 3±1 μg mg^{-1}, the other FA showed similar concentrations in green algae and phytoplankton (Table 1-6).

In contrast, the FA 18:2ω6 and 18:3ω3, which fall to the left (higher plants) side showed similar concentrations in mangrove, seagrass and green algae. Based on the mean, concentrations of 18:2ω6 ranged between 1 and 5 μg mg^{-1}, and mean concentrations of 18:3ω3 ranged between 1 and 9 μg mg^{-1} compared with the rest of the sources with concentrations of 0.1 – 0.7 μg mg^{-1}. The concentration of the FA 16:0, 22:5ω3 and 24:1ω9, which were related to red algae and 18:1ω7 and 20:3ω3 which related to green algae, with percentages falling in the lower half of PC2 (Fig. 1-3A, B), did not show the same relationship when concentration was compared.

For instance, 16:0 was significantly more abundant in the mangrove *R. mangle,* the green algae *H. opuntia*, and phytoplankton ranging between mean values of 5 and 10 μg mg^{-1} than the rest of primary producers (H=17.3 p=0.008). While, 22:5ω3 was significantly more abundant in the green algae *H. opuntia* and phytoplankton ranging between 0.1 and 0.3 μg mg^{-1} than the rest of primary producers (H=13 p=0.005: Table 1-

6). The FAs 18:1ω9, 18:4ω3, 20:4ω6 and 22:4ω3 separate the brown algae well from phytoplankton (Fig. 1-3C, D) but showed no significantly different concentrations with other primary producers. The FA 18:1ω9 had similar concentrations in phytoplankton in the dry season, brown algae *Dictyota* sp. and mangrove *R. mangle* with mean values ranging between 2 and 3 μg mg^{-1} and significantly higher than red algae *Galaxaura* sp., green algae *H. opuntia* and phytoplankton in the rainy season (H=21.2 p=0.002). However, 18:4ω3 and 20:4ω6 had a significantly higher percentage in brown algae *Dictyota* sp. (Table 1-3) and these FA showed similar concentrations between brown algae *Dictyota* sp. and green algae *H. opuntia* of 1 and 2 μg mg^{-1}, respectively. Finally, 22:4ω3 also with a high percentage in brown algae *Dictyota* sp. had similar concentrations to those in phytoplankton, around 0.1 μg mg^{-1}.

1.4.4 Trophic retention factor

TRFs were calculated as explained at the end of the data analysis section. Statistically significant relationships between trophic level and logarithmic concentrations (μg mg^{-1} dry wt) were found for all the FA considered (Table 1-7). The saturated FA, 14:0 concentrations tends to decrease with trophic level increase during the dry season from all the sources considered, and tended not to change with respect to the sources in the rainy season. In contrast, the other saturated FA, 16:0 and the polyunsaturated FA, 16:2ω4 did not show changes with respect to the source (TRF≈ 1) in both seasons, except when the source was the seagrass *T. testudinum.* From this source, 14:0, 16:0 and 16:2ω4 showed TRFs of 1.41, 2.48 and 2.06 times per trophic level, respectively, in the rainy season. The monounsaturated FA 18:1ω9 and 18:1ω7 did not show concentration changes with

respect to the primary producer (TRF≈ 1) when the sources were green algae *H. opuntia* and brown algae *Dyctiota* sp. in both seasons, and mangrove *R. mangle* and phytoplankton in the dry season. However, oleic acid,18:1ω9 had a trophic magnification with respect to the seagrass *T. testudinum* and red algae *Galaxaura* sp. in both seasons and with respect to phytoplankton in the rainy season. Also mangrove *R. mangle* showed a TRF=2.18 in the rainy season but with no significant difference to one (p=0.112). Meanwhile, *cis*-vaccenic acid 18:1ω7 just had a trophic retention from seagrass *T. testudinum* in both seasons and from mangrove *R. mangle* and phytoplankton in the rainy season. Nervonic acid 24:1ω9 was present just in green algae *H. opuntia* and red algae *Galaxaura* sp., and phytoplankton; however, this FA only showed trophic retention from red algae *Galaxaura* sp. and phytoplankton (Table 1-7). PUFA of 18 carbons trend to decrease as trophic level increases or they did not change with respect to the primary producer (TRF≤ 1). Linoleic acid 18:2ω6 showed trophic reduction (TRF< 1) mostly in the dry season from all sources, except from the red algae *Galaxaura* sp. with TRF=0.8 (p=0.172), not significantly different to one, while in the rainy season it did not change with respect to all primary producers (TRF≈ 1), except from the green algae with a trophic reduction TRF= 0.35 (p=0.001). In contrast, α-linolenic acid 18:3ω3 showed trophic reduction (TRF< 1) in both seasons from all primary producers, except from the red algae *Galaxaura* sp. and phytoplankton in the rainy season (TRF≈1). Stearidonic acid 18:4ω3 was not present in mangrove and sea grass, and also showed trophic reduction (TRF< 1) in both seasons from all marine primary producers, except from the red algae *Galaxaura sp.* with a TRF≈ 1. The HUFA of 20 carbons, 20:3ω3 and 20:5ω3 trend to decrease as trophic level increases (TRF< 1) principally from green algae *H. opuntia* in

both seasons, and they did not change with respect to the primary producer (TRF≈ 1) when the source was red algae *Galaxaura* sp. and phytoplankton. In contrast, arachidonic acid 20:4ω6 tended to increase as trophic level increases or it did not change with respect to the primary producer (TRF≥ 1). When the source was green and brown algae its concentration was similar to the sources. In contrast, trophic retention occurred when the source were red algae and phytoplankton with TRF ranging between 1.7 and 1.9. The HUFA of 22 carbons, 22:5ω3 and 22:6ω3 tended to increase as trophic level increased or they did not change with respect to the primary producer (TRF≥ 1). They did not change with respect to green algae in both seasons and with respect to phytoplankton in the dry season. In contrast, 22:5ω3 and 22:6ω3 concentration had a trophic retention with TRF ranging between 1.7 and 3.7 when the source was the red algae *Galaxaura* sp. in dry and rainy seasons, respectively, and a TRF= 1.74 when the source was phytoplankton in the rainy season (Table 1-7).

Trophic retention was evaluated for the HUFA 20:4ω6, 20:5ω3 and 22:6ω3 also considering all sources (mangrove, seagrass, brown, red and green algae, and phytoplankton) in the analysis. Concentration of 20:4ω6 increased as trophic level increased, with a positive slope of 0.93 (TRF> 1), r^2=0.36, p=0.001 in dry seasons and 0.76, r^2=0.27, p=0.001 in the rainy season (Fig. 1-4A), without significant seasonal differences in the TRF (2.54 and 2.14 respectively) $F_{1,1,97}$=1.03, p=0.313. Also, 20:5ω3 showed a positive slope of 0.34, r^2=0.36, p=0.001 in the dry season and 0.39, r^2=0.16, p=0.001 in the rainy season (Fig. 1-4B). Significant seasonal differences in 20:5ω3 TRF were observed with a higher TRF in the rainy season of 1.48 compared with 1.41 in dry seasons $F_{1,1,104}$=9.1, p=0.003. The highest TRF was recorded for 22:6ω3, the slope was

1.22, r^2=0.32, p=0.001 in the dry season and 1.26, r^2=0.42, p=0.001 in the rainy season (Fig. 1-4C), without significant seasonal differences in the TRF of 3.4 and 3.5 times per trophic level, respectively $F_{1, 1, 92}$ =0.01, p=0.93. Substitution of parameters is shown for ARA, in the dry season: [ARA]= $e^{-2.37}$ x $e^{0.93 \cdot TL}$, and in the rainy season: [ARA]= $e^{-1.68}$ x $e^{0.76 \cdot TL}$ (Fig. 1- 4A), EPA, in the dry season: [EPA]= $e^{-0.66}$ x $e^{0.34 \cdot TL}$, and in the rainy season: [EPA]= $e^{-0.2}$ x $e^{0.39 \cdot TL}$ (Fig. 1-4B), and DHA, in the dry season: [DHA]= $e^{-2.44}$ x $e^{1.22 \cdot TL}$, and in the rainy season: [DHA]= $e^{-2.49}$ x $e^{1.26 \cdot TL}$ (Fig. 1-4C).

1.5 Discussion

1.5.1 Sources of organic carbon for coral reef food webs

In the coral reef food web of of the southwest Gulf of Mexico, the δ^{13}C and δ^{15}N values of phytoplankton, macroalgae, sea grass and mangrove fell within ranges previously observed in other coral reefs ecosystems, for instance, the Florida Keys (Fourqurean and Schrlau 2003), and subtropical fringing reefs of the Ryukyu Islands (Umezawa et al. 2002). Comparison of values of δ^{13}C allowed us to distinguish the relation of each source to the food web, for instance: *R. mangle* (-29.1±2.2‰), is less enriched than the sea grass *T. testudinum* (-15.5±1.7‰) but the later had similar δ^{13}C values to *H. opuntia* (-14.4±5.5: Table 1-3).

The isotopic mixing model differentiated the contribution of these higher plants and green algae to the food web. Mangrove and phytoplankton were two sources with strong negative correlations in the pelagic food web. Mangrove contributions were high in zooplankton with 33%, small finfish *C. personatus* with 18%, and *C. hippos* juveniles with 29% (Table 1-2). Zooplankton identification was not part of the present study.

According to Okolodkov et al. (2011) the major taxa that contributed to zooplankton abundance were calanoid copepods; these dominated over other groups such as marine cladocerans (*Evadne* sp. and *Penilia* sp.), which have been recorded as the main group in similar environments. In addition, plant debris and other organic matter were detected in these samples by stereoscopic microscope, but they were not quantified. Mangrove detection in these samples could not be incorporated in zooplankton tissue but being part of suspended matter. According to Parnell et al. (2010) two sources with negative strong correlations involve one or other of the sources but not both at the same time. Zooplankton samples collected closer to the river could get more mangrove debris compared with those distant stations. Small fish *C. personatus* had a high contribution of mangrove organic carbon; apparently this contribution is underestimated in gut content studies where 91.9% of the *C. personatus* gut content was identified as copepods in the PNSAV (Santander-Monsalvo 2010). If this planktivorous fish *C.personatus* made no distinction between particles probably consuming plant debris that could explain the high contribution of mangrove organic carbon. Two of the most conspicuous primary producers in coastal marine environments in the Caribbean and tropical Atlantic Ocean are the mangrove *Rhizophora mangle* and the sea grass *T. testudinum*. While the importance of direct grazing on these plants is receiving renewed attention (Valentine and Heck 1999), it is thought that the majority of the energy flow from these plants to higher trophic levels occurs via a detrital pathway (Fourqurean and Schrlau 2003). A clear differentiation among higher plant contributions was recorded with the isotope mixing model, the sea grass *T. testudinum* had a similar contribution to phytoplankton ranging between 9 and 30% in all the food web members, and the lowest contribution was

detected in zooplankton (Table 1-2). This suggests that seagrass *T. testudinum* probably was incorporated by direct contribution from grazing and not through debris as with mangrove. The contribution of mangrove through food web transfer to fish such as the juvenile stage of jack, *C. hippos* at trophic level 3.7 is quite clear: using an enrichment of 1.1‰ for every trophic level, the expected value for $\delta^{13}C$ in the fourth trophic level if the main primary producer is mangrove is -26±2.2‰, compared with -16±2‰ if the source is only phytoplankton. The observed $\delta^{13}C$ value for carangid juveniles, was -20.3±1.2‰, more related to phytoplankton but with an incorporation of mangrove compared with adults (-14.8±0.5‰), the latter falling in the phytoplankton interval for the fourth trophic level (-16±2‰: Table 1-4). This result was confirmed by the isotope mixing model: juveniles of *C. hippos* had a mangrove contribution of 27% and 29%, in the dry and rainy seasons, respectively (Table 1-2). While adults of *C. hippos* had a mangrove contribution of 6% in the dry season; in the rainy season, adults were not detected in the captured samples. The mangrove organic carbon contribution can be explained by the consumption of invertebrates and small finfish growing in the coastal lagoons populated by mangrove such as the Mandinga lagoon, where *C. hippos* juveniles live before being recruited to the coral reef stocks. When the mixing model was able to differentiate mangrove and phytoplankton carbon, the contribution of mangrove was low: 1– 4% in the sea urchin *E. lucunter,* the surgeon fish *A. chirurgus,* and the hogfish *B. rufus*. For them the principal carbon sources were seagrass and phytoplankton, except for the sea urchin *E. lucunter.* The latter was the principal consumer of macroalgae including the foliose brown alga *Dyctiota* sp., this alga grows in living coral and can damage it, unfortunately it has low palatability for most herbivorous fish (Fong and Paul 2011), coincident with the low

contribution to the surgeon fish *A. chirurgus* carbon with 3% and 4%, in the dry and rainy seasons, respectively. Upper trophic level fish like the hogfish *B. rufus* had a greater contribution of *Dyctiota* sp. of 10% in the rainy season probably through consumption of sea urchins. Green alga *H. opuntia* had a low contribution for most of the upper members except for *E. lucunter* which had 24%, while the red alga *Galaxaura* sp. had the highest contribution of 52%. These results were coincident with six- week experiments where the sea urchin *D. antillarum* consumed considerably more *Galaxaura* sp. than any other species (Solandt and Campbell 2001). For *D. antillarum* it seems that it must feed on heavily calcified species at a greater rate than fleshier brown algal species. Also, the surgeon fish had the main contribution coming from red algae compared to green and brown algae. While sea urchin and surgeon fish ate algae by direct grazing; for *O. chrysurus* the red algae contribution (28%) could be related to its prey, the omnivore decapods crabs *Mithax* sp. and *Stenorhyncus seticornis* (Santander-Monsalvo, 2010).

1.5.2 Supply of fatty acids

The supply of 18:2ω6 and 18:3ω3 from mangrove was 5±3 and 9±3 μg mg^{-1}, respectively, while from sea grass it was 1±0.5 and 2±1 μg mg^{-1}, respectively. Vertebrates, including fish, lack the Δ12 and ω3 (Δ15) desaturases and so cannot form 18:2ω6 or LIN and 18:3ω3 or ALA, respectively, from 18:1ω9 and, therefore PUFA are essential dietary components. However, dietary LIN and ALA can, with varying efficiencies, be further desaturated and elongated in vertebrates to form HUFA, including ARA, EPA, and DHA as Bell and Tocher (2009) reported. Early nutritional studies suggested that ALA and/or LIN could satisfy the EFA requirements of freshwater fish,

whereas the ω3HUFA, EPA and DHA were required to satisfy the essential FA requirements of marine fish (Sargent et al. 2002). The supply of HUFAs for carangid *C. hippos* juveniles, as stable isotopes show, came from phytoplankton. Probably the isotope signature from mangrove is coming from 18:1ω7, the only FA that showed a significant TRF> 1 from mangrove principally in the rainy season with TRF=2.49 and p=0.010. In contrast to 18:1ω9 which had a not significant TRF of 2.18 (p= 0.112: Table 1-7). According to Dr. H. Perez-España (pers. comm.) another 45 species of fish present in the PNSAV grow up in lagoons where the mangrove is the principal higher plant, including *Megalops atlanticus* fished by sport fishermen, and several species of pargos with commercial importance (e.g., *Lutjanus synagris* and *L. analis*). There is a particular emphasis on increasing the mangrove conservation as part of the coral reef management plan avoiding urban growth on the mangrove zone to conserve coral fish populations. The loss of mangrove cover has serious consequences, for instance, the largest herbivorous fish in the Atlantic, *Scarus guacamaia*, with a recruitment dependency on mangroves has suffered local extinction after mangrove removal in some Caribbean reefs (Mumby et al. 2004).

The proportional increase of phytoplankton FA, particularly 22:6ω3 in higher trophic levels probably responds to the significantly higher biomass present in phytoplankton in the dry season of 39±14 mg m^{-3} dry wt compared to the rainy season of 14±5 mg m^{-3} dry wt (see section 1.4.1). However, acyl lipid concentrations were greater in higher trophic levels at the end of the rainy season, probably because zooplankton had a biomass peak at the end of the rainy season in September and October (Okolodkov et al. 2011). Phytoplankton biomass is probably consumed by adults of benthic invertebrates

during the dry season leading to a reproductive peak in the rainy season. That could explain why the zooplankton was composed principally of larval stages of benthic species Bivalvia (larvae), Chaetognatha, Appendicularia, Polychaeta (larvae), Gastropoda (larvae), Decapoda (larvae), Echinodermata (larvae), Hydrozoa (hydromedusae), Cladocera, and Calanoid copepods (mostly copepodite stages: Okolodkov et al., 2011). Among these taxa, the copepods are the principal food of the planktivorous fish *H. burekae* and *C. personatus* (Weaver and Rocha 2007). The cryptic fish *C. personatus* are more frequent in fringing reefs and are the most abundant fish (80%) of all individuals in Venezuelan Caribbean reefs (Rodriguez-Quintal 2010). In the PNSAV *C. personatus* contributes with a biomass of 6050 Kg km^{-2}.

The increase of the zooplankton biomass during the rainy season could explain the increase of phytoplankton carbon in *C. personatus* (68%) during the rainy season (Table 1-2). Fish feeding on plankton channels nutrients and energy from the pelagic zone to the reef environment, importing particulate organic and inorganic material to the reef when they excrete dissolved waste products while sheltering in it (Rothans and Miller 1991). This transport can explain the high proportion of 16:1ω7, 20:5ω3, and 22:6ω3, as the phytoplankton contribution in *A. chirurgus* of 23% and 29%, in the dry and rainy season, respectively (Table 1-2), as well as the high value of δ^{15}N, around 8‰ (Tables 1-4 and 1-5).

According to Hernández et al. (2008) *A. chirurgus* consumes red algae of the genera *Agardhiella*, *Centroceras*, *Ceramium*, *Galaxaura*, *Gelidiella*, *Gelidiopsis*, *Heterosiphonia*, and *Jania* (7.58%). They also consume brown algae *Dictyota* (6.05%), green algae (6.69%), and other materials (18.14%), including sand (6%). If the surgeon

fish *A. chirurgus* would consume only macroalgae its value of $\delta^{15}N$ would be around 3‰ similar to sea urchin *E. lucunter* a value of 8‰ is 3‰ higher than phytoplanton and zooplankton around 5‰ (Tables 1-3,1-4 and 1-5). The dissolved waste products from planktivorous can be deposited in sand and are incorporated into the food web by detritivorous like *A. chirurgus*.

1.5.3 Trophic retention factor

FA biomarker tracers may provide an additional advantage for studying food webs in that their signals may persist through multiple trophic transfers (Hall et al. 2006; Copeman et al. 2009). The most important long-chain (C_{20}-C_{22}) HUFA in fish are ARA, EPA, and DHA (Sargent et al. 1999). They have to be supplied to animals in their diet, although some animals can synthesize at least some of them when sufficient quantities of the LIN and ALA precursors are available. However, marine fish appear to have lost the ability to express a key elongase and/or desaturase gene (Tocher 2003). According to the results, FA composition is useful to distinguish macroalgae biomarkers when FA are used qualitatively. For instance, the high percentage of ARA in brown algae *Dyctiota* sp. and in surgeon fish *A. chirurgus* may overestimate the contribution of brown algae to the diet of surgeon fish, compared with reporting FA concentrations per unit of algal dry biomass (Table 1-6). Because there were no significant differences in the concentration per unit of algae dry weight among green and brown algae, the highest amount of ARA did not equate with its highest percentage.

In the present study, the selection of FA was based on fatty acids that clearly separated primary producers in the PCA (Fig. 1-3), those FA with higher proportions

compared with other primary producers. Most FAs separated were essential fatty acids for marine fish i.e., ARA, EPA, and DHA. All marine sources had ARA, but the brown algae *Dyctiota* sp. had the highest proportion (9±4%) but not the highest concentration, that was similar to green algae (1.7±0.7 μg mg^{-1} dry wt). With respect to green and brown algae, ARA had a TRF≈ 1 which means that ARA from green and brown algae shows no trophic accumulation or has similar concentration among top predators and primary producers. This HUFA is important in sea urchin egg development (Ciapa et al. 1995). In contrast to dietary ARA from green and brown algae, ARA was accumulated or retained from the rest of the marine primary producers increasing from 0.5 μg mg^{-1} in red algae and microphytoplankton to 2 μg mg^{-1} in the upper trophic levels. ARA is needed for finfish growth, survival, and stress resistance (Bell and Sargent 2003). Probably the required concentration of ARA in the higher tropic levels of coral reef food webs is around 2 μg mg^{-1}.

The yellowtail snapper *O. chrysurus* was the finfish with a clear seasonal change in organic carbon source. There was a quite clear relationship with macroalgae, in particular with red, and brown algae in the dry season, with contributions of 28% and 15%, respectively. The value of δ^{13}C was -13.1±0.6‰, and there was a higher proportion of ARA (7±2%) in the dry season compared with the rainy season, when red and brown algae contribution was 2% from both of them (Table 1-2), and the value of δ^{13}C was -16.6±0.7‰ more related to phytoplankton, with a lower proportion of ARA (2.7±1.3%). This change was also observed in gut contents where the diet changed from Clupeiformes fish and crustaceans in the dry season to Clupeiformes finfish and planktonic mollusks in the rainy season (Santander-Monsalvo 2010), suggesting that the Clupeiformes that

occasionally forage on floating pieces of algae (Ara et al. 2011) channel macroalgae organic carbon to pelagic top predators or that the contributions of crabs of less than 0.8% of wet wt and 17% of relative frequency according to Santander-Monsalvo (2010), was underestimated by the gut content analysis.

When all sources were considered together (data uncorrected by SIAR output contribution) to compute the trophic retention factor, the regression for EMFA showed that there is a mechanism for 'trophic magnification' not just for 22:6ω3 as proposed for cold seas (Parrish et al. 2009), but for 20:4ω6 and 20:5ω3 within the coral reef food web. In the Gulf of Mexico coral reef food web the EPA and DHA ratio is close to one in algae and phytoplankton, the principal sources of DHA (Table 1-3); however, TRF of DHA was 3.5 compared with TRF of 1.5 of EPA confirming that there is a preferential trophic retention for DHA.

The combination of the stable isotope mixing model, and the trophic retention factor for the analysis of individual FA allowed us to differentiate the contribution of each source (primary producers) to the consumer FA. TRF does not depend on the contribution from each source (Table 1-7). The non-essential FAs tend to decrease or have no change with respect to the source, but are strongly dependent on the physiological requirement of the organisms. For instance, pacific halibut, *Hippoglossus stenolepis* preferentially oxidize non-essential FA (Whyte et al. 1993). Thus, the extent to which any fatty acid is utilized for energy is largely dependent upon its dietary concentration with possibly two exceptions, the major C22 fatty acids, 22:1ω11 and DHA. Irrespective of dietary concentration, 22:1ω11 tends to be a highly oxidized, whereas DHA tends to be conserved, primarily due to it being a relatively poor substrate for β-oxidation

(Sargent,Tocher and Bell 2002). However, when the source was sea grass, non-essential FA showed significant retention across trophic levels, due to the high contribution of sea grass to the consumer's carbon, coinciding with field observations recorded in 100 publications, showing that grazing on seagrasses is widespread in the world's oceans (Valentine and Heck 1999). The PUFA 18:2ω6 and 18:3ω3 had similar tends decreasing across trophic levels. However 18:2ω6 had a TRF≈ 1 with no change with respect to all sources, except with green algae in the rainy season, while 18:3ω3 had trophic diminution in both seasons. There are 2 possibilities to explain the decrease in the slope of 18:3ω3. The first possibility is that it was difficult to assimilate because most PUFA are present in leaves, which are difficult to digest because of the high proportion of cellulose from leaves (Lawrence 1975), and the second possibility is that higher plant debris is eaten when drifting in the water column, and there is a decomposition of leaves (Wannigama et al. 1981). According to our results the second possibility is more likely because of the high TRF of 18:1ω9 and 18:1ω7 that tend to increase during mangrove leaf decomposition. As a result of this process it has been reported that leaves are more palatable (Mfilinge et al. 2003).

The HUFA EPA (20:5ω3) had a different behavior. It had a TRF> 1 which was significantly higher in the rainy season suggesting important seasonal differences related to the peak of productivity reported in the rainy season for the PNSAV and other river influenced coral reefs (Okolodkov et al. 2011). This FA showed a significant decrease across trophic levels from green and red algae when each source was considered. In contrast, ARA and DHA showed TRF≥ 1, so that it is retained or accumulated but not diminished across trophic levels even when the sources were green and brown algae. This

result is similar to the high retention of ARA reported in coastal Mediterranean lagoons (Koussoroplis et al. 2011).There are several possible mechanisms for significantly positive TRF: 1) increasing proportions of HUFA rich tissue with TL, 2) preferential consumption of HUFA rich food, 3) preferential assimilation of HUFA, and 4) preferential retention of HUFA. Here, the first two mechanisms can be partially discounted because while DHA rich neural tissue (Sargent et al. 1999) was included in some fish samples others had only muscle tissue sampled, and while chemical cues may be a determinant of food preference (Imrie et al. 1989), I am left with the fact that the slopes tell us that on average there is less HUFA in the food, and the fact that sources without HUFA like seagrass had similar contribution to phytoplankton carbon and nitrogen in upper trophic levels. I suggest that DHA and ARA are both preferentially retained. While such preferential assimilation of HUFA has been documented in crabs (Mchenga and Tsuchiya 2011) and fish (Sigurgisladottir et al. 1992), I suggest the following degrees of HUFA retention: DHA>ARA>EPA considered as essential dietary nutrients as well as essential metabolites *sensu* Parrish (2009). This is coincident with the major HUFA of polar lipids in gonads of 19 species of wild coral reef fish (Serranidae, Lutjanidae, Lethridae, Siganidae and Labridae) from Philippine which had DHA and ARA more than EPA (Suloma and Ogata 2011), they explain this incorporation because DHA is more efficient than EPA for improving growth, survival, pigmentation, and tolerance to salinity stress or reduced dissolved oxygen. DHA is also involved in the neural and visual development of larvae because it has important structural and functional roles in all membranes, but especially neural membranes (Copeman et al. 2002, Tocher 2010 and references therein).

1.6 Conclusion

This study used bulk $\delta^{13}C$ and $\delta^{15}N$ to apportion primary producer sources of organic carbon in a coral reef food web with river influence. The most probable solution was calculated using an isotopic mixing model. This study also provides fatty acid TRF calculated in a manner similar to that used for TMF of contaminants. Concentrations of the essential fatty acids 20:5ω3, 20:4ω6, and 22:6ω3 showed positive slopes across trophic levels with the slopes increasing in the order shown, suggesting an order of retention. A further refinement of the TRF approach was to estimate fatty acid TRF according to source. Using the SIAR data it was possible to estimate the contribution from each primary producer to each fatty acid in consumers. In turn, these data were used to calculate the TRF for each fatty acid from each primary producer source. Overall the results indicate phytoplankton are the major source of essential dietary nutrients for all fish studied and that dietary energy from mangroves is transferred to juvenile fish *C. hippos*, while sea grass non-essential FA are transferred to the entire food web. In addition, the sea urchin *E. lucunter* may be the only consumer of brown and green algae, while red algae were also consumed by the surgeon fish *A. chirurgus.*

1.7 Acknowledgments

Thanks to Cipriano Anaya, Yuri Okolodkov, Jacobo Santander-Monsalvo, and Sarahi Gutierrez-Villeda for their support during field work, to Jose Ake-Castillo for identification of phytoplankton, all of them from Instituto de Ciencias Marinas y Pesquerías, Universidad Veracruzana (ICIMAP-UV), to Jaime Camalich-Carpizo from Centro Interdisciplinario de Ciencias Marinas for his support to prepare isotope samples, and to Jeannette Wells from the Department of Ocean Sciences for her technical advice on chromatography. The Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico, and the Veracruz state government co-financed the Fondo Mixto (FOMIX) Project 'Fuentes orgánicas de carbono y nitrógeno y su función sobre la estructura trófica en el Sistema Arrecifal Veracruzano' (2007-2010). Analytical work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC). LCP is recipient of a Ph.D. fellowship from CONACYT (grant number 117304). LCP is grant recipient from the School of Graduate Studies, Memorial University of Newfoundland.

1.8 Bibliography

Aitchison, J. 1986. The statistical analysis of compositional data. Chapman & Hall.

Ara, R., A. Arshad, L. Musa, S. M. N. Amin, and P. Kuppan. 2011. Feeding habits of larval fishes of the family Clupeidae (*Actinopterygii: Clupeiformes*) in the estuary of River Pendas, Johor, Malaysia. J. Fish. Aquatic Sci. **6:** 816-821.

Ayukai, T. 1995. Retention of phytoplankton and planktonic microbes on coral reefs within the great Barrier reef, Australia. Coral Reefs **14:** 141-147.

Beer, S., M. Bjork, F. Hellblom, and L. Axelsson. 2002. Inorganic carbon utilization in marine angiosperm (seagrasses). Func. Plant. Biol. **29:** 349-354.

Bell, M. V., and D. R. Tocher. 2009. Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: General pathways and new directions, p. 211-236. *In* M. T. Arts, M. T. Brett and M. J. Kainz [eds], Lipids in aquatic ecosystems. Springer.

Bell, J. G., and J. R. Sargent. 2003. Arachidonic acid in aquaculture feeds: Current status and future opportunities. Aquaculture **218:** 491-499.

Christensen, V., C. J. Walters, and D. Pauly. 2005. Ecopath with ecosym: A user's guide. Fisheries Centre, University of British Columbia. (Available on line www.ecopath.org downloaded on March 2010).

Ciapa, B., D. Allemand, and G. De Renzis. 1995. Effect of arachidonic acid on Na + /H + exchange and neutral amino acid transport in sea urchin eggs. Exp. Cell. Res. **218:** 248-254.

Contreras-Espinosa, F., and B. G. Warner. 2004. Ecosystem characteristics and management for coastal wetlands in Mexico. Hydrobiol. **511:** 233-245.

Copeman, L. A., Parrish C. C., Brown J. A., and Harel M. 2002. Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): a live food enrichment experiment. Aquaculture 210:285–304.

Copeman, L. A., C. C. Parrish, R. S. Gregory, R. E. Jamieson, J. Wells, and M. J. Whiticar. 2009. Fatty acid biomarkers in coldwater eelgrass meadows: Elevated terrestrial input to the food web of age-0 Atlantic cod *Gadus morhua*. Mar. Ecol. Prog. Ser. **386:** 237-251.

DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta **45:** 341-351.

Fabricius, K. E. 2011. Factors determining the resilience of coral reefs to eutrophication: A review and conceptual model, p. 493-505. *In* Dubinsky Z, N. Stambler [eds.], Coral Reefs: An ecosystem in transition. Springer.

Fisk, A. T., K. A. Hobson, and R. J. Nortrom. 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the northwater Polynya marine food web. Environ. Sci. Technol. **35:** 732-738

Fourqurean J. W. and J. E. Schrlau. 2003. Changes in nutrient content and stable isotope ratio of C and N during decomposition of seagrasses and mangrove leaves along a nutrient availability gradient in Florida Bay, USA. Chem. Ecol. **19:** 373-390.

Fong, P., and V. J. Paul. 2011. Coral Reef Algae, p. 241-272. *In* Z. Dubinsky and N. Stambler [eds]. Coral reefs: An ecosystem in transition. Springer.

Hall, D., S. Y. Lee, and T. Meziane. 2006. Fatty acids as trophic tracers in an experimental estuarine food chain: Tracer transfer. J. Exp. Mar. Biol. Ecol. **336:** 42-53.

Harvey, H. R. 2006. Sources and cycling of organic matter in the marine water column, p. 1-25. *In* J. K. Volkman [ed]. Handbook of environmental chemistry, vol. 2, part N. Springer

Hernández, I., C. Aguilar, and G. González-Sansón. 2008. Tramas tróficas de peces de arrecifes en la región noroccidental de Cuba. II. Grupos funcionales. Rev. Biol. Trop. **56:** 1391-1401. [Trophic webs of reef fish in the Norwest region of Cuba. II. Functional groups].

Hobson, K. A., W. G. Ambrose, and P. E. Renaud. 1995. Sources of primary production, benthic-pelagic coupling, and trophic relationships within the northeast water Polynya: Insights from δ^{13}C and δ^{15}N analysis. Mar. Ecol. Prog. Ser. **128:** 1–10.

Imrie, D. W., S. J. Hawkins, and C. R. McCrohan. 1989. The olfactory-gustatory basis of food preferences in the herbivorous prosobranch *Littorina littorea* (Linnaeus). J. Moll. Stud. **55:** 217–225.

Jardine, T. D., K. Kidd, and A. Fisk. 2006. Applications, considerations, and sources of uncertainty when using stable isotope analysis in ecotoxicology. Environ. Sci. Technol. 40: 7501–7511.

Kainz, M. J., and Fisk A. T. 2009. Integrating lipids and contaminants in aquatic ecology and ecotoxicology, p. 93-113. *In* M. T. Arts, M. T. Brett and M. J. Kainz [eds.], Lipids in aquatic ecosystems. Springer.

Koussoroplis A. M., B. Alexandre, M. E. Perga, E. Koutrakis, G. Bourdier, and C. Desvilettes. 2011. Fatty acid transfer in the food web of a coastal Mediterranean lagoon:

Evidence for high arachidonic acid retention in fish. Estuar. Coast. Shelf Sci. **91:** 450–461.

Lapointe, B. E., P. J. Barile, and W. R. Matzie. 2004. Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: Discrimination of local versus regional nitrogen sources. J. Exp. Mar. Biol. Ecol. **308:** 23–58.

Lawrence J. M. 1975. On the relationship between marine plants and sea urchins. Oceanogr. Mar. Bio. Annu. Rev. **13:** 213-286.

Mchenga, I. S. S., and M. Tsuchiya. 2011. Do fatty acid profiles help to explain sesarmid crabs food choice? Eurasia. J. Biosci. 5: 91-102, doi:10.5053/ejobios.2011.5.0.11

Matthews, B., and A. Mazumder. 2005. Temporal variation in body composition (C : N) helpsexplain seasonal patterns of zooplankton d^{13}C. Freshwater Biology 50: 502–515, doi: 10.1111/j.1365-2427.2005.01336.x

McCutchan, J. H., W. M. Lewis, C. Kendall, C. C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulphur. Oikos 102: 378e390, doi: 10.1034/j.1600-0706.2003.12098.x

McLafferty, F. W. and Turecek, F. 1993. Interpretation of mass spectra. 4th Ed. University Sciences Books. Sausalito, CA. 371pp.

Mfilinge P. L., T. Meziane, Z. Bachok, and M. Tsuchiya. 2003. Fatty acids in decomposing mangrove leaves: microbial activity, decay and nutritional quality. Mar. Ecol. Progr. Ser. 265: 97-105.

Michener, R. H., and D. M. Shell. 1994. Stable isotopes ratios as tracers in marine aquatic food webs, p. 138-157. *In* K. Lajtha, and R. H. Michener [eds.], Stable isotopes in ecology and environments studies. Blackwell Scientific Publications.

Müller, C. E., A. O. De Silva, J. Small, M. Williamson, X. Wang, A. Morris, S. Katz, M. Gamberg, and D. C. G. Muir. 2011. Biomagnification of perfluorinated compounds in a remote terrestrial food chain: Lichen caribou wolf. Environ. Sci. Technol. 45: 8665-8673, doi: 10.1021/es201353v

Mumby, R. J., A. J. Edwards, J. E. Arias-González, K. C. Lindeman, P. G. Blackwell, A. Gall,I. M. Gorczynska, A. R. Harborne1, L. C. Pescod, H. Renken, C. C. C. Wabnitz, and G. Llewellyn. 2004. Mangroves enhance the biomass of coral reef fish communities in the Caribbean. Nature 427: 533-536.

Napolitano, G. E. 1999. Fatty acids as trophic and chemical markers in freshwater ecosystems, p. 21-44. *In* M. T.Arts, and B. C. Wainman [eds.], Lipids in freshwater ecosystems. Springer.

Okolodkov, Y. B., G. Campos–Bautista, I. Gárate–Lizárraga, J. A. G. González–González, M. Hoppenrath, and V. Arenas. 2007. Seasonal changes of benthic and ephiphytic dinoflagellates in the Veracruz reef zone, Gulf of México. Aquat. Microb. Ecol. 47: 223–237.

Okolodkov, Y. B., J. A. Aké-Castillo, M. G. Gutiérrez-Quevedo, H. Pérez-España, and D. Salas-Monreal. 2011. Annual cycle of the plankton biomass in the National Park Sistema Arrecifal Veracruzano, Southwestern Gulf of Mexico, p. 1-26. *In* G. Kattel [ed.], Zooplankton and phytoplankton. Nova Science.

Parnell, A. C., R. Inger, S. Bearhop, and A. L. Jackson. 2010. Source partitioning using stable isotopes: Coping with too much variation. Plos One 5: 9672, doi:10.1371

Parrish, C. C. 1987. Separation of aquatic lipid classes by Chromarod thin layer chromatography with measurements by Iatroscan flame ionization detection. Can. J. Fish. Aquat. Sci. 44: 722-731.

Parrish, C. C. 1999. Determination of total lipid, lipid classes and fatty acids in aquatic samples, p. 4-20. *In* M. T. Arts and B. C. Wainman [eds.], Lipids in freshwater ecosystems. Springer.

Parrish, C. C., D. Deibel, and R. J. Thompson. 2009. Effect of sinking spring phytoplankton blooms on lipid content and composition in suprabenthic and benthic invertebrates in a cold ocean coastal environment. Mar. Ecol. Prog. Ser. 391: 33–51

Parrish, C. C. 2009. Essential fatty acids in aquatic food webs, p. 309-326. *In* M. T. Arts, M. T. Brett, and M. J. Kainz [eds.], Lipids in aquatic ecosystems. Springer.

Pérez-España, H., J. M. Vargas-Hernández, H. Reyes-Bonilla, J. Santander-Monsalvo R. S. Goméz-Villada, M. A. Lozano-Aburto, J. Miranda-Zacarias, M. Damián-Velásquez, and A. Hernández-Romero. 2008. Caracterización ecológica y monitoreo del Parque Nacional Sistema Arrecifal Veracruzano. Primera Etapa. Informe Técnico ante la Comisión Nacional de la Biodiversidad. Centro de Ecología y Pesquerías, Universidad Veracruzana. [Ecological characterization and monitory of the National Park Coral Reef System of Veracruz].

Rodríguez-Quintal, J. G. 2010. Peces criptobentónicos de arrecifes coralinos en el Parque Nacional Archipiélago de Los Roques, Caribe de Venezuela. Rev. Biol. Trop. 58: 311-324. [Crypto-benthic fish of coral reefs in the Roques Archipelago National Park, Venezuelan Caribbean].

Rothans, T. C., and A. C. Miller. 1991. A link between biologically imported particulate organic nutrients and the detritus food web in reef communities. Mar. Biol. 110: 145-150.

R Studio package was downloaded from http://fsf.org in 01 July 2012.

Santander-Monsalvo J. 2010. Ecología trófica de los peces más abundantes del Parque Nacional Sistema Arrecifal Veracruzano. Tesis de Maestría. Instituto de Ciencias Marinas y Pesquerías. Universidad Veracruzana. [Trophic ecology of more abundant fish of the Coral Reef System of Veracruz National Park].

Sargent, J. R., G. Bell, L. McEvoy, D. Tocher, and A. Estevez. 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177: 191–199.

Sargent, J. R., D. R. Tocher, J. G. Bell. 2002. The lipids. p. 181–257. *In* J. E. Halver and R.W. Hardy [eds.], Fish nutrition. Academic Press.

Solandt, J. L., and A.C. Campbell. 2001. Macroalgal feeding characteristics of the sea urchin *Diadema antillarum* Philippi at Discovery Bay, Jamaica. Caribb. J. of Sci. 37: 32–38.

Sato, N., and N. Murata. 1988. Membrane lipids. Methods of Enzymology 167: 251–259.

Sigurgisladottir, S., S. P. Lall, C. C. Parrish, and R. G. Ackman. 1992. Cholestane as a digestibility marker in the absorption of polyunsaturated fatty acid ethyl esters in Atlantic salmon. Lipids 27: 418 – 424.

Suloma A. and H. Y. Ogata. 2011. Arachidonic acid is a major component in gonadal fatty acids of tropical coral reef in the Philippines and Japan. J. Aquac. Res. Development 2:111. doi:10.4172/2155-9546.1000111

Terrados, J., P. Ramírez-García, O. Hernández-Martínez, K. Pedraza, and A. Quiroz. 2008. State of *Thalassia testudinum* Banks ex König meadows in the Veracruz Reef System, Veracruz, Mexico. Aquat. Bot. 88: 17 – 26.

Tocher, D. R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11: 107 – 184.

Tocher D. R. 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. Aquaculture Research, 41, 717-732.

Umezawa, Y., T. Miyajima, M. Yamamuro, H. Kayanne, and I. Koike. 2002. Fine-scale mapping of land-derived nitrogen in coral reefs by $d^{15}N$ in macroalgae. Limnol. Oceanogr. 47: 1405–1416.

Valentine, J. F., and K. L. Heck. 1999. Seagrass herbivory: Evidence for the continued grazing of marine grasses. Mar. Ecol. Prog. Ser. 176: 291-302.

Wannigama, G. P., J. K. Volkman, F. T. Gillan, P. D. Nichols, and R. B. Johns. 1981. A comparison of lipid components of the fresh and dead leaves and pneumatophores of the mangrove *Avicennia marina*. Phytochemistry 20: 659-666.

Weaver, D., and L. A. Rocha. 2007. A new species of *Halichoeres* (Teleostei: Labridae) from the western Gulf of Mexico. Copeia 4: 798–807.

Whyte, J. N. C., W. C. Clarke, N. G. Ginther, and J. O. T. Jensen. 1993. Biochemical changes during embryogenesis of the Pacific halibut, *Hippoglossus stenolepis* (Schmidt). Aquaculture and Fisheries Management 24: 193-201.

Wsearch software version 32 was downloaded from http:www.wsearch.com.au in 01 February 2008.

Zavala-Hidalgo, J., S. L. Morey, and J. J. O'Brien. 2003. Seasonal circulation of the western shelf of Gulf of Mexico using a high-resolution numerical model. J. Geophys. Res. 108: 1-19

1.9 Tables

Table 1-1Taxonomic classification, functional group, fatty acid sample sizes, and trophic levels of food web species from the Veracruz coral reef ecosystem.

Sample Scientific name (trivial name)	Kingdom/Phylum/ Class/Order/Family	Functional group	Dry n	Rainy n	Trophic level
Rizhophora mangle (red mangrove)	Plantae / Tracheophyta / Angiosperm/ Myrtacea / Rhizophoracea	primary producer	4	-	1
Thalassia testudinum (sea grass)	Plantae / Tracheophyta / Angiosperm / Najadales / Hydrochartaceae	primary producer	3	3	1
Halimeda opuntia (green algae)	Plantae/ Clorophyta / Bryopsidophyceae / Bryopsidales / Halimedaceae	primary producer	7	-	1
Galaxaura sp. (red algae)	Plantae / Rhodophyta /Florideophyceae / Nemaliales / Galaxauraceae	primary producer	4	-	1
Dictyota sp. (brown algae)	Plantae / Chromalveolata / Heterokontophyta / Phaeophyceae / Dictyotales / Dictyotaceae	primary producer	5	-	1
Net tow 30 μm	Phytoplankton and Particulated Organic Matter (POM)	primary producer	8	9	1
Net tow 120 μm	Zooplankton and POM	planktivore	9	9	2.0
Echinometra lucunter (sea urchin)	Animalia / Echinodermata / Echinoidea / Echinoida / Echinometridae	herbivore	3	4	2.1
Acanthurus chirurgus (surgeon fish)	Animalia / Chordata / Actinopterygii / Beryciformes / Acanthuridae	herbivore and detritivore	4	5	2.4
Coryphopterus personatus (masked goby)	Animalia / Chordata / Actinopterygii / Perciformes / Gobidae	zooplanktivore	3	4	2.7
Halichoeres burekae (Mardi Gras wrasse)	Animalia / Chordata / Actinopterygii / Perciformes / Labridae	zooplanktivore	3	4	2.7
Bodianus rufus (hog fish)	Animalia / Chordata / Actinopterygii / Perciformes / Labridae	clams, crabs and sea urchins eater	3	5	3.4
Ocyurus crhysusrus (yellowtail snapper)	Animalia / Chordata / Actinopterygii / Perciformes / Lutjanidae	piscivore and molluscs	3	5	3.7
Caranx hippos (crevalle jack) juveniles	Animalia / Chordata / Actinopterygii / Perciformes / Carangidae	piscivores	3	4	3.7
Caranx hippos (crevalle jack) adults	Animalia / Chordata / Actinopterygii / Perciformes / Carangidae	piscivores	3	4	3.7

Table 1-2. Results from the SIAR isotope mixing model. Values are the mean contributions (%) of primary producers to food web upper levels. Values in parentheses are the 95 percentile range. Bold numbers denote significant negatively correlated sources with Pearson's *r* values obtained from the matrix plot of source proportions, *n*= sample size, and *p*= probability value of the correlation. Low correlations between sources indicates the model is performing well, when the model is struggling to differentiate between sources, it is indicated by significant correlations, and then likely solutions could involve one or other of the sources but not both at the same time.

Consumer/ source	Season	*R. mangle*	*T. testudinum*	*H. opuntia*	*Galaxaura* sp.	*Dictyota* sp.	phytoplankton	r	n	*p*
Zooplankton	dry	33 (23-45)	7 (0-25)	3 (0-25)	5 (0-29)	2 (0.2-19)	22 (0-38)	-0.69	9	0.0198
	rainy	29 (19-39)	15 (0-29)	3 (0-27)	4 (0-30)	5 (0-30)	22 (0-35)	-0.62	10	0.0279
E. lucunter	dry	1 (0-2)	11 (1-20)	4 (0-26)	52 (27-51)	15 (2-25)	3 (0-24)	-0.71	14	0.0022
	rainy	1 (0-2)	9 (1-16)	24 (1-43)	28 (4-52)	26 (1-46)	4 (0-24)	-0.83	13	0.0003
A. chirurgus	dry	1 (0-6)	30 (1-56)	2 (0-21)	15 (3-24)	3 (1-18)	29 (2-55)	-0.56	10	0.0461
	rainy	3 (1-6)	25 (0-37)	4 (0-32)	19 (0-35)	4 (0-33)	23 (0-41)	-0.31	5	0.3059
C. personatus	dry	18 (12-25)	26 (6-40)	2 (0-23)	17 (0-29)	15 (0-28)	21 (0-41)	-0.77	6	0.0366
	rainy	5 (1-16)	15 (2-40)	1 (0-5)	2 (0-18)	1 (0-8)	68 (20-86)	-0.97	18	0.0001
H. burekae	dry	17 (7-27)	23 (2-41)	3 (0-29)	4 (1-20)	3 (0-28)	23 (0-42)	-0.58	7	0.0861
	rainy	-	-	-	-	-	-	-	-	-
B. rufus	dry	4 (1-8)	28 (7-51)	13 (0-26)	13 (0-33)	3 (0-31)	25 (1-47)	-0.56	6	0.1239
	rainy	2 (0-6)	29 (9-52)	3 (1-14)	17 (1-34)	10 (0-32)	27 (1-47)	-0.58	15	0.0117
O. chrysurus	dry	1 (0-3)	25 (5-48)	4 (0-27)	28 (6-46)	15 (0-33)	16 (0-36)	-0.50	10	0.0706
	rainy	12 (4-18)	25 (4-49)	2 (0-17)	2 (0-28)	2 (0-27)	24 (0-58)	-0.86	15	0.0001
C. hippos	dry	29 (19-40)	11 (0-31)	3 (0-30)	3 (0-27)	3 (0-28)	23 (0-40)	-0.70	10	0.0121
juveniles	rainy	27 (19-34)	17 (1-34)	4 (0-22)	7 (1-20)	8 (1-20)	20 (0-36)	-0.81	9	0.0041
C. hippos	dry	6 (0-16)	24 (1-40)	16 (0-31)	21 (1-35)	22 (0-34)	22 (0-36)	-0.33	3	0.3729
adults	rainy	-	-	-	-	-	-	-	-	-

Table 1-3. Fatty acid composition and $\delta^{13}C$, $\delta^{15}N$ isotopes of primary producers of the Veracruz coral reef ecosystem. Values are mean ± 95% confidence interval. SFA= saturated FA, MUFA=monounsaturated FA, PUFA=polyunsaturated FA, HUFA=highly unsaturated FA, BFA= branched-chain FA, OFA= odd-chain FA, FAME=FA methyl ester, and AL dry wt^{-1}= acyl lipids by unit of dry weight.

