Master of Science Course

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M.Sc. Thesis in International Studies in Aquatic Tropical Ecology

Seasonal Variations in Symbiont Densities and Skeletal Phosphorus Concentrations in the Eastern Pacific (Costa Rica) Coral *Pavona clavus*

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Bremen, August 2005

Universität Bremen

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ABSTRACT

Within reef-building corals, zooxanthellae densities and chlorophyll concentrations have been suggested to reflect variations in environmental factors, such as water temperature, light levels or nutrient concentrations. In the same context, phosphorus incorporated into coral skeletons has been used to reconstruct changes in seawater phosphorus concentrations, although only on a yearly scale. Despite this, neither the effect of seasonal upwelling of cool, nutrient-enriched waters or high inputs of terrestrial runoff on the density and chlorophyll concentrations of zooxanthellae has been studied, nor is it known whether elevated seawater phosphate concentrations are reflected in coral skeletons also on a seasonal scale. This study aimed to investigate: a) the temporal pattern of the density and chlorophyll concentrations of zooxanthellae in the reef-building coral *Pavona clavus* in an upwelling and a non-upwelling region of the Pacific coast of Costa Rica over a five months period, and b) the acid-available phosphorus concentrations in skeletal cores of *P. clavus* from the upwelling region with a sub-seasonal resolution. In the upwelling region of Culebra Bay, the zooxanthellae density was decreased significantly in the dry (=upwelling) season month January 2005 compared to the period from September to December 2004, while at the non-upwelling region of the Marine National Park Ballena, the density was significantly higher during the dry season months December 2004 and January 2005 than during the rainy season months September and October 2004. Based on this, it is suggested here that generalized hypotheses on seasonal cycles of zooxanthellae populations in corals are not valid for regions where seasonal features such as upwelling or high impact of terrestrial runoff, as observed in the Marine National Park Ballena, play a major role in determining environmental conditions. On the other hand, the temporal pattern of chlorophyll concentrations showed no regional difference, but rather seems to have followed only a negative correlation with ambient light levels, which were similar at both study sites. Within the skeleton of *P. clavus* from the upwelling region, phosphorus concentrations were highest at the end of the dry season and lowest at the end of the rainy season possibly coinciding with the reproductive period of *P. clavus*. It is postulated that increased light levels and elevated seawater phosphate concentrations represent favourable conditions for the incorporation of phosphorus into the coral skeleton, while low light levels and allocation of energy from growth to reproduction seems to decrease the rate of this process.

ACKNOWLEGEMENTS

First of all, I would like to thank my supervisors PD Dr. Rubén Lara and Dr. Carlos Jiménez for their invaluable support throughout the whole project and my field supervisor Prof. Dr. Jorge Cortés for his unique way to help me by keeping things simple but professional at the same time. I would also like to thank you for all the suggestions that improved a lot the quality of this thesis.

I am grateful to all the people at the CIMAR. Each one of you made a special contribution to my enjoyable stay during my time in Costa Rica. Don Rigo, Leda and Fabian readily helped me with administrative and field-work related matters. Lab work was never boring thanks to the presence of the ever-smiling uncle Jenaro Acuña and his “nephews”: Eddy and Minor.

Without the continuous help of the students Cindy, Bernadette, Jaime and the assistance of Davis, this project would have been impossible to realize, thank you! I would like to say a special “thank-you” to Chepe for all the things that are impossible to mention here in one sentence, but especially for his friendship.

At the ZMT, I would like to thank Matthias and Dieter for their technical support and time for discussion. I would like to thank the ISATEC office and all the people that are involved in this MSc-program for their support, teaching and assistance.

I am indebted to all my study colleagues and house-mates for providing a stimulating environment and enjoyable time throughout the last two years in Bremen. I am especially grateful to king Malik, el niñote Michael, and the best cacahuate ever Conny deserves a special mention! Thank you!

Finally, I would like to thank my family Kayoko, Kaneko and Kazushiro y gracias a la niña más linda a cuyo lado siempre me sentiré en casa!

This study was financially supported by the DAAD (Deutscher Akademischer Austauschdienst) that granted me with a scholarship for the field work in Costa Rica. Different financial sources of the CIMAR and ZMT covered costs for field trip expenses, material and laboratory usage.

LIST OF FIGURES

LIST OF TABLES

TABLE OF CONTENTS

1. INTRODUCTION

Coral reefs are among the biologically most diverse, ecologically most mature and structually most complex marine ecosystems in nature (Odum 1971). Located in shallow tropical and subtropical seas, these highly productive ecosystems are often referred to as ‘oases’ in waters of very low nutrient concentrations. The maintenance of high gross net primary production in these nutrient-impoverished waters can only be sustained by highly efficient recycling of nutrients. The best described recycling system is the symbiosis between reef-building corals and unicellular algae, also known as: zooxanthellae (endosymbiotic dinoflagellates). Living in their endodermal tissues, zooxanthellae photosynthesize and transfer up to 95% of the photosynthetic production to the invertebrate host (Muscatine 1990). Another effect of the photosynthetic activity by the algae is an increased rate of calcification at high light levels, also known as light-enhanced calcification (reviewed by Barnes and Chalker 1990), which refers to the coral’s ability to precipitate calcium carbonate, the main component of coral reefs.

Understanding the relevance of this symbiotic association, low density of zooxanthellae has become an issue of increasing importance owing to incidences of bleaching events, nowadays representing the major threat to coral reefs on a global scale (Hughes *et al.* 2003). Bleaching of corals is understood as a loss of zooxanthellae and/or the loss of photosynthetic pigments (Hoegh-Guldberg and Smith 1989; Porter *et al.* 1989) and mass bleaching events have typically occurred during periods of high sea surface temperatures (SSTs) coinciding with disturbances to the El Niño Southern Oscillation (Hoegh-Guldberg 1999). In several experimental studies, exposure of corals to higher than normal temperatures or light/UV irradiance has been shown to elicit the loss of zooxanthellae (Glynn and D'Croz 1990; Lesser *et al.* 1990; Gleason and Wellington 1993), but also low temperature stress has been associated with decreased zooxanthellae densities and changes in chlorophyll concentrations (Saxby *et al.* 2003). Furthermore, studies on the effect of other factors, such as ambient nutrient concentrations (Muscatine 1990; Hoegh-Guldberg 1994) or the synergetic effect of water temperature and nutrient enrichment (Schlöder and D’Croz 2004) have added more subtle aspects to the understanding of what controls the density of zooxanthellae in corals.