FAME	*R. mangle*	*T. testudinum*	*H. opuntia*	*Galaxaura* sp.	*Dictyota* sp.	phytoplankton (dry season)	phytoplankton (rainy season)
14:0	2.5±0.7	2.6 ± 1.7	7.6 ± 1.3	4.3 ± 3.7	8.6 ± 1.4	12.6 ± 5.5	21 ± 3
15:0		0.4 ± 0.2		0.5 ± 0.1	0.6 ± 0.2	1.6 ± 0.3	1 ± 0.1
16:0	21±7	35 ± 4	31 ± 3	55 ± 6	23 ± 6	29 ± 2	27 ± 1
17:0	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	1.3 ± 0.4	0.6 ± 0.2
18:0	3.1 ± 0.9	3.3 ± 1.4	0.9 ± 0.2	1.7 ± 0.7	1.3 ± 0.4	6.7 ± 1.6	5.1 ± 1.5
20:0	0.9 ± 0.4	0.5 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.4 ± 0.2
24:0	1.1 ± 0.4	1 ± 0.9	2.4 ± 0.5	0.5 ± 0.4		0.5 ± 0.1	0.5 ± 0.1
Σ SFA	29 ± 4	44 ± 3	43 ± 3	63 ± 6	34 ± 4	53 ± 4	57 ± 2
16:1*ω*9	0.3 ± 0.2	0.8 ± 0.5	0.7 ± 0.4	0.4 ± 0.2	1.2 ± 0.9	0.5 ± 0.4	0.4 ± 0.1
16:1*ω*7	0.4 ± 0.2	1.7 ± 0.4	3.8 ± 0.7	2.6 ± 1.1	5 ± 4	8 ± 3	14 ± 2
16:1*ω*5	0.9 ± 0.6	1.6 ± 0.5	0.3 ± 0.1	0.4 ± 0.2	5 ± 1	1.4 ± 0.1	0.5 ± 0.1
18:1*ω*9	14 ± 1.5	4 ± 2	1.8 ± 0.6	3.8 ± 0.9	15 ± 1	10 ± 4	3 ± 1
18:1*ω*7	0.6 ± 0.1	1.4 ± 1	3 ± 1	3 ± 2	1.4 ± 0.9	2.3 ± 0.7	1.1 ± 0.3
20:1*ω*11			0.3 ± 0.1	2 ± 1		0.5 ± 0.2	0.3 ± 0.1
20:1*ω*9	0.4 ± 0.1		0.5 ± 0.1	1 ± 0.9	0.3 ± 0.2	0.3 ± 0.1	0.1 ± 0.01
22:1*ω*7			1.1 ± 0.3		0.3 ± 0.2		
24:1*ω*9			1.3 ± 0.3	0.8 ± 0.6		0.5 ± 0.2	0.4 ± 0.1
ΣMUFA	16 ± 1	10 ± 3	13 ± 2	14 ± 2	29 ± 4	24 ± 3	20 ± 1
16:2*ω*6			2.2 ± 0.3	0.2 ± 0.5	0.2 ± 0.1		0.2 ± 0.01
16:2*ω*4		0.4 ± 0.1		0.2 ± 0.2	0.2 ± 0.1	0.7 ± 0.1	1.1 ± 0.2
16:3*ω*4	0.3 ± 0.2	1.3 ± 0.4	5.7 ± 0.6	0.2 ± 0.1	0.7 ±0.5	0.5 ± 0.1	1 ± 0.2
16:4*ω*3					1 ± 0.9	0.4 ± 0.2	0.6 ± 0.2
18:2*ω*6	21 ± 4	18 ± 5	7.3 ± 1.1	1.7 ± 0.3	2.2 ± 0.7	3.1 ± 0.6	1.8 ± 0.2
18:3*ω*3	33 ± 9	27 ± 8	9 ± 2	1 ± 1	2.1 ± 0.6	1.1 ± 0.3	0.6 ± 0.2
18:4*ω*3			1.6 ± 0.5	0.9 ± 0.8	7 ± 2		0.9 ± 0.2
ΣPUFA	54 ± 4	46 ± 6	26 ± 1	4 ± 1	14 ± 3	5.7 ± 0.4	6.1 ± 0.8
20:2*ω*6				2 ± 1	0.6 ± 0.4	0.3 ± 0.1	0.3 ± 0.1
20:3*ω*6			0.5 ± 0.1		1.1 ± 0.8	0.1 ± 0.09	0.2 ± 0.01
20:4*ω*6			1.7 ± 0.5	2 ± 1	9 ± 4	1.1 ± 0.5	1.2 ± 0.2
20:4*ω*3			0.8 ± 0.2	0.5 ± 0.3	2.2 ± 0.3	0.3 ± 0.1	0.3 ± 0.1
20:5*ω*3			5.5 ± 1.9	9 ± 7	5.8 ± 0.6	4.1 ± 1.6	6.6 ± 0.9
22:5*ω*3			1 ± 0.2	0.7 ± 0.4		0.5 ± 0.3	0.5 ± 0.1
22:6*ω*3			5 ± 2	0.7 ± 0.6		8 ± 4	5 ± 2
ΣHUFA			15 ± 4	16 ± 5	19 ± 6	14 ± 5	15 ± 3
ΣBFA				0.4 ± 0.1		1.6 ± 0.2	1.3 ± 0.2
ΣOFA	0.3 ± 0.1	0.8 ± 0.1	0.2 ± 0.1	1.2 ± 0.2	0.8 ± 0.2	3.1 ± 0.6	1.6 ± 0.3
AL % dry wt^{-1}	2.5±0.9	1±0.4	3±2	0.4±0.2	1.5±0.5	1.4±0.5	2.3±0.4
$\delta^{13}C$	-29±2.2	-15.5±1.7	-14.4±5.5	-11.2±2.7	-14.0±2.7	-19.2±2.1	-20.3 ±0.3
$\delta^{15}N$	2.0±0.3	3.2±0.4	-0.7±1.2	-2.0±1.5	-0.1±0.7	5.1±1.4	5.5±0.5

Table 1-4. Fatty acid composition and $\delta^{13}C$, $\delta^{15}N$ isotopes of upper food web members at the end of the dry season. Values are mean ± 95% confidence interval. SFA= saturated FA, MUFA=monounsaturated FA, PUFA=polyunsaturated FA, HUFA=highly unsaturated FA, BFA= branched FA, OFA= odd-chain-branched FA, FAME=FA methyl ester and AL dry wt^{-1}= acylated lipids by unit of dry weight.

FAME	Zooplankton	*E. lucunter*	*A. chirurgus*	*C. personatus*	*H. burekae*	*B. rufus*	*O. chrysurus*	*C. hippos* adults	*C. hippos* juveniles
14:0	12±4	7.3±0.3	2±1	3±1	5±1	2±1	0.7±0.1	2.4±0.3	1±0.1
15:0	1.4±0.2	1.1±0.1	0.5±0.2	0.8±0.2	1.3±0.1	0.6±0.1	0.6±0.1	0.9±0.1	0.7±0.3
16:0	29±2	10±8	11±8	23±3	26±1	24±4	22±2	24±5	21±1
17:0	1.3±0.3	-	0.8±0.1	2.2±0.5	2.2±0.1	1.1±0.4	1±0.02	1.7±0.1	1.5±0.5
18:0	7±2	4.6±0.1	11±1	12±2	11.5±0.5	11±4	8±1	15±1	20±2
20:0	0.6±0.3	1.1±0.1	0.14±0.01	0.4±0.1	0.4±0.1	0.4±0.2	0.2±0.01	0.6±0.1	0.3±0.1
24:0	0.5±0.1	-	0.32±0.05	1.7±0.5	0.9±0.1	0.6±0.3	1.7±0.2	1.8±0.3	0.9±0.2
Σ SFA	50±4	25±8	25±9	45±6	50±2	42±8	33±2	46±6	46±2
16:1ω9	0.5±0.1	6±1	0.02±0.01	-	-	0.2±0.1	-	0.3±0.1	0.2±0.1
16:1ω7	9±4	1.8±0.1	5.2±1.6	5±1	6±1	3±1	1.5±0.3	2.5±0.5	1.1±0.3
16:1ω5	0.5±0.1	-	-	-	-	-	-	-	-
18:1ω9	7±2	3.6±0.7	7±1	7±2	9.2±0.3	9±2	7±1	13±2	7.7±0.1
18:1ω7	2.3±0.5	3.2±0.6	2.8±0.3	4±2	2.2±0.1	2.4±0.6	1.7±0.1	2.9±0.4	2.7±0.2
20:1ω11	0.5±0.2	7±1	-	0.2±0.1	0.2±0.04	0.9±0.2	0.14±0.04	1±0.1	0.2±0.04
20:1ω9	0.3±0.1	4±1	0.2±0.1	0.17±0.01	0.3±0.04	0.3±0.1	0.3±0.1	0.7±0.1	0.2±0.03
22:1ω7	0.03±0.02	2.9±0.1	-	-	-	0.3±0.1	-	0.2±0.1	0.24±0.01
24:1ω9	0.5±0.2	0.4±0.2	1.3±0.4	0.9±0.4	1.3±0.1	2±1	1.7±0.2	1.8±0.3	2.3±0.7
ΣMUFA	22±2	27±2	18±3	18±2	19±1	19±4	13±1	23±3	15±1
16:2ω6	-	0.1±0.01	0.07±0.03	0.22±0.07	-	0.1±0.03	-	-	-
16:2ω4	0.9±0.2	0.3±0.05	0.04±0.02	0.3±0.1	0.1±0.01	0.3±0.2	0.5±0.3	0.3±0.02	0.6±0.02
16:3ω4	0.5±0.1	0.4±0.1	0.2±0.1	-	-	0.1±0.01	-	0.4±0.02	0.5±0.3
16:4ω3	0.6±0.4	2.2±0.5	0.13±0.09	-	-	-	-	-	-
18:2ω6	2.9±0.6	0.9±0.4	1.7±0.5	1.4±0.4	1.2±0.2	1.1±0.1	1±0.1	0.9±0.3	0.6±0.03
18:3ω3	1.3±0.3	0.6±0.3	0.7±0.3	2±1	0.3±0.01	0.2±0.1	0.27±0.07	0.1±0.07	0.1±0.01
18:4ω3	0.7±0.2	2±1	1.1±0.4	0.5±0.3	0.4±0.01	0.1±0.01	0.2±0.04	0.1±0.03	0.1±0.01
ΣPUFA	8±1	8±2	5±2	4±1	2±0.2	1.9±0.4	1.8±0.04	1.8±0.3	1.9±0.3
20:2ω6	0.4±0.1	1±0.2	0.3±0.07	0.3±0.03	0.2±0.02	0.3±0.02	0.4±0.1	0.3±0.1	0.3±0.02
20:3ω6	0.1±0.04	0.7±0.02	0.9±0.1	0.2±0.01	0.4±0.1	0.2±0.05	0.4±0.1	0.2±0.05	0.2±0.01
20:4ω6	1.7±0.5	13±3	13±3	3.9±0.04	2.1±0.2	11±3	7.4±1.7	5.1±0.5	4.1±0.5
20:4ω3	0.3±0.1	0.4±0.1	0.11±0.03	0.3±0.1	0.2±0.01	0.2±0.02	0.3±0.2	0.1±0.04	0.14±0.01
20:5ω3	5±1	12±1	9±2	4.4±0.7	4.7±0.5	4.8±0.7	4.1±0.4	2.4±0.4	3.5±0.6

22:5ω6	0.8±0.2	0.5±0.3	2.6±0.5	1.2±0.2	1.1±0.2	2±0.4	2.9±0.3	2.1±0.6	1.8±0.3
22:5ω3	0.6±0.3	0.3±0.1	5±1	2±1	0.7±0.01	2.3±0.6	1.9±0.3	2.1±0.6	1.2±0.06
22:6ω3	7±3	0.8±0.4	18±4	18±6	18±2	13±8	33±7	14±1	24±4
ΣHUFA	17±5	30±4	52±9	31±9	28±2	35±12	50±3	28±3	36±4
ΣBFA	1.4±0.2	1.3±0.2	0.7±0.1	1.9±0.2	1.4±0.1	1.2±0.2	1.8±0.04	1.1±0.1	0.9±0.1
ΣOFA	1.6±0.3	1.6±0.2	1.6±0.2	3.7±0.4	4.4±0.1	2.4±0.7	3±1	3.5±0.2	3±1
AL% dwt^{-1}	3.2±1.4	5.5±5	3±2	5±3	8±1	3.2±0.4	3±1	3±2	3±2
$\delta^{13}C$	-21±1	-11±0.3	-14.4±0.5	-17.7±0.4	-18±2	-15±1	-13±1	-14.8±0.5	-20.3±1.2
$\delta^{15}N$	5.9±1.4	3.3±0.3	8±0.4	8.4±0.5	8±1	9±1	9.3±0.3	11±1	12±0.3

Table 1-5. Fatty acid composition and $\delta^{13}C$, $\delta^{15}N$ isotopes of food web upper members, at the end of the rainy season. Values are mean ± 95% confidence interval. SFA= saturated FA, MUFA=monounsaturated FA, PUFA=polyunsaturated FA, HUFA=highly unsaturated FA, BFA= branched FA, OFA= odd-chain FA, and AL dry wt^{-1}= acylated lipids by unit of dry weight.

FAME	Zooplankton	*E. lucunter*	*A. chirurgus*	*C. personatus*	*H. burekae*	*B. rufus*	*O. chrysurus*	*C. hippos* juveniles
14:0	21±4	8±4	3±2	8±3	10±2	8±4	6±2	3.6±0.5
15:0	1±0.1	1.5±0.3	0.6±0.1	1.3±0.5	1.4±0.2	0.8±0.1	1.2±0.7	0.7±0.1
16:0	26±2	22±2	26±3	27±15	23±3	26±3	29±2	35±3
17:0	0.7±0.3	1±0.3	0.7±0.1	4±1	1.9±0.3	1.1±0.2	2±0.7	1±0.2
18:0	5±1	4.7±0.4	8±1	16±1	9.6±0.4	10±1	11±1	11±1
20:0	0.1±0.03	0.9±0.1	0.12±0.03	0.4±0.1	0.5±0.04	0.4±0.1	0.8±0.3	0.4±0.1
24:0	0.5±0.1	0.1±0.06	0.3±0.1	1.3±0.3	0.9±0.1	0.3±0.03	0.4±0.1	0.4±0.1
Σ SFA	55±1	39±5	39±4	59±10	49±3	47±6	51±6	53±3
16:1ω9	0.3±0.1	0.4±0.1	0.1±0.01	0.03±0.01	-	0.2±0.02	1.1±0.3	0.4±0.02
16:1ω7	13±2	7±2	6±2	6±4	11±1	8.6±2.2	4.5±0.4	5.7±0.6
16:1ω5	0.4±0.06	0.3±0.1	0.3±0.1	-	-	0.3±0.03	0.21±0.04	0.14±0.01
18:1ω9	3±1	3±1	0.1±0.03	5.6±1.3	6.9±0.3	8±2	11±4	20±2
18:1ω7	1.1±0.4	3.6±0.2	2.3±0.2	4.7±2.2	2.9±0.3	3.8±0.5	3.2±0.4	3±0.2
20:1ω11	0.2±0.1	6±1	0.02±0.01	0.6±0.3	0.4±0.1	0.9±0.4	0.3±0.1	0.2±0.1
20:1ω9	0.2±0.04	2±0.6	0.3±0.2	0.3±0.1	0.3±0.1	0.4±0.1	0.7±0.1	0.5±0.1
22:1ω7	-	0.7±0.1	0.1±0.01	-	-	0.12±0.04	-	0.08±0.03
24:1ω9	0.6±0.1	0.2±0.1	1±0.2	0.6±0.1	1.2±0.1	0.4±0.1	0.9±0.2	0.4±0.2
ΣMUFA	20±1	25±3	16±2	19±8	23±2	23±2	24±3	31±2
16:2ω6	0.2±0.03	-	-	0.3±0.2	0.3±0.1	-	-	-
16:2ω4	1±0.2	0.6±0.2	0.8±0.2	0.2±0.1	0.3±0.1	0.7±0.2	0.7±0.1	0.5±0.05
16:3ω4	0.8±0.2	0.6±0.1	1±0.3	-	-	0.7±0.1	0.6±0.03	0.6±0.03
16:4ω3	0.6±0.1	2±1	0.8±0.3	-	-	0.6±0.3	0.6±0.2	0.2±0.1
18:2ω6	3±1	2±1	1.3±0.1	1±0.2	0.9±0.1	1.4±0.2	0.9±0.3	0.7±0.1
18:3ω3	0.7±0.3	1.7±0.6	0.7±0.1	1±0.3	0.2±0.02	0.3±0.1	0.3±0.1	0.3±0.1
18:4ω3	0.9±0.2	1.2±0.3	1.7±0.7	0.6±0.3	0.4±0.1	0.3±0.04	0.2±0.1	0.2±0.1
ΣPUFA	7.5±1.4	8.5±1.4	8±1	3±1	2.3±0.3	5±0.4	4.3±0.4	3.2±0.3
20:2ω6	0.3±0.04	1.6±0.3	0.1±0.03	0.3±0.1	0.2±0.02	0.4±0.1	0.2±0.1	0.1±0.04
20:3ω6	0.3±0.03	0.5±0.03	0.6±0.07	0.3±0.2	0.2±0.02	0.4±0.1	0.2±0.1	0.07±0.03
20:4ω6	1.4±0.2	11±3	9±3	2.5±0.6	2.3±0.3	7±3	3±1	1.4±0.6
20:4ω3	0.4±0.1	0.2±0.06	0.3±0.1	0.2±0.07	0.3±0.04	0.2±0.02	0.2±0.1	0.04±0.01
20:5ω3	7±1	7±2	6.3±0.6	3±0.5	5.4±0.7	5.2±0.8	2.3±0.6	2±0.7

22:5ω6	0.7±0.1	0.1±0.02	2.3±0.2	0.7±0.05	0.9±0.2	1±0.3	1.4±0.4	0.4±0.2
22:5ω3	0.6±0.1	0.3±0.1	3.9±0.6	0.8±0.6	1.1±0.2	1.9±0.9	1±0.4	0.7±0.1
22:6ω3	6±1	0.5±0.2	10±3	8±2	14±3	7±3	10±3	7±3
ΣHUFA	17±2	23±5	35±6	11±6	24±4	23±7	19±4	12±5
ΣBFA	1.5±0.2	1.2±0.3	0.28±0.05	5±1	1.2±0.2	0.8±0.1	1.2±0.4	0.7±0.1
ΣOFA	1.7±0.3	2.8±0.6	1.4±0.2	5±1	3.8±0.5	2.2±0.3	3.5±1.2	1.9±0.3
AL% dwt^{-1}	3±1	9±4	3±1	10±3	16±6	7±2	7±3	18±7
$\delta^{13}C$	-20±0.5	-3.1±1.0	-14.2±1.9	-17.0±0.4	-	-14.4±0.5	-16.6±0.7	-19.4±0.6
$\delta^{15}N$	4.9±2.3	3.3±0.3	7.9±1.2	7.9±0.6	-	10.7±0.5	10.4±0.7	14.5±0.5

Table 1-6. Concentration (μg mg^{-1} dry wt) of fatty acids selected from principal components analysis in the studied primary producers of the coral reef system of Veracruz. Values are means ± confidence interval at 95%. Values in parentheses are the medians. *H*= Kruskall-Wallis statistic, and *p*= probability value. Letters denote significant differences among columns. D= dry season, R= rainy season.

FAME	*R. mangle*	*T. testudinum*	*H. opuntia*	*Galaxaura* sp.	*Dictyota* sp.	phytoplankton D	phytoplankton R	*H*	*p*
14:0	0.7±0.3 (0.6)^{b}	0.2±0.1 (0.1)^{b}	3±2 (1.5)^{a}	0.2±0.1 (0.2)^{b}	1.1±0.4 (1.02)^{b}	1.5±0.4 (1.3061)^{a}	5±1(4.4)^{a}	25.6	<0.001
16:0	5±2 (4.4559)^{a}	2±1 (2.323)^{b}	10±8 (6.432)^{a}	2±1 (2.266)^{b}	3±1 (3.067)^{b}	4±2 (3.702)^{b}	6±2 (7.001)^{a}	17.3	0.008
16:1ω7	0.2±0.1 (0.0833)^{b}	0.2±0.1 (0.1376)^{b}	2±1 (0.8086)^{b}	0.1±0.05 (0.1053)^{b}	0.4±0.3 (0.2846)^{b}	1±0.3 (0.8293)^{a}	3±1 (2.9821)^{a}	25.3	<0.001
16:2ω4		0.02±0.01 (0.0282)^{b}	0.2±0.1 (0.0780)^{a}	0.01±0.001 (0.0086)^{b}	0.03±0.01 (0.0257)^{b}	0.1±0.05 (0.0777)^{a}	0.3±0.1 (0.2416)^{a}	26.5	<0.001
18:1ω9	3±2 (2.5067)^{a}	0.3±0.1 (0.3092)^{b}	1±0.5 (0.2446)^{b}	0.1±0.05 (0.1584)^{b}	2±1 (1.9033)^{a}	2±1 (1.1741)^{a}	0.7±0.5 (0.5481)^{b}	21.2	0.002
18:1ω7	0.2±0.1 (0.1216)^{b}	0.1±0.05 (0.0977)^{b}	1±0.4 (0.6956)^{a}	0.1±0.05 (0.1058)^{b}	0.06±0.02 (0.0568)^{b}	0.3±0.2 (0.3271)^{a}	0.3±0.2 (0.1952)^{a}	15.5	0.017
18:2ω6	5±3 (3.6989)^{a}	1±0.5 (1.1238)^{a}	3±2 (1.3703)^{a}	1±0.4 (0.0687)^{b}	0.3±0.2 (0.2752)^{b}	0.4±0.2 (0.3700)^{b}	0.4±0.1 (0.3461)^{b}	23.3	0.001
18:3ω3	9±3 (8.0296)^{a}	2±1 (1.6351)^{a}	3±2 (2.3267)^{a}	0.5±0.2 (0.0258)^{b}	0.3±0.2 (0.3359)^{b}	0.2±0.1 (0.1582)^{b}	0.1±0.05 (0.1148)^{b}	24.0	0.001
18:4ω3			0.5±0.2 (0.4515)^{a}	0.04±0.01 (0.0221)^{b}	1±0.5 (1.0618)^{a}		0.2±0.1 (0.2273)^{b}	14.3	0.002
20:3ω3			2±1 (0.1990)^{a}	0.5±0.3 (0.0134)^{b}		0.01±0.01 (0.0066)^{b}		6.9	0.031
20:4ω6			0.5±0.2 (0.5693)^{a}	0.1±0.05 (0.0713)^{b}	2±0.7 (1.6199)^{a}	0.2±0.1 (0.1000)^{b}	0.3±0.1 (0.2664)^{b}	16.2	0.003
20:5ω3			2±1 (1.6161)^{a}	0.5±0.3 (0.2865)^{b}	0.9±0.4 (0.8208)^{b}	1±0.4 (0.4411)^{b}	1.5±0.5 (1.5927)^{a}	14.8	0.005
22:4ω3					0.04±0.01 (0.0384)^{a}	0.1±0.05 (0.1125)^{a}		1.5	0.221
22:5ω3			0.3±0.1 (0.2824)^{a}	0.01±0.001 (0.0113)^{b}		0.1±0.06 (0.0499)^{b}	0.1±0.03 (0.1242)^{a}	13	0.005
22:6ω3			1.5±0.5 (1.5114)^{a}	0.02±0.01 (0.243)^{b}		2±1 (0.8251)^{a}	1.3±0.8 (1.5797)^{a}	9.3	0.025
24:1ω9			0.4±0.1 (0.3608)^{a}	0.03±0.02 (0.0326)^{b}		0.1±0.05 (0.0789)^{b}	0.1±0.06 (0.0727)^{a}	10.5	0.015

Table 1-7. Trophic retention factor (TRF) of FA selected by PCA, and corrected with the proportion of each primary producer source resulting from the SIAR mixing model. p= probability value, bold numbers denote significant regressions, if $p< 0.05$ means that slope β is significantly different from zero or that TRF= e^{β} is different to 1. Values of TRF> 1 means trophic retention, and TRF< 1 means trophic reduction.

FAME	season	*R. mangle*	*p*	*T. testudinum*	*p*	*H. opuntia*	*p*	*Galaxaura* sp.	*p*	*Dictyota* sp.	*p*	phytoplankton	p
14:0	dry	0.23	0.001	0.55	0.007	0.27	0.001	0.55	0.012	0.33	0.001	0.29	0.001
	rainy	1.12	0.782	1.41	0.035	0.47	0.003	1.04	0.844	0.61	0.060	0.73	0.112
16:0	dry	0.51	0.104	1.57	0.053	0.66	0.147	0.97	0.869	0.86	0.556	0.82	0.309
	rainy	1.56	0.273	2.48	0.001	0.71	0.240	1.18	0.505	1.001	0.996	1.32	0.083
16:1ω7	dry	0.50	0.086	0.98	0.939	0.46	0.002	0.89	0.579	0.65	0.050	0.57	0.005
	rainy	1.82	0.127	1.92	0.001	0.61	0.040	1.27	0.257	0.92	0.740	0.90	0.561
16:2ω4	dry			1.13	0.541	0.59	0.097	1.14	0.589	0.78	0.426	0.63	0.021
	rainy			2.06	0.001	0.72	0.184	1.58	0.059	1.03	0.904	1.05	0.759
18:1ω9	dry	0.51	0.078	1.81	0.005	1.11	0.617	1.58	0.019	0.87	0.595	0.93	0.711
	rainy	2.18	0.112	3.97	0.001	1.62	0.090	2.48	0.001	1.42	0.272	2.49	0.001
18:1ω7	dry	0.65	0.205	1.57	0.046	0.67	0.142	1.16	0.536	1.21	0.429	0.97	0.838
	rainy	2.49	0.010	4.15	0.001	0.92	0.807	1.71	0.082	1.73	0.071	2.13	0.001
18:2ω6	dry	0.19	0.001	0.06	0.010	0.33	0.001	0.78	0.172	0.53	0.003	0.54	0.001
	rainy	0.59	0.230	0.89	0.492	0.35	0.001	0.89	0.645	0.59	0.055	0.92	0.575
18:3ω3	dry	0.01	0.001	0.28	0.001	0.17	0.001	0.49	0.006	0.28	0.001	0.33	0.001
	rainy	0.40	0.042	0.61	0.037	0.25	0.001	0.76	0.331	0.43	0.008	0.91	0.568
18:4ω3	dry					0.23	0.001	0.51	0.030	0.22	0.001		
	rainy					0.26	0.001	0.64	0.122	0.28	0.001	0.64	0.003
20:3ω3	dry					0.40	0.007	0.78	0.366			0.87	0.396
	rainy					0.08	0.001	0.24	0.009				
20:4ω6	dry					1.04	0.875	1.75	0.083	0.89	0.732	1.71	0.003
	rainy					0.94	0.791	1.70	0.043	0.89	0.697	1.87	0.006
20:5ω3	dry					0.50	0.007	0.81	0.437	0.62	0.069	0.83	0.220
	rainy					0.42	0.001	0.77	0.311	0.57	0.035	0.85	0.241
22:4ω3	dry									0.58	0.204	0.83	0.742
	rainy												
22:5ω3	dry					1.13	0.711	2.13	0.014			1.62	0.059
	rainy					0.81	0.424	1.73	0.031			1.74	0.010
22:6ω3	dry					1.32	0.389	3.67	0.001			1.50	0.156
	rainy					1.01	0.971	2.97	0.001			1.74	0.041
24:1ω9	dry					0.93	0.802	1.75	0.010			1.45	0.046
	rainy					0.59	0.037	1.23	0.288			1.53	0.031

1.10 Figures

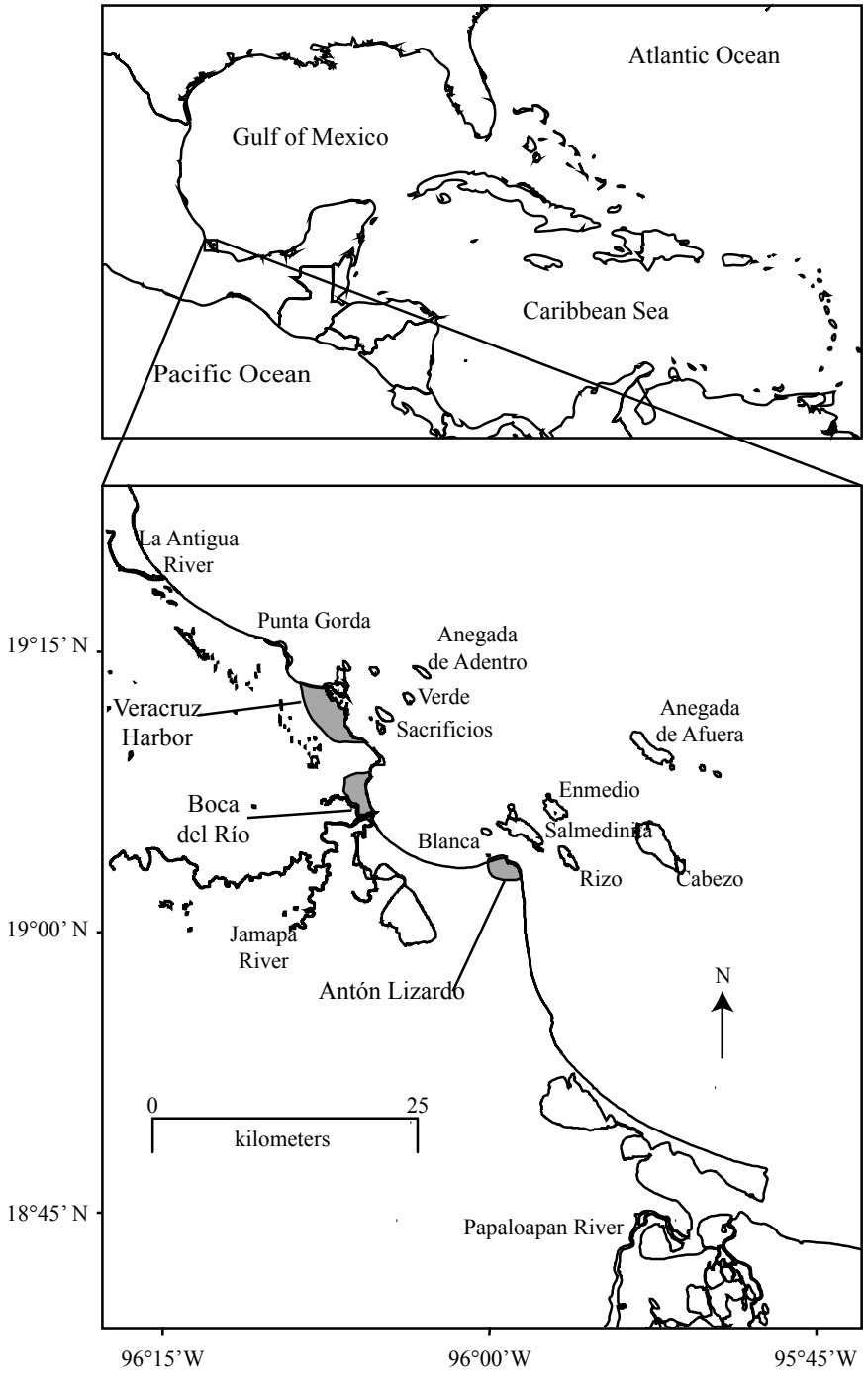

Figure 1-1. Geographical location of the coral reef system of Veracruz in the Gulf of Mexico.

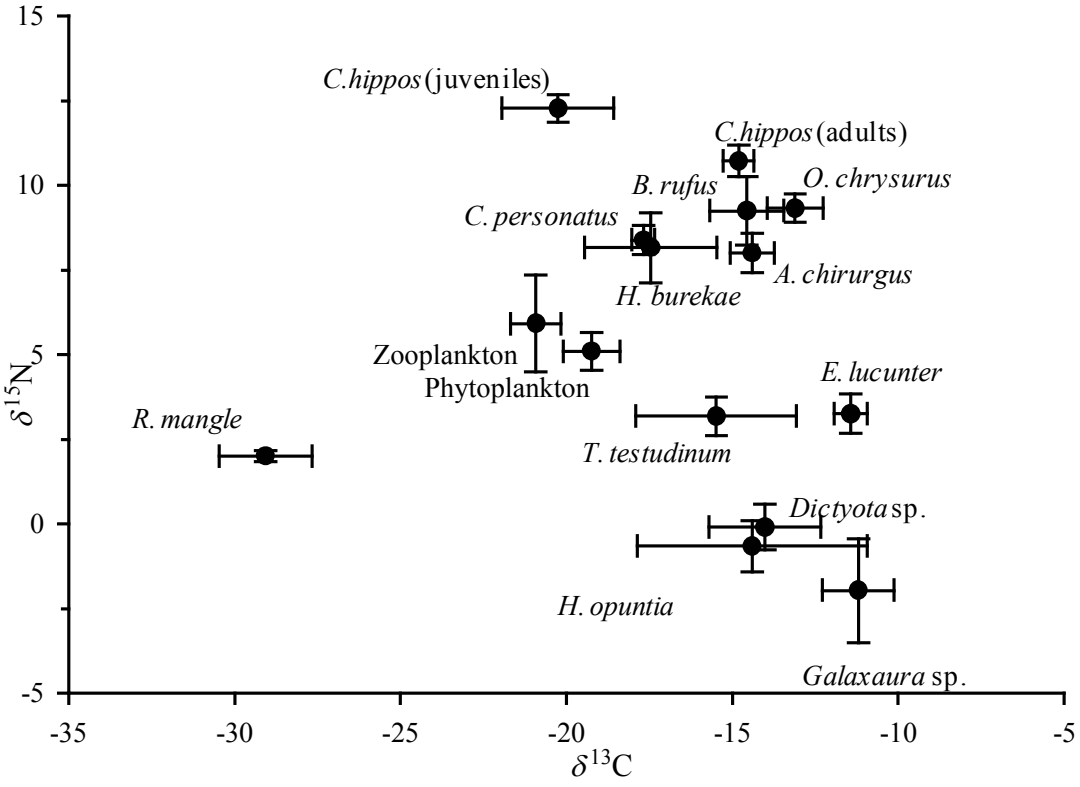

Figure 1-2. Isotopic δ^{13}C and δ^{15}N values of primary producers and subsequent consumers during the dry season in the PNSAV. Primary producers are microphytoplankton > 30 µm, red, brown, and green algae *Galaxaura* sp., *Dictyota* sp., and *Halimeda opuntia*, respectively, higher plants such as mangrove *Rizhophora mangle*, and sea grass *Thalassia testudinum*. Subsequent consumers are sea urchin *Echinometra lucunter*, and fish *Acanthurus chirurgus*, *Coryphopterus personatus*, *Halichoeres burekae*, *Bodianus rufus*, *Ocyurus chrysurus*, and *Caranx hippos*. Horizontal and vertical bars represent the corresponding standard deviation.

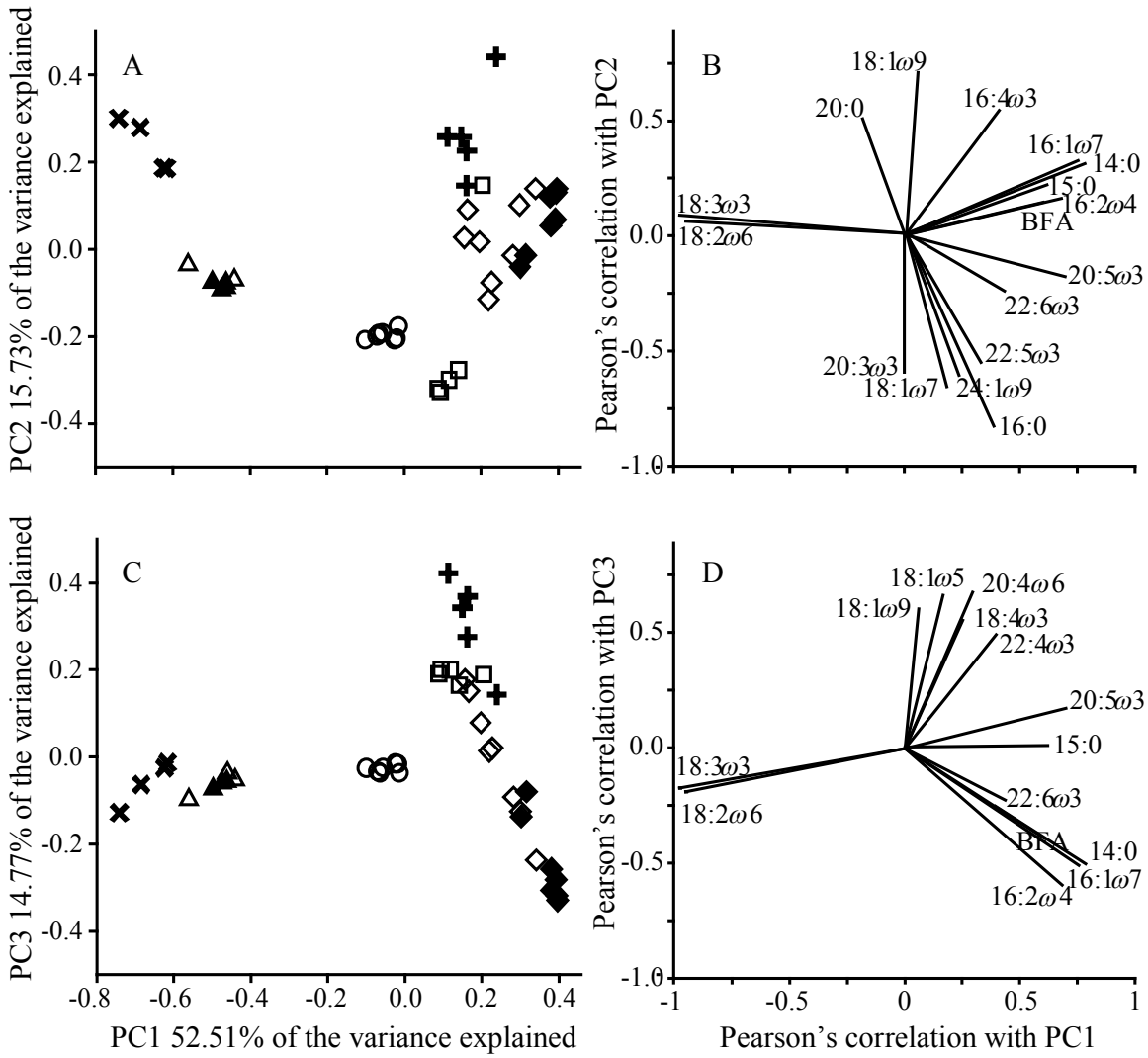

Figure 1-3. Principal components analysis, separating fatty acid biomarkers of higher plants from marine sources. A) PC1 *vs*. PC2, B) fatty acids correlated with PC1 and PC2, C) PC1 *vs*. PC3, and D) fatty acids correlated with PC1 and PC3. Crosses are mangrove, triangles are sea grass, plus signs are brown algae, squares are red algae, circles are green algae, and diamonds are phytoplankton. Open symbols are used for the dry season and filled symbols for the rainy season.

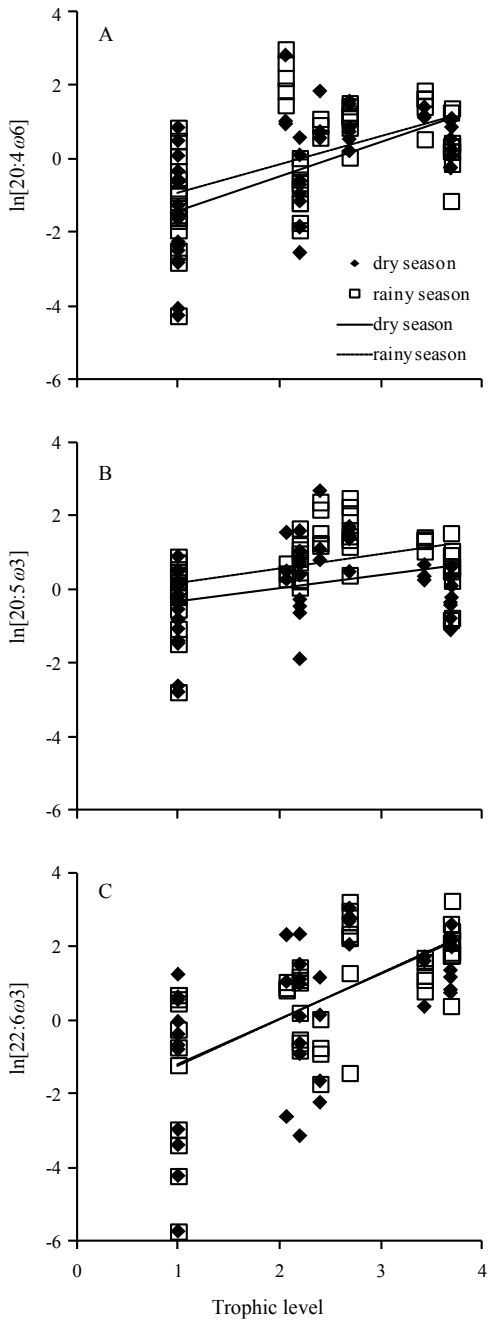

Figure 1-4. Linear regression of highly unsaturated fatty acid from all sources related to the trophic level of the food web members of the coral reef of Veracruz. The equation to calculate HUFA concentration is $[HUFA]= e^{\alpha} \times e^{\beta \cdot TL} \times C_0$, where $C_0= 1$ mg mg^{-1}. Substitution of parameters is shown for (A) ARA, (B) EPA, and (C) DHA. Filled diamonds and continuous lines are for the dry season, and open boxes and dashed lines are for the rainy season.

Chapter 2 Seasonal Shifts in C: N Ratios, Lipid Classes, Fatty Acids and Sterols in a Gulf of Mexico Coral Reef Food Web under River Influence

2.1 Abstract

Many coastal systems are experiencing increasing anthropogenic nitrogen (N) and organic carbon (OC) inputs, especially coral reefs under river influence during the rainy season. To determine how primary consumers, habitat providers such as coral, grazers and top predators respond to the N and OC enrichment, carbon: nitrogen ratios (C:N mol mol^{-1}), lipid classes (LC), fatty acids (FA), sterols (ST) and the ratio of triacylglycerol to ST (TAG:ST) were measured. They were used to evaluate nutritional quality in the six most abundant primary producers (PP) and nutritional condition in 10 ubiquitous consumers in a Gulf of Mexico coral reef under river influence. Low C:N ratios reveal nitrogen enrichment in PP and is a direct consequence of lipid and carbohydrates synthesis in the case of C and protein in the case of N. Among the LC, high ratios of TAG:ST or steryl and wax esters (SE/WE) reflect high-energy storage. These tracers helped to identify primary producers that promoted a better nutritional condition in consumers. High TAG content showed that phytoplankton and zooxanthellae could be considered to have higher nutritional quality, followed by seagrass, mangrove and macroalgae. Seasonal nutritional condition of consumers shifts depending on the species. The 10 consumers were clearly separated using non-metric multidimensional scaling (nMDS) and the greatest separation was between the coral *Montastrea cavernosa* and the sponge *Aplysina* sp. with a dissimilarity of 70%. Zooplankton, sea urchins, clams, and

five teleost fish species were in-between these two groups. ST from PP allowed the identification of major food sources of consumers in contrasting seasons. The phytoplankton sterol 24-methylenphenol was correlated with higher ratios of TAG:ST and FA characteristic of diatoms principally in grazers such as the sea urchin *Echinometra lucunter* and top predators such as the perciform fish *Bodianus rufus*, *Ocyurus chrysurus* and *Caranx hippos* suggesting a better nutritional condition in these species and high retention of OC during the rainy season. In contrast, the habitat providing coral *M. cavernosa* showed a better condition in the dry season as a result of a zooxanthellae contribution traced with gorgosterol. Therefore, a negative effect of river discharges was detected and its nutritional condition decreased making it more vulnerable to diseases, suggesting that abundance of zooplankton did not satisfy the energy demand of *M. cavernosa* during the rainy season.

2.2 Introduction

Many coastal systems are experiencing increasing anthropogenic N and OC inputs (Keuskamp et al. 2015) especially coral reefs under river influence during the rainy season. In response to anthropogenic nutrient enrichment and overfishing of herbivores, algae could grow over corals and shade those (Bellwood et al. 2004). The Gulf of Mexico coral reef named Parque Nacional Sistema Arrecifal Veracruzano (PNSAV) had a decrease of coral cover from 40-50% in the mid-1960s to 15-21% in 1999 and 19% in 2009 (Horta-Puga and Tello-Musi 2009). In some coral reefs control of macroalgae populations relies on herbivorous consumption. For instance, in the Caribbean reefs,

consumption of foliose macroalgae ranges between 15% and 40% in acanthurids and scarids, respectively (Dromard et al. 2015). However, in the PNSAV coral reef, brown algal biomass contributed 3-4% to the surgeon fish *A. chirurgus* OC, in contrast to the sea urchin *E. lucunter* (15-26%). Apparently surgeonfish consumption was low because food chains are based much more on phytoplankton and/or pelagic zooplankton, than on macroalgae (Chapter 1). A direct implication of this result is that the reduction of foliose macroalgae cannot depend only on herbivore consumption. According to the values of $\delta^{15}N$ in macroalgae in the PNSAV, the source of nitrogen is likely fertilizer (Chapter 1). In this study I will evaluate C:N ratios as indicators of limited *vs* enriched dissolved nitrogen conditions in macroalgae according to Lapointe et al. (2004). The C:N ratio also assesses the effect of nitrogen discharges on the rest of the primary producers such as phytoplankton (Bittar et al. 2013), seagrass (Duarte, 1990), and mangrove (Reef et al. 2010) and provides information on the nutritional condition of consumers at higher trophic levels (El-Sabaawi et al 2012). Reproductive status of consumers may also influence C(%) and C:N ratio in fish likely because reproduction affects lipid accumulation in somatic and reproductive tissues (Sweeting et al. 2006). This effect appeared to be largely explained by variability in quality of basal resources such as organic matter C:N (El-Sabaawi et al. 2012).

The rates at which primary production is retained by herbivores and higher trophic levels are important parameters in understanding OC transfer dynamics in these systems. According to Christensen and Pauly (1993), the overall average transfer efficiency is 9.2% from one trophic level to the next. The significance of the loss of live coral may relate to changes in habitat structure and to trophodynamic relationships (Bell and Galzin,

1984). Top predators tend to be large, long-living individuals with a slow response to environmental changes (Jennings et al., 2001). Therefore it is important to understand the effect of river discharges on the nutritional condition of the great star coral *M. cavernosa*, one of the most abundant corals in the study site and the principal habitat provider in the PNSAV (Pérez-España et al. 2012), and upper trophic levels. Whereas photosynthesis provides the main source of energy in hermatypic corals (Davies 1991), some species such as *M. cavernosa,* also use heterotrophic carbon sources, and consumption of zooplankton can provide 10-20% of their energy requirements (Porter, 1976). The degree of heterotrophy has been related to turbidity (Anthony and Fabricius, 2000): high turbidity is a common condition in coral reefs under river influence. During the rainy season, river discharge seems to increase the availability of nutrients (Terrados et al. 2008), and sea surface temperatures increase to 29-32°C (Salas-Monreal et al. 2009). Nutrients available in the water column are taken up by phytoplankton and to an unknown extent by bacteria, and are closely linked with pelagic food webs. Consequently, the zooplankton biomass undergoes a tenfold increase during the rainy season in coral reefs influenced by rivers (Okolodkov et al. 2011) increasing the food supply for zooplanktivores including heterotrophic corals. However, at the end of the rainy season corals health decreases resulting in the increase of number of disease coral colonies up to 30 compared to 7 in the dry season (Pérez-España et al. 2012). There is a gap in the knowledge of ecology for this coral: does high abundance of zooplankton satisfy the energy demand of *M. cavernosa* during the rainy season or the nutritional condition decreases making it more vulnerable to disease? How do primary consumers and top predators respond to the nutrient enrichment?

The present study includes six primary producers: mangrove, seagrass, green, red and brown algae, phytoplankton and zooxanthellae and ten consumers from different trophic levels which are functionally distinct: suspension feeders (the coral *M. cavernosa* and clam *P. carnea*), deposit feeders (the sponge *Aplisyna* sp.), grazers (the sea urchin *E. lucunter* and surgeon fish *A. chirurgus*) and active predators. The active predators include pelagic ones such as zooplankton, the most abundant fish in the PNSAV the masked goby *C. personatus*, as well as benthic active predators such as the hogfish *B. rufus* and two top predators species listed in the Mexican fishing charter, the yellowtail snapper *Ocyurus crhysurus* and the crevalle jack *Caranx hippos*.

2.2.1 Nutritional condition using C:N ratios and lipid biomarkers

The C:N ratio reveals enriched dissolved inorganic nitrogen sources that promote growth of primary producers. A basic principle in ecology is that high amount of nutrients promotes growth of primary producers and a high consumption of primary producers promotes accumulation of storage lipids which are 10-20% of OC in consumers. Lipids provide biosynthesis information, they are analytically accessible and they are conserved through the food web, therefore lipids are good biomarkers of the nutritional status of a community. However, a community is composed of different species and trophic levels, for instance, primary consumers can display a variety of responses depending on their feeding habits and the nutritional quality of primary producers (Dromard et al. 2015). Nutritional condition of food web members is an important index of health, because larval development in many marine organisms is largely dependent upon energy reserves, which correspond in most cases to triacylglycerols (TAG). When the energy derived from

exogenous feeding exceeds the immediate metabolic demands of the larvae, then the excess energy can be stored as TAG. In contrast, when this energy is insufficient to maintain the basal metabolism of the larvae, endogenous TAG is preferentially catabolized. The concentration of TAG typically declines during starvation, as in anchovy (*Engraulis mordax*), herring (*Clupea harengus*) and the Atlantic cod (*Gadus morhua*) as reviewed by Giraldo et al. (2013 and references therein). Such observations suggest that lipid components are good indicators of nutritional condition for grazer and active predator fish and other functionally distinct components of a food web. One of the advantages of the TAG:ST index is that it adjusts quickly to changes in food availability or quality compared to morphometric condition indices.

2.2.2 Lipid classes

Lipids comprise a large group of chemically heterogeneous compounds, the majority of which include esters of FA as part of their structure. The relative percentage of lipid classes helps to characterize the nutritional condition of an organism. TAG are short-term energy storage lipids, whereas wax esters (WE) are long-term energy storage lipids. During starvation stress, TAG is utilized before WE (Lee and Patton 1989). Acetone mobile polar lipids (AMPL) are a mixture of pigments and chloroplast-associated glycolipids which strongly indicates photosynthetic supply. Phospholipids (PL) and sterols (ST) are primarily membrane structural components, and dominance of these lipid classes in the lipid profile indicates a constant food source. Hydrocarbon (HC) markers include alkanes derived from algae or plant leaves, and polycyclic aromatic hydrocarbons (PAH) are derived mainly from crude petroleum and fuel spills (Parrish et al. 2000).

2.2.3 Fatty acids

FA represent the "building blocks" of lipids and are the largest constituent of neutral lipids (NL), such as TAG, SE and WE, as well as of the polar PL. Marine primary producers, including bacteria, diatoms, dinoflagellates, angiosperms and macroalgae can be distinguished by characteristic FA, as well as combinations of FA. While classes of benthic primary producers are distinct in their overall FA composition, most do not possess unique marker FA that can be used to identify their contribution to higher trophic levels, and other biomarkers such as stable isotopes are needed to identify the FA sources (Chapter 1). Unlike mammalian predators, benthic invertebrates have the capacity to significantly modify their dietary FA and thereby obscure markers of food sources (reviewed by Kelly and Scheibling 2012). Therefore the use of species specific dietary markers has been suggested, such as non-methylene interrupted FAs for benthic invertebrates (Budge et al. 2007) or sterols for primary producers (Drazen et al. 2008), as these may provide complementary results to those from FA and stable isotope analysis.

2.2.4 Sterols

Cholesterol is the main ST in most animals, and the presence of other ST may provide good trophic indicators (Drazen et al. 2008). For example, phytosterols are synthesized by algae and plants. Their main function is to regulate membrane fluidity (Rozner and Garti 2006). The ST content is a major means by which eukaryotic cells modulate and refine membrane fluidity, permeability, and the function of various membrane proteins. It has been shown that the ordering capacity provided by cholesterol is of significantly greater magnitude than that of any of cholesterol's metabolic precursors likewise; the ordering

effect provided by phytosterols differs from that of cholesterol, which may limit direct substitution of cholesterol by phytosterols in animal membranes. Even phytosterols are structurally similar to cholesterol, although they have an extra hydrophobic carbon chain at the C-24 position (Martin-Creuzburg and von Elert , 2009 and references therein). Herbivorous insects and also the crustaceans examined to date use dietary sterols to synthesize cholesterol. Therefore, most species studied are capable of dealkylating and reducing common C-24-alkyl phytosterols, such as sitosterol or stigmasterol, to cholesterol (Martin-Creuzburg and von Elert, 2009 and references therein). However, more than 200 different types of phytosterols have been reported in plant material, and not all of them are suitable as cholesterol precursors allowing me to consider them as biomarkers.

Although some animals such as fish, crustaceans and cephalopod molluscs contain cholesterol as their predominant sterol (often more than 93% of total sterols) the animals from most other phyla contain very complex mixtures often with twenty or more different sterols. In some taxa, such as bivalve molluscs or sponges, a sterol other than cholesterol may be the major constituent (Goad, 1981). The sterol composition of an animal result from the balance established among several contributory sources of sterol. The factors to consider for a sterol biomarkers are: (a) the spectrum of sterols encountered in the diet of the animal and the selectivity which the animal displays for the absorption, or excretion, of any particular compound in the mixture; (b) the assimilation by a host animal of sterols produced by symbiotic algae or other associated organisms such as bacteria or fungi in the digestive tract; (c) the capacity of an animal to modify any absorbed dietary sterol; (d) the contribution, if any, from *de novo* biosynthesis of sterols from simple precursors such

as acetyl-CoA and mevalonic acid (MVA). In addition, the nutritional condition of the animal may have a role in determining its sterol synthetic requirements. In animals with a slow rate of sterol synthesis most of the acetate (*de novo* sterol precursor) may be rapidly depleted by competing pathways increasing their incorporation into FA (Goad, 1981). For instance, the "MVA shunt" pathway (also known as the "Popjak shunt") forms a non-sterol-forming pathway to redirect flux to small molecules or FA in animal, fungal, or plant tissues (Nes 2011 and references therein).

The seasonality of river discharges to the coral reef ecosystem allowed me a unique opportunity to simultaneously assess how nutrients and turbidity variation related to season might influence organism stoichiometry across a wide range of species of different ecological function and trophic levels, conditions that modulate the transfer of basal resources. I hypothesize that macroalgae C:N ratio will reflect an anthropogenic nitrogen enrichment because C:N ratio decreases whit nitrogen fertilization (Lapointe et al. 2004). Also, I hypothesize that macroalgae will have a lower nutritional quality than seagrass, zooxanthellae and phytoplankton because of the low amount of lipids and the high amount of FFA (Nelson et al. 2002). I also predict that I will find some FA characteristic of primary producers in consumers and that there are biomarker sterols for each primary producer.

Additionally, I predict that nutritional condition will shift seasonally depending on the species, because the rainy season is related to zooplankton supply (Okolodkov et al. 2011) that influences reproduction and growth in some species. For instance coral can obtain energy from zooplankton in the rainy season but the turbidity reduces growth of zooxanthellae probably decreasing its nutritional condition. Finally, I predict that

consumer species with high nutritional condition with high proportions of TAG or another FA storage class, such as SE or WE, will correlate with primary producer sterol biomarkers. This will allow the detection of high nutritional quality food primary producers for consumers, both invertebrates and vertebrates, because the rate of synthesis of sterols *de novo* (cholesterol) will decrease due to *de novo* sterol precursors rapidly depleting by competing pathways increasing their incorporation into FA (Goad, 1981) mainly stored in TAG and SE/WE.

The goals of this Chapter were:

1) To determine dissolved nitrogen enrichment in primary producers using C:N ratios.

2) To evaluate the nutritional quality of green, red and brown macroalgae compared to mangrove, seagrass, zooxanthellae and phytoplankton, using C:N, total lipids and TAG:ST ratio.

3) To identify primary producer apportionment in coral, sponges and clams using FA.

3) To characterize the sterol biomarkers from each primary producer.

4) To assess if the seasonal nutritional condition shifts depending on the species in a coral reef food web under river influence.

5) To define relative apportioning of primary producer sources using ST tracers in the consumers, by correlation of ST biomarkers with storage and structural lipids.

2.3 Material and methods

2.3.1 Study site

The Veracruz reef system National Park (Parque Nacional Sistema Arrecifal Veracruzano: PNSAV) is located off Veracruz, Mexico, adjacent to the cities of Boca del

Rio and Anton Lizardo, in the southwest Gulf of Mexico, between 19° 02’ 24.00’’ and 19° 15’ 27.11’’ N and between 96° 12’ 01.00’’ and 95° 46’ 46.19’’ W (Diario Oficial de la Federación, 2012). It is part of a larger coral reef system that includes the Caribbean and the Gulf of Mexico (Fig. 1). A group of 13 reefs is located adjacent to Veracruz and Boca del Rio and another group of 15 reefs with larger structures is located adjacent to Antón Lizardo; both groups are divided by the Jamapa River (Fig. 1), and delimited to the North by La Antigua River and to the south by the Papaloapan River. During winter they are typically affected by frontal incursions of northerly systems locally known as ‘nortes’ which produce winds of 120 km h^{-1}, monthly precipitation of 34 ± 15 mm, and a southerly marine current. During summer, the atmospheric conditions are dominated by tropical storms from the south which produce high monthly precipitation rates of 265 ± 99 mm and northerly current systems (Zavala-Hidalgo 2003). Under sustained southerly wind conditions a cyclonic eddy develops off Veracruz Port, which enhances productivity of the area. However, under northerly wind conditions or variable southerly winds, the relatively high productivity area vanishes (Salas-Monreal et al. 2009). The temperature (28–26°C) and salinity (34–36.4 psu) variations during a tidal cycle are within the ideal range for coral colonies to grow. Finally, the PNSAV is an area of high evaporation; therefore, the salinity (>32 psu) and temperature (>25°C) values are usually higher than its surrounding ocean water (Salas-Monreal et al. 2009).

2.3.2 Sampling methods

Sampling was conducted in two seasons of three consecutive years: April-May (dry season) and September-October (rainy season) from 2007 to 2009. However, some

samples were collected only in some seasons of some years (Table 2-1). The most abundant primary producers and primary consumers were selected based on ecological studies (Okolodkov et al 2007, Terrados *et al.*, 2008, Peréz-España and Vargas-Hernández 2008). A total of 18 groups were considered in this study (Table 2-1). Macroalgae and seagrass were taken by scuba diving, while mangrove leaves were collected by hand with nitrile gloves from trees located in the Jamapa river margin in April-May 2008. Plankton was sampled (Okolodkov et al. 2011) at nine stations through the PNSAV: the mouth of the Jamapa River, the Enmedio, Cabezo, Anegada de Afuera, Anegada de Adentro, Verde, and Sacrificios reefs, one offshore station between Anegada de Adentro and Anegada de Afuera, and one offshore from Anton Lizardo village (Fig. 2-1) in April and October 2008. Collection was by net tows using 30 and 120 μm meshes to collect phytoplankton and zooplankton, respectively.

Benthic primary producers were collected in the Sacrificios and Cabezo reefs; around 5 specimens of each of the principal macroalgae: *Halimeda opuntia* (green algae), *Dictyota* sp. (brown algae) and *Galaxaura* sp. (red algae) and the more abundant seagrass *Thalassia testudinum* were taken by scuba diving. The zooxanthellae contribution was evaluated in one of the most abundant species of hermatypic corals in the PNSAV *Montastrea cavernosa* (Perez-España et al. 2012) that had been collected in the Cabezo and Blanca reefs in May 2008, and October 2009 by scuba diving. During the rainy season coral and zooxanthellae were separated by centrifugation according to Papina et al. (2003).

Samples of the sponge *Aplysina* sp. and sea urchin *Echinometra lucunter* were collected in the Rizo, Blanca and Verde reefs in September 2007 and in April 2008.

Amber pen shells, *Pinna carnea* were collected in Giote and Cabezo in April 2008 and October 2009. Fish were collected in the Blanca, Rizo, Santiaguillo and Salmedinita reefs and off Antón Lizardo, in the south and in the Verde and Anegada de Adentro reefs in the north in September-October 2007 and April-May 2008. Species locations are shown in Table 2-1. Divers went to the deepest part of the reef and followed a transect to the shallowest part of the reefs, collecting big fish with a harpoon and small fish with an anesthetic solution of eugenol as indicated by Santander-Monsalvo (2010).

Plankton samples were centrifuged to concentrate the biomass, and macroalgae were cleaned of epiphytes. Sea urchins *(E. lucunter*), amber pen shells (*P. carnea*), the great star coral (*M. cavernosa*), the tube sponge (*Aplysina* sp.), and masked goby (*Coryphopterus personatus*) were collected for subsequent analysis of whole soft tissues. Subsamples of muscle tissue were taken from the surgeon fish *Acanthurus chirurgus*, the hogfish *Bodianus rufus*, the yellowtail snapper *Ocyurus chrysurus*, and the crevalle jack *Caranx hippos*. Sex was determined when the gonad was macroscopically identified by color (white for males and orange for females); otherwise the organism was considered immature.

2.3.3 Laboratory methods

All samples were weighed wet and freeze dried on a Lyophilizer Virtis 5L for accurate dry weight determination. A 20-200 mg sample was weighed dry with an analytical balance: Heraeus (maximum weight of 60 g). Freeze-dried samples were ground in a mortar into a homogeneous fine powder. Around 2 mg of subsample was used to determine the amount of carbon and nitrogen per sample with an elemental analyzer

(EA) Costech, interfaced with continuous flow to a Finnigan Delta Plus V with Conflow IV isotope ratio mass spectrometer (IRMS). The analyzer was calibrated using 2, 5-bis-(5-tertbutyl-benzoxazol-2-yl-thiopen) (BBOT) with C: N= 11. Total lipids were extracted from the other part of the sample with chloroform: methanol: water 2:1:0.8 (Folch et al., 1957, modified by Parrish 1999), and total lipid extracts were divided in two. The first was separated into lipid classes by Chromarod thin layer chromatography (Parrish, 1987a). Concentrations of lipid classes were obtained by interpolation with a calibration curve constructed with five concentrations, ranging between 0.5 and 4.0 μg, of the following standards: nonadecane, cholesteryl sterate, 3-hexadecanone, triplamitin, palmitic acid, cetyl alcohol, cholesterol, monopalmitoyl, and phosphatidylcholine (Sigma-Aldrich). The rest of the lipid extract was derivatized to obtain methyl esters with hydrochloric acid and methanol 5:95 heated to 85°C for 2.5 hours (Sato and Murata, 1988). The rest of the fatty acid extracts was silylated with bis trimethyl silyl trifluoro acetamide (BSTFA) Supelco: 3-2024 (Copeman and Parrish, 2004). Fatty acids from zooxanthellae and suspension feeders: great star corals, sponges and amber pen shells, were recovered in pure hexane and analyzed in a gas chromatograph with a mass spectrometer as the detector (Varian CP 3800 GC-MS), on an Omegawax 250 column (Agilent Technologies) of 30 m x 0.25 mm x 0.25 μm. After injection, the temperature of the column was subjected to the following sequence: 110°C for 3 min, increased to 165°C at a rate of 30°C min^{-1}, maintained at 165°C for 2 min, increased to 209°C at a rate of 2.2°C min^{-1}, and maintained at 209°C for 18 min. The mass spectrometer detector was set at 260°C and the ion source was set at 70 eV. Fatty acid methyl ester (FAME) quantification was computed by interpolation of area peaks with a calibration curve of 37

standard fatty acids (Supelco 47885-U). The total concentration of identified FAME in the sample (mg mL^{-1}) was considered as 100%, and an individual FAME was calculated as a proportion of the total identified FAME. The fatty acid profile of the rest of the organisms was evaluated in Chapter 1 and used in this Chapter to compare with lipid class and sterol profiles.

Sterols from all samples were recovered in hexane and analyzed in a Hewlett Packard 6890 GC-MS with a DB-5 column 30 m x 0.32 mm x 0.25 μm. Peaks were identified by retention time of standards and mass spectra interpretation in a GC-MS (Appendix 1). The reference for sterol identification was Jones et al. (1994), and for other compounds it was McLafferty and Turecek (1993).

Areas were integrated with Wsearch 32 software (Wsearch 2008; version 1.6 2005). Sterol proportions were determined with a Varian gas chromatograph with a flame ionization detector (GC-FID). After injection, the temperature of the column was subjected to the following sequence: 60°C for 1 min, increased to 100°C at a rate of 25°C min^{-1}, increased to 150°C at a rate of 15°C min^{-1}, and finally to 315°C at a rate of 3°C min^{-1}. The quadrupole was set at 150°C and the MS ion source was set at 270°C and 70 eV. Areas were integrated with the Galaxy software and each sterol area was calculated as the proportion of the total area. This proportion was related to total sterols computed by adding the concentration of steryl/wax ester plus free sterol fractions of dry biomass to obtain an approximation of individual sterol concentrations per unit of dry biomass (μg mg^{-1} or mg g^{-1}). Wax esters were identified by GC-MS in the same chromatogram as sterols accounting for around 1% of total sterols in primary producers and sponges, around 10% in teleost fish, and around 20% in coral and pen shells.

2.3.4 Data analysis

A one way analysis of variance (ANOVA) was used to assess the contribution of the species by season as a predictive factor for C:N composition. Dissolved nitrogen enrichment (DNE) of primary producers was evaluated using C:N ratios from each primary producer compared with reference values characteristic of macroalgae (Lapointe et al. 2004), phytoplankton (Bittar et al. 2013), seagrass (Duarte, 1990), and mangrove (Reef et al. 2010) in DNE waters. Nutritional condition of primary producers and consumers was evaluated using total lipids per dry weight, and the condition index based on TAG: ST, analyzed with a one way ANOVA including species by season and separating primary producers, invertebrates and fish.

FA from primary producers and fish were described in a previous work (Carreón-Palau et al. 2013). In this study FA composition was compared among suspension feeders by ANOVA in order to detect significant differences in the primary producer FA. A log ratio transformation was performed to normalize the percentage of total FAs prior to the statistical test; the centered log ratio transformation consists of the division by the geometric mean of the sample followed by a log transformation according to Aitchison (1986). Sterol biomarkers were defined as the distictive sterol for each primary producer assimilated by a primary consumer or by a host, used to detect the key sources for suspension feeders including coral, clams and sponges, as well as grazers, and active predators.

Species differences of individual (univariate) lipid classes and sterols were tested independently in primary producers, invertebrates and fish with a one way ANOVA using

the Fisher statistic (*F*); residual analyses were used to test assumptions of normality and equal variance. When assumptions were violated, the Kruskall-Wallis statistic (*H*), a non parametric analysis also termed ANOVA on ranks, was used *(*α= 0.05).

Seasonal nutritional condition shift depending on species was tested with a multivariate analyses of FA and ST profiles, as well as structural and storage lipid classes: ST, PL, TAG, and SE/WE, using the PRIMER software 6.1.16 & PERMANOVA + version 1.0.6 (PRIMER-E, Plymouth, UK). Non-metric multi-dimensional scaling (nMDS) was conducted based on a Bray-Curtis similarity coefficient. No transformation were used to avoid artificial weighting of FA, ST and lipid classes that made only trace contributions to their respective profiles. Similarities among species and seasons were investigated using the similarity percentages (SIMPER) function and statistical differences were tested with two way crossed analysis using the analysis of similarity (ANOSIM) function (significance level < 5% or $p < 0.05$). Significant differences were confirmed with a permutational multivariate analysis of variance (PERMANOVA). PERMANOVA analysis allows multivariate comparisons with data without a normal distribution of residuals. The method of permutation of residuals under a reduced model was used and significant differences were tested with Marcov Chain (MC) probability values because sample size was less than 30. In this study, factors were season, species and the interaction season x species and the response variables were FA, ST and lipid class profiles. Comparison among species was pair-wise tested. The factor species included zooplankton, the coral *M. cavernosa*, sea urchin *E. lucunter*, amber pen shell *P. carnea,* sponge *Aplysina* sp., surgeon fish *A. chirurgus*, masked goby *C. personatus,* hog fish *B. rufus,* yellow tail snapper *O. chrysusrus* and jack *C. hippos*. The second factor was

the season: dry or rainy. Primary producers were not included because they were only collected in the dry or the rainy season, not both. MDS results were spliced into two plots according to season to facilitate visualization. The plot shown in Figure 3 was constructed in Sigma Plot software version 12 from data obtained with PRIMER software.

A significant main effect of species would indicate consistent differences associated with the taxonomic origin, and species without significant differences (group of species) in their lipid class, FA and ST profiles means similar physiological pathways. A significant season effect would indicate a consistent influence of the season (turbidity, nutrients, and zoopankton supply), independently of the species. A significant species x season interaction would indicate that the effect of species on lipid classes, FA and ST profile differed by season, either because the season effect might be stronger in one species than in others, or because there is extensive species variation among the sampled seasons that is not predictable in any consistent fashion by the season.

Correlation between sterol biomarkers with storage or structural lipids was assessed with the Pearson correlation statistic using the concentration per dry weight ($\mu g\ mg^{-1}$). Significant positive correlations with storage lipids (SE and TAG) mean diminution of cholesterol *de novo* biosynthesis allowing the detection of biomarkers. Significant positive correlations with structural lipids (ST and PL) means direct incorporation of biomarkers into the cellular membrane, while significant positive correlations with AMPL means that biomarkers are coming from the thylakoid membrane of PP. Pearson correlation analyses were performed with the Minitab Statistics Software (version 15.1.1.0, 2007).

2.4 Results

2.4.1 Nitrogen enrichment in primary producers and consumers using C:N ratios

C:N values are shown in Table 2-2. C:N in mangrove *R. mangle* leaves ranged from 15 to 25 reflecting enrichment of nitrogen in the ground. Also, seagrass *T. testudinum* from PNSAV had levels of organic carbon at 43±1% dry weight, but a higher proportion of nitrogen was reflected in a C:N ratio of 16±3. C:N ratios of macroalgae were also similar to those reported in nitrogen enriched conditions in Exuma Cays, Bahamas (Lapointe et al. 2004); according to these authors the effect of nitrogen enrichment promoted low C:N ratios of 19±3, while dissolved nitrogen limitation promoted high C:N ratios of 42±6 in macroalgae. In the present study, red algae *Galaxaura* sp. had the highest C:N ratio at 26±2, similar to green algae *H. opuntia*, and significantly higher than in brown algae *Dictyota* sp. ($F_{5,21}$=4.7, p=0.008) with the lowest C:N ratio of 20±1 (Table 2-2). The slight increase in C:N ratio in the coral reef phytoplankton of this study, from 10±2 in the dry season to 12±1 in the rainy season, could be explained by the increase in stored lipids from 33% of organic carbon in the dry season to 42% in the rainy season (proportions calculated from Table 2-2).

Among consumers, the C:N ratio was significantly higher during the rainy season (Table 2-2) in the sea urchin *E. lucunter* at 12±1 compared to 8.5±0.4 in the dry season ($F_{2,28}$=21.9, p<0.001), and in the masked goby *C. personatus* at 4.7±0.1*, B. rufus* at 4.1±0.1, *O. chrysusrus* at 4.0±0.1, and *C. hippos* at 5.2±0.4 compared to 3.8±0.1, 3.2±0.8, 3.7±0.1, and 3.7±0.1, respectively in the dry season ($F_{9,95}$=21.7, p<0.001).

2.4.2 Nutritional quality of macroalgae compared to other primary producers

Benthic primary producers ranged considerably in size from whole green algae *H. opuntia* at 5±1 cm to leaves of the seagrass *T. testudinum* at 35±5 cm. In contrast, the heaviest primary producer specimens were the green algae at 29±20 g due to their carbonate deposits. Phytoplankton had a minimum size of 30 μm. Primary producers collected in the PNSAV varied in C:N ratio from 10±2 in phytoplankton to 26±2 in red algae. Significantly lower C:N ratios were detected in phytoplankton, followed by seagrass at 16±3, mangrove at 20±5 and brown algae at 20±1 $F_{5,21}$=4.7, p=0.008 (Table 2-2). Total lipids and the proportion of TAG with respect to ST showed no significant differences among primary propucers. In contrast, the red algae showed significantly lower amounts of lipid per dry weight with 5±2 mg g^{-1} and phytoplankton and zooxanthellae had higher amounts in the rainy season at 51±30 mg g^{-1} and 73±13 mg g^{-1}, respectively ($F_{7, 34}$=5.72, p=0.001). According to the above mentioned results, nutritional quality of macroalgae was lower than the rest of the primary producers as I expected. In particular PNSAV primary producers had low concentrations of lipids and limited storage lipids, such as TAG and SE/WE, as well as high proportions of structural lipids, principally chloroplast associated lipids recovered in AMPL and PL (Table 2-3).

2.4.3 Nutritional condition of consumers using lipid classes

Most consumers did not show differences between seasons in terms of length; however, *P. carnea* showed significant differences in length (p<0.05), being longer in the rainy season. The weight of invertebrates varied between 0.2±0.1 g for zooplankton to 53±20 g for the amber pen shell *P. carnea*. Fish collected were juveniles for *C. hippos*, while *A.*

chirurgus, *B. rufus* and *O. chrysurus* were a mix of immature males and females in both seasons (Table 2-2). In contrast, the sex of the masked goby *C. personatus* was not recorded because of the small size. There were two species with significant seasonal length differences (showing an interval at 95% of confidence, $p<0.05$): the yellow tail snapper *O. chrysurus* and masked goby *C. personatus* (Table 2-2). The bivalve *P. carnea* showed around a 3 times higher concentrations of lipids per dry weight in the rainy season compared to the dry season, while fish increased lipid content from 1.3 to 5.8 times (Table 2-2).

Lipid classes of suspension feeders are shown in Table 2-3. In the amber pen shell *P. carnea* the AMPL fraction showed a significant increase from 8±3% in the dry season to 18±3% in the rainy season ($F_{9, 45}= 3.404$, $p= 0.003$). Sponge and coral had no significant seasonal differences, ~20%. The TAG fraction was higher in grazers and active predators suggesting a better nutritional condition and high retention of OC in the food web during the rainy season. In comparison, structural lipid proportions such as PL were significantly higher in the dry season (Table 2-3).

The great star coral *M. cavernosa* showed a significantly higher proportion of TAG: ST in the dry season of 6±3 compared to 1.4±0.5 ($F_{9, 39}=4.0$, $p=0.001$) in the rainy season (Table 2-2). Also, *M. cavernosa* showed an increase in ketones in the rainy season and significantly higher storage of SE/WE at 25±5% in the dry season, compared to 16±3% in the rainy season ($F_{9, 39}=56.2$, $p<0.001$). Similarly, the sponge *Aplysina* sp., the amber pen shell *P. carnea*, and the hog fish *B. rufus* showed significantly higher energy storage in SE/WE esters ($F_{9,39}=56.2$, $p<0.001$) compared to the rest of the food web members (Table 2-3).

2.4.4 Primary producer apportionment in coral, sponges and clams using FA

FA of mangrove leaves, seagrass, green, red and brown algae, phytoplankton and fish were analyzed and identified in a previous work (Carreón-Palau et al. 2013) while FA of suspension feeders, such as the coral *M. cavernosa,* the sponge *Aplysina* sp. and the amber sea pen *P. carnea* are shown in this study (Table 2-4). The zooxanthellae fraction of the coral had the highest proportion of saturated FA at 56±4%, with no significant differences with the whole coral in the dry season at 54±4%, or in the rainy season at 66±5%. The amber pen shell *P. carnea* had the second highest proportion at 38±3% with no seasonal differences, and finally the sponge *Aplysina* sp. had the lowest proportion, 31±3%, also with no seasonal differences.

The most abundant saturated FA was 16:0 in the coral *M. cavernosa* and the amber pen shell *P. carnea*. In corals, 16:0 showed significant seasonal differences with 36±11% in the dry season compared to 53±5% in the rainy season ($F_{6, 21}$=49.1, p<0.001), but for clams there were no seasonal differences (23±2%). For sponges the principal contributor to saturated FA was 22:0 with no seasonal differences and an unusually high proportion of 8±3% compared to the other suspension feeders with less than 0.2% (Table 2-4).

Monounsaturated FA (MUFA) ranged from 11±1% in *P. carnea* during the dry season to 25±6% in *M. cavernosa* in the dry season. The major contribution to MUFA was from 18:1ω9 in zooxanthellae and *M. cavernosa*, while 16:1ω7 was the major MUFA in the sponge *Aplysina* sp. in both seasons and in the amber pen shell *P. carnea* in the rainy season. In contrast, the long chain FA 24:1ω7 was characteristic of the sponge *Aplysina* sp. (Table 2-4).

Polyunsaturated FA (PUFA) of 16 and 18 carbons were minor contributors, ranging from 1.5±0.4% in the sponge *Aplisyna* sp. to 5±1% in zooxanthellae. The only suspension feeder with seasonal differences in PUFA was the sea pen shell *P. carnea* with 3±1% in the dry season and 5±1% in the rainy season ($F_{6, 21}$=31.7, p<0.001). PUFA showed clear differences between *M. cavernosa* and sponges and clams. For *M. cavernosa* the characteristic PUFA was 18:5ω3 with low proportion ranging from 0.1 to 0.4%, characteristic of dinoflagellates, while for sponges and clams they were 16:2ω6 and 16:3ω4, characteristic of diatoms, also ranging from 0.1 to 0.4%.

Highly unsaturated FA (HUFA) of 20 and 22 carbons ranged from 11±3% in *M. cavernosa* to 55±12% in sponges. The major contribution to HUFA was from 22:6ω3, 20:4ω6 and 20:5ω3 in zooxanthellae, *M. cavernosa* and *P. carnea*; *P. carnea* also showed a high contribution of non-methylene interrupted FA (NMI). In contrast, the major contribution of HUFA in the sponge *Aplysina* sp. came from 26:2ω6, a characteristic FA of sponges. *Aplysina* sp. was also the suspension feeder with the highest proportion of branched FA, especially in the rainy season at 15±2%, suggesting an important contribution of bacteria to the sponge diet (Table 2-4).

There was a significant difference in the essential PUFA composition among suspension feeders: the coral *M. cavernosa* had the highest proportion of 18:2ω6 at 1.2±0.2%, similar to the zooxanthellae proportion. In contrast, *P. carnea* had the highest proportion of 18:3ω3 at 0.8±0.2%. Sponges had the lowest 18:2ω6 and 18:3ω3 proportions, around 0.3±0.2 %. In contrast, the essential FA (EFA) 20:4ω6, 20:5ω3, and 22:6ω3 were 10 times higher than PUFA. The highest proportions were detected in the sea pen shell *P. carnea* with different trends. Sea pen shells had significantly higher

20:4ω6 and 20:5ω3 in the rainy season at 6±1% ($F_{6, 21}$=62.8, p<0.001) and 9±2% ($F_{6,21}$=72.8, p<0.001), respectively. In contrast, 22:6ω3 was higher in the dry season at 19±7% compared to the rainy season at 10±1% ($F_{6, 21}$=13.3, p<0.001). The coral *M. cavernosa* had intermediate proportions of 20:4ω6 at 3±1%, 20:5ω3 2±1%, and 22:6ω3 5±2% without significant seasonal differences. EFA composition of the whole coral was similar to that of zooxanthellae (Table 2-4).

2.4.5 Identifying sterol biomarkers for primary producers on coral reefs

I took four factors into consideration to determine sterols that could be used as biomarkers.

(a) The profile of sterols encountered in the PP shown in Table 2-5.

The total number of sterols identified in PP was 29; from four in the mangrove to 19 in phytoplankton. Primary producer sterols such as β-sitosterol were the main sterol in the mangrove *R. mangle* at 82±1%, seagrass *T. testudinum* at 66±2%, and green alga *H. opuntia* at 89±1%. Cholesterol was present in all primary producers except in mangrove leaves, and it was the main sterol in red algae at 30.2±0.1% and phytoplankton at 50±8%. Isofucosterol was the main sterol in brown algae at 37±1% and it was shared with green algae at much lower proportions at 1.6±0.3%, while campesterol was the main sterol in zooxanthellae at 47±11%, and it was shared with seagrass, green and red algae, and phytoplankton (Table 2-5).

The mangrove *R. mangle*, had cycloartenol at 7±2% of total sterols as a distinctive sterol, while phytoplankton had several distinctive sterols. The seagrass *T. testudinum* had six sterols, and trans-22-dehydrocholesterol was distinctive at 5±1% of total sterols, being

shared only with phytoplankton at <1%. The green alga *H. opuntia* had eight sterols; its distinctive sterols were spinasterol (1.1±0.3%) and stellasterol at 1.2±0.2%. Stellasterol was also present in phytoplankton samples at a significantly lower proportion. Similarly, the red alga *Galaxaura* sp. had three sterols shared with phytoplankton with significantly higher proportions in red algae: cholestanol (11±2%), brassicasterol (23±2%) and brassicastanol (5±1%).

The brown alga *Dictyota* sp. was composed of seven sterols, among which four sterols were distinctive: 4,24 dimethyl, 5,7 dien-3β-ol (19±2%), poriferasterol (18±1%), fucosterol (21±1%), and an isomer of fucosterol (5±1%). Phytoplankton had 19 different sterols, among which eight were distinctive: 24-mehylenphenol (2±1%), sitostanol (2±2%), 23,24 dimethylcholest 5,7-dien-3β-ol (0.7±0.8%), trans-22-dehydrocholestanol (0.6±0.5%), patinosterol (0.4±0.5%), dinosterol (0.4±0.3%), dinostanol (0.5±0.4%) and ergost 8(14)-enol (0.2±0.3%); however, they were all present at low proportions and had high variability because they came from different phytoplankton groups. Zooxanthellae had five sterols among which pregnanone (12±6%) and gorgosterol (6±2%) were distinctive (Table 2-5).

(b) Selectivity which the consumer displays for absorption or excretion of any particular compound in the mixture.

Biomarkers were selected from distinctive primary producer sterols that were detected in zooplankton, the sponge *Aplysina* sp., sea pen shell *P. carnea*, coral *M. cavernosa* and the sea urchin *E. lucunter*. Fish were not considered to define biomarkers because they can synthesize cholesterol from other sterols and absorb phytosterol to a lesser extent. For

instance, pregnanone, ergost-8(14)-enol, spinasterol, and fucosterol were distinctive for zooxanthellae, phytoplankton, green and brown algae, respectively. However, they cannot be considered as biomarkers because they were not incorporated in the zooplankton, sponge *Aplysina* sp., sea pen shell *P. carnea*, coral *M. cavernosa* or sea urchin *E. lucunter* tissues. In contrast, cycloartenol from mangrove was detected in zooplankton in the rainy season with a variable and low proportion of 0.4±0.7%, while the sea grass biomarker trans-22-dehydrocholesterol was detected in zooplankton in the dry season at 6±2%. Stellasterol from the green alga *H. opuntia* and phytoplankton was identified in zooplankton, *E. lucunter*, *P. carnea* and *Aplysina* sp. Sterols from the brown alga *Dictyota* sp., 4, 24 dimethyl 5,7 dien-3*β*-ol and poriferasterol, were detected in *E. lucunter* and the sponge *Aplysina* sp. Isofucosterol was also detected in the sponge *Aplysina* sp. In turn, brassicasterol from the red alga *Galaxaura* sp. and phytoplankton was detected in all invertebrates studied, with the highest proportions in the sponge *Aplysina* sp. ($F_{9, 39}$=29.6, p<0.001) in the dry season at 20±1% (Table 2-6).

Occelasterol was detected in phytoplankton and zooxanthellae, while 24-methylenephenol was distinctive in phytoplankton and gorgosterol in zooxanthellae (Table 2-5). Occelasterol was better incorporated in zooplankton than 24-methylenephenol, and it was probably consumed by the great star coral *M. cavernosa* increasing from 2±1% in the dry season to 5±1% in the rainy season (H= 37.46, df= 9, p< 0.001). Occelasterol was also incorporated into the masked goby *C. personatus* increasing from 2.9±0.2% in the dry season to 18±6% in the rainy season (H= 24.012, df= 7, p= 0.001, Table 2-6). The distinctive sterol from phytoplankton, 24-methylenephenol, was detected in all invertebrates studied, except in the coral *M. cavernosa,* the latter contained

gorgosterol, a distinctive sterol of zooxanthellae, with significantly higher proportions in the dry season at 20±4% compared to 9±1% in the rainy season ($F_{1, 6}$= 11.2, p= 0.016).

The green algae biomarker, stellasterol was detected in all teleost fish except in the hog fish *B. rufus.* The phytoplankton biomarker 24-methylenephenol was detected in all fish with a higher proportion in the surgeon fish *A. chirurgus* during the rainy season and in the masked goby *C. personatus* during the dry season. The brown algae biomarkers, fucosterol and isofucosterol, were detected in *B. rufus* a sea urchin predator, and fucosterol was also detected in *C. personatus* a zooplanktivore goby.

Primary producer sterols such as β-sitosterol were detected in all fish studied. In addition, the surgeon fish *A. chirurgus* was the only fish with significant seasonal differences; in the dry season it had a greater proportion of β-sitosterol at 5±2% compared to 0.5±0.3% ($F_{9, 26}$=25, p<0.001) in the rainy season (Table 2-7), coincident with the higher contribution of seagrass to the bulk carbon and nitrogen of *A. chirurgus* reported in the Chapter 1.

(c) Assimilation by a host animal of sterols produced by symbiotic algae or other associated organisms such as bacteria or fungi in the digestive tract.

Pregnanone and gorgosterol were distinctive of zooxanthellae, however only gorgosterol was incorporated or absorbed by the coral host with significant seasonal differences as mentioned above (Table 2-6). Coincident with the increase of SE/WE as a storage class (Table 2-3); occelasterol, 24-methylenephenol, brassicasterol, and cholesterol in turn, increased their proportions in the rainy season (Table 2-6).

(d) Contribution, if any, from *de novo* biosynthesis of sterols from simple precursors such as acetyl-CoA and mevalonic acid (MVA).

In the PNSAV, small percentages with no more than 5% of other sterols not identified in the primary producers were detected in zooplankton, echinoderms, clams and sponges, indicating *de novo* synthesis or biosynthesis from precursors. For instance, desmosterol was detected in the sponge *Aplysina* sp. at 0.6±0.1% with no seasonal differences. The sea urchin *E. lucunter* showed 7-dehydrocholesterol with significant seasonal differences from 0.7±0.3% in the dry season to 3.3±1.5% in the rainy season (95% confidence interval, $p<0.05$). Numerous sterols from other sources not considered in the present study were detected in the amber pen shell *P. carnea*: Sechocholesta-5(10) 6,8, triene at 2–5%, ergosterol at 1-2%, 4,23,24 trimethyl 5α-cholest 24(28) en-3β-ol at 1-3%, and 4,23,24 trimethyl-5α-cholest-7-en-3β-ol at 1% (Table 2-6). Finally, a small percentage (0.4±0.3%) of the sterol 5α-cholest-7-en-3β-ol (lathosterol) was detected in the masked goby *C. personatus*. Teleost fish showed less variety of sterols than crustaceans, mollusk, cnidarians and poriferans; the most abundant sterol was cholesterol ranging from 73±6% in *C. personatus* to 96±1% in *O. chrysurus* and *C. hippos*. Cholesterol showed significantly higher proportions in the dry season in all fish studied ($F_{9,39}=28$, $p<0.001$). In contrast, primary producer biomarkers were more abundant in the rainy season (Table 2-7).

2.4.6 Seasonal nutritional shift depending on species

A significant effect with species indicated consistent differences associated with taxonomic origin (Pseudo $F_{9,79}=42.4$, p(perm)= 0.001, p(MC)=0.001) and there were no

species without significant differences (groups of species). A significant season effect indicated a consistent influence of the season (turbidity, nutrients, and zoopankton supply), independently of the species (Pseudo $F_{1,79}$=34.3, p(perm)= 0.001, p(MC)=0.001: Fig. 2-3). However, a significant species x season interaction was observed (Pseudo $F_{9,79}$=5.8, p(perm)= 0.001, p(MC)=0.001) indicating that the effect of season on lipid classes, FA and ST profile differed by species as I hypothesized.

Species were clearly separated using non-metric multidimensional scaling (nMDS, Fig. 2-2). nMDS showed the greatest separation between the great star coral *M. cavernosa* and the sponge *Aplysina* sp. (ANOSIM R =1, p= 0.001, t=8.2, p(perm)=0.001), with the highest dissimilarity of 70% (Table 2-8). Zooplankton, sea urchins, clams, and fish were in-between these two groups. Inspection of fatty acid, sterol and lipid class profiles shows *M. cavernosa* to have about ten times the campesterol, 16:0, and SE/WE of *Aplysina* sp. (Appendix 2). The SIMPER results indicate that these three compounds are important in distinguishing between these two groups with an individual contribution of 24%, 17%, and 11%, respectively, giving a cumulative contribution to the overall dissimilarity of 51% in the great star coral *M. cavernosa*. The zooxanthellae biomarker identified in *M. cavernosa* was gorgosterol with a contribution of 5% to the dissimilarity. *Aplysina* sp. had 26:2ω6 as a characteristic FA with a 11% contribution, while 24:1ω7, as well as the brown algae sterol biomarkers 4-24 dimethyl 5,7-dien-3-β-ol and poriferasterol had low contributions of 2%, 2%, and 1%, respectively (Appendix 3).

Zooplankton, sea urchins, clams and fish were in-between coral and sponges with dissimilarities ranging between 51% and 67% to sponges, and dissimilarities ranging between 52% and 60% to coral. All species (groups) had significant differences in their

fatty acid, sterol and lipid class profiles. However, hogfish *B. rufus*, yellow tail snapper *O. chrysurus*, and jack *C. hippos* had lower dissimilarities among them ranging between 23% and 26% (Table 2-8). The main contributors to these dissimilarities were TAG, PL, 22:6ω3 and 18:1ω9. Among these, PL and 22:6ω3 had the highest average abundance in *O. chrysurus* (Appendix 3). While dissimilarities among zooplankton, clams and sea urchins ranged from 38% to 41%, the main contributors to these dissimilarities were TAG, PL, 14:0, 16:1ω7, 22:6ω3, free ST and brassicasterol. Zooplankton had the highest proportions of 14:0 and 16:1ω7, clams had the highest proportions of PL, 22:6ω3, free ST and brassicasterol suggesting a direct accumulation from phytoplankton with contributions from diatoms. Finally, the sea urchin *E. lucunter* had the highest proportion of TAG and the lowest of 22:6ω3 (Appendix 3).

Food web relationships among the hog fish *B. rufus* and its prey showed dissimilarities in the FA, ST and lipid class profile of 37% to the clam *P. carnea* and 34% to the sea urchins *E. lucunter*. Characteristic non-methylene interrupted FA 22:2 Δ7,15, and 22:2 Δ7,13 present in the clam *P. carnea* at 4% and 2%, respectively (Table 2-4) were detected in hog fish in small proportions of 0.2% (Appendix 3).

Seasonal comparisons considered the species averages of FA, ST and lipid class profiles. Significant seasonal differences were also detected with ANOSIM (R= 0.674 p= 0.001) and SIMPER analyses with 31% dissimilarity. The main contributors to seasonal dissimilarity were TAG, 16:0, 14:0, 16:1ω7, and 24-methylenephenol with higher proportions in the rainy season. Meanwhile, phospholipids, 22:6ω3, campesterol, β-sitosterol, and steryl and wax esters had higher proportions in the dry season (Fig. 2-3). hog fish *B. rufus,* yellow tail snapper *O. chrysurus* and jack *C. hippos* shifted from high

proportions of phospholipids, 22:6ω3, 24:1ω9 and 18:0 in the dry season (see lower right quadrant in Fig. 2A and C), to a higher proportion of TAG, FA and ST characteristic of phytoplankton, such as 16:3ω3, 16:2ω4, 16:1ω5, 16:1ω7 and 24-methylenephenol in the rainy season (see upper left quadrant in Fig. 2B and C). In contrast, the masked goby *C. personatus* and the surgeon fish *A. chirurgus* had less of a seasonal shift.

Significant interaction of species x season showed that the species which showed the most seasonal change was the sea urchin *E. lucunter* due to the higher proportions of TAG and 16:0 in the rainy season. In contrast, the great star coral *M. cavernosa* had higher proportions of SE and WE, gorgosterol, campesterol, and 18:1ω9 in the dry season. The species that showed the least seasonal shift were the sponge *Aplysina* sp. and the clam *P. carnea* (Fig. 2A, B and C). During the dry season all species were significantly different with p(MC)<0.025, while during the rainy season most of species were significantly different p(MC)<0.025 except for the hog fish *B. rufus* and yellowtail snapper *O. chrysurus* p(MC)=0.051 with a low dissimilarity of 16% between them (Table 2-8).

2.4.7 Accumulation and correlation of structural and storage lipid classes with sterols concentration

Total lipid concentration increased significantly in the rainy season in echinoderms and mollusks, such as *E. lucunter* and *P. carnea*, and in all the fish, except the surgeon fish (Table 2-2). The lipid classes that explain this increase were TAG in sea urchins with high variation in their concentrations, from 24±20 mg g^{-1} in the dry season to 60±44 mg g^{-1} ($F_{9, 39}$=3.8, p=0.002) in the rainy season, probably due to reproductive status. In

contrast, clams *P. carnea* had a significant increase in their SE/WE fraction, with concentrations increasing from 1.2±0.7 mg g^{-1} in the dry season to 5±2 mg g^{-1} in the rainy season ($F_{6, 26}$=175, p<0.001). Meanwhile, fish had a significant increase in TAG ($F_{9,26}$=14.7, p<0.001) except *C. personatus*. For the masked goby the AMPL fraction caused the lipid increase in the rainy season. Hog fish with sea urchins and clams in its diet had a higher concentration of SE/WE in the dry season, compared with the rainy season when they were not detected (Table 2-9).

The chloroplast associated lipids contained in the AMPL fraction were used to detect autotrophic contributions to samples (Parrish et al. 2000). The primary producers studied showed concentrations ranging from 1.2±1.1 mg g^{-1} to 15±3 mg g^{-1}. The red algae and sea grass had a significantly lower concentration than phytoplankton, green algae and zooxanthellae, while brown algae had intermediate amounts of 6±2 mg g^{-1} ($F_{6, 26}$=3.4, p=0.014). Zooplankton and the filter-feeding mollusk *P. carnea* significantly increased their AMPL concentration in the rainy season from 8±5 mg g^{-1} to 14±3 mg g^{-1} ($F_{9, 39}$=5.7, p<0.001), while sea urchins at 5±1 mg g^{-1}, sponges at 14±4 mg g^{-1} and coral at 13±9 mg g^{-1} did not show seasonal variation, suggesting a constant autotrophic contribution (Table 2-9). In contrast, fish had the opposite behaviour in the dry and rainy seasons. For instance, surgeon fish *A. chirurgus* at 5±3 mg g^{-1} and the yellow tail snapper *O. chrysurus* at 8±3 mg g^{-1} had a significant higher concentrations in the dry season, while *C. personatus* at 31±12 mg g^{-1} and *C. hippos* at 11±4 mg g^{-1} had higher concentrations in the rainy season ($F_{9, 26}$=7.0, p<0.001; Table 2-9).

The concentration of structural lipids like PL and free ST in consumer tissues did not change seasonally. The only exception was the hog fish with the highest concentration of

sterols in the dry season ($F_{9, 26}$=7.0, p<0.001) with 5±1 mg g^{-1} (Table 2-9). The increase of sterols in the hog fish is explained by the concentration of cholesterol and of fucosterol to a lesser extent. On the other hand, the phytosterol β-sitosterol had the highest concentration in the mangrove *R. mangle* at 0.8±0.1 mg g^{-1}, in second place was the seagrass and green algae with similar concentrations of 0.4±0.2 mg g^{-1}, the lowest concentration was detected in phytoplankton at 0.1±0.1 mg g^{-1} ($F_{6, 26}$=13.3, p<0.001) and finally zooxanthellae had none detected. Suspension feeders such as clams *P. carnea* and sponges *Aplysina* sp. showed an accumulation of β-sitosterol increasing their concentration between 10 and 30 times with respect to phytoplankton and between two to four times with respect to mangrove. This accumulation was significantly higher in the sponge in the rainy season ($F_{9, 39}$=38.8, p<0.001; Table 2-9). In contrast, fish had a trophic reduction in β-sitosterol with low concentrations ranging between 0.003 and 0.1 mg g^{-1}. The surgeon fish had significantly higher concentrations in the dry season, while the jack *C. hippos* had higher concentrations in the rainy season ($F_{9, 26}$=7.9, p<0.001).

Apportionment of brown algae was recorded using poriferasterol, a biomarker present in the sea urchin and sponge, while fucosterol was detected in the masked goby and hog fish. Among these, sea urchins and fish had lower concentrations than brown algae, while sponges had similar concentrations (Table 2-9), suggesting an accumulation by filter feeding.

In the present study, the whole organism was analyzed including spat tissues of *P. carnea*, *Aplysina* sp. and *M. cavernosa* and they increased their concentration of 24-methylenephenol compared to phytoplankton ($F_{9, 39}$=9.9, p<0.001). Meanwhile fish had a

similar concentration of 24-methylenecholesterol compared to phytoplankton. Similarly, gorgosterol the zooxanthellae biomarker, was detected only in the great star coral *M. cavernosa* with significantly higher concentrations in the dry season compared to the source zooxanthellae and to the whole coral in the rainy season ($F_{9, 39}$=9.9 , p<0.001) when the coral had similar concentrations to zooxanthellae (Table 2-9).

To test if precursors of *de novo* sterols such as cholesterol were directed into FA stored as TAG as has been reported by Goad (1981) and Nes (2011) and references therein, I performed a Pearson correlation test to verify the coincidence of primary producer sterol biomarkers with the highest storage lipid classes such as TAG and SE/WE, as well as the TAG:ST ratio (Table 2-10). Macroalgae and phytoplankton sterols were significantly positively correlated with structural lipids such as ST, AMPL and PL but not with storage lipids for invertebrates such as zooplankton, clam *P. carnea*, sea urchins *E. lucunter* and sponge *Aplysina* sp. The only sterol with a significant positive correlation (r= 0.865, p< 0.001) between concentrations was gorgosterol and SE/WE present in the coral *M. cavernosa* (Table 2-10). In contrast, fish had a significant positive correlation between brassicasterol from red algae and phytoplankton with storage SE/WE, as well as with structural lipids. Meanwhile, 24-methylenecholesterol from phytoplankton was the only biomarker significantly positively correlated (r=0.518, p=0.001) with TAG confirming that phytoplankton had the highest nutritional quality for the PNSAV teleost fish.

2.5 Discussion

Nitrogen is a limiting nutrient for autotrophic organisms which is a typical condition for most coral reefs (Wood, 1998). According to my results an enrichment from

anthropogenic sources was detected with C:N ratios in mangrove, seagrass, macroalgae and phytoplankton. Nitrogen is an essential element of amino acids, nucleic acids, and the main component of chlorophyll a, which is the site of carbohydrate formation (photosynthesis) from dissolved inorganic and organic C. Fixed C was stored in the primary producers biomass and was reflected in a higher C:N ratio of phytoplankton during the rainy season when the river discharge increases nutrient loads in the PNSAV i.e. phosphates (Terrados et al. 2008). Macroalgae and higher plants accumulate C as polysaccharides which are difficultly digested by animals (Dromard et al. 2015). According to this study, macroalgae also had high amounts of FFA while phytoplankton and zooxanthellae fixed C as lipids, including FA and sterols with high nutritional value. One of the major challenges that animals are frequently confronted with during their life cycle is coping with nutritional constraints. Nutrients favour brown algae over coral (Fabricius et al. 2011), and even grazers such as sea urchins and surgeon fish feed on brown algae. Surgeon fish preferred red algae probably because of their amount of cholesterol. In contrast to plants and algae, which contain a great number of phytosterols, most animals predominantly contain cholesterol. Consequently, most herbivorous species take up dietary phytosterols and metabolize them to cholesterol (Gergs et al. 2015). It is well established that dietary deficiencies in essential biochemical nutrients can limit the growth and reproduction of consumers while primary producers with high nutritional quality favour nutritional condition of consumers (Gergs et al. 2015). The seasonal shift of consumer nutritional condition depended on the species. The rainy season positively affected the nutritional condition measured as the TAG:ST ratio of zooplankton, clams and perciform fish *B. rufus*, *O. chrysurus* and *C. hippos* but negatively affected the

principal habitat provider coral M. cavernosa making it vulnerable to disease. There were three species with no effect on their nutritional condition: the sponge *Aplysina* sp., masked goby *C. personatus* and surgeon fish *A. chirurgus*. Lower nutritional condition of coral is consistent with the increase of diseases during the rainy season (Perez-España et al. 2012) and a historical cover diminution explained by the high amount of nitrogen use as fertilizers in the state of Veracruz (Tobon et al. 2011).

2.5.1 Dissolved nitrogen enrichment in primary producers using C:N ratios

In this coral reef food web of the southwest Gulf of Mexico, C:N ratios of primary producers, mangrove, seagrass, and macroalgae, fell within ranges previously observed in other coral reef ecosystems with reports of nitrogen enrichment. Mangrove values indicate enrichment of nitrogen as in *Avicenia marina* in Bold Hill Creek in Moreton Bay, Queensland, Australia. In this species, C:N ratios were lower in trees with nitrogen fertilization with a C:N value of 11 compared with 30 without nitrogen fertilization i.e. nitrogen limitation (Reef et al. 2010). A higher proportion of nitrogen reflected in C:N ratios of macroalgae were also similar to those reported in nitrogen enriched conditions in Exuma Cays, Bahamas (Lapointe et al. 2004); according to these authors the effect of nitrogen enrichment promoted low C:N ratios of 19±3, while dissolved nitrogen limitation promoted high C:N ratios of 42±6 in macroalgae. In the present study, red algae had the highest C:N ratio at 26±2, similar to green algae, and significantly higher than in brown algae *Dictyota* sp. with the lowest C:N ratio of 20±1 (Table 2-2), suggesting that brown algae biomass is increased by anthropogenic nitrogen enrichment. In the PNSAV, brown algae were consumed by sea urchins but not by surgeon fish, the latter prefers red algae

according to the results of C and N stable isotope mixing model analysis (Carreón-Palau et al. 2013), therefore brown algae biomass will not be controlled by surgeon fish. Thus, the control of brown algae biomass will be more effective if the nutrient load is reduced by tertiary treatment plants.

Similarly to macroalgae, C:N ratios of phytoplankton indicate nitrogen-replete conditions according to Bittar et al. (2013). These authors recorded an increase in C:N mol ratio of diatoms from 9±1 under nitrogen replete conditions to 14±2 after one day of N-stress and 17±1 after two days of N-stress. As well, there was a proportionally larger increase of neutral lipids such as TAG and SE/WE in response to N-stress (Bittar et al. 2013). Increased accumulation of lipids has been observed at the end of natural diatom blooms (Parrish 1987) and verified in experiments with $^{14}CO_2$ assimilation and differentiation of incorporated ^{l4}C into the main macromolecular pools: proteins, polysaccharides, and lipids (Linqvist and Lignell 1997). However, even in the rainy season when a high C:N ratio favoured lipid increases, the C:N ratios in phytoplankton were equivalent to those under N-replete conditions, as in Elser et al. (2007). These authors reported that coastal hard-bottom systems (rocky intertidal, temperate reef and coral reef macro- and microalgae) show substantial positive responses in biomass production to N and N + P, but the strongest responses, especially to N or N + P enrichment, are for phytoplankton.