Despite the progress based on these experimental results, the relevance and effect of these factors on the zooxanthellae density under gradual seasonal changes, as they occur in the natural environment, has been more difficult to determine. Under non-bleaching conditions, changes of zooxanthellae densities are suggested to be slow with low migration rates (Hoegh-Guldberg *et al.* 1987), and probably represent adjustments to changes in the environment in order to optimize the physiological performance of the symbiotic association. Only in a few studies, densities of zooxanthellae have been reported to be related to seasonal variations in physical environmental parameters like seawater temperature, solar radiation or nutrient availability. Studying *Pocillopora damicornis* from Hawaii, Stimson (1997) reported a positive correlation between zooxanthellae density and dissolved inorganic nitrate concentration and a negative one with solar radiation, but no correlation was found with seawater temperature. A long term study by Fagoonee *et al.* (1999) in Mauritius has shown a seasonal cycle of zooxanthellae density in the coral *Acropora formosa* and a positive correlation between zooxanthellae density and nitrate concentration. Fitt *et al.* (2000), working with five Caribbean species, hypothesized that the density of zooxanthellae was highest during the winter season and lowest during late summer-fall periods, coinciding with lowest and highest seawater temperatures, respectively. It is not known, however, whether the findings of these studies are also valid for regions where turbidity and nutrient levels may become periodically elevated (and seawater salinities decreased) due to increased levels of terrestrial run-off during periods of high precipitation, or where seasonal patterns of seawater temperature and nutrient concentrations are influenced by coastal upwelling.

The skeletal banding of scleractinian corals has been shown to be annual (Knutson *et al.* 1972) and to correlate with variations in environmental parameters, such as temperature and light (Highsmith 1979; Wellington and Glynn 1983). Furthermore, through the analysis of the skeletal chemical composition, local variations in e.g. seawater temperature, precipitation or influences of human activities have been possible to study (reviewed in Buddemeier and Kinzie 1976). For example, records of phosphorus in coral skeletons have been used to reconstruct ambient phosphorus levels in relation to the location and history of phosphorus pollution episodes on a yearly scale (Dodge *et al.* 1984; Kumarsingh *et al.* 1998). While the manner of phosphorus incorporation has not been established yet, these studies suggest that the coral skeleton contains records of ambient seawater phosphorus levels. However, it is not known whether seasonal variations in seawater phosphorus, as observed for example during the seasonal upwelling of nutrient-enriched waters in the Gulf of Panama (D'Croz and Robertson 1997), generate a differential signal of phosphorus concentrations in coral skeletons.

To examine the effect of seasonal and regional variations in environmental factors on the zooxanthellae density and chlorophyll concentrations, the massive coral *Pavona clavus* from an upwelling and a non-upwelling region of the eastern Pacific was studied over a five month period, covering the transition from the rainy to the dry season. Furthermore, it is aimed to assess whether variations in seawater phosphorus levels, due to seasonal upwelling, is recorded in the coral skeleton of *P. clavus*.

In order to achieve these aims, the objectives of this study are: (1) to characterize the transition from rainy to dry season by using meteorological data and monitoring several environmental parameters within and in the close proximity of two coral reefs in an upwelling and non-upwelling region of the Costa Rican Pacific coast, (2) to determine the density and chlorophyll concentrations of zooxanthellae in the coral *P. clavus* at both regions during this time, and (3) to analyze the inorganic phosphorus content in coral cores of *P. clavus* from the upwelling region on a resolution high enough to detect seasonal variations.

The working hypotheses to be tested are:

1. The density and chlorophyll concentration of zooxanthellae in the coral tissue of *P. clavus* responds to seasonal variations in environmental factors.
2. The regional difference between an upwelling and a non-upwelling region is reflected in dissimilar patterns of zooxanthellae densities and chlorophyll concentrations.
3. The seasonal variations in environmental factors that corals are exposed to is recorded in the form of differential phosphorus signals within the coral skeleton.

2. MATERIAL AND METHODS

2.1 The study region: Pacific coast of Costa Rica

The small (50,700 km^2) Central American country Costa Rica, with a population of approximately 4.0 million, is bounded to the north by Nicaragua, on the east by the Caribbean Sea, on the southeast by Panama and to the south and west by the Pacific Ocean. It lies wholly within the tropics (08ºN - 11ºN) and most regions have a rainy season from May to November and a dry season from December to April (Instituto Meteorológico Nacional (IMN), www.imn.ac.cr). In general, the country can be divided into three zones: a) the northern lowlands with the Caribbean coast, b) the central mountain range formed by the Cordillera de Guanacaste, Cordillera de Tilarán, Cordillera Central and Cordillera Talamanca, and c) the 1,160 km long, hilly Pacific coast with its numerous bays, beaches, inlets and two large gulfs: Nicoya in the north and Golfo Dulce in the south, which are enfolded by the peninsulas of Nicoya and Osa, respectively. In the southern section of the coast, precipitation is high and relatively constant over the entire year with a low during the dry season. In the northern section, precipitation is low during the dry season, while the central part represents a transition zone between those climatically distinct regions (Cortés and Jiménez 2003).

The first comprehensive study of the Pacific coast's coral reefs was published by Cortés and Murillo (1985). Since then, numerous publications followed describing coral reefs of different areas along the coast. Among those are: Isla del Caño (Guzmán 1986; Guzmán and Cortés 1989; Guzmán and Cortés 2001), Isla del Coco (Guzmán and Cortés 1992), the region of Santa Elena in the northern part (Cortés 1996/1997a) or the coastal areas of the National Park Corcovado in the southern part of the coast (Cortés and Jiménez 1996).

For the purpose of this thesis, two study sites were chosen along the Costa Rican Pacific coast. Culebra Bay (CB), located at the northern Pacific coast as a section of the Gulf of Papagayo and the Marine National Park Ballena (MNPB), which forms a part of the central Pacific coast of Costa Rica (Fig. 1). Although both regions are under the general influence of seasonal changes between the rainy the dry season, each one has its own climatic and oceanographic characteristics. Also regarding the coral communities and coral reefs, differences in species diversity, composition and abundance have been described (see below).