Nitrogen enrichment in primary producers is explained because in the state of Veracruz only 20% of their original rainforest area remains; the majority of the area was converted for planting of food crops and cattle ranching. Veracruz has an estimated 5

million head of cattle, 1.2 million hogs and 600,000 goats as well as a large number of trout farms using fertilizer (Tobón et al. 2001 and references therein)

Active predators such as zooplankton and grazers such as sea urchins had similar C:N ratios. Zooplankton reflected the C:N ratios of phytoplankton, while sea urchins had a decrease as a result of respiration and excretion compared to their principal food: seagrass and macroalgae, respectively. Zooplankton values in this study were similar to the cladoceran component of zooplankton of the Great Barrier Reef with a value of 8.8, but higher than values reported in copepods (between 4.0 and 5.5: Ikeda and McKinnon, 2012), and small mesozooplankton with C:N ratios of 5.1±0.3 in open waters of the Atlantic ocean (Laiz-Carrión et al. 2012). Meanwhile, C:N ratios of sea urchins were similar to *Echinocardium cordatum* (Thorsen, 1998). Thorsen suggested a selective assimilation of nitrogen. Even though there was a selective assimilation of nitrogen, sea urchins and fish significantly increased their C:N ratios as a consequence of lipid increase in the rainy season (Table 2-2) suggesting a better nutritional condition.

According to El-Sabaawi (2012) variability in basal resource quality can affect multiple trophic levels, not just primary consumers. Food web structure as a whole is highly stoichiometrically constrained. Even so, upper consumers are constrained in their C:N ratios as a result of excretion, pseudofeces production and respiration. Fish in the present study had a lower C:N range (3.2 to 5.2), compared to fish from open waters of the Bay of Biscay in the northeast Atlantic Ocean where C:N values ranged from 3.8 to 7.3 (Czamanski et al. 2011). Physiological studies are needed to define if the difference in the range is enough to suggest that nitrogen enrichment in the primary producers was reflected in secondary and tertiary consumers. However, there is evidence in continental

aquatic ecosystems that elemental composition of an insectivorous fish Rivulus hartii appeared to be largely explained by variability in quality of basal resources such as benthic organic matter C:N (El- Sabaawi et al. 2012).

2.5.2 Nutritional quality of macroalgae compared to other primary producers

High proportions of carbon indicate a deficiency in nitrogen and a high proportion of non assimilable carbohydrates so that phytoplankton can be considered to have higher nutritional quality, followed by seagrass, mangrove, and brown algae (Table 2-2). Lipid classes, FA, and ST have been used to understand food web dynamics and trophic ecology in marine systems (Drazen et al. 2008). For instance, red algae from southern California had the lowest concentration of lipids in similar seasons, and green algae had similar values of TAG and high proportions of PL (Nelson et al. 2002). However red algae *Galaxaura* sp. is the algae with the highest proportion of cholesterol (30%) resulting in the highest concentration (0.060 mg g^{-1} dry weight calculated from Table 2-5) suggesting that red algae *Galaxaura* sp. consumed by surgeon fish *A. chirurgus* and sea urchin *E. lucunter* reported in the Chapter 1 could be explained by selection of food with cholesterol for herbivores. Even if, brown algae had slightly less cholesterol (0.055 mg g^{-1}) than red algae, the high variation recorded in the cholesterol concentration shown in this study and the low palatability of *Dictyota* sp. for herbivorous fishes explained by its high content of unpalatable deterrent molecules (Fong and Paul, 2011) can explain its low incorporation in sea urchin and surgeon fish.

In aquatic vertebrates, sterol requirements are met by *de novo* synthesis and by dietary uptake in ratios depending on the amounts of sterols provided in the diet. Therefore, a

dietary source of sterols is presumably not essential. In contrast invertebrates need dietary cholesterol. For instance, in laboratory growth experiments with *Daphnia magna* and *D. galeata*, Martin-Creuzburg et al. (2009 and references therein) showed that growth of offspring on a sterol-free diet was significantly affected by the sterol content of the mother's diet; i.e., somatic growth rates of offspring decreased with decreasing amounts of sterols in the maternal food.

In contrast to macroalgae, phytoplankton and zooxanthellae had the highest concentration of lipids and the main lipid storage class was TAG, while in zooxanthellae it was TAG and SE/WE. Phytoplankton nutritional quality was also observed in the cholesterol concentration of 0.5 mg g^{-1} dry weight (calculated from Table 2-5), almost ten times higher than red and brown macroalgae. Similarly, zooxanthellae had almost ten times more cholesterol than phytoplankton but it is incorporated just by coral feeders. For instance, *Scarus taeniopterus*, *S. iseri*, *S. vetula* and *Sparisoma viride* were characterized by the assimilation of the coral *Orbicella annularis* in a Caribbean coral reef (Dromard et al. 2015).

On the other hand, the high proportions of FFA observed in seagrass brown and red algae, suggest low nutritional quality. In contrast, mangrove and green algae had lower proportions. The only similarity with southern California macroalgae was with brown algae which had 22% FFA. Low percentages (<5%) are expected with rapid sampling and processing of fresh algae (Nelson et al. 2002). However, if the samples had broken-down, mangrove leaves and green algae should also have had high levels of FFA because they were processed similarly. Another possibility is the lipolysis of glycolipids quantified in the AMPL fraction. The main effects of macroalgae FFA and/or glycolipids on

consumers consist of the low nutritional quality and a possible toxicity. Unsaturated FA have been implicated in toxicity towards fish and mammalian cells causing haemolysis and ichthyotoxicity. Toxic FA occurs in glycolipids or in the free form (reviewed by Parrish 2013). Pure FFA containing 20:5ω3 and 18:4ω3 have been reported as toxic for fish because cell contents released from broken cells hydrolyse in a high reactive oxygen species (ROS) environment to produce higher levels of FFA and ROS. Lipid peroxidation occurs on gill and other membranes resulting in reduced respiratory and osmoregulatory capacity and allowing the transfer of FFA and O_2^- into the consumer blood stream.

Damage to the chloride cells of the gills can also lead to reduced osmoregulatory capacity. Toxic mechanisms may occur in isolation or combination (Marshall et al. 2003). In coral reefs, FFA effects have not been reported, but methanol extracts of the brown algae *Dictyota bartayresiana*, and green algae *Halimeda opuntia* caused bleaching and suppression of photosynthetic efficiency in the Caribbean coral *Porites porites*. In addition methanolic extracts of red algae *Galaxaura filamentosa* promoted bleaching when it was in contact with *Porites cilindrica* in the Fiji islands (Douglas and Hay, 2010).

2.5.3 Identifying sterol biomarkers for primary producers on coral reefs

Stable isotopes were not analyzed in suspension feeders such as coral, sponges and clams because coral and sponges contain high amounts of carbonates, so results were too variable to be considered, and clams were collected after stable isotope analysis. Fortunately, lipid classes, FA and ST helped to characterize primary producers in suspension feeders and the rest of food web. Mangrove *R. mangle* and seagrass *T.*

testudinum had no characteristic lipid classes but 18:2ω6, 18:3ω3, and β-sitosterol showed the highest proportions. Green algae *H. opuntia* had the highest proportion of AMPL, 18:1ω7, 20:3ω3 and stellasterol. Red algae *Galaxaura* sp. shared 20:5ω3 and brassicasterol with phytoplankton. According to Rampen et al. (2010), brassicasterol previously suggested as being specific to diatoms was only the fifth most common sterol in diatoms, this sterol was absent in some of the major diatom groups, its relatively high concentrations seems to be restricted to pennate diatoms (Rampen et al., 2010). In the PNSAV, the presence of brassicasterol in red algae *Galaxaura* sp. at higher proportions than phytoplankton (Table 2-5) suggests that it is not a clear biomarker for diatoms. In contrast, 24-methylenecholesterol was present in 67% of 106 diatom cultures analyzed by Rampen et al. (2010) followed by cholesterol, campesterol, and β-sitosterol. In the PNSAV, 24-methylenecholesterol was also present in green algae *H. opuntia* but in significantly lower proportions and 24-methylenephenol was distinctive of phytoplankton (Table 2-5). These results are consistent with the main group of phytoplankton recorded in the PNSAV being centric diatoms, mainly Chaetocerotales (Okolodkov et al. 2011) as according to Rampen et al. (2010) major sterols of centric diatoms are 24-methylenecholesterol, cholesterol, campesterol, and to a lesser extent, 24-methylenephenol.

Brown algae *Dictyota* sp. had high proportions of free sterols, and fucosterol, isofucosterol, poriferasterol, and 4,24 dimethyl 5,7-dien-3-β-ol were distinctive. Also 18:4ω3 and 20:4ω3 showed higher proportions. Among the sterols mentioned only 4,24 dimethyl 5, 7-dien-3-β-ol can be considered a biomarker because fucosterol, isofucosterol

and poriferasterol have been detected in diatoms (Rampen et al. 2010). This is not unusual because diatoms and brown algae belong to the Heterokontophyta division (Delwiche, 1999). Phytoplankton of the PNSAV had the highest proportion of TAG, 14:0, 16:1ω7, 16:2ω4, and the major proportion of 24-methylenecholesterol and 24-methylenephenol in the rainy season. ST composition of phytoplankton in the PNSAV suggests that phytoplankton was comprised mainly of diatoms. Finally, zooxanthellae had high proportions of SE/WE, 16:0, 18:1ω9, 22:6ω3, and 18:5ω3 as major FA, while gorgosterol was their distinctive sterol. According to Rampen et al. (2010) gorgosterol had been detected in one diatom species, *Delphineis* sp. at 13%. However, the presence of gorgosterol in diatoms is rare, but Whiters et al. (1982) reported that zooxanthellae are capable of synthesizing gorgosterol in high concentrations. Furthermore, the levels of gorgosterol and related sterols are frequently very high when these same zooxanthellae are no longer free living but exist as symbionts of various invertebrates.

Sterol profiles allow the identification of diet sources, however β-sitosterol should not automatically be considered as a marker of higher plants, because according to the results, this sterol was more abundant in green algae, and according to Raven et al. (2010) in some diatoms it can reach from 4 to 100% of total sterols. However, information from stable isotopes can help to identify sterol origin. For instance, based on the isotopic information obtained in Chapter 1, the most likely source of β-sitosterol for *A. chirurgus* was seagrass, while for *C. hippos* it was mangrove leaves. Similarly, distinctive sterols of brown algae *Dictyota* sp. (poriferasterol and fucosterol), phytoplankton (24-methylenephenol), and zooxanthellae (gorgosterol), have been reported in some diatoms

(Raven et al. 2010). However, if not universal biomarkers, the sterols mentioned above can be considered biomarkers for this coral reef ecosystem or distinctive of the sources studied, because they were present in just one source in the present study. These results underline the need to collect the possible organic carbon sources in the ecosystem studied in order to avoid less reliable conclusions based on other ecosystems.

The inability to synthesize sterols from low molecular weight precursors such as acetate or mevalonate is widespread among crustaceans and some molluscs (Kanazawa, 2001, Gergs et al. 2015). Desmosterol had been reported as a product of 24-methylenecholesterol by side chain dealkylation in sponges (Kerr et al. 1992). According to Yoshiyama-Yanagawa et al. (2011) 7-dehydrocholesterol is produced from cholesterol by dehydrogenation of the carbons at positions 7 and 8 produced by sea urchins. The other sterol found only in sea urchins was dihydrobrassicasterol, which remained stable at around 1%. The latter is structurally similar to campesterol but with the opposite stereochemistry at C-24 (*S* in dihydrobrassicasterol and *R* in campesterol which is usually present in higher plants: O'Connel et al., 2012). A sterol originally detected in zooplankton, ergost-7-en-ol at 1.1±0.8% was identified in the surgeon fish *A. chirurgus,* the hogfish *B. rufus,* the yellow tail snapper *O. chrysusrus* and the jack cravalle *C. hippos* suggesting direct consumption of zooplankton, or an efficient trophic transfer. Occelasterol was also incorporated into the masked goby *C. personatus* increasing from the dry to the rainy season probably transferred trophically by zooplankton or by eating live coral tissue with zooxanthellae in them. The latter is less probable because we should detect gorgosterol if the source would be zooxanthellae. Finally, *C. personatus* had small proportions of lathosterol, suggesting lower capacity to metabolize Δ7-phytosterols

present in zooplankton transferred trophically from phytoplankton. Recently, Gergs et al. (2015) provide evidence that the Δ7-phytosterols present in the microalgae *Scenedesmus obliquus* cannot be metabolized to cholesterol in the invertebrate *Gammarus roeselii*, resulting in the accumulation of lathosterol in the animals and potentially in sterol-limited growth.

2.5.4 Seasonal nutritional shift depending on species using lipid class, fatty acid, and sterol profiles

Among the consumers, the coral *M. cavernosa* and the sponge *Aplysina* sp. showed the highest dissimilarity of 70%. *M. cavernosa* had significantly higher proportions of 18:1ω9, campesterol, gorgosterol and SE/WE. Of these compounds the sterols were the most convincing for showing a bigger proportion from zooxanthellae in the dry season, in comparison to 18:1ω9 the most common monoene in the biosphere and SE/WE that is unlikely to be transferred intact. *M. cavernosa* also had a better TAG:ST condition index in the dry season probably because in the rainy season turbidity increases periodically in the Jamapa and Papaloapan plumes (personal observation) and temperature increases from 29 to 31°C at the sea surface. Decreasing cell density of zooxanthellae has been documented in Japanese coral reefs as a result of high temperatures. In healthy corals, lipids account for 18 to 38% dry tissue weight (Yamashiro et al. 2005), and according to these authors *Goniastrea aspera* (Faviidae) had 10% of lipid per dry tissue weight under bleached conditions. This is over twice the 5% per dry tissue weight of *M. cavernosa* under normal conditions, but this is a river influenced coral reef. In addition, SE/WE of *G. aspera* decreased from 30% under normal conditions to 15% under bleached

conditions. This is similar to what was observed in *M. cavernosa* which had a significant decrease from 25% in the dry season to 16% in the rainy season, suggesting that *M. cavernosa* experienced a decrease in zooxanthellae concentrations even if a bleaching event was not detected. Also gorgosterol decreased from 20% of total sterols in the dry season to 9%, and there were higher proportions of ketones in the rainy season. The occurrence and generation of long chain (32 to 34 carbons) ketones have been reported to serve some type of energy storage function in the algal cell of haptophytes (Eltgroth et al. 2005). In contrast, shorter chain (16 to 18 carbons) ketones have been reported in fire coral *Millepora* sp., which was the first report for corals, suggesting that the compounds very likely originate from endosymbiotic bacteria and/or algae (Al-Lihaibi 2007). In the present study, ketones are present in zooxanthellae in a similar proportion as in the host coral, and mass spectrometry analysis confirmed the presence of shorter chain ketones of 18 carbons in zooxanthellae (Appendix 1) as the source for coral. Unfortunately, zooxanthellae were separated from the whole coral in the rainy season only, precluding comparison in the dry season when the whole coral had lower proportions of ketones. Further investigation is necessary in this species. In addition, *M. cavernosa* had higher proportions of TAG (21%) than *G. aspera* (11% under normal conditions and 7% under bleached conditions) with no significant seasonal change, probably because of zooplankton consumption that accounts for 10 to 20% of *M. cavernosa* energy according to Muscatine and Porter (1977). However, the TAG:ST ratio observed in the PNSAV during the dry season was not reached by zooplankton consumption in the rainy season. Therefore, abundance of zooplankton did not satisfy the energy demand of *M. cavernosa* during the rainy season and the nutritional condition decreased making it more

vulnerable. This low nutritional condition could explain the number of disease coral colonies reported by (Perez-España et al. 2012) which increased up to 30 in the rainy season compared to 7 in the dry season.

Contributions of seagrass were detected by the presence of β-sitosterol in coral *M. cavernosa*, clam *P. carnea*, surgeon fish *A. chirurgus*, sea urchin *E. lucunter*, yellow tail snapper *O. chrysurus* and sponge *Aplysina* sp. Active and passive suspension feeders like corals, clams and sponges probably feed on suspended particulate and dissolved matter, coinciding with the seagrass apportionment in coral reported by Lai et al. (2013). For grazers and active predators the explanation is an efficient trophic transfer from primary consumers that feed on seagrass. Among the suspension feeders, clams reflected phytoplankton changes in their FA composition: *P. carnea* collected in the dry season showed a significantly higher proportion of 22:6ω3 suggesting high dinoflagellate consumption; in contrast 16:1ω7 and 20:5ω3 had higher proportions in the rainy season suggesting diatom consumption. Diatoms are typically rich in 20:5ω3, but deficient in 22:6 ω3, whereas dinoflagellates contain high 22:6 ω3 and low 20:5ω3 (Anderson and Pond, 2000)

Zooplankton had a bigger contribution from phytoplankton than from red algae, the other source with high proportions of brassicasterol, which could explain the high proportion of TAG. Trophic transfer of zooplankton was detected using 24-nordehydrocholesterol in masked goby *C. personatus.* These results are consistent with the stable isotope analysis of the Chapter 1. Also, *C. personatus* reached higher lipid contents in the rainy season but this increase was not related to the proportion of storage

lipids such as TAG, rather the increase in lipids was related to AMPL. This class is a mixture of pigments and chloroplast-associated glycolipids and thus strongly indicates photosynthetic organisms in sediment samples (reviewed by Parrish et al. 2000). This suggests either an increase in photoautotroph food sources probably detected in the stomach from recent food intake since the whole organism was analyzed, or the biosynthesis of sphingolipids and pigments, also soluble in acetone according to Mishima and Hayasi (1978). In contrast, an increase in the proportion of trans-22-dehydrocholesterol, the characteristic sterol of seagrass and trace percentages of 24-nordehydrocholesterol detected in mangrove were detected in *C. personatus* in the dry season. These results suggest contributions from phytoplankton in the rainy season *versus* mangrove and seagrass in the dry season, also reported in the Chapter 1 on the basis of C and N stable isotope results.

Results of multivariate comparison of lipid, sterol and fatty acid profiles among species, showed that fish were the most similar species; hogfish *B. rufus*, yellow tail snapper *O. chrysurus*, and jack *C. hippos* suggesting that the low dissimilarity among fish taxa is related to similar physiology and phylogeny more than similar diets.

Finally, the sponge *Aplysina* sp. had a high proportion of PL and the highest proportion of sterols. In addition, this sponge had high proportions of branched FA and 22:0. In contrast, common polyunsaturated FA from the ω6 and ω3 series, such as 20:4ω6, 20:5ω3 and 22:6ω3, are quite rare in sponge PL, that instead usually contain very long chains from 22 to 34 carbons (Denis et al. 2009). For instance, the characteristic FA 26:2ω6 accounts for 32% of total FA on average but it can reach up to 50% in *Aplysina* sp. in the PNSAV. This genus has been reported to be mixotrophic with a small

proportion of the tissue containing symbiotic cyanobacteria (Wilkinson and Chesire 1990). Probably the 26:2ω6 detected in the present study is biosynthesized by sponges from 18:2ω6 usually abundant in some groups of cyanobacteria. According to Zimmerman et al. (1989), long chain FA and ST are not detected in the cultured microbial symbionts of sponges. Therefore, nutritional constraints on cyanobacteria contributions are related to deficiency in sterols, more than in polyunsaturated FA.

The high diversity of sterols in sponges could be explained by the high capacity to filter feed (reviewed by Usher 2008) or because they are more varied in the range of symbioses formed than corals. Symbionts include cyanobacteria and eukaryotic rhodophytes, diatoms, dinoflagellates and chlorophytes (Usher 2008 and references therein). However, the high proportion of branched FA generally associated with bacteria (Zimmerman et al. 1989) suggests that filter feeding on suspended organic matter is probably the main way that the sponge *Aplysina* sp. obtained sterols; sponges are known to retain particles as small as 0.1 μm (Leys & Eerkes-Medrano 2006).

Seasons were compared considering all species pooled. The main contribution to seasonal dissimilarity was due to stored carbon such as TAG, 16:0, 14:0, 16:1ω7, from diatoms revealed by 24-methylenephenol with higher proportions in the rainy season. While, structural lipids such as PL, 22:6ω3, campesterol, β-sitosterol, had higher proportions in the dry season revealing dinoflagellate and seagrass contributions to the food web. However the significant interaction of species and season showed that species that drive the seasonal shift were *B. rufus*, *O. chrysurus* and *C. hippos* shifting from high percentage of phospholipids, 22:6ω3, 24:1ω9 and 18:0 in the dry season to a higher percentage of TAG and FA characteristic of diatoms in the rainy season. Higher trophic

level species such as *C. hippos* were collected in the juvenile stage, so that changes were related to diet rather than reproductive physiology. In teleost fish, most of the evidence shows that they have a fast growth rate followed by increased storage, principally in species at higher (cold-temperate) latitudes where productivity varies greatly on seasonal scales. Far less is known about energy allocation of juvenile fishes at lower (warm-temperate) latitudes, where growing seasons are longer, periods of low winter resources are shorter, and temporal patterns of productivity are less variable. The increase in TAG in *C. hippos* during the rainy season suggests that there is an increase in productivity due to river discharge reflected in higher trophic levels independently of sexual maturation.

In addition, sexual maturity is closely related to the amount of lipids in liver and muscle, and can explain the increase in TAG in *O. chrysurus* which increased almost twice in size and almost four times in wet weight from the dry to the rainy season. Sexual maturation also influenced variation in sea urchins with around six times more TAG in the rainy season than in the dry season. This result is consistent with the tenfold increase in TAG proportions in the gonad in *Paracentrus lividus* related to temperature and gonadic maturation (Montero-Torreiro and García-Martínez, 2003). In contrast, the small masked goby *C. personatus* did not show changes in storage lipids with phospholipids, 18:0, 16:0, and 22:6ω3 as major contributors to its intra-specific dissimilarity, probably because they live around three weeks as adults. These results are consistent with the proxy life history data based on a study of *Coryphopterus kuna* which has a 60-day pelagic larval life and matures rapidly. They are sexually active in as few as three weeks and live for about two months after settlement. (Tornabene et al. 2015). Therefore, individuals of *C. personatus* analyzed in the PNSAV during dry and rainy seasons could

belong to different cohorts with similar nutritional condition in both seasons but higher accumulation of lipids during the rainy season when coincident with the high zooplankton biomass reported by Okolodkov et al. (2011). Recently, *C. personatus* has been identified as a primary prey item of the invasive lionfish (*Pterois volitans*) in the Bahamas and likely to be so in the remainder of its range. This species is easily targeted by the lionfish given its small, shallow body and demersal habits. In the Bahamas, a 65% decline in lionfish prey biomass over a period of two years was observed (Green *et al.* 2012). Therefore *C. personatus* was included as vulnerable in the IUCN red list of threatened species (Tornabene et al. 2015). In the PNSAV the first report of lion fish was in December 2011 and there was a second report on January 2012 suggesting the establishment of the population (Santander-Monsalvo et al. 2012). Because *C. personatus* trophically transfers OC from phytoplankton through zooplankton, it is an important connection to local top predators. I suggest monitoring its population as a measure of health of this coral reef along with the coral *M. cavernosa*.

2.5.5 Accumulation and correlation of structural and storage lipid classes with primary producer source sterols in the coral reef food web

Sterol biosynthesis in invertebrates does not to take place or proceeds at a slow rate. For instance, the sterol components of sponges are likely to vary with habitat. Similarly, sea scallops and oysters may be capable of sterol biosynthesis (Kanazawa, 2001 and references therein); however, the incorporation of unmodified dietary phytosterols was detected after a 6-week feeding period reflecting the diet composition, principally in spat tissues of scallops according to Kanazawa (2001 and references therein). Concentration of

sterols in the whole organism of clam *P. carnea* and coral *M. cavernosa* suggests differential sterol retention depending on feeding habits: filter feeding *versus* symbiotic apportionment. While *M. cavernosa* depends on zooxanthellae contribution, *P. carnea* reflects the available phytoplankton. On the other hand, because fish can synthesize cholesterol from acetyl-Co A and mevalonic acid (Leaver et al. 2008) they can maintain constant concentrations of cholesterol, in contrast to crustaceans, molluscs, sponges, clams and coral whose capacity to synthesize cholesterol is limited to incorporation and modification of dietary sterols.

According to my results, precursors of *de novo* sterols such as cholesterol were directed into FA stored as TAG as have been reported by Goad (1981*)* and reviewed by Nes (2011) during the rainy season. This confirms that phytoplankton is the primary producer with highest nutritional quality that promoted a better condition in teleost fish, while for coral it was zooxanthellae which promoted a better nutritional condition during the dry season. Therefore, nitrogen enrichment promoted a good nutritional condition in teleost fish and a slow response to environmental changes in top predators as found by Jennings et al., (2001). In contrast, nitrogen enrichment did not promoted a better nutritional condition in surgeon fish *A. chirurgus* and masked goby *C. personatus.*

2.6 Conclusion

Macroalgae, mangrove, seagrass and phytoplankton C:N ratios reflected an anthropogenic nitrogen enrichment. Therefore, the low C:N ratio was detected also in upper trophic levels suggesting that nitrogen enrichment in the primary producers was reflected in secondary and tertiary consumers. Brown and red macroalgae had high

proportion of FFA and low TAG:ST ratios. Therefore, they can be considered to have the lower nutritional quality followed by green algae, mangrove, and seagrass. In contrast, phytoplankton and zooxanthellae can be considered to have higher nutritional quality. Energy storage was observed in the TAG fraction of hervibores/detritivores, and carnivorous fish suggesting a better nutritional condition and high retention of OC in the food web during the rainy season. Sterols from primary producers allowed the identification of the main sources in the dry and rainy seasons. Red and green algae sterols were shared with phytoplankton making their identification difficult. In contrast, brown algae distinctive sterols were found in sea urchins and sponges. Cholesterol showed significantly higher proportions in the dry season in all fish studied as a *de novo* sterol for fish. In contrast, primary producer biomarkers were more abundant in the rainy season, as expected, probably because precursors of *de novo* sterols such as cholesterol were used in the synthesis of FA stored as TAG, the major lipid class in rainy seasons for fish. In contrast to teleost fish, the great star coral *Montastrea cavernosa* showed a better nutritional condition in the dry season as a result of a zooxanthellae contribution traced with gorgosterol and this correlated with the storage lipid SE/WE. The phytoplankton sterol 24-methylenphenol was correlated with higher contents of storage lipid TAG and FA characteristics of diatoms while structural lipid proportions such as phospholipids were significantly higher in the dry season and correlated with free sterols and 22:6ω3 a FA characteristic of dinoflagellates. In addition, an increase in the proportion of trans-22-dehydrocholesterol, the characteristic sterol of seagrass and trace percentages of 24-nordehydrocholesterol from mangrove were detected in the dry season. However, the seasonal shift of nutritional condition depended on the species. Rainy seasons positively

affected the nutritional condition of zooplankton, clams and perciform fish *B. rufus*, *O. chrysurus* and *C. hippos* but negatively affected the principal habitat provider coral *M. cavernosa* making it vulnerable to disease. There were three species with no effect on their nutritional condition: sponge *Aplysina* sp., masked goby *C. personatus* and surgeon fish *A. chirurgus*. Lower nutritional condition of coral is consistent with the increase of diseases during the rainy season and a historical cover diminution explained by the high amount of nitrogen use as fertilizers in the state of Veracruz in particular and in the Gulf of Mexico in general.

2.7 Acknowledgments

Thanks go to Cipriano Anaya, Yuri Okolodkov, Jacobo Santander-Monsalvo, and Sarahi Gutierrez-Villeda all from Instituto de Ciencias Marinas y Pesquerías, Universidad Veracruzana (ICIMAP-UV) for their support during field work, to Rene Rebollar Prudente from the Chromatography lab at Centro de Investigaciones Biológicas del Noroeste (CIBNOR), to Jaime Camalich-Carpizo from Centro Interdisciplinario de Ciencias Marinas for his support in preparing samples for carbon and nitrogen ratios, and to Linda Windsor and Jeanette Wells from the Core Research Equipment & Instrument Training Network (CREAIT) at Memorial University of Newfoundland (MUN) for their technical advice on sterols and lipid classes, respectively. The Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico, and the Veracruz state government cofinanced the Fondo MixtoProject ‘‘Fuentes orgánicas de carbono y nitrógeno y su función sobre la estructura trófica en el Sistema Arrecifal Veracruzano’’ (Fomix-Veracruz 37567). Analytical work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC). L.C.P. was recipient of a Ph.D. fellowship from CONACYT (grant number 117304) and from the School of Graduate Studies at MUN

2.8 Bibliography

Al-Lihaibi S. S. 2007. Aldehyde and ketone compounds from marine hydrocoral identified by gas chromatography-mass spectrometry. Electr. J. Nat. Subs., 2, 1-4.

Anderson T. R. and D.W. Pond. 2000. Stoichiometric theory extended to micronutrients: comparison of the roles of essential fatty acids, carbon and nitrogen in the nutrition of marine copepods. Limnol. Oceanogr. 45: 1162-1167.

Anthony K. R. N., and K. E. Fabricius. 2000. Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity. J. Exp. Mar. Biol. Ecol. 252: 221–253

Bell J. and R. Galzin 1984. Influence of live coral cover on coral-reef fish communities. Mar. Ecol. Prog. Ser. 15:265–274

Bellwood, D. R., Hughes T. P., Folke C, Nyström M. 2004. Confronting the coral reef crisis. Nature 429: 827-833

Bittar T. B., Y. Lin, L. R. Sassano, B. J. Wheeler, S. L. Brown, W. P. Cochlan, and Z. I. Johnson. 2013. Carbon allocation under light and nitrogen resources gradients in two model marine phytoplankton. J. Phycol. 49: 523-535.

Budge S. M., A. M. Springer, S. J. Iverson, and G. Sheffield. 2007. Fatty acid biomarkers reveal niche separation in an Arctic benthic food web. Mar. Ecol. Prog. Ser. 336: 305–309.

Copeman L. A. and C. C. Parrish. 2004. Lipids classes, fatty acids, and sterols in seafood from Gilbert Bay, southern Labrador. J. Agric. Food Chem., 52(15): 4872–4881.

Christensen V. and D. Pauly. 1993. Flow characteristics of aquatic ecosystems, p.338-352. *In* V. Christensen and D. Pauly (eds.) Trophic models of aquatic ecosystems. ICLARM Conf. Proc. 26, 390 p.

Czamanski M., A. Nugraha, P. Pondaven, M. Lasbleiz, A. Masson, N. Caroff, R. B., P. Tréguer. 2011. Carbon, nitrogen and phosphorus elemental stoichiometry in aquacultured and wild-caught fish and consequences for pelagic nutrient dynamics. Mar. Biol. 158:2847–2862 DOI 10.1007/s00227-011-1783-7

Davies P. S., 1991. Effect of daylight variations on the energy budgets of shallow-water corals. Mar. Biol. 108, 137-144.

Delwiche C. F. 1999. Tracing the thread of plastid diversity through the tapestry of life. Am. Nat. 154: S164-S177

Denis C., G. Wielgosz-Collin, A. Bretéché, N. Ruiz, V. Rabesaotra, N. Boury-Esnault, J. M. Kornprobst, and G. Barnathan. 2009. New 17-Methyl-13-Octadecenoic and 3,16-Docosadienoic Acids from the Sponge Polymastia penicillus. Lipids 44: 655–663. DOI 10.1007/s11745-009-3291-9

De Troch M., P. Boeckx, C. Cnudde, D. Van Gansbeke, A. Vanreusel, M. Vincx, M. J. Caramujo. 2012. Bioconversion of fatty acids at the basis of marine food webs: insights from a compound-specific stable isotope analysis. Mar. Ecol. Prog. Ser. 465: 53–67 doi: 10.3354/meps09920

Diario Oficial de la Federación 2012. Diario Oficial México. Tercera Sección p. 1-14. Jueves 29 de Noviembre de 2012.

Douglas B. R. and M. E. Hay. 2010. Chemically rich seaweeds poison corals when not controlled by herbivores. PNAS 107(21): 9683-9688. Doi:10.1073/pnas.0912095107

Drazen, J. C., C. F. Phleger, M. A. Guest, and P. D. Nichols. 2008. Lipid, sterols and fatty acid composition of abyssal holothurians and ophiuroids from the North-East Pacific Ocean: Food web implications. Comp. Biochem. Physiol. Part B 151: 79–87

Dromard C. R., Y. Bouchon-Navaro, M. Harmelin-Vivien, and C. Bouchon. 2015. Diversity of trophic niches among herbivorous fishes on a Caribbean reef

(Guadeloupe, Lesser Antilles), evidenced by stable isotope and gut content analyses. J. Sea Res. 95: 124–131.

Duarte, C. M. 1990. Sea grass nutrient content. Mar Ecol Prog Ser 67: 201-207.

Elser, J. J. M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Letters, 10: 1135–1142 doi: 10.1111/j.1461-0248.2007.01113.x

El-Sabaawi, R. W., , T. J. Kohler, E. Zandona, J. Travis, M. C. Marshall, S. A. Thomas, D. N. Reznick, M. Walsh, J. F. Gilliam, C. Pringle, A. S. Flecker. 2012. Environmental and organismal predictors of intraspecific variation in the stoichiometry of a neotropical freshwater fish. PLoS ONE 7(3): e32713. doi:10.1371/journal.pone.0032713

Eltgroth, M. L., R. L. Watwood, and G. V. Wolfe. 2005. Production and cellular localization of long-chain neutral lipids in the Haptophyte algae *Isochrysis galbana* and *Emiliania huxleyi*. J. Phycol. 41:1000-1009.

Fabricius, K. E. 2011. Factors determining the resilience of coral reefs to eutrophication: A review and conceptual model, p. 493-505. In Dubinsky Z, N. Stambler [eds.], Coral Reefs: An ecosystem in transition. Springer.

Fraser A. J. 1989. Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. Can. Fish. Aquat. Sci., 46: 1869-1873.

Folch, J., M. Lees and G. H. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Gergs R., N. Steinberger, B. Beck, T. Basen, E. Yohannes, R.Schulz and D. Martin-Creuzburg. 2015. Compound-specific $\delta^{13}C$ analyses reveal sterol metabolic constraints in an aquatic invertebrate. Rapid Commun. Mass Spectrom. 29: 1789–1794

Giraldo C., P. Mayzaud, E. Tavernier, J. O. Irisson, F. Penot, J. Becciu, A. Chartier, M. Boutoute and P. Koubbi. 2013. Lipid components as a measure of nutritional condition in fish larvae (*Pleuragramma antarcticum*) in East Antarctica. Marine Biology, 160:877–887 doi 10.1007/s00227-012-2141-0Goad L. J. 1981. Sterol biosynthesis and metabolism in marine invertebrates. Pure App. Chem. 51: 837-852.

Hooper T. and C. C. Parrish. 2009 Profiling neutral lipids in individual fish larvae by using short column gas chromatography with flame ionization detection. Limnol. Oceanogr.: Methods 7: 411–418

Horta Puga, G. y J. L. Tello Musi. 2009. Sistema Arrecifal Veracruzano: condición actual y programa permanente de monitoreo: Primera Etapa. Universidad Nacional Autónoma de México. Facultad de Estudios Superiores Iztacala. Informe final SNIB-CONABIO

proyecto No. DM005. México D. F. [Coral reef system of Veracruz: actual condition and permanent monitoring program: Firts stage]

Ikeda T. and A. D. McKinnon. 2012. Metabolism and chemical composition of zooplankton and hyperbenthos from the Great Barrier Reef waters, North Queensland, Australia. Plankton Benthos Res 7(1): 8–19.

Jennings, S., M.J. Kaiser, J.D. Reynolds. 2001. Marine Fisheries Ecology. Blackwell Science, London.

Jones, G. J., Nichols, P. D., Shaw, P. M. 1994. Analysis of microbial sterols and hopanoids. In: Goodfellow, M., O'Donnell, A. G. eds. Chemical methods in prokaryotic systematics. Wiley, New York

Kanazawa A., 2001. Sterols in marine invertebrates. Fisheries Science, 67: 997–1007.

Karlson, R. H. 1999. Dynamics of coral communities. Kluwer Academic Publishers, Dordrecht

Kelly J. R., and R. E. Scheibling, 2012. Fatty acids as dietary tracers in benthic food webs. Mar Ecol Prog Ser 446: 1-22.

Kerr R. J., S. L. Kerr, S. Malik, and C. Djerassi. 1992. Biosynthetic studies in marine lipids 38. Mechanism and scope of sterols side chain dealkylation in sponges. Evidence of concurrent alkylation and dealkylation. J. Am. Chem. Soc. 114:299-303.

Keuskamp J. A., I. C. Feller, H. J. Laanbroek, J. T.A. Verhoeven, and M. M. Hefting. 2015. Short- and long-term effects of nutrient enrichment on microbial exoenzyme activity in mangrove peat. Soil Biol. Bioch. 81: 38-47

Lai S., L. G. Gillis, C. Mueller, T. J. Bouma, J. R. Guest, K. S. Last, A. D. Ziegler, P. A. Todd. 2013. First experimental evidence of corals feeding on seagrass matter. Coral Reefs. DOI 10.1007/s00338-013-1062-9

Laiz-Carrión, R., J. M. Quintanilla, A. P. Torres, F. Alemany, and A. García. 2013. Hydrographic patterns conditioning variable trophic pathways and early life dynamics of bullet tuna *Auxis rochei* larvae in the Balearic Sea. Mar. Ecol. Prog. Ser. 475: 203–212.

Lapointe, B. E., P. J. Barile, C. S. Yentsch, M. M. Littler, and B. Kakuk. 2004. The relative importance of nutrient enrichment and herbivory on macroalgal communities near Norman's Pond Cay, Exumas Cay, Bahamas: a natural enrichment experiment. J. Exp. Mar. Biol. Ecol. 298: 275-301.

Leaver M. J., L. A. N. Villeneuve, A. Obach, L. Jensen, J. E. Bron, D. R. Tocher, and J. B. Taggart. 2008. Functional genomics reveals increases in cholesterol biosynthetic genes and highly unsaturated fatty acid biosynthesis after dietary substitution of fish oil with

vegetable oils in Atlantic salmon (*Salmo salar*). BMC Genomics, 9:299, doi:10.1186/1471-2164-9-299.

Lee R. F. and J. S. Paton, 1989 Alcohol and waxes. Wax esters versus triacylglycerols in myctophid fishes from the Southern Ocean. In ACKMAN, R.G., ed. Marine biogenic lipids, fats, and oils. Boca Raton, FL: CRC Press, 73-102.

Leys S. P. and Eerkes-Medrano D. I. 2006. Feeding in a calcareous sponge: Particle uptake by pseudopodia. Biol. Bull. 211: 157-171.

Lindqvist, K., and R. Lignell. 1997. Intracellular partitioning of $^{14}C0_2$ in phytoplankton during a growth season in the northern Baltic. Mar. Ecol. Prog. Ser. 152: 41-50.

Marshall J. A., P. D. Nichols, B. Hamilton, R. J. Lewis, G. M. Hallegraeff. 2003. Ichthyotoxicity of *Chattonella marina* (Raphidophyceae) to damselfish (*Acanthochromis polycanthus*): the synergistic role of reactive oxygen species and free fatty acids. Harmful Algae 2: 273–281.

McLafferty, F. W. and Turecek, F. 1993. Interpretation of mass spectra. 4th Ed. University Sciences Books. Sausalito, CA. 371pp.

Montero-Torreiro M. F. and P. García-Martínez. 2003. Seasonal changes in the biochemical composition of body components of the sea urchin, *Paracentrotus lividus*, in Lorbe. (Galicia, north-western Spain). J. Mar. Biol. Ass. U.K. 83: 575-581.

Mishima Y. and A. Hayasi. 1978. Studies of sphingolipids of *Barnea* (*Umitakea*) *dilatata japonica*. I. Cerebroside and ceramide aminoethylphosphonate. J. Japan Oil Chem. Soc. 27(2):92-97.

Munday P. L. and B. W. Molony. 2002. The energetic cost of protogynous versus protandrous sex change in the bi-directional sex-changing fish *Gobiodon histrio*. Mar. Biol. 141: 1011–1017. doi: 10.1007/s00227-002-0904-8

Muscatine L. and J. W. Porter. 1977. Reef corals: Mutualistic symbioses adapted to nutrient-poor environments. BioScience, 27: 454-460.

Nelson, M. M., C. F. Phleger, and P. D. Nichols. 2002. Seasonal Lipid Composition in Macroalgae of the Northeastern Pacific Ocean. Bot. Mar. 45: 58-65.

Nes, W. D. 2011. Biosynthesis of cholesterol and other sterols. Chem. Rev. (111): 6423–6451 dx.doi.org/10.1021/cr200021m

O'Connell N. M., Y. C. O'Callaghan, N. M. O'Brien, A. R. Maguire, F.O. McCarthy. 2012. Synthetic routes to campesterol and dihydrobrassicasterol: a first reported synthesis of the key phytosterol dihydrobrassicasterol. Tetrahedron 68: 4995-5004.

Okolodkov, Y. B., G. Campos-Bautista, I. Gárate-Lizárraga, J. A. G. González-González, M. Hoppenrath, and V. Arenas. 2007. Seasonal changes of benthic and ephiphytic dinoflagellates in the Veracruz reef zone, Gulf of México. Aquat. Microb. Ecol. 47: 223–237, doi:10.3354/ame047223

Okolodkov, Y. B, J. A. Aké-Castillo, M. G. Gutiérrez-Quevedo, H. Pérez-España, and D. Salas-Monreal. 2011. Annual cycle of the plankton biomass in the National Park Sistema Arrecifal Veracruzano, southwestern Gulf of Mexico, p. 1–26. In G. Kattel [ed.], Zooplankton and phytoplankton. Nova Science.

Papina, M., T. Meziane, R. van Woesik. 2003. Symbiotic zooxanthellae provide the host-coral Montipora digitata with polyunsaturated fatty acids. Comp. Biochem. Physiol. Part B 135:533–537

Parrish, C. C. 1987a. Separation of aquatic lipid classes by Chromarod thin layer chromatography with measurements by Iatroscan flame ionization detection. Can. J. Fish. Aquat. Sci. 44: 722–731, doi:10.1139/f87-087

Parrish, C. C. 1987b. Time series of particulate and dissolved lipid classes during spring phytoplankton blooms in Bedford Basin, a marine inlet. Mar. Ecol. Prog. Ser. Vol. 35: 129-139.

Parrish, C. C. 1999. Determination of total lipid, lipid classes and fatty acids in aquatic samples, p. 4–20. In M. T. Arts and B. C. Wainman [eds.], Lipids in freshwater ecosystems. Springer.

Parrish, C. C., T. A. Abrajano, S. M. Budge, R. J. Helleur, E. D. Hudson, K. Pulchan Y C. Ramos. 2000. Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. in: P. Wangersky (Ed.) The handbook of environmental chemistry. Vol. 5, Part D. Springer-Verlag, Berling. 193-223 Pp.

Parrish C. C. 2013. Lipids in Marine Ecosystems. ISRN Oceanography, 16 pages

Pérez-España, H., J. Santander-Monsalvo, J. Bello-Pineda, R.S. Gómez-Villada, J. A. Ake-Castillo, M. A. Lozano-Aburto, M. A. Román-Vivés, M. Marín-Hernández. 2012. Caracterización ecológica del Parque Nacional Sistema Arrecifal Veracruzano. In: Sánchez, A.J., Chiappa-Carrara, X., Brito-Pérez, R. (Eds), Recursos acuáticos costeros del sureste, Vol. II. FOMIX Yucatán-RECORECOS-UNAM Sisal, Mérida, Yucatán, México, pp. 581-601.[Ecological characterization of the Coral Reef System of Veracruz National Park]

Perissinotto, R., P. Mayzaud, P. D. Nichols, J. P. Labat. 2007. Grazing by *Pyrosoma atlanticum* (Tunicata, Thaliacea) in the south Indian Ocean. Mar Ecol Prog Ser 330: 1-11.

Porter, J. W. 1976. Autotrophy, heterotrophy, and resource partitioning in Caribbean reef-building corals. Amer. Natur. 110: 731-742.

Rampen, S. W., B. A. Abbas, S. Schouten, and J. S. S. Damsté. 2010. A comprehensive study of sterols in marine diatoms (Bacillariophyta): Implications for their use as tracers for diatom productivity. Limnol. Oceanogr., 55(1): 91–105

Reef R., M. C. Ball, I. C. Feller, and C. E. Lovelock. 2010. Relationships among RNA: DNA ratio, growth and elemental stoichiometry in mangrove trees. Funct. Ecol. 24:1064–1072

Salas-Monreal D. , D. A Salas-de-León, M. A. Monreal-Gómez and M. L. Riverón-Enzástiga. 2009. Current rectification in a tropical coral reef system. Coral Reefs) 28:871–879 DOI 10.1007/s00338-009-0521-9

Santander-Monsalvo 2010. Ecología trófica de los peces más abundantes del Parque Nacional Sistema Arrecifal Veracruzano. Tesis de Maestría. Instituto de Ciencias Marinas y Pesquerías, Universidad Veracruzana. [Trophic ecology of more abundant fish of the Coral Reef System of Veracruz National Park.]

Sharpe R. L., M. Drolet and D. L. MacLatchy. 2006. Investigation of de novo cholesterol synthetic capacity in the gonads of goldfish (*Carassius auratus*) exposed to the phytosterol beta-sitosterol. Reprod. Biol. and Endocrinol., 4:60 doi:10.1186/1477-7827-4-60.

Sweeting, C. J., N. V. C. Polunin, and S. Jennings. 2004. Tissue and fixative dependent shifts of δ^{13}C and δ^{15}N in preserved ecological material. Rapid Comm. in Mass Spectrom. 18:2587–2592

Terrados, J. P. Ramírez-García, O. Hernández-Martínez, K. Pedraza, and A. Quiroz. 2008. State of *Thalassia testudinum* Banks ex König meadows in the Veracruz Reef System, Veracruz, Mexico. Aquat. Bot. 88: 17–26, doi:10.1016/j.aquabot.2007.08.003

Thorsen, M. S. 1998. Microbial activity, oxygen status and fermentation in the gut of the irregular sea urchin *Echinocardium cordatum* (Spatangoida: Echinodermata). Mar. Biol., 132: 423-433.

Tobón, W., C. Martínez-Garza and J. Campo. 2011. Soil responses to restoration of a tropical pasture in Veracruz, South-Eastern Mexico. J. Trop. For. Sci., 23(3): 338-344

Tornabene, L., J. Brenner, J. Williams, T. Camarena-Luhrs, and R. Robertson. 2015. *Coryphopterus personatus*. The IUCN Red List of Threatened Species 2015: e.T185960A69807937. Downloaded on 08 January 2016.

Usher K. M. 2008. The ecology and phylogeny of cyanobacterial symbionts in sponges. Mar. Ecol. 29: 178–192. doi:10.1111/j.1439-0485.2008.00245.x

Whiters, N., W. C. M. C. Kokke, W. Fenical, and C. Djerassi. 1982. Sterol patterns of cultured zooxanthellae isolated from marine invertebrates: Synthesis of gorgosterol and 23-desmethylgorgosterol by aposymbiotic algae. Proc. Natl. Acad. Sci. USA 79: 3764-3768.

Wildish D. and D. Kristmanson. 1997. Benthic suspension feeders and flow. Cambridge University Press.

Wilkinson C.R., and Cheshire A.C. 1990. Comparisons of sponge populations across the Barrier Reefs of Australia and Belize: evidence for higher productivity in the Caribbean. Mar. Ecol. Prog. Ser., 67: 285–294.

Yamashiro H., H. Oku, and K. Onaga. 2005. Effect of bleaching on lipid content and composition of Okinawan corals. Fish. Sci. 71: 448–453

Yoshiyama-Yanagawa T., S. Enya, Y. Shimada-Niwa, S. Yaguchi, Y. Haramoto, T. Matsuya, K. Shiomi, Y. Sasakura, S. Takahashi, M. Asashima, H. Kataoka, and R. Niwa. 2011. The Conserved Rieske Oxygenase DAF-36/Neverland Is a Novel Cholesterol-

metabolizing Enzyme. J. Biol. Chem. 2011, 286:25756-25762 doi: 10.1074/jbc.M111.244384

Zavala-Hidalgo J., S. L. Morey, and J. J. O'Brien. 2003. Seasonal circulation of the western shelf of Gulf of Mexico using a high resolution numerical model. J. Geophys. Res. 108: 1–19.

Zimmerman, M. P., F. C. Thomas, J. E. Thompson, C. Djerassi, H. Streiner, E. Evans and P. T. Murphy. 1989. The Distribution of Lipids and Sterols in Cell Types From the Marine Sponge *Pseudaxinyssa* sp. Lipids 24: 210-216

2.9 Tables

Table 2-1. Taxonomic classification, sample size for lipids, and sample size of C and N in parentheses of primary producers and consumers collected in the PNSAV.

Sample Scientific name (trivial name)	Kingdom/Phylum/ Class/Order/Family	Dry season n	Rainy season n	Location dry season	Location rainy season
Rhizophora mangle (red mangrove)	Plantae / Tracheophyta / Angiosperm/ Myrtacea / Rhizophoracea	4(4)	-	Lagos de Moreno	-
Thalassia testudinum (sea grass)	Plantae / Tracheophyta / Angiosperm / Najadales / Hydrochartaceae	3(4)	-	Rizo	Cabezo
Halimeda opuntia (green algae)	Plantae/ Chlorophyta / Bryopsidophyceae / Bryopsidales / Halimedaceae	5(4)	-	Cabezo	-
Galaxaura sp. (red algae)	Plantae / Rhodophyta /Florideophyceae / Nemaliales / Galaxauraceae	4(3)	-	Cabezo	-
Dictyota sp. (brown algae)	Plantae / Chromalveolata / Heterokontophyta / Phaeophyceae / Dictyotales / Dictyotaceae	4(4)	-	Cabezo	-
Zooxanthellae	Dinoflagellates/*Simbiodinium* sp.	-	4(0)	-	Blanca
30 µm net tow	Phytoplankton	9(5)	9(4)		
120 µm net tow	Zooplankton	9(4)	9(4)		
Pinna carnea (amber pen shell)	Animalia / Mollusca /Bivalvia Pterioida/ Pinnidae	7(0) I, F	4(0) I, F	Giote and Cabezo	Giote and Cabezo
Aplysina sp. (sponge)	Animalia / Porifera / Demospongiae / Verongida /Aplysinidae	6(0)	2(0)	Giote and Blanca	Verde and Cabezo
Montastrea cavernosa (great star coral)	Animalia / Cnidaria / Scleractinia / Faviidae	7(0)	4(0)	Cabezo and Blanca	Cabezo and Blanca
Echinometra lucunter (sea urchin)	Animalia / Echinodermata / Echinoidea / Echinoida / Echinometridae	3(14) I	4(13) F	Blanca	Cabezo
Acanthurus chirurgus (surgeon fish)	Animalia / Chordata / Actinopterygii / Beryciformes / Acanthuridae	4(10) M, F	5(5) M, F	Salmedina	Rizo
Coryphopterus personatus (masked goby)	Animalia / Chordata / Actinopterygii / Perciformes / Gobidae	3(6)	5(18)	Blanca	Verde and Anegada Adentro
Bodianus rufus (hog fish)	Animalia / Chordata / Actinopterygii / Perciformes / Labridae	3(7) I, M	5(15) I, M	Anegada Adentro	Anegada Adentro
Ocyurus chrysurus (yellowtail snapper)	Animalia / Chordata / Actinopterygii / Perciformes / Lutjanidae	3(10) I, M, F	5(15) M, F	Verde	Santiaguillo
Caranx hippos (crevalle jack)	Animalia / Chordata / Actinopterygii / Perciformes / Carangidae	3(10) I	4(9) I	Mandinga Lagoon	Mouth of Jamapa river

Table 2-2. Size and nutritional quality of primary producers and consumer nutritional condition studied in the PNSAV. C= Carbon, N= Nitrogen, TAG= Triacylglycerols, ST= Sterols. Values are means ± 95% of confidence interval (CI). Superscript letters denote significant differences. Multiple pair's comparison was done among species of primary producers, invertebrates, and fish independently. *F*= Fisher statistic, *p*= Tuckey's family error probability value.

Food web member	Season	Standard length(cm)	Wet weight (g)	C: N mol ratio	Total lipids (mg g^{-1} dry wt)	TAG: ST ratio
Primary producers						
R. mangle (leaves)	dry	8 ± 2^{a}	0.4 ± 0.1^{a}	20 ± 5^{a}	24 ± 17^{b}	0.8 ± 0.7^{a}
T. testudinum (leaves)	dry	35 ± 5^{b}	2 ± 1^{b}	16 ± 3^{ab}	10 ± 4^{a}	1.5 ± 1.4^{a}
H. opuntia (whole)	dry	5 ± 1^{c}	29 ± 20^{c}	22 ± 8^{a}	36 ± 19^{b}	13 ± 11^{a}
Galaxaura sp. (whole)	dry	12 ± 3^{a}	22 ± 3^{c}	26 ± 2^{a}	5 ± 2^{a}	3.2 ± 2.7^{a}
Dictyota sp. (whole)	dry	9 ± 2^{a}	15 ± 2^{c}	20 ± 1^{a}	25 ± 7^{b}	0.4 ± 0.3^{a}
Phytoplankton (per m^3)	dry	0.00003^{d}	0.04 ± 0.01^{d}	10 ± 2^{b}	27 ± 5^{b}	1.9 ± 0.2^{a}
Phytoplankton (per m^3)	rainy	0.00003^{d}	0.02 ± 0.01^{d}	12 ± 1^{b}	51 ± 30^{bc}	20 ± 19^{a}
Symbionts						
Zooxanthellae (5±2 cm^2)	rainy	-	0.17 ± 0.03^{e}	-	73 ± 13^{c}	2.1 ± 1.2^{a}
Statistic		CI 95%	CI 95%	$F_{6,21}$=4.7	$F_{7,34}$=5.7	$F_{7,34}$=1.2
p		<0.05	<0.05	0.008	0.001	0.314
Invertebrates						
Herbivores						
E. lucunter	dry	4.2 ± 0.3^{a}	34.2 ± 7.8^{ab}	8.5 ± 0.4^{a}	63 ± 52^{ab}	6 ± 5^{a}
E. lucunter	rainy	-	-	12 ± 1^{b}	102 ± 38^{b}	25 ± 14^{c}
Planktivores/detritivores						
P. carnea	dry	13 ± 2^{b}	17 ± 9^{a}	-	27 ± 7^{a}	0.7 ± 0.6^{a}
P. carnea	rainy	20 ± 4^{c}	53 ± 20^{b}	-	75 ± 9^{b}	1.6 ± 0.4^{a}
Aplysina sp.	dry	21 ± 6^{c}	24 ± 5^{a}	-	64 ± 17^{b}	1.0 ± 0.9^{a}
Aplysina sp.	rainy	30 ± 10^{c}	28 ± 11^{a}	-	77 ± 27^{ab}	0.4 ± 0.1^{a}
Planktivores						
Zooplankton (per m^3)	dry	0.00012^{d}	0.2 ± 0.1^{c}	-	36 ± 18^{a}	9 ± 6^{bc}
Zooplankton (per m^3)	rainy	0.00012^{d}	0.7 ± 0.3^{d}	12 ± 2^{b}	36 ± 11^{a}	7 ± 2^{b}
M. cavernosa (piece)	dry	12.1 ± 5.9^{ab}	17 ± 9^{a}	-	49 ± 9^{a}	6 ± 3^{b}
M. cavernosa (piece)	rainy	5.3 ± 2.3^{a}	49 ± 19^{b}	-	36 ± 18^{ab}	1.4 ± 0.5^{a}
Statistic		CI 95%	CI 95%	$F_{2,28}$=21.9	$F_{9,45}$=3.8	$F_{9,45}$=4.0
p		<0.05	<0.05	0.001	0.002	0.001
Teleost fish						
Herbivores/detritivores						
A. chirurgus	dry	21 ± 2^{a}	262 ± 64^{a}	3.6 ± 0.1^{ab}	35 ± 20^{a}	1.1 ± 0.4^{a}
A. chirurgus	rainy	23 ± 1^{a}	292 ± 42^{a}	3.8 ± 0.1^{ab}	29 ± 10^{a}	5 ± 2^{a}
Planktivores						
C. personatus	dry	3.0 ± 0.1^{b}	0.21 ± 0.02^{b}	3.8 ± 0.1^{b}	57 ± 21^{a}	0.5 ± 0.3^{a}
C. personatus	rainy	2.3 ± 0.2^{c}	0.16 ± 0.03^{b}	4.7 ± 0.1^{c}	114 ± 15^{c}	1.2 ± 0.6^{a}
Molluscivores/Echinivores						
B. rufus	dry	21 ± 3^{a}	211 ± 62^{a}	3.2 ± 0.8^{a}	58 ± 5^{a}	1.1 ± 0.4^{a}
B. rufus	rainy	24 ± 3^{a}	254 ± 59^{a}	4.1 ± 0.1^{b}	76 ± 17^{b}	50 ± 33^{b}
Piscivores						
O. chrysurus	dry	19 ± 2^{a}	91 ± 24^{c}	3.7 ± 0.1^{ab}	34 ± 11^{a}	0.3 ± 0.2^{a}
O. chrysurus	rainy	35 ± 3^{d}	375 ± 82^{a}	4.0 ± 0.1^{b}	74 ± 14^{b}	27 ± 20^{b}
C. hippos juvenile	dry	24 ± 1^{a}	176 ± 31^{d}	3.7 ± 0.1^{b}	31 ± 17^{a}	4 ± 3^{a}
C. hippos juvenile	rainy	22 ± 1^{a}	140 ± 6^{d}	5.2 ± 0.4^{c}	181 ± 65^{c}	68 ± 38^{b}
Statistic		CI 95%	CI 95%	$F_{9,95}$=21.7	$F_{9,45}$=10.3	$F_{9,45}$=5.0
p		<0.05	<0.05	<0.001	<0.001	0.001

Table 2-3. Lipid class composition of primary producers and consumers collected in the PNSAV. D= dry, R= rainy, HC= Hydrocarbons, SE/WE= Steryl and/or wax esters, KET= Ketones, TAG= Triacylglycerols, FFA=Free fatty acids, ALC=Alcohols, ST=Sterols, AMPL=Acetone Mobile Polar Lipids, PL=Phospholipids. Values are means ± 95% confidence interval. Superscripts letters denote significant differences among rows. *F*= Fisher statistic, *p*= probability value. D= dry season and R= rainy season.

	HC	SE/WE	KET	TAG	FFA	ALC	ST	AMPL	PL
Primary producers									
R. mangle D	10±4^{a}	-	2±2^{a}	3±3^{a}	0.6±0.3^{a}	2±1^{a}	3±1^{a}	32±7^{ab}	47±2^{a}
T. testudinum R	4±3^{ab}	0.1±0.1^{a}	1±2^{a}	6±6^{a}	20±3^{b}	6±5^{a}	4±2^{a}	25±9^{b}	40±4^{a}
Macroalgae									
H. opuntia D	1±1^{b}	0.2±0.2^{a}	0.4±0.4^{a}	6±4^{a}	1.5±0.3^{a}	1±1^{a}	0.7±0.5^{a}	44±7^{a}	46±7^{a}
Galaxaura sp. D	2±3^{ab}	0.3±0.5^{a}	1±1^{a}	3±1^{a}	35±13^{b}	3±2^{a}	1±1^{a}	27±10^{b}	29±11^{a}
Dyctiota sp. D	4±1^{ab}	0.3±0.5^{a}	1±1^{a}	5±4^{a}	23±4^{b}	3±3^{a}	15±6^{b}	23±4^{b}	26±7^{a}
PhytoplanktonD	4±1^{ab}	0.7±0.8^{a}	1±1^{a}	13±8^{ab}	31±12^{b}	4±3^{a}	12±5^{b}	6±1^{c}	29±9^{a}
Phytoplankton R	13±6^{a}	-	2±1^{a}	27±13^{b}	18±5^{b}	1.0±1^{a}	2±1^{a}	20±5^{b}	18±5^{ab}
Zooxanthellae R	9±3^{a}	14±4^{b}	14±7^{b}	18±10^{ab}	7±1^{a}	3±1^{a}	7±1^{b}	20±6^{b}	8±2^{b}
$F_{7, 26}$	3.86	29.4	14.60	2.48	5.85	0.98	8.10	16.66	8.67
p	0.004	< 0.001	<0.01	0.040	<0.001	0.466	<0.001	<0.001	<0.001
Invertebrates									
Herbivores									
E. lucunter D	7±5^{ab}	0.4±0.1^{d}	3 ±1^{b}	11±1^{a}	6±5^{ab}	0.7±0.3^{b}	4±1^{b}	13±8^{ab}	44±10^{c}
E. lucunter R	3±3^{b}	1±1^{cd}	3±2^{b}	64±10^{b}	12±4^{ab}	3±3^{b}	3±1^{b}	4±2^{a}	15±9^{b}
Planktivores/detritivores									
P. carnea D	7±4^{a}	5±3^{cd}	1 ±1^{b}	12±3^{a}	0.9±0.8^{a}	1.4±0.9^{b}	12±4^{ac}	8±3^{a}	57±12^{c}
P. carnea R	5±1^{a}	6±2^{c}	8 ±2^{a}	14±2^{a}	7±5^{a}	6±3^{a}	9±2^{ac}	18±3^{b}	26±8^{b}
Aplysina sp.D	1±1^{b}	0.6±0.5^{d}	1±1^{b}	13±9^{a}	9±6^{ab}	0.5±0.1^{b}	14±3^{c}	19±7^{b}	43±10^{bc}
Aplysina sp.R	1±1^{b}	3.5±0.1^{c}	2 ±1^{b}	6±1^{a}	21±8^{ab}	0.8±0.2^{b}	16±1^{c}	22±3^{b}	31±10^{b}
Planktivores									
Zooplankton D	4±3^{ab}	1±1^{cd}	1 ±1^{b}	39±13^{b}	22±10^{b}	-	7±2^{ab}	13±8^{ab}	20±4^{b}
Zooplankton R	7±2^{a}	1±1^{cd}	1 ±1^{b}	25±6^{b}	16±3^{ab}	0.7±0.4^{b}	4±1^{b}	22±6^{b}	25±4^{b}
M. cavernosa D	1±0.2^{b}	25±5^{b}	1±1^{b}	21±7^{a}	5±2^{a}	0.5±0.3^{b}	4±1^{ab}	25±10^{b}	19±4^{b}
M. cavernosa R	8±2^{a}	16±3^{a}	14±7^{a}	12±2^{a}	8±5^{a}	6±2^{a}	9±3^{ac}	16±7^{ab}	11±3^{a}
$F_{9, 45}$	5.58	56.18	14.63	4.55	3.83	14.99	15.26	3.40	16.72
p	<0.01	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001	0.003	<0.001
Teleost fish									
Herbi/detritivores									
A. chirurgus D	12±2^{a}	0.4±0.1^{a}	2±2^{a}	6±4^{a}	17±1^{a}	0.8±0.3^{a}	5±2^{a}	14±2^{a}	44±4^{a}
A. chirurgus R	2±2^{b}	0.1±0.1^{a}	1±1^{a}	32±11^{b}	17±1^{a}	-	7±3^{a}	4±2^{b}	35±8^{a}
Planktivores									
C. personatus D	4±3^{b}	0.3±0.3^{a}	2±1^{a}	2±1^{a}	2±2^{b}	0.3±0.2^{a}	5±1^{a}	6±4^{b}	78±3^{b}
C. personatus R	4±3^{b}	0.3±0.2^{a}	1±1^{a}	6±3^{a}	6±3^{b}	0.2±0.1^{a}	5±3^{a}	30±10^{c}	48±9^{a}
Invertebrativores									
B. rufus D	2±1^{b}	3.8±0.4^{b}	6±1^{b}	10±5^{a}	8±3^{b}	3.0±0.1^{b}	9±1^{a}	5±1^{b}	36±10^{a}
B. rufus R	1±1^{b}	0.1±0.1^{a}	1±1^{b}	63±10^{c}	6±3^{b}	1±1^{a}	2±1^{b}	3±2^{b}	24±6^{a}
O. chrysurus D	4±1^{b}	0.4±0.3^{a}	0.4±0.3^{b}	2±1^{a}	10±9^{ab}	0.2±0.1^{a}	8±4^{ab}	4±3^{b}	71±7^{b}
O. chrysurus R	3±2^{b}	0.1±0.1^{a}	0.1±0.1^{b}	44±19^{b}	8±6^{ab}	1±1^{a}	3±2^{b}	10±6^{ab}	32±9^{a}
C. hippos D	8±6^{b}	1±1^{a}	0.4±0.3^{b}	12±8^{a}	9±4^{ab}	1±1^{a}	5±3^{ab}	9±7^{ab}	56±6^{b}
C. hippos R	1±0.2^{b}	0.1±0.1^{a}	0.1±0.1^{b}	74±10^{c}	4±4^{ab}	1±1^{a}	1±1^{b}	7±5^{ab}	11±3^{c}
$F_{9, 30}$	4.08	64.05	10.76	25.83	5.23	4.89	4.36	9.29	25.49
p	0.002	<0.001	<0.001	<0.001	<0.01	<0.001	0.001	<0.001	<0.001

Teleost fish									
Herbivores/detritivores									
A. chirurgus D	12±2[a]	0.4±0.1[a]	2±2[a]	6±4[a]	17±1[a]	0.8±0.3[a]	5±2[a]	14±2[a]	44±4[a]
A. chirurgus R	2±2[b]	0.1±0.1[a]	1±1[a]	32±11[b]	17±1[a]	-	7±3[a]	4±2[b]	35±8[a]
Planktivores									
C. personatus D	4±3[b]	0.3±0.3[a]	2±1[a]	2±1[a]	2±2[b]	0.3±0.2[a]	5±1[a]	6±4[b]	78±3[b]
C. personatus R	4±3[b]	0.3±0.2[a]	1±1[a]	6±3[a]	6±3[b]	0.2±0.1[a]	5±3[a]	30±10[c]	48±9[a]
Molluscivores/Echinivores									
B. rufus D	2±1[b]	3.8±0.4[b]	6±1[b]	10±5[a]	8±3[b]	3.0±0.1[b]	9±1[a]	5±1[b]	36±10[a]
B. rufus R	1±1[b]	0.1±0.1[a]	1±1[b]	63±10[c]	6±3[b]	1±1[a]	2±1[b]	3±2[b]	24±6[a]
Piscivores									
O. chrysurus D	4±1[b]	0.4±0.3[a]	0.4±0.3[b]	2±1[a]	10±9[ab]	0.2±0.1[a]	8±4[ab]	4±3[b]	71±7[b]
O. chrysurus R	3±2[b]	0.1±0.1[a]	0.1±0.1[b]	44±19[b]	8 ±6[ab]	1±1[a]	3±2[b]	10±6[ab]	32±9[a]
C. hippos D	8±6[b]	1±1[a]	0.4±0.3[b]	12±8[a]	9±4[ab]	1±1[a]	5±3[ab]	9±7[ab]	56±6[b]
C. hippos R	1.2±0.2[b]	0.1±0.1[a]	0.1±0.1[b]	74±10[c]	4±4[ab]	1±1[a]	1±1[b]	7±5[ab]	11±3[c]
$F_{9, 28}$	4.08	64.05	10.76	25.83	5.23	4.89	4.36	9.29	25.49
p	0.002	<0.001	<0.001	<0.001	<0.01	<0.001	0.001	<0.001	<0.001

Table 2-4. Fatty acid (FA) composition of symbiotic zooxanthellae and suspension feeders (FA > 0.2 %) studied in the PNSAV. Values are mean ± 95% confidence interval. SFA= saturated FA, MUFA= monounsaturated FA, PUFA= polyunsaturated FA, HUFA= highly unsaturated FA, BFA= branched FA, OFA= odd chain FA, and AL= Acyl lipids per dry weight. Superscript letters denote significant differences among columns, *df*= degree of freedom, *F*= Fisher statistics and *p*= Tukey's family error *p* value. FAME=FA methyl ester

	Zooxanthellae	*M. cavernosa*		*Aplysina* sp.		*P. carnea*		*df*	*F*	*p*
FAME	rainy	dry	rainy	dry	rainy	dry	rainy			
14:0	2.4 ± 0.3^{b}	3 ± 1^{b}	4 ± 1^{ab}	2 ± 1^{b}	3 ± 1^{b}	2 ± 1^{b}	6 ± 1^{a}	6, 21	27.7	<0.001
16:0	42 ± 3^{ab}	36 ± 11^{b}	53 ± 5^{a}	4 ± 1^{d}	7 ± 3^{cd}	22.3 ± 0.1^{b}	23 ± 2^{ab}	6, 21	49.1	<0.001
17:0	0.2 ± 0.1^{c}	0.2 ± 0.1^{c}	0.2 ± 0.1^{c}	0.2 ± 0.1^{bc}	0.4 ± 0.2^{b}	3 ± 1^{a}	1.9 ± 0.1^{a}	6, 21	63.0	<0.001
18:0	10 ± 1^{a}	13 ± 5^{a}	8 ± 2^{a}	1.5 ± 0.4^{b}	3 ± 2^{b}	8 ± 2^{a}	7 ± 1^{a}	6, 21	44.3	<0.001
20:0	1.1 ± 0.3^{a}	1 ± 1^{a}	0.7 ± 0.3^{a}	0.6 ± 0.3^{a}	0.9 ± 0.1^{a}	0.5 ± 0.2^{a}	0.5 ± 0.1^{a}	6, 21	5.2	0.002
22:0	0.2 ± 0.1^{b}	0.2 ± 0.1^{b}	0.1 ± 0.04^{b}	8 ± 3^{a}	9 ± 3^{a}	0.1 ± 0.01^{b}	0.1 ± 0.01^{b}	6, 21	135.3	<0.001
24:0	1.0 ± 0.1^{b}	0.1 ± 0.1^{b}	0.1 ± 0.1^{b}	4 ± 1^{a}	5 ± 3^{a}	0.1 ± 0.01^{b}	0.1 ± 0.01^{b}	6, 21	41.8	<0.001
Σ SFA	56 ± 4^{ab}	54 ± 6^{ab}	66 ± 5^{a}	22 ± 7^{c}	31 ± 11^{bc}	38 ± 3^{b}	39 ± 4^{b}	6, 21	8.8	<0.001
16:1ω7	2.2 ± 0.3^{b}	3 ± 1^{b}	2.8 ± 0.3^{b}	3 ± 1^{b}	5 ± 2^{b}	1.3 ± 0.4^{c}	6 ± 1^{a}	6, 21	57.0	<0.001
18:1ω9	10 ± 1^{a}	13 ± 3^{a}	10 ± 1^{a}	2 ± 1^{b}	2 ± 1^{b}	1.7 ± 0.3^{d}	5 ± 1^{b}	6, 21	113.7	<0.001
18:1ω7	2 ± 1^{a}	2 ± 1^{a}	1.5 ± 0.3^{a}	0.7 ± 0.2^{b}	2 ± 1^{a}	2.1 ± 0.3^{ab}	0.2 ± 0.1^{c}	6, 21	37.8	<0.001
20:1ω9	3 ± 1^{a}	4 ± 1^{a}	2 ± 1^{a}	0.3 ± 0.1^{b}	0.1 ± 0.01^{c}	4 ± 2^{a}	3 ± 1^{a}	6, 21	106.3	<0.001
24:1ω9	0.3 ± 0.1^{ab}	0.6 ± 0.3^{a}	0.1 ± 0.01^{b}	-	-	0.1 ± 0.01^{c}	0.1 ± 0.01^{c}	4, 15	19.5	<0.001
24:1ω7	-	-	-	3 ± 1^{b}	7 ± 3^{a}	-	-	1, 8	50.6	<0.001
7Me16:1ω10	0.4 ± 0.2^{ab}	0.1 ± 0.1^{b}	0.3 ± 0.1^{a}	0.9 ± 0.3^{a}	2 ± 1^{a}	-	-	4, 18	7.7	<0.001
ΣMUFA	18 ± 2^{bc}	25 ± 6^{b}	18 ± 2^{bc}	14 ± 4^{bc}	23 ± 9^{a}	11 ± 1^{c}	20 ± 1^{b}	6, 21	6.1	<0.001
16:2ω6	-	-	-	0.1 ± 0.01^{a}	0.1 ± 0.01^{a}	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	3, 12	3.1	0.073
16:2ω4	-	-	-	0.3 ± 0.1^{a}	0.1 ± 0.01^{a}	0.2 ± 0.1^{a}	0.4 ± 0.2^{a}	3, 12	2.1	0.188
18:2ω6	1.2 ± 0.2^{a}	1.1 ± 0.2^{ab}	1.2 ± 0.1^{a}	0.2 ± 0.1^{c}	0.3 ± 0.2^{c}	1.1 ± 0.2^{b}	2.0 ± 0.1^{a}	6, 21	29.5	<0.001
18:3ω3	0.3 ± 0.01^{ab}	0.3 ± 0.1^{ab}	0.3 ± 0.1^{ab}	0.2 ± 0.1^{b}	0.1 ± 0.05^{b}	0.6 ± 0.1^{a}	0.8 ± 0.2^{a}	6, 21	3.5	0.015
18:4ω3	3 ± 1^{a}	3 ± 1^{ab}	2.5 ± 0.2^{a}	0.2 ± 0.1^{c}	0.1 ± 0.05^{c}	0.6 ± 0.2^{b}	1.0 ± 0.1^{b}	6, 21	76.5	<0.001
18:5ω3	0.1 ± 0.05^{ab}	0.4 ± 0.2^{a}	0.2 ± 0.1^{ab}	-	-	-	-	3, 10	37.1	<0.001
ΣPUFA	5 ± 1^{a}	5 ± 1^{a}	4.6 ± 0.3^{a}	1.5 ± 0.4^{c}	1.0 ± 0.4^{c}	3 ± 1^{b}	5 ± 1^{a}	6, 21	31.7	<0.001
Σ20:2NMI	-	-	-	-	-	0.7 ± 0.2^{a}	0.6 ± 0.1^{a}	1, 5	2.2	0.188
20:2ω6	0.7 ± 0.1^{a}	0.9 ± 0.1^{a}	0.4 ± 0.2^{a}	0.3 ± 0.1^{b}	-	0.4 ± 0.1^{b}	0.3 ± 0.2^{ab}	5, 20	20.6	<0.001
20:4ω6	5 ± 1^{a}	3 ± 1^{b}	3.1 ± 0.1^{b}	0.6 ± 0.3^{c}	0.8 ± 0.3^{c}	5 ± 1^{b}	6 ± 1^{a}	6, 21	62.8	<0.001
20:4 ω3	0.7 ± 0.3^{a}	0.3 ± 0.1^{b}	0.5 ± 0.2^{a}	0.2 ± 0.1^{bc}	0.2 ± 0.1^{bc}	0.2 ± 0.1^{bc}	0.1 ± 0.01^{c}	6, 21	19.0	<0.001
20:5ω3	3 ± 1^{bc}	2 ± 1^{bc}	1.8 ± 0.3^{c}	0.3 ± 0.1^{d}	0.4 ± 0.2^{d}	7 ± 2^{b}	9 ± 2^{a}	6, 21	76.8	<0.001
22:2Δ7,13	-	-	-	-	-	4 ± 1^{a}	1.8 ± 0.3^{a}	1, 5	2.5	0.188

22:2 Δ7,15	-	-	-	-	-	5±3[a]	3±1[a]	1, 5	1.0	0.368
22:4ω6	2±1[a]	3±1[ab]	1.1±0.3[b]	0.8±0.3[c]	0.6±0.1[c]	2±1[bc]	1.1±0.1[b]	6, 21	18.7	<0.001
22:5ω3	0.3±0.1[b]	0.4±0.1[b]	0.3±0.1[b]	-	-	1.8±0.3[a]	1.2±0.3[a]	4, 15	12.3	<0.001
22:6ω3	7±4[ab]	5±2[b]	4±1[b]	2±1[bc]	2±1[c]	19±7[d]	10±1[a]	6, 21	13.3	<0.001
26:2ω6	-	-	-	50±14[a]	23±25[a]	-	-	1, 8	0.5	0.497
ΣHUFA	20±5[b]	16±2[b]	11±3[b]	55±12[a]	28±22[ab]	38±8[a]	29±4[ab]	6, 21	12.9	<0.001
ΣBFA	0.7±0.3[b]	0.2±0.1[c]	0.6±0.2[b]	7±2[a]	15±2[a]	0.8±0.1[b]	1.2±0.1[b]	6, 21	29.0	<0.001
ΣOFA	0.3±0.2[b]	0.2±0.1[b]	0.3±0.1[b]	2±1[a]	3±1[a]	4±1[a]	2.9±0.1[a]	6, 21	74.1	<0.001
AL mg g^{-1}	50±10[a]	48±9[a]	37±18[a]	59±16[a]	61±24[a]	20±5[b]	54±9[a]	6, 21	24.2	<0.001

Table 2-5. Sterol composition of primary producers collected in the PNSAV. Values are means ± 95% confidence interval. (H)= higher than all other, (S)= specific sterols, C= carbons and Δ= position of double bonds. Superscript letters denote significant differences among columns. TS= Concentration of total sterols per dry weight. *df*= degree of freedom, *F*= Fisher statistics and *p*= Tukey's family error *p* value

Sterol	Short formula	*R. mangrove*	*T. testudinum*	*H. opuntia*	*Galaxaura* sp.	*Dictyota* sp.	Phytoplankton	Zooxanthellae	*df*	*F*	*p* value
Pregnanone (S)	$C_{27}\Delta^{0}$	-	-	-	-	-	-	12±6	-	-	-
24-Nordehydrocholesterol (H)	$C_{26}\Delta^{5,22}$	3 ± 1^{a}	0.7 ± 0.2^{ab}	-	-	-	0.06 ± 0.01^{b}	-	2, 14	35.0	<0.001
Trans-22-dehydrocholesterol (H)	$C_{27}\Delta^{22}$	-	5 ± 1^{a}	-	-	-	0.60 ± 0.01^{b}	-	1, 11	16.0	0.002
Occelasterol	$C_{27}\Delta^{5,22}$	-	-	-	-	-	7 ± 3^{a}	6 ± 3^{a}	1, 10	0.3	0.594
Trans-22-dehydrocholestanol (S)	$C_{27}\Delta^{22}$	-	-	-	-	-	0.6±0.5	-	-	-	-
Patinosterol (S)	$C_{27}\Delta^{22}$	-	-	-	-	-	0.4±0.5	-	-	-	-
Cholesterol (H)	$C_{27}\Delta^{5}$	-	3 ± 1^{a}	0.9 ± 0.1^{a}	30.2 ± 0.1^{b}	1.3 ± 0.4^{a}	50 ± 8^{c}	29 ± 4^{b}	5, 23	45.0	<0.001
Cholestanol (H)	$C_{27}\Delta^{0}$	-	-	-	11 ± 2^{a}	-	5 ± 1^{b}	-	1, 11	53.0	<0.001
Brassicasterol (H)	$C_{28}\Delta^{5,22}$	-	-	-	23 ± 2^{a}	-	5 ± 1^{b}	-	1, 11	30.7	<0.001
Brassicastanol (H)	$C_{28}\Delta^{22}$	-	-	-	5 ± 1^{a}	-	0.9 ± 0.7^{b}	-	1, 11	33.2	<0.001
Ergost-8(14)-enol (S)	$C_{28}\Delta^{24(28)}$	-	-	-	-	-	0.2±0.3	-	-	-	-
Stellasterol (H)	$C_{28}\Delta^{7,22}$	-	-	1.2 ± 0.2^{a}	-	-	0.7 ± 0.6^{a}	-	1, 13	1.04	0.328
24-Methylenecholesterol (H)	$C_{28}\Delta^{5,22}$	-	-	0.9 ± 0.2^{a}	-	-	6 ± 1^{b}	-	1, 12	27.7	<0.001
Campesterol (H)	$C_{28}\Delta^{5}$	-	9.4 ± 0.4^{ab}	4 ± 2^{a}	14 ± 1^{b}	-	9 ± 2^{ab}	47 ± 11^{c}	4, 20	77.5	<0.001
Stigmasterol (H)	$C_{29}\Delta^{5}$	8.1 ± 2.7^{a}	16 ± 1^{b}	1.2 ± 1.0^{c}	-	-	3 ± 2^{c}	-	3, 18	44.2	<0.001
23,24 dimethylcholest 5,7-dien-3-*β*-ol (S)	$C_{29}\Delta^{5,7}$	-	-	-	-	-	0.7±0.8	-	-	-	-
24-Methylenephenol (S)	$C_{28}\Delta^{7,24(28)}$	-	-	-	-	-	2±1	-	-	-	-
4,24 dimethyl 5,7-dien-3-*β*-ol (S)	$C_{29}\Delta^{5,7}$	-	-	-	-	19±2	-	-	-	-	-
Poriferasterol (S)	$C_{29}\Delta^{5,22}$	-	-	-	-	18±1	-	-	-	-	-
Spinasterol (S)	$C_{29}\Delta^{7,22}$	-	-	1.1±0.3	-	-	-	-	-	-	-
β-Sitosterol (H)	$C_{29}\Delta^{5}$	82 ± 1^{a}	66 ± 2^{b}	89 ± 1^{a}	17 ± 1^{c}	-	8 ± 7^{c}	-	4, 21	166.8	<0.001
Fucosterol (S)	$C_{29}\Delta^{5,24}$	-	-	-	-	21±1	-	-	-	-	<0.001
Sitostanol (S)	$C_{29}\Delta^{0}$	-	-	-	-	-	2±2	-	-	-	-
Isomer of fucosterol (S)	$C_{29}\Delta^{5,24}$	-	-	-	-	5±1	-	-	-	-	-
Dinosterol (S)	$C_{30}\Delta^{22}$	-	-	-	-	-	0.4±0.3	-	-	-	-

Dinostanol (S)	$C_{30}\Delta^{0}$	-	-	-	-	-	0.5±0.4	-	-	-	-
Gorgosterol (S)	$C_{30}\Delta^{5}$	-	-	-	-	-	-	6±2	-	-	-
Isofucosterol (H)	$C_{29}\Delta^{5,24}$	-	-	1.6±0.3[a]	-	37±1[b]	-	-	1, 7	281.3	<0.001
Cycloartenol (S)	$C_{30}\Delta^{5}$	7±2	-	-	-	-	-	-			-
TS (mg g^{-1} dry wt)		0.9±0.2[a]	0.4±0.3[a]	0.4±0.3[a]	0.2±0.1[a]	4±3[a]	1.0±0.3[a]	15±4[b]	6, 26	54.6	<0.001

Table 2-6. Sterol composition of zooplankton, coral, sea urchins, clams and sponges collected in PNSAV. Values are means ± 95% confidence intervals. Superscript letters denote significant differences among columns. TS= Concentration of total sterols by dry weight. *df*= degree of freedom, *F*= Fisher statistics or *H*= Kruskal-Wallis statistic, and *p*= Tukey's family error *p* value. When not specified statistics confidence interval were used to determine significant differences

Species	Short formula	Zooplankton		*M. cavernosa*		*E. lucunter*		*P. carnea*		*Aplysina* sp.				
Sterol		dry	rainy	dry	rainy	dry	rainy	dry	rainy	dry	rainy	*df*	*F or H*	*p* value
24-Nordehydrocholesterol	$C_{26}\Delta^{5,22}$	0.9±0.2^{a}	-	-	-	-	-	3±1^{b}	5±3^{b}	-	-			<0.05
Trans-22-dehydrocholesterol	$C_{27}\Delta^{22}$	6±2	-	-	-	-	-	-	-	-	-	-	-	-
24-Nor-22,23 methylenecholest-5-en-3-*β*-ol	$C_{27}\Delta^{5}$	-	1±1^{a}	-	-	-	-	2±1^{ab}	2.5± 0.4^{b}	-	-			0.056
Occelasterol	$C_{27}\Delta^{5,22}$	2±1^{a}	11±3^{c}	2±1^{a}	5±1^{b}	2±1^{a}	2±1^{a}	9±2^{c}	3±1^{a}	5±1^{b}	2±1^{a}	9	24	0.001
Trans-22-dehydrocholestanol	$C_{27}\Delta^{22}$	2.5±0.4	-	-	-	-	-	-	-	-	-	-	-	-
Cholesterol	$C_{27}\Delta^{5}$	51±5^{a}	45± 7^{a}	14± 4^{b}	26± 4^{c}	85±2^{d}	69±5^{d}	38±10^{a}	46±12^{a}	25±1^{c}	38±3^{a}	9, 39	28.1	<0.001
Cholestanol	$C_{27}\Delta^{0}$	3± 1^{ab}	4±1^{a}	-	-	-	-	2±1^{b}	2±1^{ab}	-	-			<0.050
Desmosterol	$C_{27}\Delta^{5,24}$	-	-	-	-	-	-	-	-	0.6±0.1^{a}	0.7±0.1^{a}			>0.050
7-Dehydrocholesterol	$C_{28}\Delta^{5,7}$	-	-	-	-	0.7±0.3^{a}	3±2^{b}	-	-	-	-			>0.050
Secocholesta-5(10), 6,8 triene	$C_{27}\Delta^{5,6,8}$	-	-	-	-	-	-	2 ±1^{a}	5±2^{a}	-	-			>0.050
Brassicasterol	$C_{28}\Delta^{5,22}$	6±2^{a}	6±1^{a}	1±1^{b}	6±2^{a}	3±1^{b}	4±1^{ab}	14±2^{c}	8±3^{ac}	20±1^{d}	16±2^{c}	9, 39	29.6	0.001
Brassicastanol	$C_{28}\Delta^{22}$	2±1^{a}	-	-	-	-	-	0.9±0.5^{a}	3±2^{a}	-	-			>0.05
Ergost-7-enol	$C_{28}\Delta^{7}$	-	1.1±0.8	-	-	-	-	-	-	-	-			>0.05
Ergosterol	$C_{28}\Delta^{5,7,22}$	-	-	-	-	-	-	1.3±0.3^{a}	2.3±0.3^{b}	-	-			<0.05
Stellasterol	$C_{28}\Delta^{7,22}$	-	2±1^{b}	-	-	0.4±0.2^{a}	0.6±0.2^{a}	2±1^{b}	0.3±0.1^{a}	1.9±0.4^{b}	1.5±0.1^{b}	8	17	0.009
24-Methylenecholesterol	$C_{28}\Delta^{5,24}$	3±1^{a}	6±3^{a}	-	-	-	-	3±3^{a}	3±2^{a}	-	-	3, 22	2.8	0.061
Dihydrobrassicasterol	$C_{28}\Delta^{5}$	-	-	-	-	0.6±0.1^{a}	1.2±0.8^{a}	-	-	-	-			>0.05
Campesterol	$C_{28}\Delta^{5}$	4±1^{a}	8±3^{b}	53±5^{c}	45±4^{c}	2.1±0.2^{a}	5±2^{b}	6±2^{ab}	4±3^{ab}	4.6±0.4^{ab}	5.3±0.1^{ab}	9, 37	116	<0.001

Stigmasterol	$C_{29}\Delta^{5}$	0.5±0.2^{a}	2±1^{a}	0.3±0.2^{a}	2±1^{a}	2±1^{a}	1±1^{a}	2±1^{ab}	3±1^{b}	6.5±0.2^{c}	7±1^{c}	9, 40	36.5	<0.001
23-24 dimethylcholest 5,7-dien-3-*β*-ol	$C_{29}\Delta^{5,7}$	2.1±0.4	-	-	-	-	-	-	-	-	-	-	-	-
24-Methylenephenol	$C_{28}\Delta^{7,24(28)}$	-	5±2^{a}	0.2±0.1^{b}	2±2^{a}	0.6±0.3^{a}	5±3^{b}	2.4±0.4^{a}	3±2^{a}	2.3±0.4^{a}	1.7±0.2^{a}	8, 30	2.7	0.025
4-24 dimethyl 5,7-dien-3-β-ol	$C_{29}\Delta^{5,7}$	-	-	-	-	0.5± 0.1^{a}	1.3±1.6^{a}	-	-	4.9±0.1^{b}	5±1^{b}			<0.050
Poriferasterol	$C_{29}\Delta^{5,22}$	-	-	-	-	0.4±0.1^{a}	0.7±0.3^{a}	-	-	4±1^{b}	3.8±0.1^{b}	3, 11	47	0.001
β-Sitosterol	$C_{29}\Delta^{5}$	6±2^{ab}	6±2^{ab}	1.1±0.8^{a}	3±1^{ab}	3.1±0.2^{ab}	5±1^{ab}	10±6^{ab}	4±2^{a}	15.4±0.3^{b}	18.3±0.3^{c}	9, 39	12.5	0.001
Sitostanol	$C_{29}\Delta^{0}$	1.2± 0.4^{a}	0.1±0.1^{a}	2±1^{a}	2±1^{a}	-	-	-	-	-	-			>0.05
4, 24 dimethyl 5α cholestan 3-β-ol	$C_{29}\Delta^{0}$	-	-	-	-	-	-	0.3± 0.1^{a}	3±3^{a}	-	-			>0.05
Isomer of fucosterol	$C_{29}\Delta^{5,24}$	-	-	-	-	-	-	-	-	1.6±0.3^{a}	1.8±0.2^{a}			>0.05
4,23,24 trimethyl 5α cholest 24(28)-en-3*β*-o**l**	$C_{30}\Delta^{24(28)}$	-	-	-	-	-	-	3± 2^{a}	2±1^{a}	-	-			>0.05
Dinosterol	$C_{30}\Delta^{22}$	1.0±0.4^{a}	0.1±0.2^{b}	0.3±0.2^{b}	0.6±0.2^{a}	-	-	-	-	-	-			<0.050
Dinostanol	$C_{30}\Delta^{0}$	-	0.3±0.5^{a}	7±6^{a}	1±1^{a}	-	-	-	-	-	-			>0.050
4,23,24 trimethyl-5α-cholest-7-en-3*β*-ol	$C_{30}\Delta^{7}$	-	-	-	-	-	-	0.4±0.1^{a}	0.9±0.2^{b}	-	-			<0.050
Gorgosterol	$C_{30}\Delta^{5}$	-	-	20±4^{a}	9±1^{b}	-	-	-	-	-	-	1, 6	11.2	0.016
Isofucosterol	$C_{29}\Delta^{5,24}$	-	-	-	-	0.7±0.2^{a}	2±1^{a}	-	-	-	-			>0.050
Fucosterolb	$C_{29}\Delta^{5,24}$									1.6±0.3^{a}	1.8± 0.4^{a}	1, 5	0.346	0.582
Cycloartenol	$C_{30}\Delta^{24}$	-	0.4±0.7	-	-	-	-	-	-	-	-			
TS (mg g^{-1} dry wt)		3±1^{a}	2±1^{a}	13±2^{b}	9±2^{b}	3±3^{a}	3±1^{a}	12±3^{b}	5±2^{a}	10±4^{b}	15±4^{b}	9, 39	13.3	<0.001

Table 2-7. Sterol composition of fish collected in the PNSAV. Values are means ± 95% confidence intervals. Superscript letters denote significant differences among columns. Boldface compounds originate in zooplankton (Zoo) and are present only in one fish taxon (Tax). TS= Concentration of total sterols per dry weight. *df*= degree of freedom, *F*= Fisher statistics or *H*= Kruskal-Wallis statistic, and *p*= Tukey's family error *p* value. When not specified statistics confidence interval were used to determine significant differences

Sterol/ season	Short formula	*A. chirurgus*		*C. personatus*		*B. rufus*		*O. chrysurus*		*C. hippos*		*df*	*F*	*p value*
		dry	rainy	dry	rainy	dry	rainy	dry	rainy	dry	rainy			
24-Nordehydrocholesterol	$C_{26}\Delta^{5,7}$	-	-	0.5 ± 0.1^{a}	0.6 ± 0.3^{a}	0.8 ± 0.7^{a}	4 ± 2^{ab}	0.3 ± 0.2^{a}	3 ± 2^{b}	0.6 ± 0.3^{a}	0.3 ± 0.2^{a}			<0.05
Trans 22 dehydrocholesterol	$C_{27}\Delta^{22}$	-	-	3 ± 2^{a}	0.8 ± 0.6^{a}	-	-	-	-	-	-			>0.05
Occelasterol	$C_{27}\Delta^{5,22}$	-	-	2.9 ± 0.2^{a}	18 ± 6^{b}	0.2 ± 0.1^{c}	0.4 ± 0.1^{d}	0.3 ± 0.2^{c}	0.9 ± 0.7^{d}	0.10 ± 0.03^{e}	0.4 ± 0.2^{d}	7	24	0.001
Cholesterol	$C_{27}\Delta^{5}$	92 ± 1^{a}	84 ± 5^{b}	83 ± 2^{b}	73 ± 6^{c}	94 ± 1^{a}	84 ± 5^{b}	96.1 ± 1.1^{d}	86 ± 4^{b}	96.3 ± 0.1^{d}	85 ± 1^{b}	9, 39	28.1	<0.001
Cholestanol	$C_{27}\Delta^{0}$	-	-	1.9 ± 0.1^{a}	1.7 ± 0.5^{a}	0.9 ± 0.2^{b}	1.0 ± 0.5^{ab}	1.0 ± 0.2^{a}	2 ± 1^{a}	0.6 ± 0.1^{b}	1.7 ± 0.6^{a}			<0.05
Brassicasterol	$C_{28}\Delta^{5,22}$	-	-	0.42 ± 0.03^{a}	0.5 ± 0.2^{a}	0.4 ± 0.3^{a}	0.5 ± 0.4^{a}	0.2 ± 0.1^{a}	0.4 ± 0.2^{a}	0.07 ± 0.04^{b}	1.0 ± 0.4^{a}	7, 29	2.8	0.020
Ergost-7-enol (Zoo)	$C_{28}\Delta^{7}$	**0.6 ± 0.3^{a}**	**4 ± 2^{b}**	**-**	**-**	**0.5 ± 0.2^{a}**	**4 ± 1^{b}**	**0.6 ± 0.3^{a}**	**0.6 ± 0.3^{a}**	**0.3 ± 0.1^{a}**	**0.5 ± 0.3^{a}**			
Stellasterol	$C_{28}\Delta^{7,22}$	0.9 ± 0.3^{a}	2.4 ± 1.8^{b}	0.8 ± 0.2^{a}	0.6 ± 0.1^{a}	-	-	0.5 ± 0.1^{a}	0.9 ± 0.6^{a}	0.2 ± 0.1^{a}	0.24 ± 0.04^{a}	8	17	0.009
24- Methylenecholesterol	$C_{28}\Delta^{5,24}$	-	-	1.8 ± 0.1^{a}	0.8 ± 0.2^{a}	0.4 ± 0.1^{a}	4 ± 2^{b}	0.5 ± 0.1^{a}	3 ± 1^{b}	0.3 ± 0.1^{a}	5 ± 1^{b}	7, 28	12.7	<0.001
Campesterol	$C_{28}\Delta^{5}$	1.2 ± 0.5^{a}	6 ± 2^{b}	2.1 ± 0.1^{a}	2.1 ± 0.3^{a}	0.8 ± 0.4^{a}	0.6 ± 0.4^{a}	0.31 ± 0.04^{c}	0.8 ± 0.4^{a}	0.5 ± 0.1^{c}	2 ± 1^{a}	9, 35	14.5	<0.001
5 α-Cholest-7-en-3β-ol (Tax) (Lathosterol)	$C_{27}\Delta^{7}$	-	-	**0.3 ± 0.1^{a}**	**0.4 ± 0.3^{a}**	-	-	-	-	-	-			>0.050
Stigmasterol	$C_{29}\Delta^{5}$	-	-	0.34 ± 0.01^{a}	0.12 ± 0.06^{b}	0.2 ± 0.1^{a}	1.4 ± 1.0^{cd}	0.2 ± 0.2^{a}	2 ± 1.5^{cd}	0.6 ± 0.1^{c}	1.2 ± 0.2^{d}			
24-Methylenephenol	$C_{28}\Delta^{7,24(28)}$	0.9 ± 0.5^{a}	3 ± 1^{b}	1.8 ± 0.2^{b}	0.3 ± 0.2^{a}	0.3 ± 0.1^{a}	0.3 ± 0.2^{a}	0.3 ± 0.1^{a}	0.6 ± 0.4^{a}	0.14 ± 0.05^{a}	0.3 ± 0.1^{a}			
β-Sitosterol	$C_{29}\Delta^{5}$	5 ± 2^{a}	0.5 ± 0.3^{b}	1.3 ± 0.1^{b}	1.0 ± 0.3^{b}	0.4 ± 0.1^{b}	0.3 ± 0.2^{b}	0.04 ± 0.02^{b}	0.6 ± 0.5^{b}	0.20 ± 0.04^{b}	3 ± 1^{c}			
Fucosterol	$C_{29}\Delta^{5,24}$	-	-	0.40 ± 0.02^{a}	-	0.08 ± 0.03^{b}	0.09 ± 0.02^{b}	-	-	-	-			
Sitostanol	$C_{29}\Delta^{0}$	-	-	-	0.2 ± 0.1	-	-	-	-	-	-			
Dinosterol	$C_{30}\Delta^{22}$	-	-	-	-	0.08 ± 0.03^{a}	0.03 ± 0.01^{b}	-	-	-	-			
Isofucosterol	$C_{29}\Delta^{5,24}$	-	-	-	-	0.04 ± 0.02^{a}	0.3 ± 0.2^{b}	-	-	-	-			
TS (mg g^{-1} dry wt)		2 ± 1^{ab}	2 ± 1^{ab}	3 ± 2^{ab}	6 ± 3^{b}	7 ± 1^{b}	1.4 ± 0.7^{a}	3 ± 2^{ab}	2 ± 1^{ab}	2 ± 1^{ab}	2 ± 1^{ab}			

Table 2-8. Dissimilarity matrix (%) based on lipid class, fatty acid and sterol profiles of zooplankton, coral, sea urchins, clams, sponges and fish collected in the PNSAV. Mcav= *M. cavernosa*, Zoo= Zooplankton, Pcar= *P. carnea*, Chip= *C. hippos*, Bruf= *B. rufus*, Achi= *A. chirurgus*, Cper= *C. personatus*, Eluc= *E. lucunter*, Ochr= *O. chrysurus*, and Aply= *Aplysina* sp. Bold numbers denote average intra-specific dissimilarity computed as 100 – average species similarity.

Average dissimilarity between/within groups considering both seasons

Species	Mcav	Zoo	Pcar	Chip	Bruf	Achi	Cper	Eluc	Ochr	Aply
Mcav	**16**	50	52	54	55	56	59	59	59	70
Zoo		**19**	38	45	39	39	45	40	45	58
Pcar			**14**	42	37	32	36	41	35	51
Chip				**12**	23	35	43	39	26	67
Bruf					**15**	26	39	34	23	65
Achi						**16**	37	38	28	57
Cper							**23**	53	32	60
Eluc								**22**	42	61
Ochr									**18**	62
Aply										**22**

Average dissimilarity between/within groups within level 'dry' of factor 'season'

Species	Mcav	Zoo	Pcar	Chip	Bruf	Achi	Cper	Eluc	Ochr	Aply
Mcav	**18**	51	55	59	58	62	61	62	67	70
Zoo		**20**	39	41	37	41	41	41	47	57
Pcar			**14**	36	34	35	32	41	35	44
Chip				**11**	16	19	17	31	16	60
Bruf					**9**	15	21	28	17	59
Achi						**11**	20	25	21	56
Cper							**7**	32	27	55
Eluc								**12**	35	57
Ochr									**14**	58
Aply										**23**

Average dissimilarity between/within groups within level 'rainy' of factor 'season'

Species	Mcav	Zoo	Pcar	Chip	Bruf	Achi	Cper	Eluc	Ochr	Aply
Mcav	**10**	48	42	52	57	53	56	55	54	61
Zoo		**16**	28	41	34	31	39	33	35	50
Pcar			**13**	44	37	30	34	36	36	57
Chip				**7**	16	28	38	29	18	65
Bruf					**11**	20	32	25	16	62
Achi						**11**	29	30	19	55
Cper							**19**	40	27	55
Eluc								**18**	31	58
Ochr									**14**	58
Aply										**23**

Table 2-9. Concentration of storage and structural lipids (mg g^{-1} dry wt). Values are mean ± standard deviation. SE/WE= Steryl and wax esters, TAG= Triacylglycerols, ST= Free sterols, AMPL= Acetone mobile polar lipids, PL= Phospholipids, SIT= β-Sitosterol, POR= Poriferasterol, FUC= Fucosterol, MEP= Methylenephenol, MEC= Methylenecholesterol, GOR= Gorgosterol, CHOL= Cholesterol D= dry season, and R= rainy season. *F*= Fisher statistic, *p*= probability value.