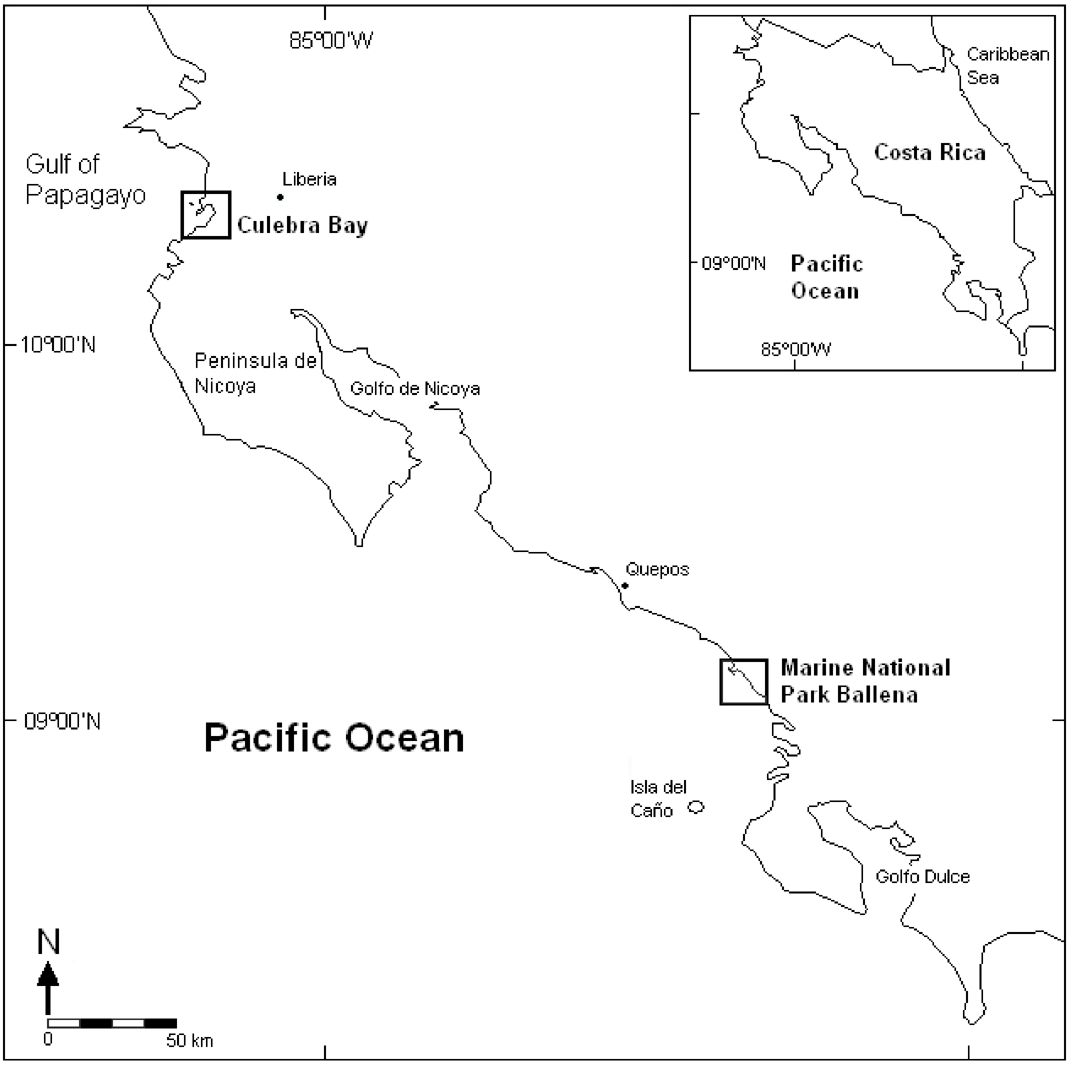


Figure 1. Location of the study sites Culebra Bay as part of the Gulf of Papagayo in the northern section and the Marine National Park Ballena in the central section of the Costa Rican Pacific coast.

2.1.1 Study site A: Culebra Bay, northern Pacific coast

Culebra Bay (CB) covers an area of ~20 km^2 and consists of a series of bays of small to large dimensions and can be divided into an outer and an inner section (Fig. 2). The bays are separated by a number of extensions formed by adjacent mountains and rocky shores. The shoreline is fringed by large sections of beaches, among which Playa Panamá, Playa Hermosa and Playa del Coco are the most important ones. Also mangrove forests from smaller in the external to larger extensions in the inner littoral can be found together with two estuaries: Estero Iguanita in the inner and Estero Palmares in the external littoral.

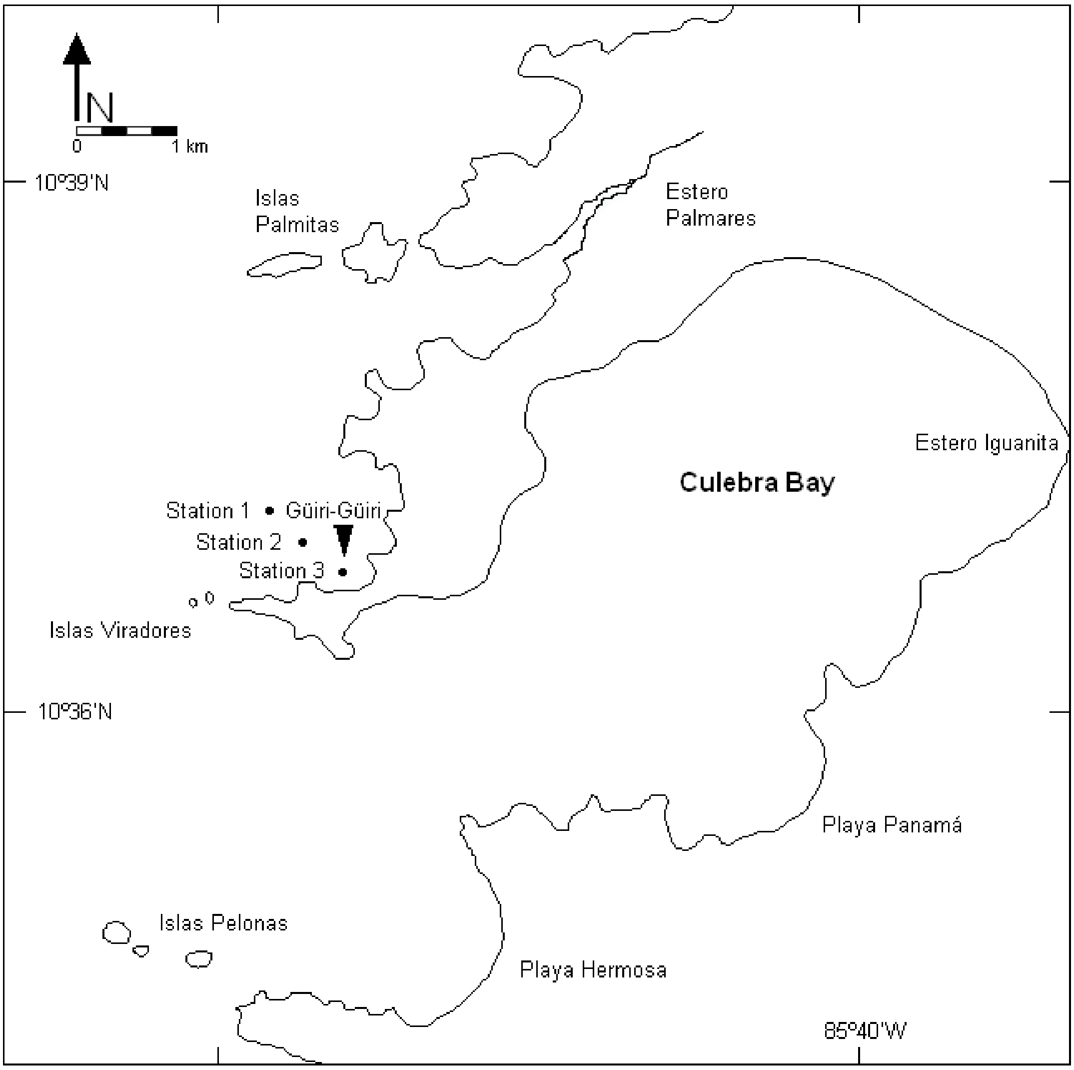


Figure 2. Map of Culebra Bay showing the location of the coral reef Güiri-Güiri and the three water sampling stations.