Primary producers	SE/WE	TAG	ST	AMPL	PL	SIT	POR FUC	MEP MEC	GOR	CHOL
R. mangle D	0.0±0.01[a]	0.6±0.5[a]	0.7±0.4[a]	8±7[ab]	11±8[ab]	0.8±0.1[c]				
T. testudinum R	0.01±0.01[a]	0.8±1.1[a]	0.4±0.2[a]	2±1[a]	4±2[a]	0.3±0.2[a]b				0.02±0.01[a]
H. opuntia D	0.01±0.01[a]	1.3±1.1[a]	0.6±0.5[a]	14±3[b]	18±8[b]	0.4±0.2[b]				0.02±0.01[a]
Galaxaura sp. D	0.1±0.07[a]	1.1±0.6[a]	0.2±0.2[a]	1.2±1.1[a]	2±1[a]	0.1±0.1[a]				0.10±0.06[a]
Dictyota sp. D	0.1±0.08[a]	1.4±0.8[a]	4±2[b]	6±2[ab]	6±2[a]		0.9±0.5			
Phytoplankton R		8±3[b]	0.9±0.5[a]	9±3[ab]	8±2[a]	0.1±0.1[a]		0.02±0.01		0.4±0.3[b]
Symbionts										
Zooxanthellae R	8.2±1.5[b]	10±5[b]	5.1±1.5[b]	15±3[b]	7±1[a]				0.8±0.3	4±1[c]
$F_{6, 26}$	174.95	15.31	12.81	3.37	6.72	13.27	16.52	3.79	42.88	146.11
p	< 0.001	< 0.001	< 0.001	0.014	< 0.001	< 0.001	<0.001	0.008	<0.001	<0.001
Invertebrates	SE/WE	TAG	ST	AMPL	PL	SIT	POR	MEP	GOR	CHOL
Herbivores										
E. lucunter D	0.2±0.1[a]	24±20[ab]	3±2[ab]	5±1[a]	24±18[b]	0.1±0.1[a]	0.01± 0.01[a]	0.01±0.01[a]		3±2[a]
E. lucunter R	0.4±0.3[a]	60±44[b]	3±1[a]	3±1[a]	13±10[ab]	0.2±0.1[a]	0.2 ± 0.1[a]	0.1±0.1[b]		2±1[a]
Planktivores/detritivores										
P. carnea D	1.2±0.7[a]	2±2[a]	3±2[ab]	2.4±1.5[a]	15±3[ab]	1.1±0.5[ab]		0.3±0.1[b]		4±2[bc]
P. carnea R	5±2[b]	4±2[a]	7±2[b]	14±3[b]	20±6[ab]	0.2±0.1[a]		0.1±0.1[b]		2±1[a]
Aplysina sp. D	0.5±0.4[a]	8±7[a]	10±3[b]	13±8[b]	26±6[b]	1.7±0.6[b]	0.6±0.1[b]	0.3±0.01[b]		3±1[b]
Aplysina sp. R	2.3±0.9[ab]	4±3[a]	11±4[b]	14±4[b]	20±15[ab]	3.4±0.1[c]	0.7±0.4[b]	0.3±0.1[b]		7±1[c]
Planktivores										
Zooplankton D	0.4±0.5[a]	13±10[a]	3±2[a]	1.4±1.1[a]	7±6[a]	0.3±0.2[a]				1.7±1.6[a]
Zooplankton R	0.2±0.2[a]	9±7[a]	2±1[a]	7.6±4.5[b]	8±4[a]	0.1±0.1[a]		0.1±0.1[b]		0.7±0.5[a]
Symbionts										
M. cavernosa D	11±4[c]	10±5[a]	1.7±0.4[ab]	13±9[b]	10±5[a]	0.2±0.1[a]		0.02±0.01[a]	2.3±0.4[a]	1.6±1.2[a]
M. cavernosa R	5.0±2.0[b]	4±2[a]	3±1[ab]	5±1[ab]	3.4±2.5[a]	0.3±0.1[a]		0.2±0.1[b]	0.9±0.1[b]	2.6±0.5[ab]
$F_{9, 39}$	31.56	4.38	10.09	5.67	5.15	38.79	72.32	9.92	13.28	7.02
p	< 0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	<0.001	<0.001	<0.001
Fish	SE/WE	TAG	ST	AMPL	PL	SIT	FUC	MEC	GOR	CHOL
Herbivores/ detritivores										
A. chirurgus D	0.2±0.1[a]	1.9±1.3[a]	1.9±0.7[a]	5.0±2.7[a]	14.9±9.0[a]	0.100[a]		0.02±0.01[a]		1.6±0.7[a]
A. chirurgus R	0	9.5±4.3[b]	2.2±0.5[a]	0.6±0.2[b]	10.9±0.7[a]	0.010[b]		0.06±0.02[b]		1.9±0.5[a]

Planktivores										
C. personatus D	0.3±0.3[a]	1.4±1.3[a]	2.6±1.2[ab]	3.8±0.1[a]	44.7±23.8[b]	0.050[b]	0.02±0.002[a]	0.07±0.01[a]		3.4±0.5[ab]
C. personatus R	2.5±1.7[a]	6.1±5.1[a]	4.6±1.9[b]	31±12[c]	48.3±18.6[b]	0.050[b]		0.04±0.02[a]		3.5±1.3[b]
Molluscivores/Echinivores										
B. rufus D	2.2±0.4[a]	5.7±2.9[a]	5.0±0.7[b]	3.0±0.6[a]	20.5±4.5[a]	0.030[b]	0.01±0.002[a]	0.03±0.01[a]		6.8±1.0[c]
B. rufus R	0	47±18[b]	1.4±0.8[a]	2.4±2.2[a]	17.6±3.9[a]	0.004[b]	0.002±0.001[b]	0.06±0.01[b]		1.1±0.6[a]
Piscivores										
O. chrysurus D	0.2±0.1a	0.5±0.2a	2.8±1.6[ab]	7.6±2.6[c]	24.6±10.4[a]	0.0013[b]		0.02±0.01[a]		2.9±1.6[ab]
O. chrysurus R	0.1±0.04[a]	36.0±22.0[b]	1.7±0.6[a]	2.6±1.9[a]	21.4±5.6[a]	0.0090[b]		0.04±0.01[a]		1.5±0.7[a]
C. hippos D	0.2±0.1[a]	3.8±2.5[a]	1.5±1.2[a]	2.6±1.9[a]	17.1±7.1[a]	0.003[b]		<0.005[a]		1.6±1.2[a]
C. hippos R	0	135±53[c]	2.4±0.6[a]	11.4±4.3[c]	19.8±7.1[a]	0.070[a]		0.04±0.01[b]		1.9±0.6[ab]
$F_{9, 26}$	1.07	14.69	4.86	7.0	5.20	7.89	119.28	4.92	-	10.69
p	0.416	< 0.001	0.001	< 0.001	< 0.001	< 0.001	<0.001	0.001	-	<0.001

Table 2-10 Pearson's correlation and (*p* values) among concentration (mg g^{-1} dry wt.) of biomarker and *de novo* sterols and storage and structural lipid classes. Asterisk denote significant correlations.

Biomarker		Invertebrates (Zooplankton, clam, sea urchin, coral and sponge)							Teleost fish (masked goby, surgeon fish, hog fish, yellowtail snapper and jack)						
Sterol	Source	WE/SE	TAG	ST	AMPL	PL	TAG:ST	Result	WE/SE	TAG	ST	AMPL	PL	TAG:ST	Result
Cycloartenol	*R. mangle*	-0.080 (0.585)	-0.041 (0.780)	-0.095 (0.515)	-0.033 (0.822)	-0.056 (0.704)	-0.017 (0.908)	no	-	-	-	-	-	-	-
Trans-22-dehydrocholesterol	*T. testudinum*	-0.118 (0.441)	0.091 (0.532)	0.123 (0.399)	-0.261 (0.070)	-0.073 (0.620)	-0.055 (0.706)	no	0.225 (0.187)	-0.166 (0.333)	0.459* (0.005)	0.271 (0.110)	0.598* (0.001)	-0.197 (0.250)	no
Stellasterol	*H. opuntia*	-0.181 (0.213)	-0.153 (0.295)	0.687* (0.001)	0.373* (0.008)	0.473* (0.001)	-0.266 (0.064)	no	0.148 (0.390)	-0.286 (0.090)	0.506* (0.002)	0.132 (0.443)	0.215 (0.208)	-0.392* (0.018)	no
Brassicasterol	*Galaxaura* phytoplankton	-0.081 (0.579)	-0.209 (0.150)	0.742* (0.001)	0.352* (0.013)	0.488* (0.001)	-0.350* (0.014)	no	0.407* (0.014)	0.052 (0.761)	0.744* (0.001)	0.498* (0.002)	0.384* (0.021)	-0.066 (0.702)	yes
Poriferasterol	*Dictyota* sp.	-0.120 (0.412)	-0.128 (0.382)	0.794* (0.001)	0.458* (0.001)	0.516* (0.001)	-0.234 (0.105)	no	-	-	-	-	-	-	-
Fucosterol	*Dictyota sp.*	-0.115 (0.431)	-0.156 (0.286)	0.795* (0.001)	0.466* (0.001)	0.510* (0.001)	-0.257 (0.074)	no	0.225 (0.188)	-0.122 (0.480)	0.406* (0.014)	-0.169 (0.326)	-0.080 (0.641)	-0.119 (0.489)	no
4-24 dimethyl 5,7 dien-3-β-ol	*Dictyota sp.*	-0.123 (0.401)	-0.115 (0.431)	0.795* (0.001)	0.460* (0.001)	0.512* (0.001)	-0.220 (0.128)	no	-	-	-	-	-	-	no
24-methylenephenol	phytoplankton	-0.070 (0.597)	-0.061 (0.678)	0.555* (0.001)	0.309* (0.031)	0.314 (0.208)	-0.195 (0.179)	no	-0.005 (0.715)	-0.292 (0.085)	0.153 (0.373)	-0.158 (0.357)	0.166 (0.332)	-0.333* (0.047)	no
Gorgosterol	zooxanthellae	0.865* (0.001)	-0.086 (0.569)	-0.200 (0.160)	0.099 (0.499)	-0.217 (0.134)	-0.010 (0.945)	yes	-	-	-	-	-	-	-
β-sitosterol	*R.mangle*, *T. testudinum* and *H. opuntia*	-0.081 (0.586)	-0.155 (0.288)	0.782* (0.001)	0.345* (0.015)	0.462* (0.001)	-0.307* (0.032)	no	0.021 (0.487)	0.141 (0.412)	0.215 (0.207)	0.262 (0.123)	0.167 (0.332)	-0.067 (0.696)	no
Occelasterol	Phytoplankton and zooxanthellae	0.186 (0.200)	-0.217 (0.135)	0.311* (0.029)	0.354* (0.012)	0.238 (0.100)	0.263 (0.068)	no	0.205 (0.230)	-0.124 (0.472)	0.644* (0.001)	0.683* (0.001)	0.543* (0.001)	-0.177 (0.301)	no
24-Methylenecholesterol	Phytoplankton and *H. opuntia*	-0.200 (0.169)	-0.055 (0.705)	-0.023 (0.877)	-0.097 (0.509)	-0.025 (0.862)	-0.162 (0.266)	no	-0.090 (0.603)	0.518* (0.001)	0.182 (0.287)	0.191 (0.264)	0.177 (0.302)	0.263 (0.121)	yes
Cholesterol	De novo, *Galaxaura*	0.011 (0.650)	0.047 (0.748)	0.620* (0.001)	0.156 (0.286)	0.517* (0.001)	-0.254 (0.074)	no	0.280 (0.098)	-0.283 (0.094)	0.816* (0.001)	0.150 (0.384)	0.317 (0.059)	-0.427* (0.009)	yes

2.10 Figures

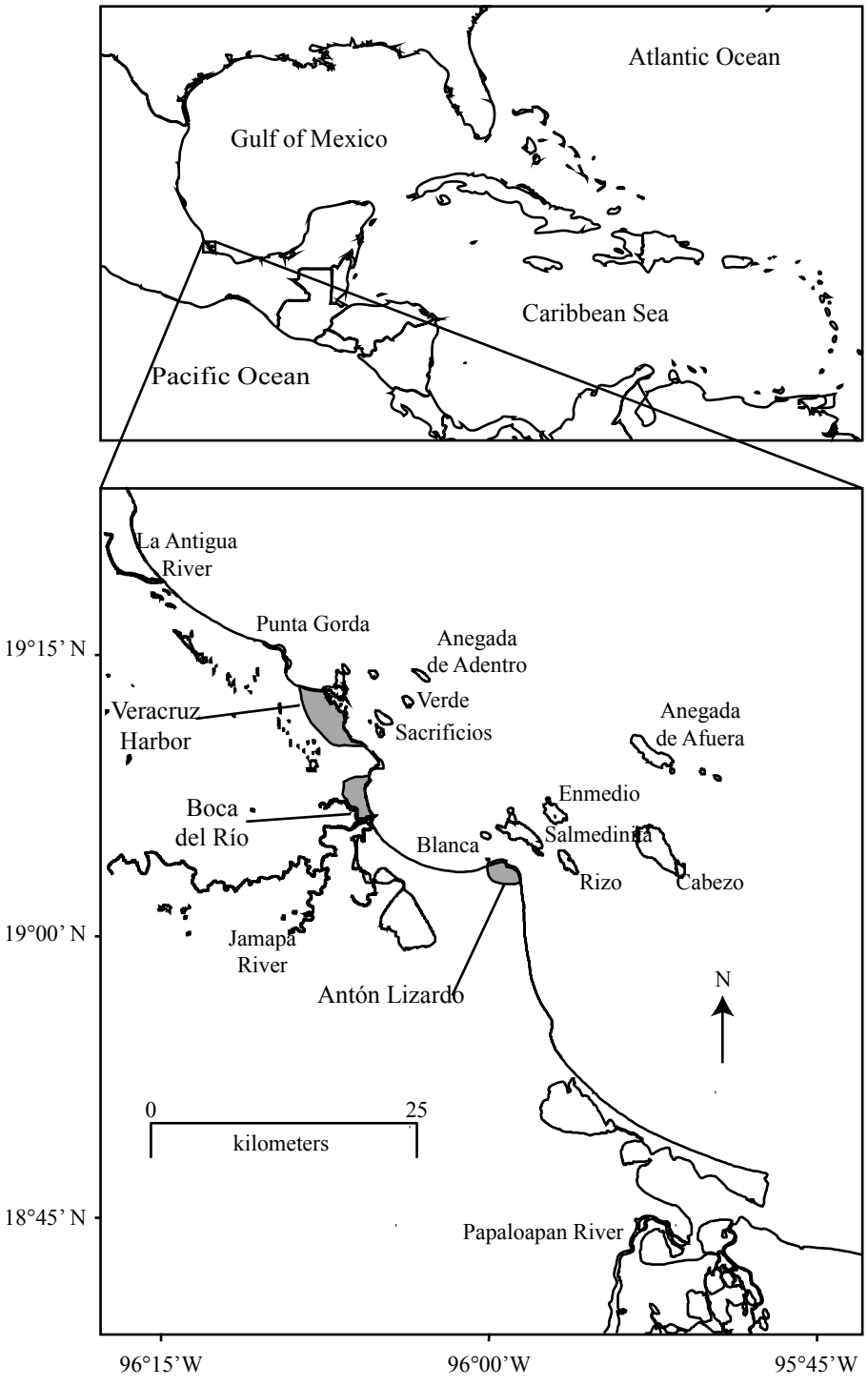

Figure 2-1. Location of the coral reef system of Veracruz in the Gulf of Mexico

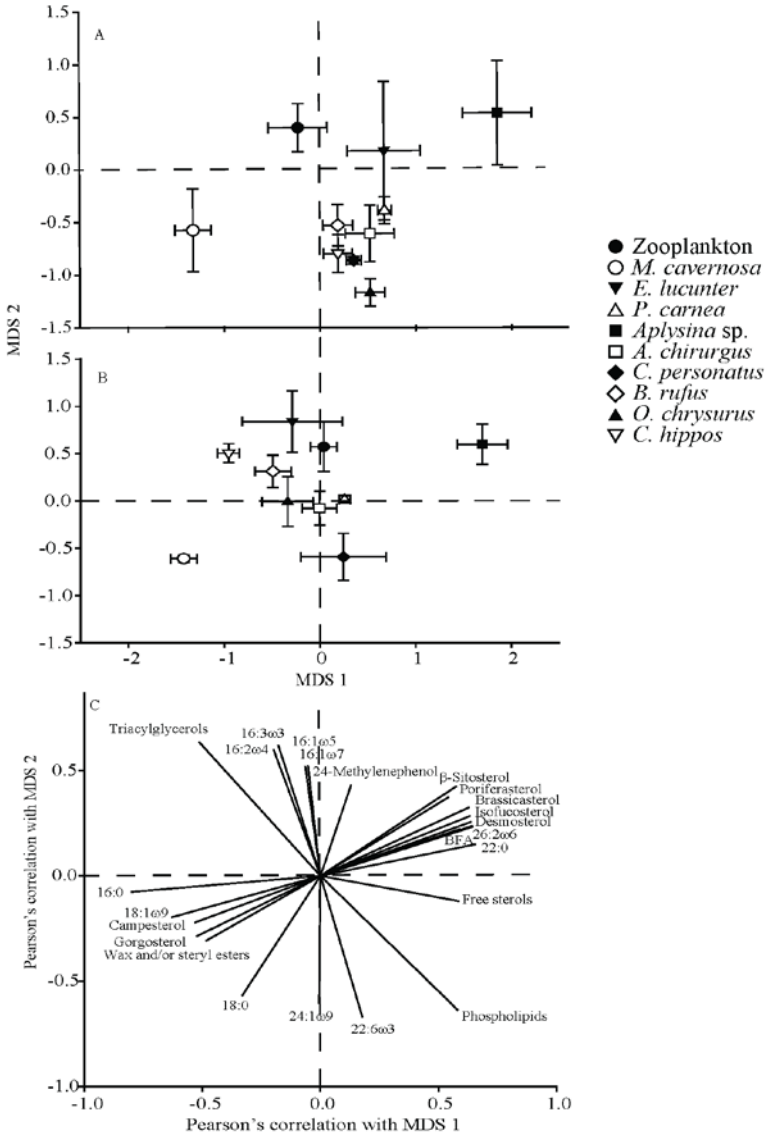

Figure 2-2. Scatter plot of non-metric multidimensional scaling (nMDS) using Bray-Curtis distance matrix for fatty acid data (expressed as a percentage of total fatty acids), sterols (expressed as percentage of total sterols) and structural and storage lipid classes (expressed as percentage of total lipids) of ten of the most abundant consumers in the coral reef ecosystem of Veracruz. Axis scales are arbitrary in nMDS. A) Scores plotted from dry season results, B) Scores plotted from rainy season results, and C) Pearson's correlation with MDS 1 and MDS 2 with values higher than 0.5. Broken lines separate the quadrants

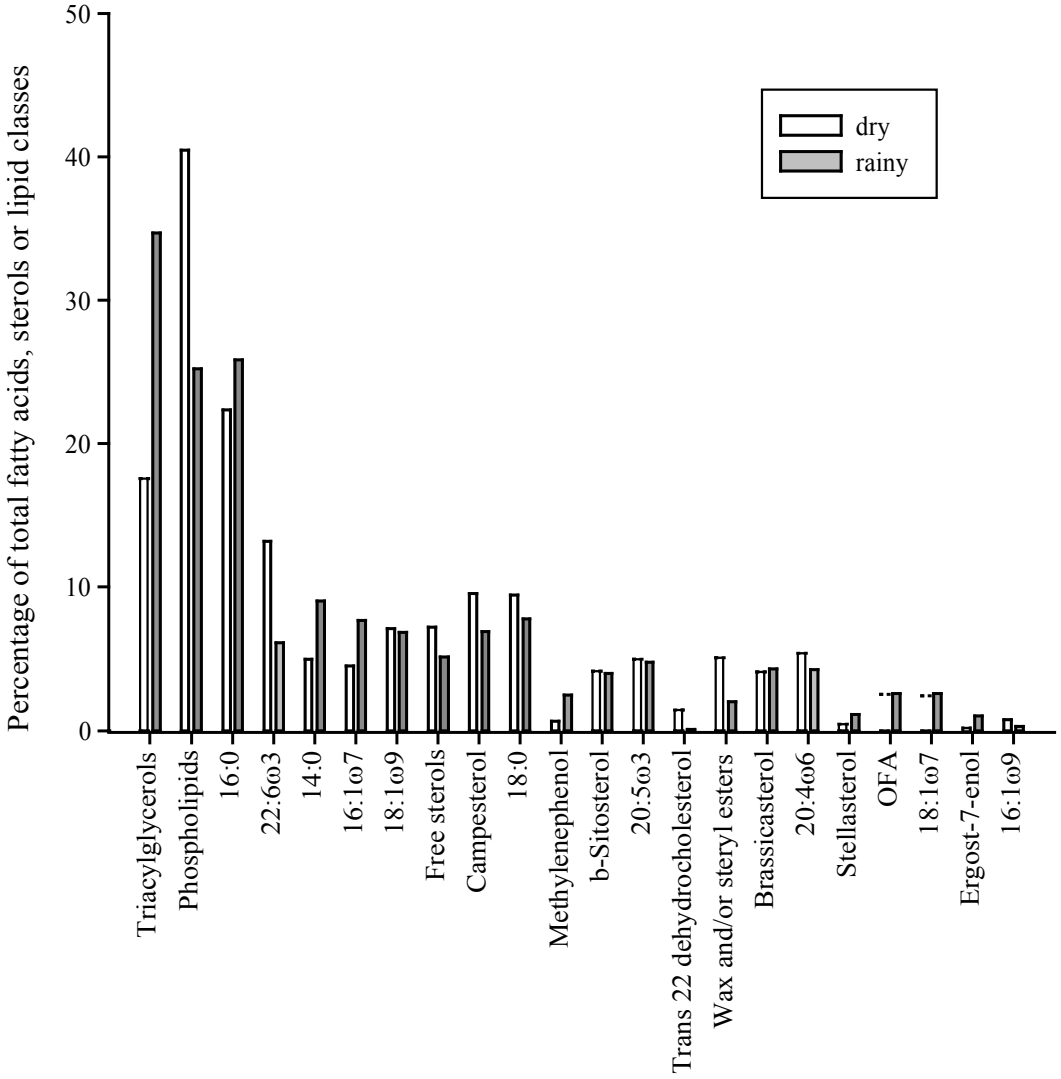

Figure 2-3. Average contribution of compounds primarily providing the discrimination between dry and rainy seasons considering all species of consumers collected in the coral reef ecosystem of Veracruz. Seasonal fatty acid, sterol and lipid class profiles showed a significant SIMPER dissimilarity of 31.4% (ANOSIM R= 0.67 p= 0.001). Cut off for low contribution of 90%.

Chapter 3 Urban Sewage Organic Carbon in the Suspended Particulate Matter of a Gulf of Mexico Coral Reef under River Influence

3.1 Abstract

The nutitional quality of suspended particulate matter SPM and the degree of human fecal pollution in a gulf of Mexico coral reef system were evaluated using lipid classes, FA and sterols in two contrasting seasons: dry and rainy. SPM had high proportions of triacylglycerols and saturated and monounsaturated FA, however it was considered poor quality because it had low proportions of essential and highly unsaturated FA. Urban sewage organic carbon was traced with coprostanol. The reference value of coprostanol that denoted contamination was set using two samples from the sewage treatment plant (STP) of Carcamo de la Zamorana in Boca del Rio city near the coral reef system of Veracruz, and it was contrasted with one Jamapa River station and nine marine stations including six coral reefs. The concentration of coprostanol in the SPM was 3621 ± 98 ng L^{-1} comprising 26% of total sterols. During the dry season, the Jamapa river was contaminated upstream with human feces with coprostanol at 1823 ng mL^{-1}, the 5β-coprostanol: cholesterol ratio at 0.5, and 5β-coprostanol: (5α-cholestanol+5β-coprostanol) at 0.7. In contrast, marine stations had concentrations of coprostanol lower than the limit, ranging between 6 and 28 ng mL^{-1}. During the rainy season a dilution effect was detected in the river, however significantly higher concentrations of coprostanol in the marine stations were detected ranging between 15 and 215 ng mL^{-1}, higher than the regulation limit for tropical marine coastal waters (30 ng mL^{-1}). Among the reefs, the Sacrificios was

more vulnerable to human-fecal pollution, and offshore reefs Anegada de Adentro, Verde, and Anegada de Afuera had a lower degree of contamination. Finally, only three stations were clearly uncontaminated during both seasons including two reefs: Enmedio and Cabezo, both located in the southern part of the PNSAV, and the site located offshore of the Jamapa River, with no presence of coprostanol. Results suggest that contamination in the rainy season comes from Anton Lizardo village and/or from the Papaloapan River. A monitoring program is necessary as part of the management program of the marine protected area PNSAV.

3.2 Introduction

Detritus can be broadly defined as any form of non-living organic matter, including different types of plant tissue (e.g. leaf litter, dead wood, aquatic macrophytes, algae), animal tissue (carrion), dead microbes, faeces (manure, dung, faecal pellets, guano, frass), as well as products secreted, excreted or exuded from organisms (e.g. extracellular polymers, nectar, root exudates and leachates, dissolved organic matter, extracellular matrices, mucilage). The relative importance of these forms of detritus in terms of origin, size and chemical composition, varies across ecosystems (Moore et al. 2004). In coral reefs, detritus discharged from rivers is considered allochthonous. When detritus is mostly algal in origin and produced *in situ* it is referred to as autochthonous, and is composed mainly of dead filamentous algae and phytoplankton, and secondarily of fleshy macroalgae, coralline algae, cyanobacteria, phytoplankton and sea grasses. Non-algal detritus is mostly congealed coral mucus bound with other particulate material (Alongi 1988). On reef slopes and crests, the material is mostly coral mucus while over reef flats

and lagoons, the material is mostly algae and fecal matter. This material, by itself, has high carbon content. However, it acts as a substrate for bacteria, ciliates, cyanobacteria, and other microorganisms that coat the particles. Bacteria can convert dissolved organic material into particulate organic material by aggregating it in particles. This provides a substantially enriched particle replete with lipids and proteins in the suspended particulate matter. As such, detritus becomes a very nutritious food source for many organisms. Lipids, essential and highly unsaturated FA and sterols, principally cholesterol, can be used to evaluate detritus nutritional quality for SPM consumers such as suspension and filter feeders, for example bivalves, sponges and coral (Anthony, 1999). However, in coral reefs under river influence, the discharge of rivers and urban sewage has been recognized as a major environmental problem since 1972. Regions where sewage pollution of coral reefs has been documented include the Red Sea (Walker and Ormond 1982), the wider Caribbean (Rodriguez, 1981), the Caroline Islands (Amesbury et al., 1976) and Hawaii (Smith et al. 1981). Biological tertiary sewage treatment on a wide scale was suggested as a measure to improve water quality to a level where recovery is possible. To date, these reefs have proved to be very resilient once the stressor has been removed (Bahr et al. 2015).

The coastal zone is the main source of foreign economic input for Mexico, and federal policies are adjusting to improve its environmental condition. The coral reef system of Veracruz is a marine protected area named Parque Nacional Sistema Arrecifal Veracruzano (PNSAV). It is the largest in the southern Gulf of Mexico, and untreated wastewater discharges (both domestic and industrial) from adjacent cities had been considered one of the major environmental problem (Ortiz-Lozano et al. 2005). The coral

reef system is located on a terrigenous platform receiving a discharge of 2.7 $x10^9$ m^3 $year^{-1}$ directly from the Jamapa River and 44.7 $x10^9$ m^3 $year^{-1}$ indirectly from the Papaloapan River (Comision Nacional del Agua 2011), the eighth and the second largest Mexican rivers flowing into the Gulf of Mexico, respectively (Comision Nacional del Agua 2011). A high sedimentation rate of 2 kg m^{-2} day^{-1} has been recorded in the reef area (Pérez-España et al. 2012). To determine the effect of river discharges on the quality of the reef SPM in the Gulf of Mexico, quality tracers such as lipid classes, FA and sterols were used. Among the lipid classes FFA and HC denote low quality. The uncombined FA or nonesterified fatty acids found in an organism usually come from the breakdown of a triglyceride (triacylglycerol), while HC markers include alkanes derived from algae or plant leaves, and polycyclic aromatic hydrocarbons (PAH) derived mainly from crude petroleum and fuel spills. PAH can also be found in combustion products of fuels such as heating oil, gasoline and wood. Coastal sediments act as the ultimate reservoirs for these compounds when they are transported unaltered through the water column (Parrish et al. 2000).

Coprostanol constitutes about 60% of the total sterols in human faeces (Ferezou et al. 1978; Leeming et al. 1996), and has been proposed as a specific indicator of anthropogenic sewage, especially in waters offshore from urbanized areas, although care must be taken in the interpretation of coprostanol concentrations in areas remote from human influences since other sources are possible (Walker et al. 1982). Coprostanol is formed in the gut of higher mammals including humans by the stereospecific reduction of the double bond of cholesterol (O'Leary et al. 1999), and mammals such as pigs, sheep, cows and cats have coprostanol in their faeces, but total concentrations and most

importantly, amounts relative to other sterols are very reduced compared to human faeces (Leeming et al. 1996).

Work using both faecal sterols and bacterial indicators has demonstrated that domestic pets, livestock, birds and native animals can be a major source of bacterial contamination in some urban creeks and rivers (Leeming et al. 1997). However, in the context of tracing anthropogenic sewage from major sewage outfalls, analyses of coprostanol referenced to its isomer 5α-cholestanol are usually sufficient to define the extent of contamination (O'Leary et al. 1999). Human waste is the principal source of 5β-coprostanol in the environment, comprising around 2-6% of the dry solids in raw, untreated sewage. This relatively high concentration and its stability allow its use in the assessment of the faecal matter in samples, especially SPM. Since 5β-coprostanol is formed from cholesterol in the digestive tract of humans by microbial biohydrogenation of cholesterol, the ratio of the product over reactant can be used to indicate the degree of human derived sewage contamination due to its persistence and scarcity in the natural environment (Leeming et al. 1996). Raw untreated sewage typically has a 5β-coprostanol / cholesterol ratio of ~10 which decreases through a sewage treatment plant (STP) down to ~2 in the discharged liquid wastewaters, thus undiluted STP wastewaters can be identified by this high ratio. As the faecal matter is dispersed in the environment, the ratio will decrease as more (non-faecal) cholesterol from animals is encountered. Grimalt and Albaiges (1990) suggested that sediment samples from the fresh/brackish water systems and the surrounding marine waters of the Ebro Delta with a 5β-coprostanol / cholesterol ratio greater than 0.2 should be considered as contaminated by faecal material.

Another indicator of human faecal contamination is the proportion of the two isomers 5α-cholestanol and 5β-coprostanol. 5α-cholestanol is formed naturally in the environment by bacteria and generally does not have a faecal origin. Samples with ratios > 0.7 may be contaminated with human faecal matter; samples with values < 0.3 can be considered uncontaminated. Samples with ratios between these two cut-offs cannot be readily categorized on the basis of this ratio alone (Grimalt et al. 1990, Bujagic et al. 2016).

Standard sanitary regulations for water quality vary among countries probably owing to greater survival of bacteria in warmer tropical waters, for instance in Japan, 1000 cells of *E. coli* per 100 mL is the limit for bathing water, whereas in Canada the limit is 200 cells per 100 mL and in the European Union the limit is 2000 cells per 100 mL (Isobe et al. 2002 and references therein). These authors proposed 30 ng L^{-1} as limit of coprostanol concentrations corresponding to 1000 cells of *E. coli* per 100 mL in tropical waters of Vietnam. The same limits were used as a reference for tropical coral reefs in the present chapter because it is consistent with the limits for indirect contact accepted by the Mexican regulation NOM-003-ECOL-1997(Diario Oficial de la Federacion 2003).

Studies indicate that the removal of coprostanol from sewage by the activated sludge process appears to be principally due to biodegradation (Dutka et al. 1974 and references therein). The removal efficiency depends on the type of sewage treatment plant. For instance, for coprostanol used as marker for sewage and agricultural contamination, a tertiary plant had a removal of 99%, while a secondary treatment plant had a removal of 86% according to Furtula et al. (2012). Thus the finding of any traces of coprostanol, and perhaps cholesterol in raw water would be indicative of relatively fresh fecal pollution and therefore the presence of potential health hazards (Dutka et al. 1974).

The seasonality of river discharges to the coral reef ecosystem provided an opportunity to assess how load variation related to season, conditions that modulate the fate of sewage organic carbon, might influence SPM nutritional quality and the degree of contamination across a range of locations including coral reefs in the PNSAV.

I hypothesize that southern locations will be affected by the Jamapa river with lower nutritional quality and higher amounts of coprostanol during the dry season when the current flows southward, and that the opposite occurs during the rainy season when northern locations will receive lower quality and more sewage contamination under the influence of the Papaloapan and the Jamapa rivers when the current flows northward.
In order to test this hypothesis the objectives of this chapter are:
1) To evaluate the nutritional quality of particulate organic matter in a Gulf of Mexico coral reef under river influence using lipid class, fatty acid and sterol profiles, during the dry and rainy season and
2) To determine the degree of human faecal pollution in the particulate organic matter of Veracruz coral reefs during dry and rainy seasons using the coprostanol/cholesterol and coprostanol/(coprostanol + cholestanol) ratios and the coprostanol concentration previously suggested for tropical environments.

3.3 Material and methods

3.3.1 Study site

The PNSAV is located off Veracruz, Mexico. It is adjacent to the cities of Veracruz, Boca del Rio and Anton Lizardo, in the Southwest Gulf of Mexico, between 19° 02’ 24.00’’ and 19° 15’ 27.11’’ N and between 96° 12’ 01.00’’ and 95° 46’ 46.19’’ W (Diario

Oficial de la Federación 2012), and it is part of a larger coral reef system that includes the Caribbean and the Gulf of México (Fig. 3-1). A group of 13 reefs is located adjacent to the Veracruz and Boca del Rio cities in the north and another group of 15 reefs with larger structures is located adjacent to Antón Lizardo village in the south; both groups are divided by the Jamapa River (Fig. 1), and delimited to the North by La Antigua River and to the south by the Papaloapan River. During winter they are typically affected by frontal incursions of northerly systems locally known as 'nortes' which produce winds of 120 km h^{-1} and monthly precipitation is 34±15 mm. During spring, precipitation decreases which is therefore was considered the dry season and the marine current runs southward increasing the influence of La Antigua river in the northern reefs and from the Jamapa river in the southern reefs. During summer (the rainy season), the atmospheric conditions are dominated by tropical storms from the south which produce high monthly precipitation rates of 265±99 mm and the current systems runs northward (Zavala-Hidalgo 2003) increasing the influence of the Jamapa river to the northern reefs and the Papaloapan river to all the PNSAV but principally to the southern reefs.

3.3.2 Sampling methods

The study was carried out during dry and rainy seasons in May and October 2008, respectively. Stations include two stations in the Boca del Rio city sewage system, one upstream station in the Jamapa River, and nine stations in the PNSAV. Water collection was part of the zooplankton and phytoplankton net tows sampling of Chapter 1, therefore no replicates were collected in order to complete the cruise track of the vessel in the same day. One sample of 4 L was collected at each station located in the Zamorana channel and

pumping sump before and after the sewage treatment plant (STP) in May 2008 as a reference for contamination source. The PNSAV stations included three reefs from the northern part (Anegada de Adentro, Isla Verde and Sacrificios); three reefs from the southern part (Anegada de Afuera, Cabezo and Enmedio); one station at the mouth of the Jamapa River, one offshore from the Jamapa River (between Anegada de Adentro and Anegada de Afuera reefs) and one in front of Antón Lizardo village. Locations were organized according to the distance to the pumping sump (Carcamo de la Zamorana) that releases water to the mangrove forest named Lagos de Moreno after passing through the sewage treatment plant (STP) that received domestic sewage water from the channel named La Zamorana. This station was named 'before STP' and it was located 0.44 km upstream. The river station was located 7.4 km upstream, while the river mouth station was located 7.0 km down the Jamapa River, and the station offshore from the river was located 10.6 km from the source (Fig. 3-1). Northern reefs were significantly closer to the sewage point at 15.8±1.5 km, while the southern reefs were significantly further, located at 26.1±6.4 km (Table 3-1).

One water sample, taken from a boat using a plastic bottle, was collected from the surface at each station. Bottle samples were kept on ice in the dark for around five hours until arrival at the laboratory. Between 0.5 and 4 L of seawater were filtered under low vacuum through Whatman glass fiber filters (GF/F) with a diameter of 47 mm and a nominal pore size of 0.7 μm. It was not possible to take subreplicates because of the concentration limit of the gas chromatograph mass detector (GC-MS) of 10 μg mL^{-1}.

Filters were previously ashed at 480°C for 24 hours and weighed until constant weight according to Saliot et al. (2002).

3.3.3 Laboratory methods

After rinsing with distilled water, GF/F filters were freeze dried on a Lyophilizer Virtis 5L. Freeze-dried samples were weighed in order to measure dry SPM concentration (Zhu and Lee 1997). Then the filters were lipid extracted with chloroform: methanol: water 2:1:0.8 (Folch et al., 1957 modified by Parrish 1999), and total lipid extracts were divided in two. The first one was separated into lipid classes by Chromarod thin layer chromatography (Parrish, 1987). Concentrations of lipid classes were obtained with calibration curves constructed with five concentrations, ranging between 0.5 and 4.0 µg, of the following standards: nonadecane, cholesteryl sterate, 3-hexadecanone, tripalmitin, palmitic acid, cetyl alcohol, cholesterol, monopalmitin, and phosphatidylcholine (Sigma-Aldrich). The rest of the lipid extract was derivatized to obtain methyl esters with hydrochloric acid and methanol 5:95 heated to 85°C for 2.5 hours (Sato and Murata, 1988). FAME were recovered in 0.5 mL of pure hexane and analyzed in a gas chromatograph with a flame ionization detector (GC-FID) Agilent 6890, with an Omegawax 250 column (Agilent Technologies) of 30 m x 0.25 mm x 0.25 µm (Appendix 1). FAME quantification was computed by adding the areas, each sum was considered as 100%, and an individual FAME was calculated as a proportion of the total identified FAME. After injection of FAME hexane extracts were dried with nitrogen and silylated with 100 µL of bis-trimethyl silyl-trifluoro acetamide (BSTFA) Supelco: 3-2024 (Copeman and Parrish, 2004).

Sterols from all samples were recovered in 0.1 mL of hexane and analyzed in a Hewlett Packard 6890 GC-MS with a DB-5 column 30 m x 0.32 mm x 0.25 µm. Peaks were identified by retention time of standards and mass spectra interpretation in a GC-MS (Appendix 2). Areas were integrated with Wsearch 32 software (Wsearch 2008; version 1.6 2005). Sterol proportions were determined with a Varian GC-FID. Areas were integrated with the Galaxie software and each sterol area was calculated as the proportion of the total area. This proportion was related to the free sterol fraction of dry biomass to obtain coprostanol concentration per unit of dry biomass (ng mg^{-1}) and to the filtered volume (ng L^{-1}).

3.3.4 Data analysis

Seasonal changes in depth of Sechi disk visibility (as a proxy of turbidity), salinity, temperature, and SPM concentration were determined with the one way ANOVA Fisher statistic (F) using Minitab software version 15. Location groups of stations had four levels: source, north, middle and south, while season had two levels dry *vs* rainy seasons. Lipid class, fatty acid and sterol profiles (Tables 3-2, 3-3 and 3-4, respectively) were used in the non-metric multidimensional scaling (nMDS) analyses (Figs. 3-2 and 3-3). Multivariate analyses of lipid class, fatty acid, and sterol profiles of all stations were performed using PRIMER software version 6.1.16. and PERMANOVA^{+} version 1.0.6 (PRIMER-E, Plymouth, UK). Non-metric multi-dimensional scaling (MDS) was conducted based on a Bray-Curtis similarity coefficient. No transformation was used to avoid artificial weighting of fatty acids, sterols and lipid classes that made only trace contributions to their respective profiles. Similarities among locations by season were

investigated using the similarity percentages (SIMPER) function. SIMPER identifies the compounds primarily providing the discrimination between two observed station clusters. Statistical differences were tested with one way analysis in dry and rainy seasons independently using the analysis of similarity a permutation-based hypothesis testing (ANOSIM), which tests for differences between groups of (multivariate) samples from different seasons and locations (significance level < 5% or $p < 0.05$). Significant differences were confirmed with a permutational multivariate analysis of variance (PERMANOVA). PERMANOVA analysis allows multivariate comparisons with data without a normal distribution of residuals. The method of permutation of residuals under a reduced model was used. Hierarchical cluster analysis was used to detect stations with 70% of similarity.

Samples were grouped according to location (Table 3-1), except for the source group which was split into sewage and river upstream; groups were defined as follows: 1) sewage with before and after treatment stations, 2) river upstream, 3) north with Sacrificios, Anegada de Adentro and Isla Verde reefs, 4) middle with Jamapa mouth and Jamapa offshore stations, and 5) south with Enmedio, Anegada de Afuera, and Cabezo reefs, and Anton Lizardo offshore.

Polluted localities were detected using coprostanol concentration per filterd volume (L) and per mg of SPM, and the coprostanol: cholesterol and coprostanol: [coprostanol+cholestanol] ratios. For the latter, the limits to consider stations as polluted were 0.2 and 0.7, respectively. Stations with coprostanol: [coprostanol+cholestanol] ratios lower than 0.3 were considered uncontaminated according to Grimalt et al. (1990). Limits of the coprostanol concentration were set according to Isobe et al. (2002).

Those stations higher than 30 ng L^{-1} were plotted as $\log_{10}$ of the concentration to facilitate viewing small concentrations (Fig. 3-5). The ratio coprostanol: cholesterol was plotted against the coprostanol: [coprostanol+cholestenol] ratio (Fig. 3-6).

3.4 Results

3.4.1 Nutritional quality of suspended particulate matter using lipid classes, fatty acids and sterols

Salinity was significantly different in the sewage channel and upstream river compared to the other locations at zero salinity units, except in the river upstream at 5 in the dry season, while it ranged between 25 and 29 practical salinity units (PSU) in the Jamapa River mouth, and from 34 to 37 PSU in the north and south reefs with no significant differences (Table 3-1). Temperature was significantly higher in the dry season in the sewage source and river locations at 32°C compared with 27 to 29°C in the middle, north and south stations in the dry and rainy seasons ($F_{3,8}$=80.37, p= 0.001, Table 3-1). Turbidity exhibited significant differences between sewage and river (n=3) at 1.8±1.0 m compared to northern stations (n=3) at 8.3±1.4 m and southern stations (n=4) at 24.1±10.4 m in the dry season ($F_{3,8}$=7.36, p=0.011). In the dry season the river mouth and the offshore stations had intermediate turbidity (visibility) 13.3±1.8 m, while in the rainy season the river mouth had high turbidity with visibility to a depth of 1.4 m in contrast to the low turbidity detected in the station offshore from the river with 34.4 m visibility ($F_{3,8}$=4.18, p=0.047). Concentrations of SPM were significantly higher in the group named ‘source’ that includes before and after STP and upstream stations at

26.5±8.8 mg L^{-1} and the group including the 'south' stations at 12.8±7.1 mg L^{-1} compared with northern reefs at 1.4±1.3 mg L^{-1} in the dry season ($F_{3,8}$=8.34, p=0.008). Unfortunately, sewage samples were not collected in the rainy season, however in the upstream river station, SPM decreased from 21.5 mg L^{-1} in the dry season to 3.5 mg L^{-1} in the rainy season (Table 3-1).

Loads of lipids in the SPM decreased from 1.7 mg L^{-1} in the sewage channel before treatment, with TAG and FFA as major lipid classes, to 1.0 mg L^{-1} after treatment, with PL and HC as major lipid classes (Table 3-2). The lowest SPM lipids of 0.02 mg L^{-1} and 0.01 mg L^{-1} were recorded at Anton Lizardo offshore in the dry season and in the upstream river station in the rainy season, respectively. In addition, marine stations had significantly higher proportions of hydrocarbons 18.9±7.5% of total lipids in the dry season than in the rainy season 7.3±3.7% ($F_{1, 16}$= 7.43, p= 0.015). This was especially true in Anton Lizardo offshore with ten times more in the dry season at 44% compared to 4% in the rainy season. The source of HC could be related to the army base located in Anton Lizardo town. In contrast the lipid concentration was lower in the dry season at 0.04±0.02 mg L^{-1} than in the rainy season with 0.11±0.05 mg L^{-1} ($F_{1,16}$=6.71, p=0.02). In addition, FA 16:0 had significantly higher proportions in the rainy season (Table 3-3). Highly unsaturated FA was present in low proportions while saturated FA were present in higher proportions (Table 3-3). Sewage had a higher proportion of coprostanol and stigmasterol, while the river, middle, northern and southern stations had higher proportions of 24-methylenecholesterol and brassicasterol (Table 3-4).

There were no significant differences among location groups in the dry season (ANOSIM R= 0.117, p= 0.225). SIMPER results showed that lipid class, FA, and sterol

profiles had high variability among stations considered in the sewage and middle location groups resulting in low internal similarities at 53% and 55%, respectively. While north and south location groups had higher intra-group similarities at 78% and 61%, respectively. The similarity matrix showed lower similarities among sewage and river, middle, north and south stations from 44 to 58%. In contrast, river and north stations had the highest similarity at 71% (Table 3-6).

Sewage samples were characterized by high proportions of cholesterol, FFA, coprostanol, phospholipids and *cis* vaccenic 18:1ω7. However, there were some differences. The sample collected upstream of the STP station showed higher proportions of FFA, as well as stigmasterol and 18:3ω3 characteristic of plant material or green algae. While the sample collected downstream of the STP station showed higher proportions of phospholipids, cholesterol and 18:1ω9. The river upstream station was characterized by high proportions of campesterol, triacylglycerols, 24-methylenecholesterol, 16:0 and 18:0. A similarity, derived from a hierarchical cluster analysis, of 70% was detected between stations based on distance to the coast rather than a northern *vs* southern pattern (Figs. 3-1 and 3-2). For instance, the river mouth and Anton Lizardo offshore were grouped; Verde and Sacrificios reefs were grouped, as well as Anegada de Adentro and Enmedio (Fig. 3-2). Compounds with Pearson correlations with MDS 1> 0.6 that characterize the latter were 24-methylenecholesterol, and AMPL often associated with chloroplasts. The distant reef Anegada de Afuera was separated also by arachidonic acid (20:4ω6), while the river offshore and Cabezo reef were grouped with high proportions of TAG and free sterols (Fig. 3-2).

As in the dry season, in the rainy season there were no significant differences among location groups (ANOSIM R= 0.051, *p*= 0.385). Sewage locations were the same as in the dry season with a low internal similarity of 53%. SIMPER results showed that the internal similarity of middle locations was 60%. While north and south locations had higher intra-group similarities of 77% and 64%, respectively. The similarity matrix showed lower similarities among sewage and river, middle, north and south stations around 58%. In contrast, river *vs* north and river *vs* south stations had higher similarities of 82% and 72%, respectively (Table 3-5). Unlike in the dry season, according to the cluster analysis in the rainy season, the stations were not grouped by the distance to the coast. The after STP station was grouped with the river offshore station at 70% similarity with high proportions of phospholipids and the monounsaturated FA 18:1ω9. Verde and Anegada de Afuera reefs were grouped also at 70% with high proportions of 18:2ω6 with a Pearson correlation with MDS 1 of 0.4. Anton Lizardo offshore was separated, and the rest of the stations were grouped with 70% similarity with high proportions of sitosterol, campesterol, TAG, and 24-methylenecholesterol (Fig. 3-3).

According to the PERMANOVA anaysis there were no significant differences between seasons with *p*(perm)= 0.058 and the interaction between location and season was not significant with a *p*(perm)= 0.689. However a sigificant difference was detected among locations between sewage and north with a similarity of 59% or a dissimilarity of 41% *p*(perm)= 0.022. The compounds with higher contributions to the significant separation were FFA, coprostanol, phospholipids, 18:1ω7, cholesterol, stigmasterol, HC and 18:3ω3 for the sewage location. In contrast higher levels of TAG, AMPL, campesterol, 18:0, 24-methylenecholesterol, 16:0, brassicasterol and campestanol were

detected in the north part of the PNSAV (Fig. 3-4). Among these compounds coprostanol and cholesterol were better biomarkers of sewage, while 24-methylenecholesterol and campesterol were significantly higher in the north part of the PNSAV when univarariate analyses were performed (Table 3-4).

3.4.2 Human fecal pollution in the particulate organic matter of the Veracruz coral reefs

Different indicators of human fecal pollution were computed to identify polluted stations. Total sterol decreased from 646 ng mg^{-1} before the STP to 386 ng mg^{-1} after treatment, and coprostanol from 168 ng mg^{-1} to 100 ng mg^{-1} suggesting a removal efficiency of 60%. Stations from the sewage system had high concentrations of coprostanol of 3621 ± 98 ng L^{-1}. River upstream had half the concentration at 1823 ng L^{-1} showing a 1:1 dilution in the river during the dry season. While in the rainy season a strong dilution was recorded in the river upstream giving a concentration of 9 ng L^{-1}. In contrast, the river mouth had a high concentration of 215 ng L^{-1} (Table 3-6). This is an order of magnitude lower than sewage stations, so this is not high (as it scales with the sewage) but compared to the limit of coprostanol suggested by Isobe et al. (2002) in the tropical waters of Vietnam which is 30 ng L^{-1}, it allows me to consider the river mouth contaminated. Ratios used in this work provide source information. Cholesterol is predominantly reduced to coprostanol in humans, so to measure this relationship the ratio coprostanol: cholesterol is used, whereas in the environment its reduction to cholestanol (5a-cholestan-3b-ol) is preferential. So to measure this relationship the ratio coprostanol: [coprostanol+cholestenol] is used. The coprostanol: cholesterol ratio decreased from 0.9

before the STP to 0.5 after the STP similar to that in the river upstream station. During the dry season the river mouth, Anegada de Adentro, and Anton Lizardo offshore were at the limit (0.2), and the rest were considered uncontaminated. In contrast in the rainy season only the river mouth was contaminated (Table 3-6). Using the coprostanol: [coprostanol+cholestenol] ratio (Fig. 3-6), there was no difference between the stations before and after treatment coinciding with the coprostanol concentrations. The river upstream station was contaminated according to all indicators in the dry season. There were three stations uncontaminated in both seasons: two reefs Enmedio and Cabezo, and the river offshore station with no presence of coprostanol (Fig. 3-6).

In order to obtain a limit value expressed in terms of dry biomass (ng mg^{-1}) instead of concentration per volume (ng L^{-1}), a significant regression ($F_{1,17}$= 13.8, p= 0.002, r^2= 44.8) and a Pearson correlation (r= 0.952, p< 0.001) was computed between the concentration per dry weight of SPM versus the concentration related to volume (Eq. 1). In a first attempt I had three extreme outliers that were having a large effect on the slope and intercept. Also the relationship between the two variables was quite different at the lower end of both axes. Since the extrapolation I did (30ng L^{-1}) is on the lower end of my first graph, I removed the outliers on the extreme end, and I recalculated the regression equation and re-extrapolated the 30ng L^{-1} value.

Equation 1

Cop DW (ng mg^{-1}) = 2.48 + 0.0999 Cop V (ng L^{-1})

Where Cop DW is the concentration of coprostanol per dry weight expressed in ng per mg, and Cop V is the concentration of coprostanol per volume expressed as ng per L of filtered water. In Mexico 1000 cells of *E. coli* per 100 mL is the limit for bathing water

coliforms per 100 mL equivalent to 30 ng L^{-1} of coprostanol. Doing the substitution in equation 1 of 30 ng L^{-1} the limit value was 5.5 ng mg^{-1} of SPM (Fig. 3-7 and Table 3-6).