Besides the Gulf of Tehuantepec (Mexico) and the Bay of Panama (Panama), the Gulf of Papagayo is one of the three regions in the central eastern Pacific where wind-driven upwelling of cool, nutrient-enriched waters occurs. At CB, the direction and velocity of the wind has an essential influence on the prevailing oceanographic conditions. During the dry season, NE Trade Winds, associated with high pressure systems in the Gulf of Mexico and the Caribbean Sea, channel through a land passage between the Guanacaste volcanic mountain rage and the region of Lake Nicaragua causing strong wind jets to blow offshore (Fig. 3). This in turn induces a displacement of superficial waters and a decrease in water temperatures of up to 10ºC due to upwelled cold bottom water within a few hours, often maintaining low temperatures (<21ºC) for several days during several months (Stumpf and Legeckis

1977; Clarke 1988; Legeckis 1988; McCreary *et al*. 1989). From May to November, western and south-western winds become more dominant representing the origin for the rainy season during this period.

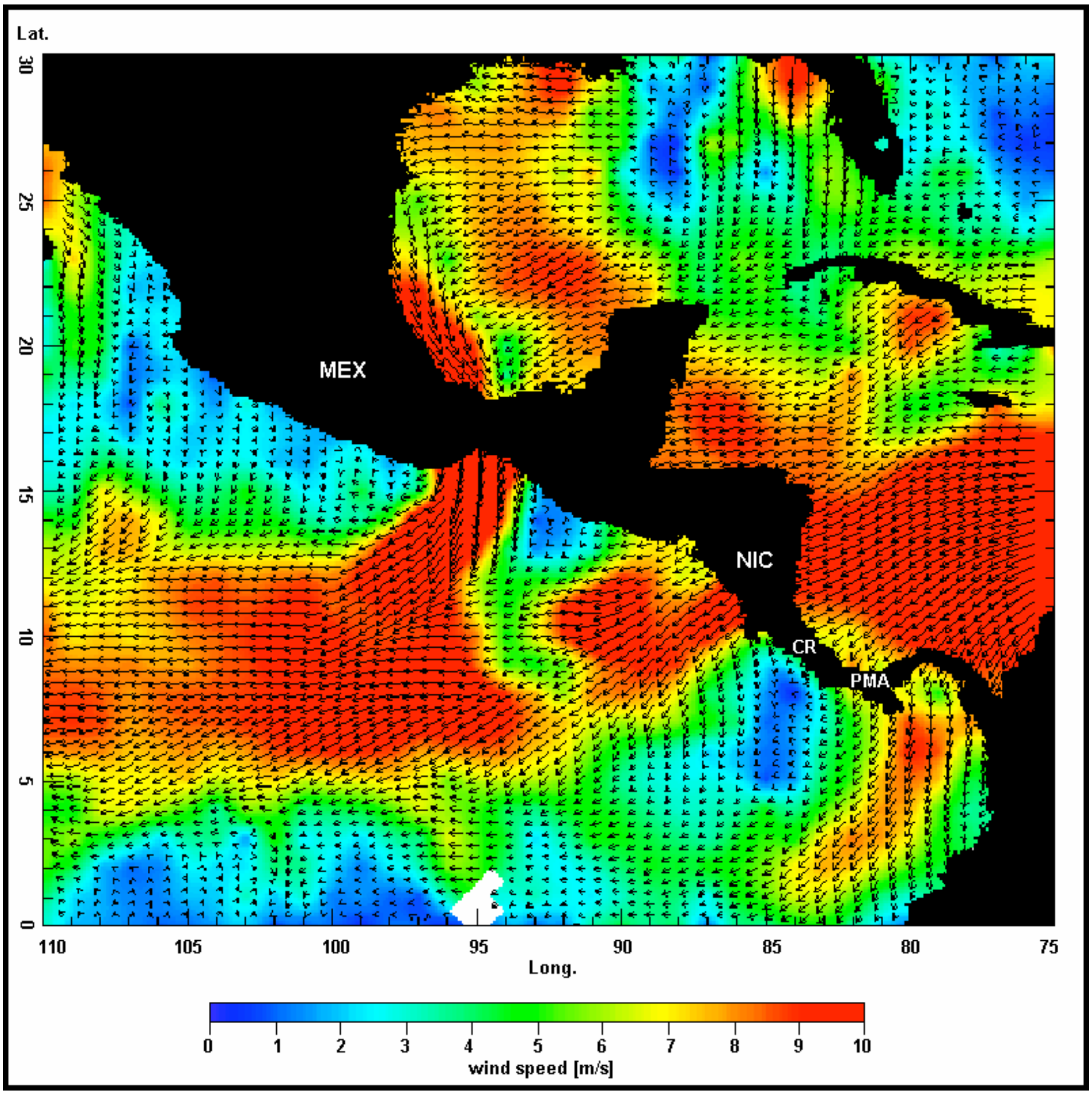


Figure 3. NSCAT image of the wind jets off Central America (6-7 March 1997) showing how NE tradewinds pass over the main land from the Caribbean through three principle passages at the Gulf of Tehuantepec, Mexico (MEX), the Gulf of Pagapayo, Costa Rica (CR) and the Bay of Panama, Panama (PMA). Black arrows indicate the direction of the wind fields, the wind speed (in ms^{-1}) is represented by the color scale at the bottom.

The coral communities and coral reefs of CB were first described by Cortés and Murillo (1985) and later intensively studied by Jiménez and others (Jiménez 1997; Jiménez 1998; Jiménez 2001a; Jiménez 2001b; Jiménez *et al*. 2001; Jiménez 2002; Jiménez and Cortés 2003b; Jiménez and Cortés 2003a). Sixteen species of reef-building corals and four species of azooxanthellate corals have been identified on basalts or sand. Among these are rare species, such as *Leptoseris papyracea* or

Fungia (Cycloseris) curvata. The main reef builder of this region are branching *Pocillopora* spp. especially in areas protected from wave motion and shallow waters (<10 m), while at greater depths, the massive species *Pavona clavus, Porites lobata and Pavona gigantea* become increasingly dominant (Jiménez 2001a). The susceptibility of *Pocillopora* spp. to cool water and poor light penetration during seasonal upwelling have been suggested to be limiting factors for coral growth and reproduction in the Gulfs of Papagayo and Panama, and thus could play an important role in structuring the species dominance in this region (Glynn 1977; Jiménez 2001b). Other studies that have been conducted in this region include for example the description of the reef fish community (Dominici 1999) and among ongoing projects are studies dealing with the reproduction of *P. clavus* (Bezy in prep.), the competition between algal and coral growth (Nivia in prep.) or the growth dynamics of the green algae *Caulerpa sertularioides* (Fernández and Cortés 2005).

One of the live coral reefs, locally known as Güiri-Güiri, is located in the northern external shores of the bay (Fig. 2). It was selected as the sampling site for coral fragments, since it is comprised almost exclusively of large colonies of *P. clavus* at depths ranging from 2 to 13 m in an area of ~1,100 m^2. *Sensu* Jiménez (1998), it can be divided into a shallow (2 - 6 m), an intermediate (6 - 9 m) and a deep (10 - 15 m) zone.