3.5 Discussion

3.5.1 Nutritional quality of suspended particulate matter using lipid classes, fatty acids and sterols

The concentration of SPM in river and sewage before treatment were similar to that reported in coral reef lagoons in the Great Barrier Reef in Australia, around 20 mg L^{-1} (Anthony, 1999). These concentrations are below the limits set by the Mexican environmental law NOM-001-SEMARNAT-1996 at 150 mg L^{-1} (DOF 2003). After treatment, the SPM was twice as concentrated. However, a clear dilution was observed once the sewage water arrived at the river mouth. As I hypothesized, SPM of the Jamapa River was similar to southern reefs in the dry season. Also, similar SPM concentrations were detected in the southern reefs, consistent with a coastal current running to the south in the dry season (Zavala-Hidalgo, 2003),

Another quality measure is the concentration of fats and oils (total lipids), with a limit set at 15 mg L^{-1} by the Mexican regulation NOM-001-SEMARNAT-1996 (DOF, 2003). This concentration was between 1 and 1.7 mg L^{-1} for the sewage SPM, suggesting a low contribution of fats and oils in waste water. However, the high proportion of FFA suggests an active triacylglycerol breakdown probably due to bacteria or detergents. Bacteria TAG breakdown is a common process in sewage in treatment plants resulting in FFA (50–55%) and fatty soaps (26–32%), these are not only the main components, but they were also easily separable from the starting waste (Pastore et al. 2015). The amount

of FFA and the FA profile are important because it could be a sustainable source of biofuels. The chemical activation of this fatty waste could be accomplished by converting the starting soaps into the respective FFA using formic acid as activator, coproducing the relevant formates. The activated fatty matter can then be converted into biofuel through direct esterification (di Bitonto et al. 2016). The respective FFA have a different profile (mainly oleic acid) with respect to the soapy fraction (saturated fatty acids are dominant) coincident with the FA profile of the treatment plant of this study. However, chloroplast-associated lipids such as AMPLs at 5% suggest that FA 18:2ω6 and 18:3ω3 apportionments are coming not just from waste fats and oils (vegetable oils) but from green microalgae (Chlorophyta) which has 18:3ω3 as the main FA (Rivero-Rodriguez et al. 2007).

The ANOSIM statistic R was low for both seasons, R= 0.117 in the dry season and 0.051 in the rainy season, suggesting no differences among groups; and the probability value was not significant. The PERMANOVA analysis confirmed that there were no significant differences between seasons, and in the interaction of season and locations. There are two reasons that could explain the lack of significant interaction (Table 3-5). The first is that the number of replicates (stations) for each location was low and it is necessary to increase the sampling sites. The second reason that explains a low R was likely because lipid class, fatty acid and sterol profiles were not related to the geographical location i.e. north *vs* south. In fact, the groups formed in the dry season were related to the distance to the coast. The second reason seems more probable because this grouping is consistent with the separation of two water masses proposed by Ortiz-Lozano et al. (2009): 1) The, shore line entrainment volume (SEV) which includes the river

mouth, Anton Lizardo offshore, Verde and Enmedio reefs, and 2) the offshore entrainment volume (OEV) coincident with the river offshore and Cabezo. During the rainy season, marine stations were more similar among middle, north and south reefs, and the river upstream, suggesting a mixed condition probably related to the flow increase of the river.

However, when both seasons were considered in the analysis there was a significant difference between sewage and the reefs of the north. The quality of SPM can explain the significant difference, in particular 24-methylenecholesterol and campesterol were not present in sewage and can reach around 10% and 5%, respectively in the northern reefs, suggesting diatom contributions to the SPM. Diatom contributions to northern reefs SPM was confirmed with significantly higher proportions of 20:5ω3 (1.7%) than the other locations (Table 3-3). In contrast, the proportion of 20:5ω3 in southern reefs (0.5%) suggests that there are no living cells but decomposing organic matter in which sterols are more stable. In contrast, coprostanol and FFA can reach around 30% in the sewage stations.

During both seasons, the river upstream station had sterols characteristic of diatoms such as 24-methylenecholesterol and brassicasterol. According to Chapter 2, the sterol 24-methylenecholesterol is distinctive for phytoplankton, and this sterol has been especially reported in centric diatoms (Bittar et al. 2013). This result is coincident with the diatom consortium isolated from the Sordo River located in Xalapa, Veracruz (Olguín et al. 2013). The consortium was formed mainly of a population of *Nitzchia frustulum* and to a lesser extent of *Navicula* sp. It showed a significantly higher specific growth rate when cultivated in water from the river compared to cultures in synthetic modified diatom

medium. The diatom consortium was able to remove 96% of ammonia nitrogen, 60% of nitrates and 95% of phosphates from the polluted river (Olguín et al. 2013).River diatoms could arrive at the coral reefs and be consumed by the coral reef community. Corals can consume SPM and the assimilation efficiency of corals is inversely related to the SPM concentration. Low concentrations of 1 mg L^{-1} allow higher assimilation efficiency according to Anthony (1999), and a range between 1.4 and 6.4 mg L^{-1} was detected in the northern reefs in both seasons and in the southern reefs in the rainy season.

According to my results, the SPM in the dry season is characterized by chloroplast associated lipids, sterols such as 24-methylenecholesterol (diatoms) and TAG mainly composed of saturated and monounsaturated FA. In contrast, during the rainy season the southern reefs had significantly higher concentrations of SPM at 13±7 mg L^{-1}, composed of TAG, 24-methylenecholesterol, and plant sterols such as sitosterol and campesterol. Sitosterol is also produced by microalgae; however, according to Butler et al. (2000) carbon-stable isotope evidence suggests that most sitosterol in the ocean is related to terrestrial sources. According to Zavala-Hidalgo et al. (2003) the marine current runs north during the rainy season and the terrestrial tracers could be transported from the Anton Lizardo village and/or from the Papaloapan River. A broader monitoring network is necessary in future research programs to include the Papaloapan River effect.

3.5.2 Human fecal pollution in the particulate organic matter of the Veracruz coral reefs

The coprostanol concentration was used as a proxy for human-fecal pollution for two reasons: Firstly, because compared to bacteria, which are highly affected by natural

factors such as temperature, salinity, and sunlight (Chou and Liu 2004; Devane et al. 2006), sterols are more resistant to environmental stress, which make them more suitable as indicators (Leeming et al. 1997; Tyagi et al. 2009 and references therein). Secondly, because it can be related to coliforms in tropical waters (Isobe et al. 2002).

Waste water discharge from Boca del Rio and Veracruz to the Jamapa River was 34 x 10^6 m^3 y^{-1} (Sistema de Agua y Saneamiento Metropolitano de Veracruz, 2005) while the treatment plant treated a volume of 4.8 x 10^6 m^3 y^{-1} in 2009, 14% of the waste water (INEGI Anuario Estadístico de Veracruz de Ignacio de la Llave, 2010). This deficient treatment was detected in the samples collected before and after STP even if there were no replicates to perform a statistical analysis. Total sterol decreased with a removal efficiency of 60%. However, no decrease in the coprostanol concentration by volume was observed because biomass concentration increased almost twice from 21 mg L^{-1} before the STP to 37 mg L^{-1} after the STP, suggesting that the treatment plant was overloaded. As a consequence, half the coral reefs had some degree of coprostanol present.

The removal efficiency is inversely related to the amount of biomass according to Furtula et al. (2012). Therefore, after treatment, coprostanol concentration surpassed the limit set at 30 ng L^{-1} by almost 120 times, revealing a deficient treatment.

The upstream river water was also contaminated in the dry season by 60 times the limit. However, this high concentration was not detected at the river mouth; the latter could be explained by the re-mineralization of organic matter and subsequent CO_2 emission from the water column in the mangrove forest which has been suggested by Borges et al. (2005). In contrast, during the rainy season the river was not contaminated upstream, so probably the rain had a dilution effect. However, the river mouth, Sacrificios

reef and Anton Lizardo offshore were contaminated. The presence of coprostanol in Sacrificios reef and the Jamapa River mouth is coincident with the current circulation to the north in the rainy season, suggesting that the PNSAV has different sources of contamination depending on the season. During the dry season from the Boca del Rio STP and Jamapa River, and in the rainy season it comes from from Anton Lizardo village and/or from the Papaloapan River.

During the development of this thesis, sampling was conducted to obtain phytoplankton, zooplankton and SPM, therefore the number of stations and the sample size was constrained to the points of intended movement for the research vessel in one day. Therefore statistical comparison within stations was not performed. Because the significance level is very dependent on the number of replicates in the comparison, I suggest designing a monitoring program with a broader area, but collecting only water samples and performing manual filtration on board. A total of 28 reefs should be sampled as well, to increase the number of samples per station before and after the sewage treatment plant of Boca del Rio and Veracuz cities.

3.6 Conclusion

Despite the presence of a treatment plant in the discharge of the Carcamo de la Zamorana to the Jamapa River, the total sterols and coprostanol removal efficiency in 2008 was low. However, during the rainy season a dilution effect allowed the reduction of waste water pollution, except in the littoral current zone with contamination from the Jamapa River and Antón Lizardo town. This current reached most of the beaches, decreasing their quality for tourism resulting in low values of the sustainability index

especially in Boca del Rio according to sustainability criteria reported by Arceo and Granados-Barba (2010). Also, the Sacrificios reef was more vulnerable to human fecal pollution and Anegada de Adentro, Verde and Anegada de Afuera reefs had some degree of contamination. A monitoring program is necessary as part of the management program of the marine protected area PNSAV. This monitoring program would evaluate if the recently (January 2013) inaugurated treatment plant in the Veracruz harbor-Boca del Rio region with tertiary treatment will reduce the levels of anthropogenic pollution from Boca del Rio and Veracruz cities. In addition, the construction of a tertiary treatment plant in Anton Lizardo village is necessary.

3.7 Acknowledgments

Thanks go to Cipriano Anaya and Yuri Okolodkov from the Instituto de Ciencias Marinas y Pesquerías, Universidad Veracruzana (ICIMAP-UV), for the field support, to Guadalupe Bautista from the Aquarium of Veracruz for the lab vacuum facilities, to Bertha Olivia Arredondo Vega from the Laboratory of Biotechnology of Microalgae at the Centro de Investigaciones Biologicas del Noroeste (CIBNOR), for the lyophilizer facilities and to Linda Windsor and Jeanette Wells from the Core Research Equipment & Instrument Training Network (CREAIT) at Memorial University of Newfoundland (MUN) for their technical advice on sterols and lipid classes, respectively. The Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico, and the Veracruz state government cofinanced the Fondo Mixto (FOMIX) Project ‘‘Fuentes orgánicas de carbono y nitrógeno y su función sobre la estructura trófica en el Sistema Arrecifal Veracruzano’’ (Fomix-Veracruz 37567). Analytical work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC). L.C.P. was a recipient of a Ph.D. fellowship from CONACYT (grant number 117304) and from the School of Graduate Studies at MUN.

3.8 Bibliography

Alongi D. M. 1988. Detritus in coral reef ecosystems: Fluxes and fates, p. 29–36. In J. H. Choat et al [eds.], Proceedings of the sixth international coral reef symposium, Townsville, Australia, Vol 1. Executive committee.

Anthony K. N. R., 1999. Coral suspension feeding on fine particulate matter. J. Exp. Mar. Biol. Ecol., 232: 85–106

Amesbury, S. S., Tsuda, R. T. Randall, R. H. Birkeland, C. E., Cushing, F. A. 1976. Limited current and underwater biological survey of the Donitsch Island sewer outfall site, Yap, western Caroline Islands, University of Guam Marine Laboratory, Technical Report No. 24.

Arceo, P. and Granados-Barba A. 2010. Evaluating sustainability criteria for a marine protected area in Veracruz, Mexico. Ocean Coast. Manage. 53: 535-543.

Bahr K. D., P. L. Jokiel and R. J. Toonen. 2015. The unnatural history of Kāneʻohe Bay: coral reef resilience in the face of centuries of anthropogenic impacts. http://dx.doi.org/10.7717/peerj.950

Bittar T. B., Y. Lin, L. R. Sassano, B. J. Wheeler, S. L. Brown, W. P. Cochlan, and Z. I. Johnson. 2013. Carbon allocation under light and nitrogen resources gradients in two model marine phytoplankton. J. Phycol. 49: 523-535.

Borges, A.V., B. Delille, and M.Frankignoulle. 2005. Budgeting sinks and sources of CO_2 in the coastal ocean: Diversity of ecosystems counts. Geophys. Res. Lett. 32, L14601. doi:10.1029/2005GL023053.

Bujagic I. M., S. Gruji, Z. Jaukovi, M. Lausevic. 2016. Sterol ratios as a tool for sewage pollution assessment of river sediments in Serbia. Environ. Pollut. 213: 76-83. http://dx.doi.org/10.1016/j.envpol.2015.12.036

Butler E, J. Parslow, J. K. Volkman , S. Blackburn, P. Morgan., J. Hunter, L. Clementson, N. Parker, R. Bailey, K. Berry, P. Bonham, A. Featherstone, D. Griffin, H. Higgins, D. Holdsworth, and V. Latham, R. Leeming, T. McGhie, D. McKenzie, R. Plaschke, A. Revill, M. Sherlock, L. Trenerry, A. Turnbull, R. Watson, L. Wilkes, 2000. Huon Estuary Study – Environmental research for integrated catchment management and aquaculture. FRDC Final Report Project No. 96/284. Fisheries Research and Development Corporation, Canberra

CNA, 2011. Atlas del Agua en México 2011. Comisión Nacional del Agua, Gobierno Federal. México, D.F. 133 pp. [Water National Comission] (available at

http://www.conagua.gob.mx/CONAGUA07/Publicaciones/Publicaciones/SGP-18-11.pdf). (consulted on 5 December 2012).

Chou, C. C., and Liu, Y. P. 2004. Determination of fecal sterols in the sediments of different wastewater outputs by GC MS. Intern. J. Environ. Anal. Chem., 84: 379–388.

Copeman L. A. and C. C. Parrish. 2004. Lipids classes, fatty acids, and sterols in seafood from Gilbert Bay, southern Labrador. J. Agric. Food Chem., 52(15): 4872–4881.

Devane, M., Saunders, D., and Gilpin, B. 2006. Faecal sterols and fluorescent whiteners as indicators of the source of faecal contamination. Chem. New Zealand, 74–77.

Diario Oficial de la Federación, Diario Oficial México a 14 de enero de 1998. Norma Oficial Mexicana NOM-003-ECOL-1997 [Mexican Regulation for contaminant limits].

Diario Oficial de la Federación 2012. Diario Oficial México. Tercera Sección p. 1-14. Jueves 29 de Noviembre de 2012.

di Bitonto L., A. Lopez, G. Mascolo, G. Mininni, C. Pastore. 2016. Efficient solvent-less separation of lipids from municipal wet sewage scum and their sustainable conversion into biodiesel. Renewable Energy 90: 55-61. http://dx.doi.org/10.1016/j.renene.2015.12.049

Dutka B. J., A. S. Y. Chau and J. Coburn. 1974. Relationship between bacterial indicators of wáter pollution and fecal sterols. Water Research (8): 1047-1055.

Furtula V., J. Liu, P. Chambers, H. Osachoff, C. Kennedy and Joanne Harkness. 2012. Sewage treatment plants efficiencies in removal of sterols and sterol ratios as indicators of fecal contamination sources. Water Air Soil Pollut, 223:1017–1031 DOI 10.1007/s11270-011-0920-8

Folch, J., M. Lees and G. H. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226:497-509.

Grimalt, J.O. and Albaigés, J. 1990.Characterization of the depositional environments of the Ebro Delta (western Mediterranean) by the study of sedimentary lipid markers. Mar. Geol., 95: 207-224

Grimalt J. O., P. Ferninder, J. M. Bayona, and J. Albaigis. 1990. Assessment of fecal sterols and ketones as indicators of urban sewage inputs to coastal waters. Environ. Sci. Technol., 24(3): 357-363.

INEGI 2010. Anuario Estadistico de Veracruz de Ignacio de la Llave. Instituto Nacional de Estadistica Geografia e Informatica [Statistical Anuarium of Veracruz de Ignaciao de la Llave].

Isobe, K. O. T.Mitsunori, N. M. P. Zacaria, H. Nguyen. L. Y. Minh, and H. Takada. 2002. Quantitative application of fecal sterols using gas chromatography-mass spectrometry to investigate fecal pollution in tropical waters: western Malaysia and Mekong Delta, Vietnam. Environ. Sci. Tech., 36: 4497-4507

Leeming, R., Ball, A., Ashbolt, N., and Nichols, P. 1996. Using faecal sterols from humans and animals to distinguish faecal pollution in receiving waters. Water Res., 30: 2893–2900.

Leeming, R., Latham, V., Rayner, M., and Nichols, P. 1997. Detecting and distinguishing sources of sewage pollution in Australian inland and costal eaters and sediments. ACS Symposium Series, 671, 306–319.

Moore,J. C., E. L. Berlow, D. C. Coleman, P. C. de Ruiter, Q. Dong, Al.Hastings, N. C. Johnson, K. S. McCann, K. Melville, P. J. Morin, K. Nadelhoffer, A. D. Rosemond, D. M. Post, J. L. Sabo, K. M. Scow, M. J. Vanni and Diana H. Wall. 2004. Detritus, trophic dynamics and biodiversity. Ecol. Lett. 7: 584–600. doi: 10.1111/j.1461-0248.2004.00606.x

O'Leary, T., R. Leeming, P. D. Nichols and J. K. Volkman. 1999. Assessment of the sources, transport and fate of sewage-derived organic matter in Port Phillip Bay, Australia, using the signature lipid coprostanol. Mar. Freshwater Res. 50: 547-556

Olguín, E. J., A. Mendoza, R. E. González-Portela and E. Novelo. 2013. Population dynamics in mixed cultures of *Neochloris oleoabundans* and native microalgae from water of a polluted river and isolation of a diatom consortium for the production of lipid rich biomass. New Biotechnol, 30(6): 705-715.

Ortiz-Lozano, L., A. Granados-Barba, V. Solís-Weissa, M. A. García-Salgado. 2005. Environmental evaluation and development problems of the Mexican Coastal Zone. Ocean Coast. Manage. 48: 161‑176

Ortiz-Lozano L. A. Granados-Barba, I. Espejel. 2009. Ecosystemic zonification as a management tool for marine protected areas in the coastal zone: Applications for the Sistema Arrecifal Veracruzano National Park, Mexico. Ocean Coast. Manage. 52: 317–323

Parrish, C. C. 1987. Separation of aquatic lipid classes by Chromarod thin layer chromatography with measurements by Iatroscan flame ionization detection. Can. J. Fish. Aquat. Sci. 44: 722–731, doi:10.1139/f87-087

Pastore, C. M. Pagano, A. Lopez, G. Mininni, and G. Mascolo. 2015. Fat, oil and grease waste from municipal wastewater: characterization, activation and sustainable conversion into biofuel. Water Sci. Technol. 71 (8) 1151-1157; DOI: 10.2166/wst.2015.084

Pérez-España, H., J. Santander-Monsalvo, J. Bello-Pineda, R.S. Gómez-Villada, J. A. Ake-Castillo, M. A. Lozano-Aburto, M. A. Román-Vivés, M. Marín-Hernández. 2012. Caracterización ecológica del Parque Nacional Sistema Arrecifal Veracruzano. In: Sánchez, A.J., Chiappa-Carrara, X., Brito-Pérez, R. (Eds), Recursos acuáticos costeros del sureste, Vol. II. FOMIX Yucatán-RECORECOS-UNAM Sisal, Mérida, Yucatán, México, pp. 581-601. [Ecologycal characteriztion of coral reef system of Veracruz PNSAV].

Rodríguez S., A. R. Beaumont, M. C. Lora-Vilchis. 2007. The effect of microalgal diets on growth, biochemical composition, and fatty acid profile of *Crassostrea corteziensis* (Hertlein) juveniles. Aquaculture 263 (2007) 199–210.

Rodríguez, A. 1981. Marine and coastal environmental stress in the wider Carbibbean region. Amblo 10: 283-294

Saliot A., C. C. Parrish, N. Sadouni, I. Boulobassi, J. Fillaux and G. Cauwet. 2002. Transport and fate of Danube delta terrestrial organic matter in the northwest black sea mixing zone. Mar. Chem. 79: 243-259.

Sato N. and N. Murata. 1988. Membrane lipids. Meth. Enzymol. 167: 251–259, doi:10.1016/0076-6879(88)67027-3

Sistema de Agua y Saneamiento Metropolitano de Veracruz, 2005. Análisis fisicoquímico, bacteriológico y de gasto de las plantas de tratamiento del área de Veracruz. Dirección de Operación Hidráulica [Physicochemichal, bacteriological and cost analyses of sewage tretment plants in the Veracruz area].

Smith, S. V., W. J. Kirnmerer, E. A. Laws, R. E. Brock, T. W. Walsh. 1981. Kaneohe Bay sewage diversion experiment: perspectives on ecosystem responses to nutritional perturbation. Pacif. Sci. 35:279-402.

Tyagi, P., D. R. Edwards and S. M. Coyne. 2009. Distinguishing between human and animal sources of fecal pollution in waters: a review. Inter. J. Water. 5: 15–34.

Walker, R. W., C. K. Wun, and W. Litsky. 1982. Coprostanol as an indicator of fecal pollution, Paper No. 2402, Massachusetts Agriculture Experiment Station, University of Massachusetts, Amherst.

Walker, D. I., and R. F. G. Ormond. 1982. Coral death from sewage and phosphate pollution at Aqaba, Red Sea. Mar. Pollut. Bull. 13: 21-25

Wsearch software version 32 was downloaded from http: www.wsearch.com.au in 01 February 2008.

Zavala-Hidalgo, J., S. L. Morey, and J. J. O'Brien. 2003. Seasonal circulation of the western shelf of Gulf of Mexico using a high-resolution numerical model. J. Geophys. Res. 108: 1-19.

Zhu, C.J. and Y.K. Lee. 1997. Determination of biomass dry weight of marine microalgae. J. Appl. Phycol. 9: 189-194.

3.9 Tables

Table 3-1. Distance to the sewage source, depth of Sechi disk (turbidity or visibility), salinity, temperature, and suspended particulate matter (SPM) in the PNSAV. STP= Sewage treatment plant. Letters denote significant differences among location groups. *F*= Fisher statistic, and *p*= probability value, significant differences with $p < 0.05$.

Station	Location group	Latitude North	Longitude West	Distance to source (Km)	Salinity		Temperature (°C)		Sechi Disk Depth (m)		SPM (mg L^{-1})	
					Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy
Before STP	Sewage	19°7'22.8"	96°7'49.8"	0.44	0	-	32	-	1.5	-	21.3	-
After STP	source	19°7'9.48"	96°7'53.62"	0	0	-	32	-	1.0	-	36.5	-
River upstream	(n=3)	19°5'42.5"	96°8'22.2"	7.4	5	0	32	28	3.0	0.8	21.5	3.5
Source mean				2.6[a]	1.7[a]	-	32[a]	-	1.8[a]	-	26.5[a]	-
SD				4.2	2.9	-	0	-	1.0	-	8.8	-
Jamapa mouth	Middle	19°6'9.6"	96°4'57.2"	7.0	25	29	29	25	14.5	1.4	10.1	10.4
Jamapa offshore	(n=2)	19°10'26.9"	96°1'1.3"	10.6	36	35	29	29	12.0	34.4	9.5	5.3
Middle mean				8.8[ab]	30.5[b]	32.0[b]	28.8[b]	27.0[b]	13.3[ab]	17.9[ab]	9.8[ab]	7.8[b]
SD				2.6	7.8		0.4	2.8	1.8	23.4	0.5	3.4
Sacrificios	North	19°10'7.1"	96°5'33.9"	14.3	36	37	29	29	7.5	15.8	2.9	5.9
Anegada de Adentro	(n=3)	19°14'16.5"	96°4'16.1"	15.7	37	36	29	29	7.5	41.1	0.4	4.4
Isla Verde		19°11'27.3"	96°3'24"	17.3	35	34	29	29	10.0	23.5	0.8	6.3
North mean				15.8[b]	36.0[b]	35.7[b]	29.0[b]	28.8[b]	8.3[ab]	26.8[b]	1.4[b]	5.5[b]
SD				1.5	1.0	1.5	0	0.3	1.4	13.0	1.3	1.0
Enmedio	South	19°7'48"	95°56'41.4"	21.8	35	37	29	29	35.0	32.3	8.6	6.3
Anegada de Afuera	(n=4)	19°10'39.5"	95°52'14.3"	23.8	35	35	28	29	11.5	29.3	6.1	7.2
Cabezo		19°2'43.1"	95°49'25.4"	35.6	37	36	28	29	30.0	24.1	13.9	6.1
Anton Lizardo		19°3'2.88"	95°56'28.7"	23.1	35	35	29	28	20.0	24.7	22.2	6.1
South mean				26.1[b]	35.5[b]	35.8[b]	28.5[b]	28.4[b]	24.1[b]	27.6[b]	12.8[a]	6.4[b]
SD				6.4	1.0	1.0	0.6	0.5	10.4	3.9	7.1	0.5
$F_{(3,8)}$				16.41	80.37	289.6	17.96	0.96	7.36	4.18	8.34	1.84
p				0.001	< 0.001	< 0.001	0.002	0.470	0.011	0.047	0.008	0.241

Table 3-2. Lipid class composition of suspended particulate matter (GF/F 0.7 µm) collected in the PNSAV. BT= before treatment, AT=after treatment, HC= Hydrocarbons, SE/WE= Steryl and/or wax esters, KET= Ketones, TAG= Triacylglycerols, FFA=Free fatty acids, ALC=Alcohols, ST=Sterols, AMPL=Acetone mobile polar lipids, PL=Phospholipids, TL= Total lipids, dw= dry weight. SD= Standard deviation. Superscript letters denote significant differences (α=0.05)

Season	Station	HC	SE/WE	KE	TAG	FFA	ALC	ST	AMPL	PL	TL µg mg^{-1} dw	TL mg L^{-1}
Dry	Sewage BT	4	0	0	10	54	2.2	0.8	5	25	78	1.7
	Sewage AT	24	0	0	2	3	1.0	1.4	9	78	28	1.0
	Source mean (SD)	14 (14)^{a}			6(6)^{a}	28(36)^{a}	2(1)^{a}	1.1(0.4)^{a}	7(2)^{a}	52(38)^{a}	53(36)^{a}	1.4(0.4)^{a}
	River upstream	3^{b}	0.2^{a}	0	42^{a}	5^{a}	0.5^{a}	2.8^{a}	22^{a}	25^{a}	66^{a}	1.4^{a}
	Jamapa mouth	19	0	0	9	21	0	0.4	36	16	8	0.08
	Jamapa offshore	13	0.6	3.8	40	9	6	1.8	13	13	4	0.04
	Middle mean (SD)	16(4)^{a}	0.3(0.4)^{a}	1.9(2.7)^{a}	24(22)^{a}	15(8)^{a}	3(4)^{a}	1.1(1.0)^{a}	24(16)^{a}	15(2)^{a}	6(3)^{b}	0.06(0.03)^{b}
	Sacrificios	16	1.2	0	31	9	1.3	3.0	21.7	17	11	0.03
	Anegada Adentro	6	0	0	16	14	0.7	0.8	10.1	53	57	0.02
	Isla Verde	11	1.3	2.0	18	7	0.7	2.2	31.3	27	19	0.02
	North mean (SD)	11(5)^{a}	0.8(0.7)^{a}	0.7(1.2)^{a}	21(8)^{a}	10(4)^{a}	0.9(0.3)^{a}	2.0(1.1)^{a}	21(11)^{a}	32(19)^{a}	29(24)^{ab}	0.02(0.01)^{b}
	Enmedio	14	0	0	4	23	0	0.8	1	58	2	0.02
	Anegada Afuera	31	0	0	3	27	0.9	0.9	28	9	13	0.08
	Cabezo	17	10	1	25	5	1.3	1.1	12	27	2	0.03
	Anton Lizardo	44	0	0	9	22	1.4	0.9	8	16	1	0.02
	South mean (SD)	26(13)^{a}	3(5)^{a}	0.2(0.4)^{a}	10(10)^{a}	19(10)^{a}	0.9(0.6)^{a}	0.9(0.1)^{a}	12(11)^{a}	28(22)^{a}	4(6)^{b}	0.02(0.01)^{b}
Rainy	River upstream	14.8^{a}	0.1^{a}	0.02	21.5^{a}	33.5^{a}	2.7^{a}	0.5^{a}	11.3^{a}	15.5^{a}	22.0	0.01^{b}
	Jamapa mouth	3.5	4.9	1.4	19	48	0.3	3.6	8	11	28	0.25
	Jamapa offshore	3.6	1.0	0.1	8	8	1.1	1.0	3	75	8	0.04
	Middle mean (SD)	3.6(0.1)^{a}	3.0(2.8)^{a}	0.8(0.9)^{a}	14(8)^{a}	28(29)^{a}	0.7(0.6)^{a}	2.3(1.8)^{a}	6(4)^{a}	43(45)^{a}	18(15)^{ab}	0.15(0.15)^{b}
	Sacrificios	3	0	0.2	34	30	0.4	0.7	19	13	38	0.2
	Anegada Adentro	9	0	0.1	26	43	0.1	0.9	7	14	24	0.1
	Isla Verde	4	0.1	0.4	18	17	0.1	1.6	20	40	10	0.1
	North mean (SD)	5(3)^{a}	0.03(0.06)^{a}	0.2(0.1)^{a}	26(8)^{a}	30(13)^{a}	0.2(0.2)^{a}	1.1(0.5)^{a}	7(10)^{a}	22(15)^{a}	24(14)^{ab}	0.1(0.1)^{b}
	Enmedio	9	0.4	0	10	36	2.5	3	20.9	18	7	0.05
	Anegada Afuera	8	0.1	0	12	9	1.0	1	2.8	67	16	0.10
	Cabezo	21	3.9	0.2	33	13	0	9	10.1	10	4	0.03
	Anton Lizardo	4	0.6	0	69	12	0	3	5.5	7	19	0.12
	South mean (SD)	11(7)^{a}	1.3(1.8)^{a}	0.1(0.1)^{a}	31(27)a	17(13)^{a}	0.9(1.2)^{a}	4.0(3.7)^{a}	10(8)^{a}	25(28)^{a}	12(7)^{ab}	0.08(0.04)^{b}
	$F_{8,13}$	1.98	0.41	0.92	1.36	0.64	0.91	0.97	1.05	0.44	3.01	30.22
	p	0.132	0.892	0.528	0.257	0.730	0.537	0.499	0.453	0.876	0.038	<0.001

Table 3-3. Fatty acid (FA) composition of suspended particulate matter (GF/F 0.7 μm) collected in the coral reef ecosystem of Veracruz. BT= Before treatment, AT=After treatment, BFA= Branched FA, AL= Acyl lipids (μg mg^{-1} dry weight), SD= Standard deviation. Superscript letters denote significant differences (α=0.05).

Season	Station	16:0	18:0	16:1ω9	16:1ω7	18:1ω9	18:1ω7	18:2ω6	18:3ω3	20:4ω6	20:5ω3	22:6ω3	BFA	AL
Dry	Sewage BT	17	5	0.7	14.7	7	27	3	7.9	0.5	0.6	0.7	4	73
	Sewage AT	31	1	7.0	0.2	22	0.0	3	0.1	0.1	0.1	0.1	5	20
	Source mean (SD)	24(10)^{b}	3(3)^{a}	4(4)^{a}	7(10)^{a}	15(11)^{a}	14(19)^{a}	3(0)^{a}	4(6)^{a}	0.3(0.3)^{a}	0.4(0.4)^{ab}	0.4(0.4)^{a}	5(1)^{a}	47(37)^{a}
	River upstream	33^{ab}	11	6.3	4.6	13	1	6	0.6	0.1	0.6^{a}	1.1	7	62^{a}
	Jamapa mouth	35	6	0.4	17	5	4	2	1	0.2	2	1	5	7
	Jamapa offshore	28	11	2.6	4	17	2	16	1	0.1	1	1	4	3
	Middle mean (SD)	32(5)^{ab}	9(4)^{a}	2(2)	11(9)^{a}	11(8)^{a}	3(1)^{a}	9(10)^{a}	1(0)^{a}	0.2(0.1)^{a}	1.5(0.7)^{a}	1(0)^{a}	5(1)^{a}	5(3)^{b}
	Sacrificios	33	11	6	5	13	1.4	6	1	0.1	1	1	7	9
	Anegada Adentro	30	13	3	6	17	2.5	7	1	0.1	1	1	5	53
	Isla Verde	29	8	1	8	22	0.1	3	1	0.5	2	5	6	16
	North mean (SD)	31(2)^{ab}	11(3)^{a}	3(3)^{a}	6(2)^{a}	17(5)^{a}	1(1)^{a}	5(2)^{a}	1(0)^{a}	0.2(0.2)^{a}	1.3(0.6)^{a}	2(2)^{a}	6(1)^{a}	26(24)^{ab}
	Enmedio	36	11	0.3	8.2	10	3	1	1	0.2	0.8	1.5	9	1.9
	Anegada Afuera	20	0	0.1	0.1	2	0	5	4	3.2	0.0	0.0	0	8.8
	Cabezo	23	9	2.6	2.8	22	2	25	2	0.0	0.4	0.2	4	1.3
	Anton Lizardo	30	12	7.1	5.1	12	2	4	1	0.1	0.5	1.2	10	0.4
	South mean (SD)	27(7)^{b}	8(5)^{a}	2.5(3.3)^{a}	4(3)^{a}	12(8)^{a}	2(1)^{a}	9(11)^{a}	2(1)^{a}	0.9(1.6)^{a}	0.4(0.3)^{b}	0.7(0.7)^{a}	6(5)^{a}	3(4)^{b}
Rainy	River upstream	44^{a}	9	0.1	13	13	0	3^{a}	2	0.3^{a}	2^{a}	1^{a}	4	18^{ab}
	Jamapa mouth	39	39	4	1	4	1	1	0.3	0.0	0.0	0.3	7	24
	Jamapa offshore	38	14	5	5	19	2	4	0.5	0.0	0.7	0.3	4	7
	Middle mean (SD)	39(1)^{ab}	27(18)^{a}	5(1)^{a}	3.0(2.8)^{a}	12(11)^{a}	1.5(0.7)^{a}	3(2)^{a}	0.4(0.1)^{a}		0.4(0.5)^{ab}	0.3(0)^{a}	6(2)^{a}	16(12)^{ab}
	Sacrificios	39	31	5	3	9	1	4	1	0.0	0	0	4	36
	Anegada Adentro	42	9	4	10	14	2	3	2	0.0	1	1	5	22
	Isla Verde	43	2	3	7	20	3	9	2	0.2	1	1	4	9
	North mean (SD)	41(2)^{a}	13(16)^{a}	4(1)^{a}	7(4)^{a}	14(6)^{a}	2(1)^{a}	5(3)^{a}	2(1)^{a}	0.1(0.1)^{a}	0.7(0.6)^{ab}	0.7(0.6)^{a}	4(1)^{a}	22(14)^{ab}
	Enmedio	41	31	8	0	8	0.1	1	0.2	0.0	0.3	0.2	5	6
	Anegada Afuera	28	9	4	5	25	2.7	16	1.5	0.0	0.5	0.4	3	15
	Cabezo	34	13	10	5	16	1.3	3	0.5	0.3	0.9	0.6	6	2
	Anton Lizardo	34	7	0	1	0	39	11	0.3	0.0	0.3	0.3	2	18
	South mean (SD)	34(5)^{ab}	15(11)^{a}	6(4)^{a}	2.8(2.6)^{a}	12(11)^{a}	11(19)^{a}	8(7)^{a}	0.6(0.6)^{a}	0.1(0.2)^{a}	0.5(0.3)^{b}	0.4(0.2)^{a}	4(2)^{a}	10(8)^{ab}
	$F_{8,13}$	3.04	0.91	0.63	0.98	0.15	0.51	1.25	0.91	0.42	2.97	1.08	0.33	2.64
	p	0.036	0.546	0.739	0.491	0.994	0.828	0.347	0.539	0.888	0.040	0.432	0.96	0.058

Table 3-4. Sterol fraction composition (% total sterols) of suspended particulate matter (GF/F 0.7 μm) collected in the coral reef ecosystem of Veracruz. TS= Total sterols (ng mg^{-1} dry weight), SPT= Sewage plant treatment and SD= Standard deviation. Superscript letters denote significant differences (α=0.05). *F*= Fisher's statistic and *p*= probability value.

Station	Coprostanol		Cholesterol		Cholestanol		24-methylene-cholesterol		Brassicasterol		Campesterol		Stigmasterol		Sitosterol		Total Sterols (ng mg^{-1})	
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy
Before SPT	26	-	30	-	8	-	0	-	2	-	4	-	22	-	6	-	646	-
After SPT	26	-	54	-	6	-	0	-	1	-	3	-	2	-	6	-	386	-
Source mean	26^{a}		42^{ab}		7^{a}		0^{b}		2^{a}		4^{c}		12^{a}		6^{a}		516^{b}	
SD	0	-	17	-	1	-		-	1	-	1		14	-	0	-	184	-
Jamapa upstream	5^{b}	2^{b}	9^{b}	41^{ab}	2^{a}	7^{a}	12^{a}	8^{a}	3^{a}	9^{a}	49^{a}	11^{bc}	3^{a}	7^{a}	0^{a}	10^{a}	1880^{a}	108^{bc}
Jamapa mouth	5	2	22	63	7	3	7	5	10	3	15.5	3.0	7	3	15	12	37	1024
Jamapa offshore	0	0	58	71	26	16	0	0	16	13	0.0	0.0	0	0	0	0	77	76
Middle mean	2^{b}	1^{b}	40^{ab}	67^{a}	16^{a}	10^{a}	3^{ab}	3^{ab}	13^{a}	8^{a}	8^{bc}	2^{c}	4^{a}	2^{a}	8^{a}	6^{a}	57^{c}	550^{bc}
SD	3	1	25	6	13	9	5	4	4	7	11	2	5	2	11	8	28	670
Sacrificios	3	2	30	35	16	9	7	10	4	5	12	12	9	8	8	12	342	255
Anegada Adentro	6	1	29	44	9	8	7	5	6	12	17	5	8	5	7	6	426	211
Isla Verde	0	3	36	45	7	5	9	6	12	7	14	5	0	5	9	11	407	148
North mean	3^{b}	2^{b}	31^{b}	41^{ab}	11^{a}	7^{a}	8^{a}	7^{a}	7^{a}	8^{a}	15^{b}	7^{bc}	6^{a}	6^{a}	8^{a}	10^{a}	392^{b}	205^{bc}
SD	3	1	4	6	4	2	1	3	4	4	3	4	5	2	1	3	44	54
Enmedio	0	0	22	55	8	5	5	6	5	3	19	6	11	5	19	11	19	206
Anegada Afuera	3	2	27	41	4	9	8	7	4	5	16	6	4	8	12	9	122	126
Cabezo	0	0	48	47	8	7	5	7	6	5	9	5	7	5	11	9	20	331
Anton Lizardo	4	3	24	37	18	9	9	9	6	6	12	11	15	5	11	8	7	571
South mean	2^{b}	1^{b}	30^{b}	45^{ab}	10^{a}	7^{a}	7^{a}	7^{a}	5^{a}	5^{a}	14^{b}	7^{bc}	9^{a}	6^{a}	13^{a}	9.0^{a}	42^{c}	309^{bc}
SD	2	2	13	5	7	1	2	1	1	1	4	3	5	2	1	0.4	63	223
$F_{8,13}$	32.81		2.99		0.74		4.08		2.15		13.05		0.8		1.25		8.34	
p	<0.001		0.039		0.660		0.012		0.106		<0.001		0.617		0.347		<0.001	

Table 3-5. Similarity matrix (%) based on SIMPER analyses of lipid class, fatty acid and sterol profiles of suspended particulate matter collected in the different locations of the coral reef ecosystem of Veracruz. Bold numbers denote similarity equal or higher than 70%. No significant differences were detected among location groups whitin seasons.

Season	Dry (ANOSIM R= 0.117, p= 0.225)					Rainy (ANOSIM R= 0.051, p= 0.385)				
Location group	Sewage	River	Middle	North	South	Sewage	River	Middle	North	South
Internal similarity	53	-	55	78	61	53	-	60	77	64
Sewage	-	44	53	58	55	-	60	56	58	56
River		-	60	**71**	55		-	65	**82**	**72**
Middle			-	**71**	63			-	68	64
North				-	69				-	**72**
South					-					-

Table 3-6. Faecal pollution indicators for the coral reef system of Veracruz. C= Contaminated, CBL= Contaminated below the limit, and UC= Uncontaminated. A significant regression ($F_{(1,20)}$= 194, p< 0.001) was computed to obtain the limit concentration of coprostanol per dry weight, the equation is Cop (ng mg^{-1})= 4.54 + 0.0367 Cop (ng L^{-1}), and substituting 30 ng L^{-1} the limit is 5.7 ng mg^{-1}. Bold numbers are up to or equal to the limit in the corresponding indicator. SD= Standard deviation. Superscript letters denote significant differences (α=0.05). F= Fisher's statistic and p= probability value.

Station	coprostanol ng L^{-1}		coprostanol/cholesterol		Coprostanol/ (coprostanol+cholestanol)		coprostanol ng mg^{-1}		Result	
	dry	rainy	dry	rainy	dry	rainy	dry	rainy	dry	rainy
Before STP	**3571**	-	**0.9**	-	**0.8**	-	**168**	-	C	-
After STP	**3671**	-	**0.5**	-	**0.8**	-	**100**	-	C	-
Source mean	**3621**^{a}	-	**0.7**^{a}	-	**0.8**^{a}	-	**134**^{a}	-		
SD	71	-	0.3	-	0	-	48	-		
River upstream	**1823**^{b}	9^{c}	**0.5**^{a}	0.1^{ab}	**0.7**^{a}	0.2^{ab}	**85**^{a}	3^{b}	C	UC
River mouth	18	**215**	**0.2**	**0.4**	0.4	0.4	2	**21**	CBL	C
River offshore	0	0	0.0	0.0	0.0	0.0	0	0	UC	UC
Middle mean	9^{c}	**108**^{c}	0.1^{b}	**0.2**^{b}	0.2^{b}	0.2^{b}	1^{b}	**11**^{b}		
SD	13	152	0.1	0.3	0.3	0.3	1	15		
Sacrificios	28	**35**	0.1	0.1	0.2	0.2	**10**	**6**	CBL	C
Anegada de Adentro	9	13	**0.2**	0.0	0.4	0.2	**26**	3	CBL	UC
Verde	0	28	0.0	0.1	0.0	0.4	0	5	UC	CBL
North mean	12^{c}	25^{c}	0.1^{b}	0.1^{b}	0.2^{b}	0.3^{b}	12^{b}	5^{b}		
SD	14	11	0.1	0.1	0.2	0.1	13	2		
Enmedio	0	0	0.0	0.0	0.0	0.0	0	0	UC	UC
Anegada de Afuera	25	15	0.1	0.0	0.4	0.2	4	2	CBL	UC
Cabezo	0	0	0.0	0.0	0.0	0.0	0	0	UC	UC
Anton Lizardo	6	**111**	**0.2**	0.1	0.2	0.3	0.3	**18**	CBL	C
South mean	8^{c}	**32**^{c}	0.1^{b}	0.03^{b}	0.2^{b}	0.1^{b}	1^{b}	5^{b}		
SD	12	53	0.1	0.1	0.2	0.2	2	9		
F(8,13)	1088		5.73		3.39		18.02			
p	< 0.001		0.003		0.025		< 0.001			
Limit to consider contaminated	30		0.2		0.7		5.5			
Reference	Isobe et al. 2002		Grimalt et al. 1990				This work			

3.10 Figures

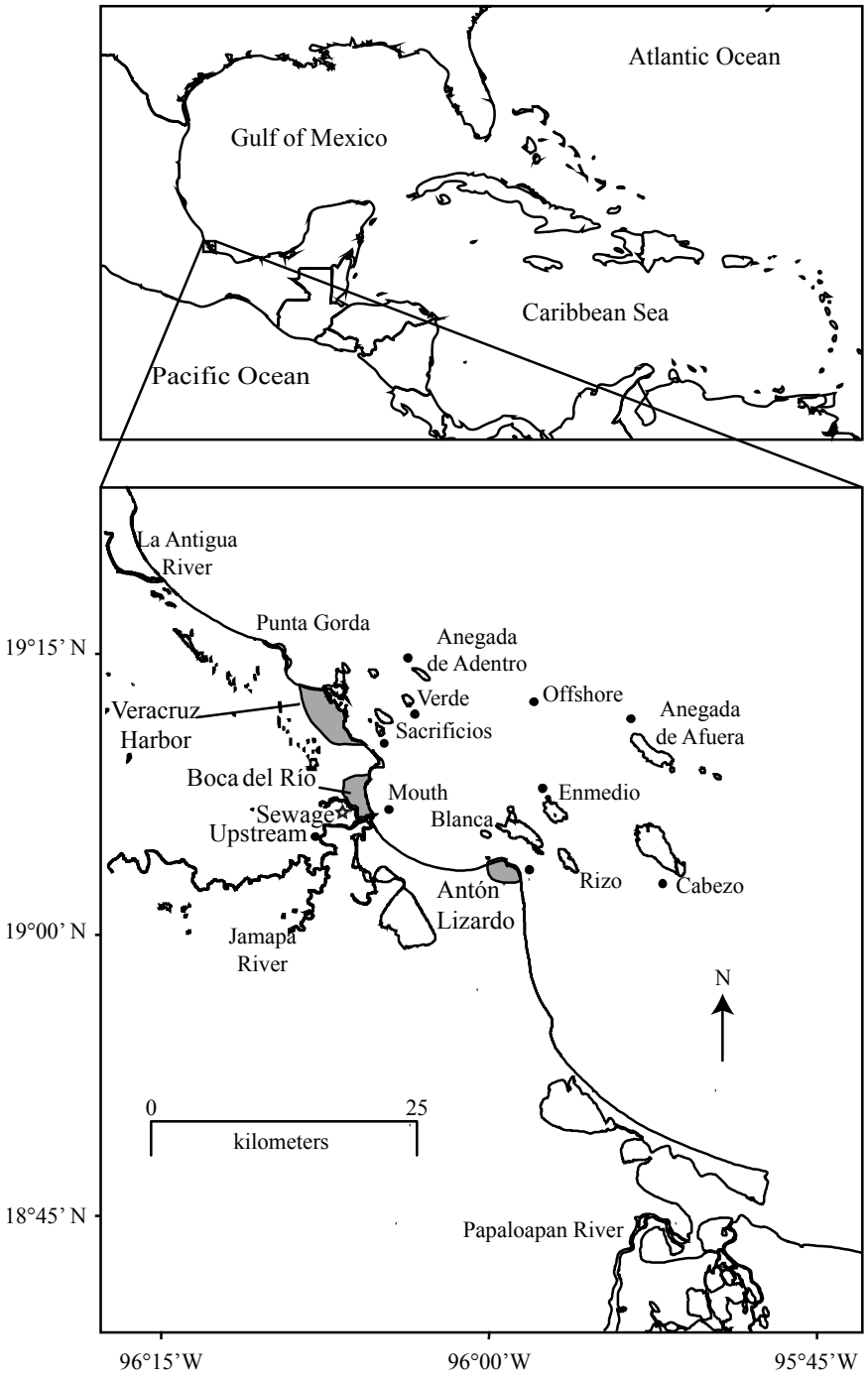

Figure 3-1. Map of the Veracruz coral reef system (PNSAV). Star denotes sewage treatment plant and dots denote water sampling sites.

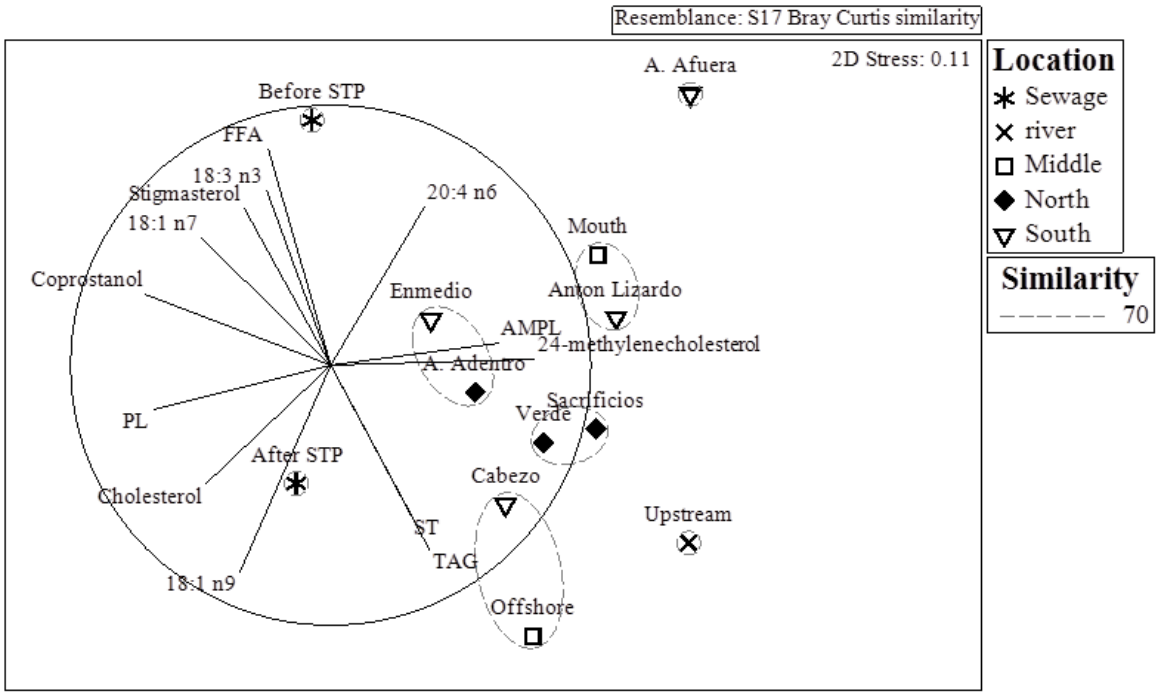

Figure 3-2. Scatter plot of non-metric multidimensional scaling (nMDS) using Bray-Curtis distance matrix for fatty acid data (expressed as a percentage of total fatty acids), sterols (expressed as percentage of total sterols) and lipid classes (expressed as percentage of total lipids) of particulate supended matter in the dry season of the coral reef ecosystem of Veracruz. Axis scales are arbitrary in nMDS (ANOSIM R= 0.117 and p= 0.225). Only variables with Pearson's correlations with MDS 1 and MDS 2 > 0.6 are plotted. Contours group locations with 70% similarity based on hierarchical cluster analysis.