2.1.2 Study site B: Marine National Park Ballena, central Pacific coast

The Marine National Park Ballena (MNPB) is located in the central section of the Pacific coast, between the mouth of Río Higuerón and Punta Piñuelas (Fig. 4). Covering a marine area of >5,000 ha, it consists of large sections of sandy beaches, separated by small river mouths and rocky headlands. Small areas of mangrove forests extend toward the interior. Further to the south, the big river mouth Boca Coronado can be found. Due to its location, the MNPB is virtually protected from strong winds by the high mountain range of the Cordillera de Talamanca, which stretches all along the central part of Costa Rica. For this reason, coastal upwelling is prevented in the central Pacific region. High precipitation during the rainy season combined with human activities, such as construction and deforestation, are responsible for high sediment discharges.

The coral communities and coral reefs along the central Pacific section of Costa Rica were first reported and described by Glynn *et al*. (1983) and Cortés and Murillo (1985). Later, more studies on coral organisms followed (Soto and Bermúdez 1990; Jiménez 1995; Cortés 1996/1997b) and also the effects of the El Niño events of 1991/92 and 1997/98 were investigated by Jiménez and Cortés (2001; 2003a).

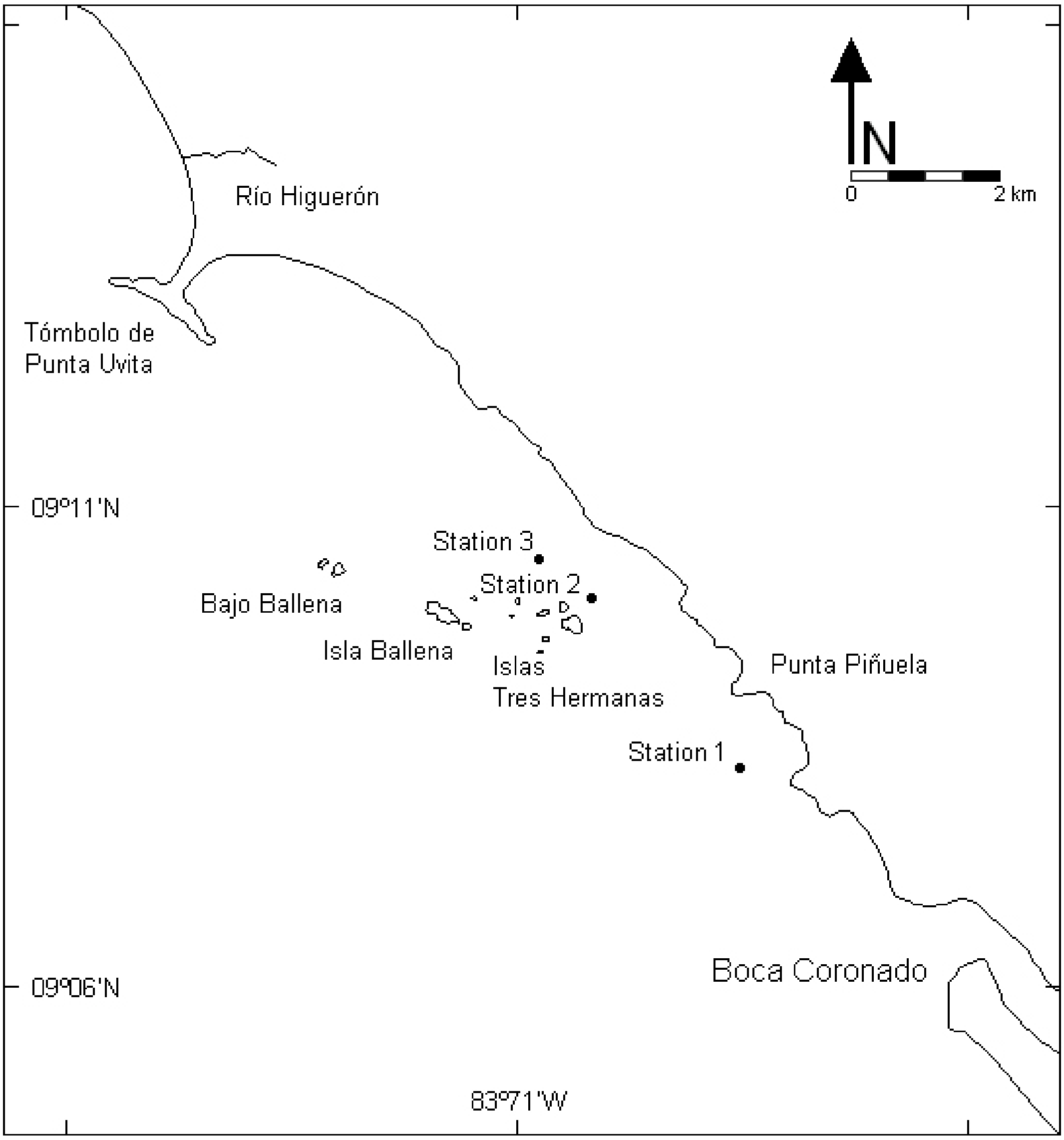


Figure 4. Map of the Marine National Park Ballena. The location of the coral reef Tres Hermanas and the three water sampling stations are indicated.

Since 2001, more intensive studies on the coral communities and coral reefs of the whole MNPB (Alvarado 2004) and the natural and anthropogenic impacts on the same have been carried out (Alvarado *et al*. 2005). Twelve reef-building and five azooxanthellate scleractinian corals have been identified so far. The coral *Porites lobata* is the most abundant species followed by *P. clavus*. Although pocilloporid corals belong to the main reef builders in other areas of the south central section of the Costa Rican Pacific coast, like at Isla del Caño or in the Corcovado National Park, these species exhibit a very low coverage in the MNPB. The synergetic effect of high turbidity and low larval recruitment rates is suggested to be the main cause of the scarcity of these species, added to the impacts of recent El Niño events (Alvarado *et al*. 2005).

The coral reef Tres Hermanas is the most developed one within the MNPB and is almost exclusively build up by large colonies of *P. lobata*, but in some sections *P. clavus* is also abundant (Alvarado 2004). This is the reason why it was chosen as the coral sampling site for the MNPB, since in this way, the requirements for the interregional comparison of the same coral species (*P. clavus*), was given.

2.2 Data collection

2.2.1 Meteorological data

Meteorological data of rainfall, temperature, sun hours and wind speed (only Liberia) from the closest stations to CB and the MNPB (Liberia: 10º35'N, 85º32'W and Damas: 09º29'N, 84º12'W, respectively) were provided by the IMN of Costa Rica. Using these data, climate diagrams were constructed in order to identify the duration of rainy and dry season in each region. In these types of diagrams after Walter and Lieth (1960), seasonal curves of mean monthly temperature (ºC) and monthly rainfall (mm) are plotted by scaling one degree of temperature equal to two millimeters of rainfall, based on assumption that the monthly potential evapotranspiration equals twice the mean monthly temperature. The time of the seasonal transitions can be determined according to the intersections of the two curves. Additionally, figures illustrating sun hours and wind speeds were produced.

2.2.2 Recording of water temperatures

Sub-surface water temperatures are continuously recorded in the coral reefs Güiri-Güiri (7 m depth) at Culebra Bay and Tres Hermanas (8 m depth) in the Marine National Park Ballena with underwater temperature data loggers (Hobotemp, Onset Computer Corp.) within the frame of other ongoing projects. The data for the fieldwork period of this study were provided by courtesy of Dr. Jorge Cortés, Dr. C. Jiménez and Lic. J.J. Alvarado.

2.2.3 Collection of seawater samples

Monthly seawater samples were taken at three stations at and in the proximity of the coral reefs at both study sites (Figs. 2 and 4). Exact position of each sampling station was recorded with a hand-held GPS (Global Positioning System) device. At each station, the vertical visibility in the water column was determined with a Secchi disk. Replicate water samples were taken at surface and one meter above bottom using Niskin bottle. At depths >16 m, samples were taken at 16 m, since this was the length of the rope tied to the Niskin bottle. Water temperature, oxygen concentration and salinity were measured directly in the Niskin bottle or in the surface water with a oxygen meter (WTW-Oxy92) or conductivity meter (WTW-LF96), respectively.

In the field laboratory, one liter of water sample was filtered (Whatman GF/C) and an aliquot of 50 mL was transferred to polyethylene bottles and conserved with 150 μL of a mercury chloride solution (35 g/L). All samples were frozen at –18ºC and transported to the laboratory in San José within the next 48 hours, where they were stored at –20ºC. Finally, all water samples were transported to Germany and analyzed in the Center of Tropical Marine Ecology (ZMT) in Bremen.

2.2.4 Collection of coral samples and isolation of zooxanthellae

Replicate fragments (8.4 ± 3.4 cm^2) of the scleractinian coral *P. clavus* were collected using SCUBA from CB and the MNPB during monthly fieldtrips from September 2004 to January 2005. Once a month, coral fragments (n=6-9) were chiseled off from non-shaded areas of upper surfaces of colonies at a depth between 7 and 9 m. The lesions caused by the sampling procedure have been observed to recover within approximately 4 to 6 month (J.J. Alvarado, pers. comm.). Coral samples were placed in pre-labeled plastic bags and transported in seawater to the field laboratory, where they were wrapped in aluminum foil and frozen in liquid nitrogen or stored in a freezer (-18ºC). Transportation to the laboratory in San José took place within 48 hours after collection, where all samples were stored at -20°C before further processing.

At the laboratories of the Centro de Investigación en Ciencias del Mar y Limnología (CIMAR), the tissue covering the coral fragments was removed using an artist's airbrush with 5 – 30 mL cool (4ºC) zooxanthellae buffer (ZB) containing 0.4 M

NaCl, 20 mM EDTA and 20 mM Tris-HCl at pH = 7.6. The slurry was collected in 15 ml centrifuge tubes on ice and homogenized by adding a cool Dithiothreitol (DTT) / Tween 20 - solution (final concentrations: 100 mM DTT and 0.05 % (v/v) Tween 20) to ensure the release of zooxanthellae and digestion of remaining coral mucus. Remaining skeletal debris was removed by letting the particles settle to the bottom of the centrifuge tube and passing the supernatant to a clean one. Then the tube was centrifuged at 4000 rpm for 15 min and re-suspended in 5 mL ZB. The first washing step was repeated once or twice to obtain a pellet of zooxanthellae. A final washing step was done only with ZB in order to remove the remaining DTT / Tween 20, before the pellet was re-suspended in 5 mL ZB resulting in a homogeneous cell suspension.

2.2.5. Coral core sampling

Between January 29th and 31st of 2000, coral cores (25 cm length, 5 cm diameter) of the massive species *P. clavus* were collected using an underwater pneumatic drill from each of the three different depth zones in the coral reef Güiri-Güiri at CB and provided for this work by courtesy of Dr. C. Jiménez.

2.3 Laboratory analyses

2.3.1 Determination of dissolved inorganic nutrient concentrations

Dissolved inorganic silicate, ammonia and phosphate concentrations were determined on a hand spectrophotometer using 5 cm cells. Nitrite and nitrate concentrations were analyzed using a continuous flow auto-analyzer. Both measurements followed the colorimetric methods after Grasshoff *et al*. (1983).

2.3.2 Determination of suspended matter, total and organic carbon and particulate organic nitrogen concentrations

In order to determine the amount of suspended matter per liter seawater, the dry weight (24 h at 60ºC) of glass fiber filters (Whatman GF/C) with suspended matter after filtration of seawater samples was subtracted from the dry weight before filtration (4 h at 450ºC). For total particulate carbon (Ctot) and particulate organic nitrogen (PON) analyzes, filters with suspended matter were dried (60ºC, 24 h) and cut into four equal-sized pieces. One quarter of each filter was wrapped into tin cups (10 x 10 mm) for Ctot and PON analyzes and another quarter for particulate organic carbon (POC) was acidified in silver cups (10.5 x 9 mm) with 1 N HCl and dried overnight, before they were analyzed on an elemental analyzer (CE Instruments NA 2100). Data were subsequently normalized to the whole filter size and expressed in μmol per liter (μM) seawater.

2.3.3 Determination of zooxanthellae densities and chlorophyll concentrations

The surface area of the coral fragments was determined by the using the aluminum foil method of Marsh (1970). An aliquot of 100 μL of the homogenate was preserved by adding 100 μL of formalin (38%) and the total cell number of zooxanthellae was determined by 16 replicate counts on a haemocytometer. After correcting for homogenate volume and surface area, the densities of zooxanthellae were determined and calculated per cm^2 coral surface area.

For the analyses of chlorophyll concentrations, the homogenate was centrifuged (3000 rpm, 10 min) to pellet the algae. The centrifuge tubes were then wrapped in aluminum foil and frozen overnight. The next day 10 mL of acetone (100%) was added and the pellet ground by using a glass stick to help break open the cells and extract the pigments. After several cycles of freezing, centrifuging and grinding, the samples were centrifuged again (4000 rpm, 15 min) and the absorbance of the supernatant was read at 630 nm, 663 nm and 750 nm on a spectrophotometer (Shimadzu UV-160A). Concentrations of chlorophyll *a* and *c* were determined per cm^2 and per cell using the equations of Jeffrey and Humphrey (1975) after correcting for homogenate volume, surface area of the coral fragments and total number of cells.

2.3.4 Determination of inorganic phosphorus concentrations in coral core slabs

Slabs (approx. 0.5 cm thick) were cut along the axis of each core using a circular geological saw and each slab was X-rayed. The radiographs were used to obtain positive black and white prints, which were photocopied on transparency films. Using these films, annual growth bands, generally consisting of one main high density band (sometimes with one or more interceptions of narrow low density bands) and one low density band, were identified and sectioned into four equidistant segments, resulting in four sub-seasonal samples. Lines were drawn with a pencil, parallel to the growth bands and perpendicular to the growth axis of the coral, in order to mark the segments to be sampled. The slabs were cleaned for 30 minutes in an ultrasonic bath and dried at 40ºC for 48 hours prior to further processing. From each segment, approximately 200 mg of skeletal powder was sampled with a precision drill using a 2 mm diamond grinding bit. 40 ± 1 mg of coral powder was dissolved in 6 ml of hydrochloric acid (1%) and shaken until complete dissolution (45–60 min). Phosphorus concentrations were subsequently determined according to modified standard methods by Grasshoff *et al.* (1983). It was necessary to replace the sulphuric acid by milli-Q water in order to avoid too low pH values. Calibration curves ($r^2>0.99$) were obtained by using standard solutions and coral powder was replaced by 40 mg of phosphate-free calcium carbonate. Absorbance was read at 880 nm on a spectrophotometer (Perkin Elmer 552) using a 5 cm cell.