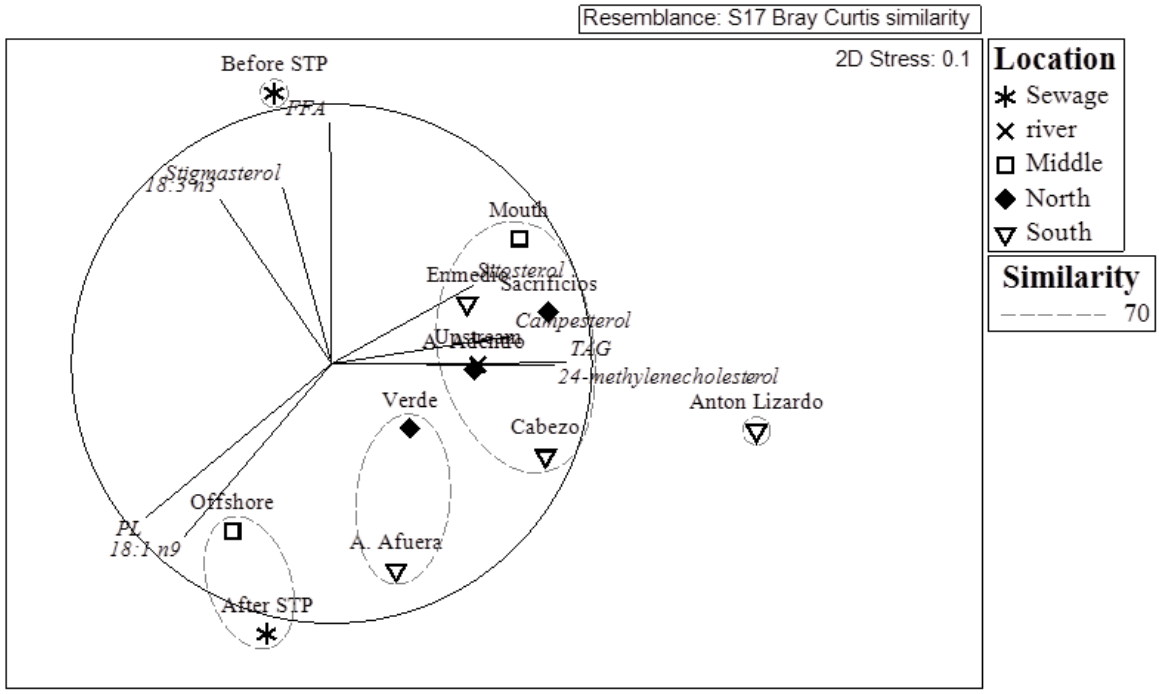

Figure 3-3. Scatter plot of non-metric multidimensional scaling (nMDS) using Bray-Curtis distance matrix for fatty acid data (expressed as percentage of total fatty acids), sterols (expressed as percentage of total sterols) and lipid classes (expressed as percentage of total lipids) of particulate suspended matter in the rainy season of the PNSAV. Axis scales are arbitrary in nMDS (ANOSIM R= 0.051 and p= 0.385). Only variables with Pearson's correlations with MDS 1 and MDS 2 > 0.6 are plotted. Contours group locations with 70% similarity based on hierarchical cluster analysis.

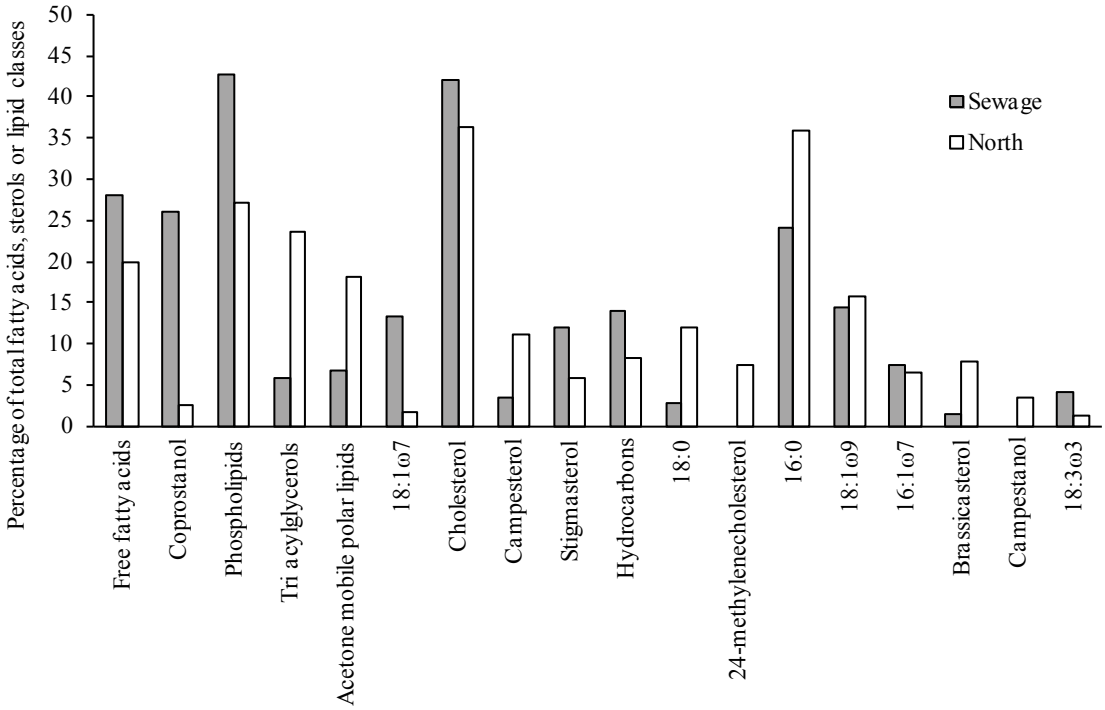

Figure 3-4 Average contribution of compounds primarily providing the discrimination between sewage and north locations considering both seasons of the suspended particulate matter collected in the PNSAV. Fatty acid, sterol and lipid class profiles showed a significant SIMPER dissimilarity of 41% (PERMANOVA t=1.6 p= 0.022). Cut off for low contribution of 90%.

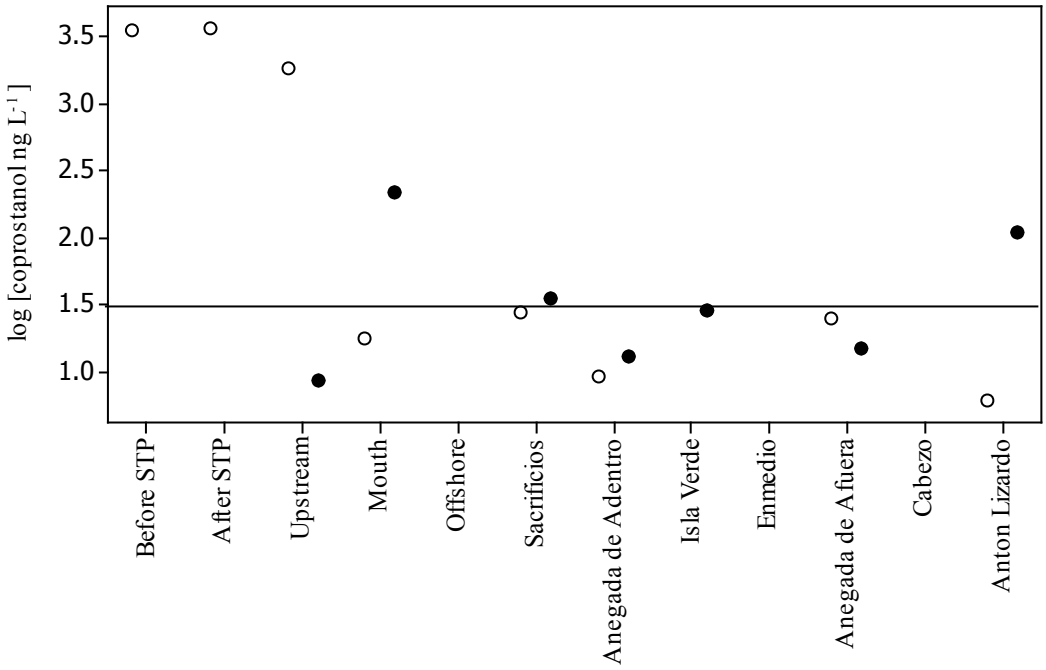

Figure 3-5. Logarithm of the coprostanol concentrations (ng L^{-1}) of water samples collected in the PNSAV in contrasting seasons. Before and after sewage treatment plant (STP). The line denotes coprostanol concentration limit set at Log_{10} (30)= 1.5 corresponding to 1000 fecal coliforms per 100 mL of water in tropical ecosystems according to Isobe *et al*. (2002). Open circles correspond to the dry season and filled circles to the rainy season. Stations without circles had no detectable coprostanol.

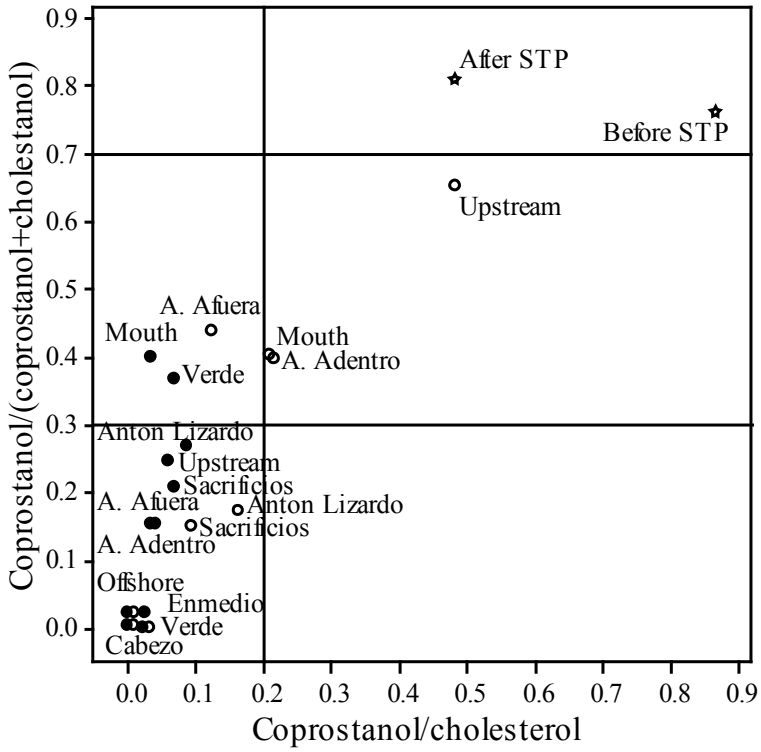

Figure 3-6. Coprostanol: cholesterol and coprostanol: [coprostanol+cholestanol] ratios. The limits to consider stations polluted were 0.2 and 0.7, respectively. Stations with coprostanol: [coprostanol+cholestanol] ratio lower than 0.3 were considered uncontaminated according to Grimalt et al. (1990). Stars are the coprostanol sources before and after sewage treatment plant (STP). Open circles correspond to the dry season and filled circles to the rainy season.

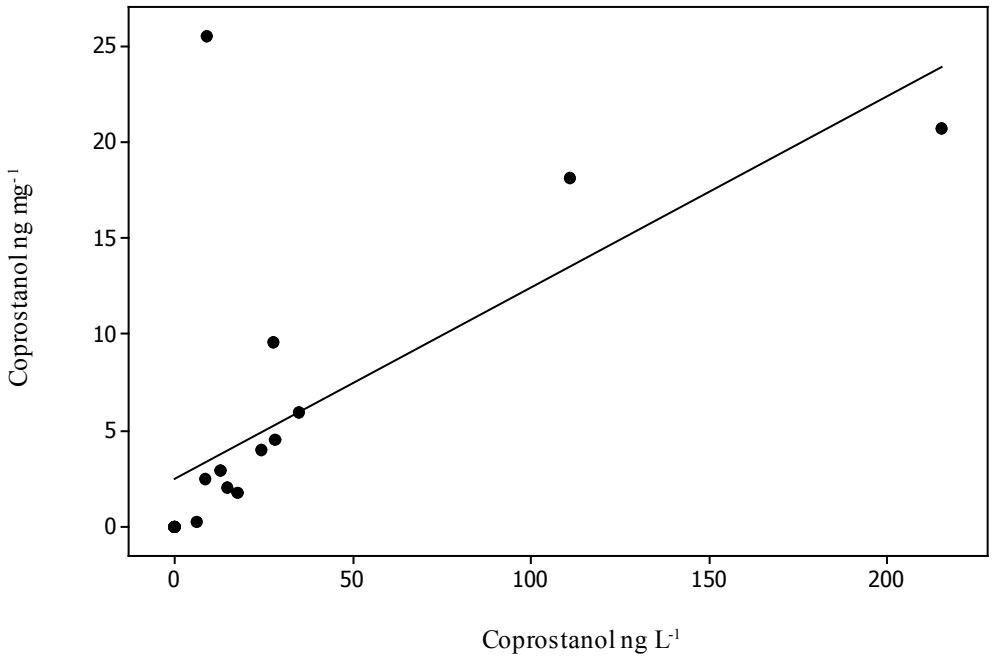

Figure 3-7. Significant regression between the coprostanol concentration per dry weight of suspended particulate matter versus the concentration related to volume Cop DW (ng mg^{-1}) = 2.48 + 0.0999 Cop V (ng L^{-1}). $F_{(1,17)}$= 13.8, p=0.002 0.001,R^2= 44.8, Pearson r= 0.669.

IV Summary and Conclusion

My approach to assessing the degree to which coral reef ecosystems have been influenced by terrestrial and anthropogenic inputs is based on distinguishing between natural and anthropogenic sources of organic carbon. Natural sources implied primary producers while the anthropogenic source analyzed was sewage discharge. The major waste components contributing to reduced water quality is the nutrient load and fecal material that originates from sources such as municipal waste sewage of defficient treatment plants.

Among the natural sources of organic carbon, mangrove (terrestrial) was distinguished from seagrass, phytoplankton and macroalgae (marine) using a carbon and nitrogen stable isotope mixing model. Mangrove and seagrass were not detected using their essential fatty acids (FA): 18:2ω6 and 18:3ω3, because those FA had a significant trophic reduction making it impossible to detect mangrove and seagrass sources in the top consumers of the coral reef food web. However, using the carbon and nitrogen stable isotope mixing model, mangrove showed a contribution of 29 – 33% in zooplankton, 17% in the masked goby *Coryphopterus personatus*, and in higher trophic level fish such as *Ocyurus chrysurus* it was 12% in the dry season. The most important finding is that juveniles of the crevalle jack *Caranx hippos* had a mangrove contribution of 29 and 27% of their bulk carbon in the dry and rainy seasons, respectively, confirming the importance of mangrove as an organic carbon source in the recruitment period of fish populations. On the other hand, the non-essential FAs tend to decrease or have no change with respect to

the source from most primary producers, but non-essential FAs are strongly dependent on the physiological requirement of the consumers. For instance, teleosts preferentially oxidize non-essential fatty acids (Whyte et al. 1993). However according to the trophic retention factor, when the source was seagrass, those FAs showed significant retention across trophic levels, due to the high contribution of seagrass to the consumer's carbon, coinciding with field observations recorded in 100 publications, showing that grazing on sea grasses is widespread in the world's oceans (Valentine and Heck 1999). In contrast, marine sources such as phytoplankton, green, red, and brown macroalgae could be overestimated if we use only highly unsaturated FA such as 20:5ω3, 20:4ω6, and 22:6ω3 as trophic biomarkers, principally in high trophic levels, because according to the trophic retention factor they were trophically retained with rates from 1.4 to 3.5 times per trophic level. Therefore, high proportions or concentrations of DHA in a top predator, for example *O. chrysurus* (trophic level=3.7) with 33% of total FA or 10 μg mg^{-1} dry weight, could mean a high apportionment of marine sources but also more steps in the food web. Therefore, carbon and nitrogen stable isotopes are necessary to evaluate both terrestrial and marine source contributions of organic carbon and fatty acids with more accuracy in coral reefs and in coastal marine ecosystems in general.

From marine sources of organic carbon in general and FA in particular, macroalgae and seagrass had a higher contribution in the dry season, while the phytoplankton contribution was higher in the rainy season. The sea urchin *E. lucunter* was the principal consumer of macroalgae including the foliose brown alga *Dyctiota* sp., this alga grows in living coral and can damage it, and unfortunately it has low palatability for most herbivorous fish (Fong and Paul 2011). This is consistent with its low contribution to the

surgeon fish *Acanthurus chirurgus* carbon at 3% and 4%, in the dry and rainy seasons, respectively. Upper trophic level teleosts like the hog fish *Bodianus rufus* had a greater contribution of *Dyctiota* sp. around 10% in the rainy season; probably through consumption of sea urchins. This fish could be a key species in the control of sea urchin populations, which can erode coral while feeding on macroalgae, suggesting it should be protected. A direct implication of this result is that the reduction of foliose macroalgae cannot depend on herbivore consumption, because it is not palatable to herbivore fish, and sea urchins erode coral while consuming brown algae. Therefore, decreasing nutrient load with tertiary treatment seems the best solution. Although I did not evaluate nutrient loads, I evaluated δ^{15}N and C:N ratios as indicators of nitrogen source and limited *vs* enriched dissolved nitrogen conditions in macroalgae. The results of Chapter 1 showed values of δ^{15}N coincident with fertilizer sources of nitrogen according to Umezawa et al. (2002), while in the results of Chapter 2, C:N ratios of macroalgae were similar to those reported in nitrogen enriched conditions in Exuma Cays, Bahamas (Lapointe et al. 2004); according to these authors the effect of nitrogen enrichment promoted low C: N ratios of 18±3, while dissolved nitrogen limitation promoted high C:N ratios of 42±6 in macroalgae. In the present study, green algae had the highest C: N ratio at 22±2, similar to red algae, and significantly higher than in brown algae *Dictyota* sp. with the lowest C: N ratio of 17±1, confirming that macroalgae biomass is increased by anthropogenic nitrogen enrichment.

Nutritional quality of primary producers and nutritional condition of consumers were evaluated in order to understand the importance of the apportionment of primary producers in dry and rainy seasons to active predators such as teleost fish, grazers such as

sea urchin, and suspension feeders such as clams, sponges and coral including the great star coral *Montastrea cavernosa*, the most abundant coral in the PNSAV. To evaluate the nutritional quality from primary producers: mangrove, seagrass, green, red and brown macroalgae, phytoplankton and zooxanthellae to pelagic and benthic consumers I used C:N ratios, lipid classes, fatty acids and sterol profiles. Results of C:N ratios and triacylglycerol (TAG) content, showed that phytoplankton and zooxanthellae can be considered to have higher nutritional quality, followed by seagrass, mangrove and macroalgae. Unfortunately, C and N stable isotopes were not analyzed in suspension feeders. However, tracing of primary producer lipids in clams, sponges and corals was possible using sterols from primary producers, allowing the identification of the main sources.

The phytoplankton sterol 24-methylenphenol was correlated with higher contents of triacylglycerols and fatty acids characteristic of diatoms principally in zooplankton, clams and perciform fish *Bodianus rufus*, *Ocyurus chrysurus* and *Caranx hippos* suggesting a better nutritional condition and high retention of organic carbon during the rainy season. In contrast, structural lipid proportions such as phospholipids, essential fatty acids such as 22:6ω3, terrestrial sterols such as campesterol and β-sitosterol, free sterols, and steryl and wax esters were significantly higher in the dry season. The great star coral *M. cavernosa* showed a better nutritional condition in the dry season as a result of a zooxanthellae contribution traced with gorgosterol. In addition, *M. cavernosa* had higher proportions of TAG (20.5±7.3%) than a coral of the same family *Goniastrea aspera* (11% under normal conditions and 7% under bleached conditions) with no significant seasonal change, probably because of zooplankton consumption that, according to Muscatine and Porter

(1977), accounts for 10 to 20% of *M. cavernosa* energy. However, the TAG: ST ratio of 6.4±3.4 observed in the dry season was not reached by zooplankton consumption in the rainy season, when a reduction of the water quality resulted in a significant decrease in body condition with a TAG:ST ratio of 1.4±0.5.

The quality of suspended particulate matter (SPM) and the degree of human fecal pollution in the Veracruz coral reef system were evaluated using lipid classes, fatty acids (FA) and sterols. SPM had high proportions of triacylglycerols, saturated and monounsaturated FA, however it was considered poor quality because it had low proportions of essential and highly unsaturated FA. During the 2008 dry season, removal efficiency of the sewage treatment plant of Carcamo de la Zamorana was ~59%. During the dry season, the river was contaminated upstream with human feces. In contrast, during the rainy season a dilution effect allowed the reduction of waste water pollution, except in the zone of the littoral current. Where values of coprostanol higher than the limit for tropical marine coastal waters (30 ng L^{-1}) were reached at the river mouth (215.3 ng L^{-1}), the Sacrificios reef (34.9 ng mL^{-1}), and offshore from Anton Lizardo (110.9 mg L^{-1}). The presence of coprostanol in Sacrificios reef and the Jamapa River mouth is coincident with the current circulation to the north in the rainy season. Also, offshore reefs: Anegada de Adentro, Verde and Anegada de Afuera had some degree of contamination below the regulation limit (coprostanol <30 ng mL^{-1}). Finally, only three stations were clearly uncontaminated during both seasons: Two reefs Enmedio and Cabezo, both in the southern part of the PNSAV, and the river offshore, with no presence of coprostanol. This result could be explained by the distance to the source of contamination.

A monitoring program is necessary as part of the management program of the PNSAV marine protected area, as well as the building of a treatment plant in Anton Lizardo village. Coprostanol concentration in suspended particulate matter can be used as an indicator of fecal pollution and $\delta^{15}N$ and C:N ratios as indicators of nitrogen source and limited *vs* enriched dissolved nitrogen conditions in macroalgae. These indicators are suggested for evaluation of the recently (January 2013) inaugurated treatment plant in the Veracruz harbor-Boca del Rio region with tertiary treatment.

IV.1 Bibliography

Fong P. and V. J. Paul. 2011. Coral Reef Algae. *In:* Coral Reefs: An Ecosystem in Transition Zvy Dubinsky and Noga Stambler Editors. Springer Science+Business Media B.V. pp. 521.

Lapointe, B. E., P. J. Barile, AND W. R. Matzie. 2004. Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: Discrimination of local versus regional nitrogen sources. J. Exp. Mar. Biol. Ecol. 308: 23–58, doi:10.1016/j.jembe.2004.01.019

Muscatine L. and J. W. Porter. 1977. Reef corals: Mutualistic symbioses adapted to nutrient-poor environments. BioScience, 27: 454-460.

Umezawa, Y., T. Miyajima, M. Yamamuro, H. Kayanne, and I. Koike. 2002. Fine-scale mapping of land-derived nitrogen in coral reefs by $d^{15}N$ in macroalgae. Limnol. Oceanogr. 47:1405–1416, doi:10.4319/lo.2002.47.5.1405

Valentine, J. F., and K. L. Heck. 1999. Seagrass herbivory: Evidence for the continued grazing of marine grasses. Mar. Ecol. Prog. Ser. 176: 291–302, doi:10.3354/meps176291

Whyte, J. N. C., W. C. Clarke, N. G. Ginther, and J. O. T. Jensen. 1993. Biochemical changes during embryogenesis of the Pacific halibut, *Hippoglossus stenolepis* (Schmidt). Aquaculture and Fisheries Management, 24: 193–201.

V Appendices

Appendix 1. Compounds detected in a Varian 3800 gas chromatograph mass spectrometer detector (MSD) with a Omegawax 250 column 30 m x 0.25 mm x 0.25 µm with helium as the carrier gas. MW=Molecular weight, RT= Retention time, FID= Flame Ionization Detector and ME= Methy ester.

RT MSD	RT FID	Compound	MW	Key identification ions	MS File	Structure
9.28	5.19	14:0	242	74, 211, 199	E5 30 micras	tetradecanoic acid ME
9.9	5.33	*iso* 14:0	256	74, 225, 213, 241, 201, 200, 191	E5 30 micras	13-methyl tridecanoic acid ME
10.14	5.52	*anteiso* 14:0	256	74, 225, 213, 241, 227	E5 30 micras	12-methyl tridecanoic acid ME
11.02	5.72	15:0	256	74, 225, 213	E5 30 micras	pentadecanoic acid ME
11.54		C_{13}-alcohol	200	55, 69, 83, 97, 111	McavOZR2	1-tridecanol
12.07	5.99	*iso*15:0	270	74, 239, 227, 255, 215, 214, 205	Achi 681	14-methyl pentadecanoic acid ME
12.16		C_{12}-aldehyde	230	55, 75, 83, 97	McavOZR2	dodecanal dimethyl acetal
12.37		C_{13}-aldehyde	244	55, 75, 83, 97	McavOZR2	tridecanal dimethyl acetal
13.13	6.22	16:0	270	74, 239, 227,	E5 30 micras	hexadecanoic acid ME
13.59	6.38	16:1ω9	268	55, 237, 225, 152	Eluc 937	cis-7 hexadecenoic acid ME
13.7	6.42	16:1ω7	268	55, 237, 225, 152	Eluc 938	palmitoleic Acid ME
13.99	6.51	16:1ω5	268	55, 237, 225, 152	E7 120 micras	cis-11 hexadecenoic acid ME
14.07		18:1-ether	282	55, 71, 82, 96	McavOZR2	ether methyl-1-octadecenyl
14.32		*iso* 16:0	284	74, 253, 241, 168, 229, 228, 219	E7 120 micras	15-methyl hexadecanoic acid ME
14.68		*anteiso* 16:0	284	74, 253, 241, 168, 255	E7 120 micras	14-methyl hexadecanoic acid ME
15.09		16:2ω6	266	67, 81, 96, 235, 234, 223, 192, 150	E5 30 micras	cis 10-hexadecadienoic acid ME
15.32		phytanic acid	326	101, 74, 295, 283, 111	E7 120 micras	hexadecane, 2,6,10,14-tetramethyl-
15.49		17:0	284	74, 253, 241, 168	Atri OC M4	heptadecanoic acid ME

Appendix 1. Continued

RT MSD	RT FID	Compound	MW	Key identification ions	MS File	Structure
15.87		C_{18} -n-alkanone	268	58, 71, 85, 96, 210, 236, 225	McavOZR2	2-octadecanone
15.94		16:3ω4	264	79, 232, 221, 190, 148	E7 120 micras	cis-6, 9,12 hexadecatrienoic acid ME
16.79		7-Me-16:1ω-10	282	251, 250, 239, 166, 267	Aply 220	7-methyl cis-7-hexadecenoic acid ME
16.96	7.41	17:1	282	55, 251, 239, 208, 166	E7 120 micras	cis-10-heptadecenoic acid ME
17.06		C_{16}-alcohol	240	55, 69, 83, 97, 111	McavOZR2	hexadecen-1-ol 9 trans
17.22		monounsaturated aldehyde	284	55, 75, 97	McavOZR2	hexadecenol diacethyl acetal
17.41	7.76	16:4ω3	262	79, 231, 230, 219, 188, 146, 108	E5 30 micras	cis 4, 7, 10, 13-hexadecatrtraenoic acid ME
17.49		C-n-aldehyde	?	55, 75, 83	McavOZR2	dodecanal dimethyl acetal
17.76		C_{16}-alcohol	240	55, 67, 82, 96, 109	McavOZR2	11-hexadecen-1-ol
18.15	8.31	18:0	298	74, 267, 255, 224, 182	E7 120 micras	octadecanoic acid ME
18.26	8.41	18:1ω11	296	55, 265, 264, 253, 222, 180	E7 120 micras	cis-7 octadecenoic acid ME
18.49	8.6	18:1ω9	296	55, 265, 264, 253, 222, 180	E7 120 micras	cis-9 octadecenoic acid ME
18.68	8.7	18:1ω7	296	55, 265, 264, 253, 222, 180	E7 120 micras	cis-11 octadecenoic acid ME
18.89		18:1ω6	296	55, 265, 264, 253, 222, 180	E7 120 micras	cis-12 octadecenoic acid ME
19.26	8.96	18:1ω5	296	55, 265, 264, 253, 222, 180	E7 120 micras	cis-13 octadecenoic acid ME
20	9.33	18:2ω6	294	67, 81, 95, 262, 251, 220, 178, 150	Mang 1003a	cis 9, 12 octradecadienoic acid ME
20.88	10.26	18:3ω6	292	79, 261, 249, 218, 176, 150	E7 120 micras	6,9,12-octadecatrienoic acid ME
21.02		19:0	312	74, 281, 269, 238, 196	E7 120 micras	nonadecanoic acid ME
21.9	10.54	18:3ω3	292	79, 261, 249, 218, 176, 108	Mang 1003a	cis 9, 12 15 octadecatrienoic acid ME
22.82	11.21	18:4ω3	290	79, 259, 258, 247, 216, 174, 108	E7 120 micras	cis 6, 9, 12, 14 0ctadecatetraenoic acid ME

Appendix 1. Continued

RT MSD	RT FID	Compound	MW	Key identification ions	MS File	Structure
23.42		C17-alcohol	254	55, 67, 82, 96, 109	McavOZR2	11-heptadecen-1-ol
24.06	12.16	20:0	326	74, 295, 283, 252, 210	Cper298	eicosanoic acid ME
24.12		C17:0 hydroxy acid ME	286	55, 71, 83, 97, 111, 227	Atri 624M	hexadecanoic acid, 2-hydroxy-, methyl ester
24.41		18:5ω3	288	79, 256, 245, 214, 172	McavOZR2	cis 3, 6, 9, 12, 15 octadecapentaenoic acid ME
24.51		20:1ω11	324	55, 293, 292, 281, 250, 208	E7 120 micras	cis 9 eicosenoic acid ME
24.62	12.67	20:1ω9	324	55, 293, 292, 281, 250, 208	E7 120 micras	cis 11 eicosenoic acid ME
24.87	12.91	20:1ω7	324	55, 293, 292, 281, 250, 208	Atri OC M4	cis 13 eicosenoic acid ME
25.04		20:2 Δ 5 , 11 NMI	322	67, 81, 91, 291, 290, 279, 248, 206	Atri OC M4	cis 5, 11 eicosadienoic acid ME
25.23		20:2 Δ 5 , 12 NMI	322	67, 81, 91, 291, 290, 279, 248, 206	Atri OC M4	cis 5, 12 eicosadienoic acid ME
25.39		20:2 Δ 5 , 13 NMI	322	67, 81, 91, 291, 290, 279, 248, 206	Atri OC M4	cis 5, 13 eicosadienoic acid ME
25.75		Cn-aldehyde	?	55, 75, 83	McavOZR2	dodecanal dimethyl acetal
26.07	14.05	20:2ω6	322	67, 81, 91, 291, 290, 279, 248, 206, 150	Atri OC M4	cis 11, 14 eicosadienoic acid ME
26.52		20:2ω3	322	67, 81, 91, 291, 290, 279, 248, 206, 108	Atri OC M4	cis 14, 17 eicosadienoic acid ME
26.87		20:3ω6	320	79, 288, 277, 246, 204, 150	Atri OC M4	cis 8, 11, 14 eicosatrienoic acid ME
27.13	15.19	21:0	340	74, 309, 297, 266, 224, 150	Atri OC M4	heneicosanoic acid ME
27.58	15.73	20:4ω6	318	79, 286, 275, 244, 202, 150	Atri OC M4	cis 5. 8, 11, 14 eicosatetraenoic acid ME
28.1	17.42	20:4ω3	318	79, 286, 275, 244, 202, 108	Atri OC M4	cis 8, 11, 14, 17 eicosatetraenoic acid ME
29.03	18.12	20:3ω3	320	79, 288, 277, 246, 204, 108	Atri OC M4	cis 11, 14, 17 eicosatrienoic acid ME
29.85	18.4	20:5ω3	316	79, 284, 273, 242, 200, 108	Atri OC M4	cis 5. 8, 11, 14, 17 eicosapentaenoic acid ME
30.59	19.38	22:0	354	74, 323, 311, 280, 238	Atri OC M4	docosanoic acid ME
30.86	19.83	22:1ω13	352	55, 321, 320, 309, 278, 236	Atri OC M4	cis 9 docosenoic acid ME
31.13	20.02	22:1ω11	352	55, 321, 320, 309, 278, 236	Atri OC M4	cis 11 docosenoic acid ME
31.6	20.23	22:1ω9	352	55, 321, 320, 309, 278, 236	Atri OC M4	cis 13 docosenoic acid ME

Appendix 1. Continued

RT MSD	RT FID	Compound	MW	Key identification ions	MS File	Structure
32.04		22:1ω7	352	55, 321, 320, 309, 278, 236	Atri 624M	cis 15 docosenoic acid ME
32.09		22:2 Δ 7 , 13 NMI	352	67, 81, 95, 321,320, 309, 278	Atri 624M	cis 7, 13 docosadienoic acid ME
32.39		22:2 Δ 7 , 15 NMI	352	67, 81, 95, 321,320, 309, 278	Atri 624M	cis 7, 15 docosadienoic acid ME
34.28	22.55	22:2ω6	350	67, 81, 95, 319, 318, 307, 276, 234, 150	Atri 624M	cis 13, 16 docosadienoic acid ME
35.1	23.83	23:0	368	74, 337, 325, 294, 252	Cper 298	tricosanoic acid ME
36.03	24.12	22:4ω6	346	79, 314, 303, 272, 230, 159	Cper 298	cis 7, 10, 13, 16 docosatetraenoic acid ME
37.62	25.69	22:5ω6	344	79, 312, 301, 270, 228, 150	Cper 298	cis 4, 7, 10, 13, 16 docosapentaenoic acid ME
39.9	27.18	22:5ω3	344	79, 312, 301, 270, 228, 108	Cper 298	cis 7, 10, 13, 16, 19 docosapentaenoic acid ME
41.02	27.99	24:0	382	74, 351, 339, 308, 266	Cper 298	tetracosanoic acid ME
41.8	28.34	22:6ω3	342	79, 310, 299, 268, 226, 108	Cper 298	cis 4, 7, 10, 13, 16, 19 docosahexaenoic acid ME
42.45	28.49	24:1ω9	380	55, 349, 348, 337, 306, 264	Cper 298	cis 15 tetracosenoic acid ME

Note 1. Chromatographic conditions were helium flow 0.9 ml min^{-1} and injector temperature 250°C. After injection, the temperature of the column was subjected to the following sequence: 110°C for 3 min, increased to 165°C at a rate of 30°C min^{-1}, maintained at 165°C for 2 min, increased to 209°C at a rate of 2.2°C min^{-1}, and maintained at 209°C for 18 min. The mass spectrometer detector was set at 260°C and ion source was set at 70 eV. Comparison of the retention time in a mass spectrometer detector (RT MSD) with a flame ionization detector (RT FID) Agilent 6890 was set for chapter 3. The GC column was a ZB wax+ (Phenomenex, U.S.A.). The column length was 30 m with an internal diameter of 0.32 mm. The column temperature began at 65°C and held this temperature for 0.5 minutes. The temperature ramped to 195°C at a rate of 40°C min^{-1}, held for 15 minutes then ramped to a final temperature of 220°C at a rate of 2°C min^{-1}. This final temperature was held for 0.75 minutes. The carrier gas was hydrogen and flowed at a rate of 2 ml min^{-1}. The injector temperature started at 150°C and ramped to a final temperature of 250°C at a rate of 120°C min^{-1}. The detector temperature stayed constant at 260°C.

Appendix 2. Compounds detected in a Hewlett Packard 6890 gas chromatograph mass spectrometer detector (MSD) with a DB-5 MS column 30 m x 0.25 mm x 0.25 µm with helium as the carrier gas. RT= Retention time, MW=Molecular weight.

RT MSD	RT FID	Compound	MW	Key identification ions	MS File	Structure
4.71	7.93	octanal dimethyl acetal	174	75, 111, 143, 174	LCP3155f	octane, 1,1-dimethoxy
5.58	8.48	nonanal dimethyl acetal	188	75, 109, 157, 188	LCP3155f	nonane, 1,1-dimethoxy
6.47	9.43	decanal dimethyl acetal	202	75, 97, 171, 201	LCP3155f	nonane, 1,1-dimethoxy
12.34	15.62	pregnanone	494	75, 117, 129, 314, 402, 446, 458	McavOCT4 Z	pregnan-11-one, 17-hydroxy-3,20-bis[(trimethylsilyl)oxy]
12.66	15.63	tetradecyl oxy	286	55, 75, 83, 103, 271, 286	LCP3155f	tetradecyl tert-butyldimethylsilyl ether
17.7	20.89	hexadecyl oxy	314	55, 75, 83, 103, 299, 314	LCP3155f	hexadecyl tert-butyldimethylsilyl ether
19.02	22.13	octadecyl oxy	342	55, 75, 83, 103, 242, 327, 342	LCP3155f	octadecyl tert-butyldimethylsilyl ether
23.23	26.16	hexanoic acid, heptadecyl (wax ester)	354	57, 75, 83, 97, 117, 283, 311, 354	LCP3183c	hexanoic acid, heptadecyl ester
23.36	26.43	dodecane dimethoxy (from wax esters)	230	55, 75, 83, 97, 199, 230	LCP3155f	dodecane 1, 1-dimethoxy
24.01	26.41	dodecosane	310	57, 71, 85, 295, 281	LCP3183i	n-dodecosane
26.72	29.02	tricosane	324	57, 71, 85, 309, 295	LCP3183i	n-tridecosane
29.34	31.56	tetracosane	338	57, 71, 85, 309, 323	LCP3183i	n-tetracosane
31.88	34.02	pentacosane	352	57, 71, 85, 337, 323	LCP3183i	n-pentacosane
34.35	36.41	hexacosane	366	57, 71, 85, 337, 351	LCP3183i	n-hexacosane
36.77	39.54	heptacosane	380	57, 71, 85, 351, 365	LCP3183i	n-heptacosane
41.86	44.54	24-nordehydrocholesterol	442	69, 97, 211, 353, 366, 442	LCP3162a	24-norcholesta-5, 22E-dien-3β-ol
46.92	49.02	coprostanol*	460	75, 215, 257, 355, 370, 403, 445, 460	LCP4335b	5β-cholestan-3β-ol TMS

*Retention time delayed respec to samples of Chapter 2 inyected in 2009 because samples of Chapter 3 were inyected in 2013

Appendix 2. Continued

RT MSD	RT FID	Compound	MW	Key identification ions	MS File	Structure
44.4	47.37	24-Nor-22, 23 methylenecholest-5-en-3-*β*-ol	456	55, 69, 111, 129, 327, 366, 456	LCP3183e	24-Nor-22, 23 methylenecholest-5-en-3*β*-ol
44.81	47.1	occelasterol	456	55, 69, 111, 129, 255, 327, 351, 366, 441, 456	LCP3183i	27-nor-24-methylcholest-5, 22E-dien-3*β*-ol
45.01	48.23	trans 22 dehydrocholestanol	458	73, 91, 345, 374, 458	LCP3174c	5a-cholest-22E-en-3*β*-ol
45.01	48.25	patinosterol	458	69, 81, 107, 257, 345, 374, 389, 404, 443, 458	LCP3174d	27-nor-24-methyl-5a-cholest-22E-en-3*β*-ol
45.6	48.28	cholesterol	458	73, 129, 329, 353, 368, 443, 458	LCP3183i	cholesta-5-en-3*β*-ol
45.75	48.44	cholestanol	460	75, 215, 305, 355, 370, 403, 445, 460	LCP3183i	5*α*-cholestan-3*β*-ol
45.98	49.39	desmosterol	456	69, 129, 253, 327, 343, 366, 441, 456	LCP3155c	cholesta-5,24-dien-3*β*-ol
45.99	49.28	7-dehydrocholesterol	456	75, 182, 351, 368, 386, 441, 456	LCP3160b	cholesta-5,7-dien-3*β*-ol
46.39	49.12	brassicasterol	470	69, 129, 255, 340, 365, 380, 455, 470	LCP3183i	24-methylcholesta-5,22E-dien-3*β*-ol
46.45	50.13	brassicastanol	472	75, 109, 257, 345, 374, 458, 472	LCP3176a	24-methyl-5*α*-cholest-22E-en-3*β*-ol
46.64	49.29	ergost-7-enol	472	213, 229, 255, 343, 367, 378, 457, 472	LCP3183i	24-methyl-5*α*-cholest-7-en-3*β*-ol
46.84	50.31	ergosterol	468	69, 75, 131, 253, 327, 342, 363, 378, 468	LCP3160d	24-methylcholesta-5,7,22E-trien-3*β*-ol
47.39	50.05	stellasterol	470	73, 129, 213, 229, 255, 343, 455, 470	LCP3183i	cholesta-7, 22E-dien-3*β*-ol
47.4	50	dihydrobrassicasterol	472	75, 209, 267, 343, 386, 472	LCP3160b	24*β*-methylcolest-5-en-3*β*-ol
47.45	50.1	ergost-8(14)-enol	470	75, 91, 229, 255, 343, 378, 457, 470	LCP3174e	24-methyl-5a-cholest-24(28)-en-3*β* -ol
47.54	50.19	24- methylenecholesterol	470	253, 257, 296, 341, 365, 386, 455, 470	LCP3183i	24-methylcholesta-5, 24(28)-dien-3*β* -ol
47.72	50.37	campesterol	472	73, 129, 255, 343, 367, 382, 457, 472	LCP3169b	24*α*-methylcholest-5-en-3*β* -ol
48.18	51.53	lathosterol	458	73, 255, 303, 353, 443, 458	LCP3166b	5*α* Cholest-7-en-3*β* -ol
48.24	51.18	stigmasterol	484	83, 129, 255, 355, 379, 394, 484	LCP3183i	24-ethylcholesta-5, 22E-dien-3*β* -ol
48.34	51.34	23-24 dimethylcholest 5, 7-dien-3 *β*-ol	486	98, 129, 283, 342, 381, 433, 486	LCP3169b	23-24 dimethylcholest 5, 7-dien-3*β*-ol

Appendix 2. Continued

RT MSD	RT FID	Compound	MW	Key identification ions	MS File	Structure
48.73	51.44	24-methylenephenol	470	75, 131, 213, 253, 365, 386, 455, 470	LCP3183i	24-methyl-5a-cholesta-7, 24(28)-dien-3β-ol
49.05	52.49	4-24 dimethyl 5, 7-dien-3-β-ol	484	69, 129, 283, 343, 355, 379, 394, 469, 484	LCP3169a	4-24 dimethyl 5, 7-dien-3-β-ol
49.14	52.61	poriferasterol	484	69, 129, 283, 343, 355, 379, 394, 469	LCP3169a	24-ethylcholesta-5, 22Z-dien-3β-ol
49.14	52.02	spinasterol	484	55, 73, 129, 213, 255, 343, 372, 469, 484	LCP3166e	24-ethyl-5α-cholesta-7, 22E-dien-3β-ol
49.49	52.04	β-Sitosterol	486	73, 129, 255, 357, 381, 396, 471, 486	LCP3183i	24-ethylcholesta-5-en-3β-ol
49.42	52.94	fucosterol	484	55, 73, 129, 257, 281, 296, 355, 371, 386,	LCP3169a	24-ethylcholesta-5,24(28)E-dien-3β-ol
49.61	52.22	sitostanol	488	75, 218, 229, 358, 381, 398, 488	LCP3174c	24-ethyl-5α-cholestan-3β-ol
49.76	53.2	unidentified isomer of fucosterol	484	73, 129, 281, 296, 386, 469, 484	LCP3169a	24-ethylcholesta-5,24(28)Z-dien-3β-ol
49.78	53.16	4, 23, 24 trimethyl 5α cholestenol	500	57, 283, 297, 359, 373, 387, 485, 500	LCP3160d	4, 23, 24 trimethyl-5α-cholest-24(28)-en-3β-ol
49.87	52.55	Dinosterol	500	69, 359, 457, 500	LCP3155f	4,23,24-trimethyl-5a-cholest-22E-en-3β-ol
50.15	52.87	Dinostanol	502	57, 73, 487, 397, 502	LCP3155f	4,23,24-trimethyl-5a-cholestan-3β-ol
50.8	53.68	4, 23, 24 trimmethylcholestenol	500	143, 227, 243, 269, 395, 410, 485, 500	LCP3160d	4, 23, 24 trimmethyl-5a-cholest-7-en-3β-ol
50.23	53.74	Isofucosterol	484	55, 73, 129, 257, 281, 296, 386, 469, 484	LCP3169a	24-ethylcholesta-5,24(28)Z-dien-3β-ol
50.5	53.82	Cycloartenol	498	73, 95, 109, 189, 369, 393, 483, 498	LCP3155a	9,19-Cyclo-24-lanosten-3β-ol
51.86	55.36	Gorgosterol	498	73, 129, 255, 343, 386, 408, 483, 498	LCP3155f	Gorgost-5-en-3β-ol

Note 2. Chromatographic conditions were helium flow 1.2 ml min^{-1} and injector temperature 250 °C. After injection, the temperature of the column was subjected to the following sequence: 60°C for 1 min, increased to 100°C at a rate of 25°C min^{-1}, increased to 150°C at a rate of 15°C min^{-1}, and finally at 315°C at a rate of 3 °C min^{-1}. The quadrupole was set at 150°C and MS ion source was set at 270°C and 70 eV. Identification was based on Jones et al. 1994 and NIST Search 2.0. Comparison of retention time of mass spectrometer detector (RT MS) with a gas chromatograph with a flame ionization detector (RT FID) Agilent 6890, was set with a DB-5 column 30 m x 0.32 mm x 0.25 µm using the same chromatographic method.

Appendix 3. Results from the SIMPER analysis of Chapter 3. Values are the average similarity by species of compounds that contribute to species similarity, their average abundance, and percentage contribution to the similarity. Values in parentheses are average dissimilarity percentage to great star coral *M. cavernosa.*

Consumer	Average similarity (%)	Compounds	Average abundance (%)	Contribution to similarity (%)	Cumulative contribution (%)	Primary producers source according to sterol biomarkers
M. cavernosa Great star coral (00.00)	83.9	Campesterol	49.6	23.5	23.5	Zooxanthellae and phytoplankton trophic transferred by zooplankton
		16:0	41.9	16.5	40.0	
		Wax and/ or steryl esters	22.5	10.9	50.8	
			15.6	7.3	58.2	
		Gorgosterol	17.3	7.0	65.2	
		Triacylglycerols	14.9	6.8	72.0	
		Phospholipids	12.2	5.6	77.6	
		18:1ω9	1.2	< 1		
		24-methylenephenol				
Zooplankton (50.42)	81.2	16:0	27.0	17.1	17.1	Phytoplankton
		Triacylglycerols	31.1	15.8	32.9	
		Phospholipids	22.4	12.9	45.8	
		14:0	17.6	9.9	55.6	
		16:1ω7	12.1	6.8	62.4	
		20:5ω3	6.2	3.8	66.2	
		Brassicasterol	6.2	3.5	69.7	
		Campesterol	6.3	3.1	72.8	
		24-Methylenephenol	2.8	1.3	74.1	
		Ergost-7-enol	1.1	< 1		
		24-nordehydrocholesterol	0.9	< 1		
P. carnea Amber pen shell (51.55)	86.4	Phospholipids	41.6	20.7	20.7	Phytoplankton (diatoms), particulate matter containing sea grass and green algae, and zooplankton
		16:0	22.8	13.5	34.2	
		22:6ω3	14.2	7.3	41.5	
		TAG	12.7	6.6	48.1	
		Brassicasterol	11.0	5.6	53.7	
		ST	10.5	5.4	59.1	
C. hippos Cravalle jack (53.56)	87.8	Triacylglycerols	47.4	28.3	28.3	Mangrove and sea grass trophic transferred by invertebrates, and phytoplankton trophic transferred by finfish
		16:0	28.4	17.3	45.6	
		Phospholipids	30.5	15.4	60.9	
		18:1ω9	15.2	9.1	70.0	
		22:6ω3	11.8	4.7	74.7	
		β-sitosterol	1.6	< 1		
		24-methylenephenol	0.1	< 1		
B. rufus Hogfish (55.36)	84.8	Triacylglycerols	42.9	27.8	27.8	Phytoplankton trophic transferred by the amber pen shell and brown algae trophic transferred by sea urchins
		16:0	24.2	15.0	42.8	
		Phospholipids	28.3	14.6	57.4	
		18:0	9.9	5.9	63.2	
		22:6ω3	12.0	5.1	68.3	
		18:1ω9	7.7	4.4	72.7	
		22:2 □□□□□□ □□□□□□□□□□□	1.0	< 1		
			0.6	< 1		
		24-methylenephenol	0.3	< 1		
		Fucosterol	0.1	< 1		

Appendix 3. Continued

Consumer	Average similarity	Compounds	Average abundance (%)	Contribution to similarity (%)	Cumulative contribution (%)	Source according to sterol biomarkers
A. chirurgus Surgeon fish (56.38)	84.3	Phospholipids	41.1	25.8	25.8	Sea grass, red algae and phytoplankton trophic transferred by zooplankton
		16:0	19.6	10.1	35.9	
		Triacylglycerols	18.9	9.4	45.4	
		22:6ω3	13.4	7.8	53.2	
		20:4ω6	10.7	6.1	59.3	
		Campesterol	3.4	1.6	61.9	
		β-sitosterol	3.0	1.7	63.6	
		Ergost-7-enol	2.5	1.1	64.7	
		24-methylenephenol	1.85	< 1		
C. personatus Masked goby (58.52)	77.4	Phospholipids	55.2	30.8	30.8	Zooplankton, and particulate matter containing sea grass, red and brown algae.
		18:0	14.9	11.8	42.7	
		16:0	19.6	8.1	50.7	
		22:6ω3	12.5	7.1	57.8	
		Campesterol	2.1	1.5	58.3	
		Trans 22 dehydrocholesterol	1.8	< 1		
			0.6	< 1		
E. lucunter Sea urchin (58.57)	78.16	Triacylglycerols	41.2	19.8	19.8	Sea grass and macroalgae
		16:0	17.6	11.8	31.6	
		Phospholipids	25.5	10.9	42.5	
		20:4ω6	11.8	7.0	49.5	
		20:5ω3	8.7	5.0	54.5	
		14:0	7.8	4.3	58.8	
		β-sitosterol	4.4	2.7	61.5	
		Campesterol	3.7	2.3	63.8	
O. chrysusrus Yellow tail snapper (58.99)	82.0	Phospholipids	46.6	24.0	24.0	Sea grass and macroalgae trophic transferred by invertebrates, and phytoplankton transferred by finfish
		16:0	26.6	17.9	41.8	
		Triacylglycerols	28.1	15.8	57.6	
		22:6ω3	19.7	9.0	66.6	
		18:0	9.1	5.8	72.4	
		18:1ω9	8.9	5.0	77.4	
		14:0	3.8	2.5	79.9	
		16:1ω7	3.4	2.5	82.4	
		24-methylenephenol	0.5	< 1		
		β-sitosterol	0.3	< 1		
Aplysina sp. Tube sponge (70.13)	77.8	Phospholipids	34.6	17.3	17.3	Phytoplankton, particulate matter containing sea grass, and brown algae.
		26:2ω6	32.0	10.6	27.9	
		β-sitosterol	16.3	9.8	37.7	
		Brassicasterol	16.7	8.7	46.3	
		Free sterols	15.2	8.0	54.4	
		Branched FA	10.9	5.6	60.0	
		22:0	9.9	5.4	65.4	
		Triacylglycerols	10.8	3.1	68.5	
		Campesterol	4.8	3.1	71.6	
		4-24 dimethyl 5,7-dien-3-β-ol	4.9	2.7	74.3	
			4.8	2.3	76.6	
		24:1ω7	4.0	2.2	78.8	