2.4 Statistical analyses

Mann-Whitney's Rank Sum Test was used to determine differences in dissolved inorganic nutrient concentrations or particulate matter between the rainy and the dry season. One-way ANOVAs were conducted to test differences in zooxanthellae densities or chlorophyll concentrations between the sampling months or between the three depth zones at the coral reef Güiri-Güiri, respectively. When a significant difference between the groups was detected, a post-hoc test was performed at a level of $p<0.05$ (Tukey or Student Neumann Keuls).

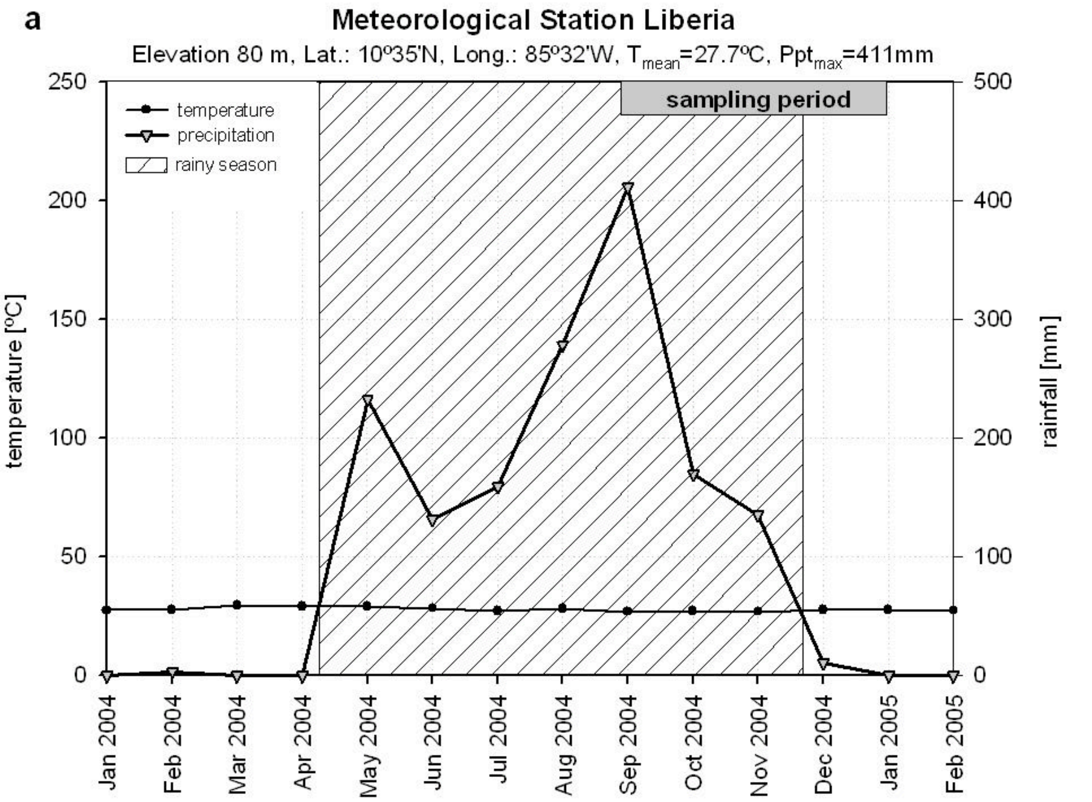
3. RESULTS AND DISCUSSION

3.1 Seasonality at the study sites Culebra Bay and Marine National Park Ballena

In the year 2004, the annual mean temperature at the meteorological station of Liberia was 27.7ºC (range: 21.0 – 31.7ºC) and the hottest month with monthly mean temperatures over 28ºC were those from March to June. The annual total rainfall was 1,528 mm with a monthly mean of 127 mm and a maximum of 411 mm in September. The amount of rainfall follows a characteristic seasonal pattern, where the driest months extend from December to April (Fig. 5a). From May to November rainfall was high (>100 mm) with two maxima in May and September and a low in June and July. The low rainfall period, generally occurring around July, is a dry period that is known as the Veranillo (little summer) de San Juan (Ramírez 1983).

Based on long-term data records at the station of Liberia, the months from May to October are considered to comprise the rainy season and the months from December to March the dry season, while November and April can be regarded as transitional months, during which rainfalls do occur, although with less regularity (IMN www.imn.ac.cr). The data presented here are consistent with this and since rainfall in November 2004 was relatively high it was regarded as part of the rainy season 2004 within the frame of this study.

At the meteorological station of Damas, the annual mean temperature of the year 2004 was 26.7ºC, only 1ºC lower than at the station of Liberia, although with a wider range of daily mean temperatures (16.6 – 31.5ºC). Mean monthly temperatures varied between 25.4ºC and 28.1ºC and the highest temperatures were recorded from April to June. The MNPB is considered to be located in an area of moderate to high rainfall. The total annual rainfall of 4,051 mm in the year 2004 was more than 2.5 times higher than in the area of CB and the monthly mean was 338 mm. In contrast to the region of CB, the period of higher rainfall (>100 mm per month) already began in mid-March and lasted until the beginning of December 2004 (Fig. 5b). Similar to the region of CB, the first maximum of rainfall was in May (723 mm), whereas the second was not in September, but in October (793 mm). As in the northern part, the less humid months of the rainy season were June and July.



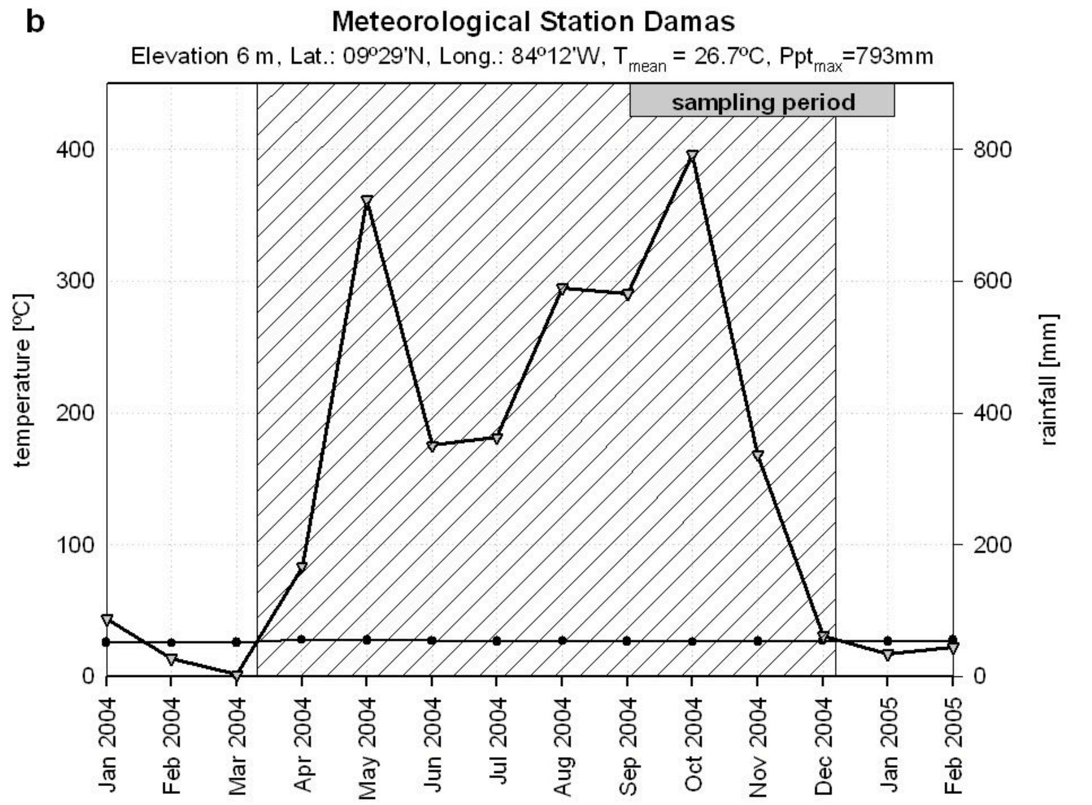


Figure 5. Climate diagram after Walter and Lieth (1960) showing monthly mean temperature (ºC) and monthly rainfall (mm) recorded at the meteorological stations of Liberia (a) and Damas (b) from January 2004 to February 2005. The duration of the rainy season was determined according to the intersections of the two curves.

According to long-term data from the central Pacific coast, the rainy season generally extends from April to December, although April and December are also considered as transitional months between the rainy and the dry season. In December 2004, rainfall was very low, thus it was regarded as part of the dry season 2004/2005. Usually there is no reduction of rainfall during July and August like in the northern region. However, in 2004, there was a low between June and July and furthermore, rainfall was very low in December 2004, thus, it will be considered as part of the dry season 2004/2005.

Summarizing the results of this section, it can be observed that both regions are characterized by alternating dry and rainy seasons. In the northern part, rainfall is lower and the duration of the rainy season is a little shorter than in the central section of the coast. In the region of CB, the transition from rainy to dry season was around end-November 2004, and at MNPB around the beginning of December 2004. The rainy season of 2004 started later at CB (April) than at MNPB (March). Based on these results, the main requirement for this study, that is to cover the transition between rainy and dry season, was fulfilled during the period of the field work (end-September 2004 to end-January 2005). The transition from rainy to dry season is chosen to be for both regions at the beginning of December.

3.2 Climatic and oceanographic aspects during the sampling period

The data for mean daily sun hours, mean surface water salinity (from all sampling stations), monthly rainfall, sub-surface water temperature and mean wind speed (only for CB) are presented in the Figures 6a-d.

At CB, mean daily sun hours were increasing from September 2004 to February 2005. In the months from December 2004 to February 2005, mean daily sun hours were higher than the annual mean (7.7 h d^{-1}) of 2004 (Fig. 6a). In the generally cloudier region of the MNPB, mean monthly sun hours per day never exceeded the yearly mean of 5.8 hours per day during the rainy season, but from December 2004 on, values increased to up to 8.6 hours per day in February 2005 (Fig. 6a). Rainfall at CB decreased from September, the month of maximum rainfall, to November and was lower than 10 mm during the arid northern dry season (Fig. 6b), while at the MNPB rainfall peaked only in October, before it dropped to less than 65 mm during dry season. An inverse relation between the amount of rainfall and seawater surface salinity could be observed for all monthly fieldtrips to both study sites, except in September at CB, where salinity was higher than expected in the wettest month of this region. This could be explained by the fact that the day of sampling was proceeded by a couple of days without rainfall (data not shown), thus the data collected in September are likely to represent a drier than normal day of the rainy season. The range of salinities (19.1 – 31.5) was substantially higher than the one at CB (31.7 – 33.7), indicating that rainfall and associated terrestrial runoff play a major role among the factors that determine the oceanographic conditions in the region of the MNPB. During the period of September 2004 to January 2005, mean monthly sub-surface water temperatures decreased from 28.8 ± 0.7ºC to 25.84 ± 1.7ºC in the intermediate section of the coral reef Güiri-Güiri. Standard deviations and the range of temperatures were increasing substantially towards the dry season, reaching minimum temperatures as low as 19.3 to 19.8ºC (Fig. 6c). At the same time, mean monthly wind speeds were higher in the dry season (15.3 – 20.6 km h^{-1}) than in the rainy season (6.7 – 9.6 km h^{-1}), which was expected due to the intensification of the NE Trade Winds during the dry season (Fig. 6d). In the MNPB, the water temperature followed a different pattern (Fig. 6c). Here, monthly mean temperatures first decreased from September (28.5 ± 0.6ºC) to October (27.7 ± 0.9ºC), but then increased from October 2004 to January 2005 (29.9 ± 0.3ºC).

The meteorological and oceanographic patterns reported in this section are congruent with former reports on the seasonality governing CB as part of the northern section of the Costa Rican Pacific coast and MNPB as part of the central section (Cortés and Jiménez 2003). Apart from lower rainfall and more sun hours per day, the most important influence on the oceanographic conditions at CB is the occurrence of wind driven seasonal upwelling of cold waters (Legeckis 1988), which is prevented at MNPB due to its protection by the Cordillera Talamanca. This is reflected in the data presented in this study. At CB, seawater temperatures were decreasing accompanied by increasing wind speeds towards the dry season. In accordance to the observed negative trend from the rainy to the dry season, Jiménez (2001) reported superficial seawater temperatures (SSTs) of 27.0 ± 0.1ºC during the rainy season and 22.9 ± 0.3ºC during the dry season. At a depth of 7 m the mean temperature was 27.1ºC (range: 20.5 - 31.6ºC) and 25.8ºC (range: 9.9 – 31.5ºC) at 12 m depth. In the present study, increasing standard deviations and a wider range of minimum and maximum temperatures towards the dry season were probably due to increasing frequencies of upwelling cold water fronts that reached the coral reef. It is plausible that corals at Güiri-Güiri are faced with cold water stress during those months. In fact, Jiménez (2001) reported light coral bleaching two weeks after an upwelling event and cold water stress on corals is also documented under natural and experimental conditions (Glynn *et al*. 1983; Saxby *et al*. 2003).

Different to the conditions at CB, daily sun hours and ambient temperature are the main factors in the MNPB that determine seawater temperatures throughout the entire year, since seasonal upwelling is absent in this region. In January 2005, sub-surface water temperatures were already as high as 29.98 ± 0.29ºC, while April is expected to be the hottest month of the year. Thus, as opposed to the situation at CB, high temperature rather than cool temperature stress can be expected as a potential stress factor for the corals of the MNPB during the dry season.

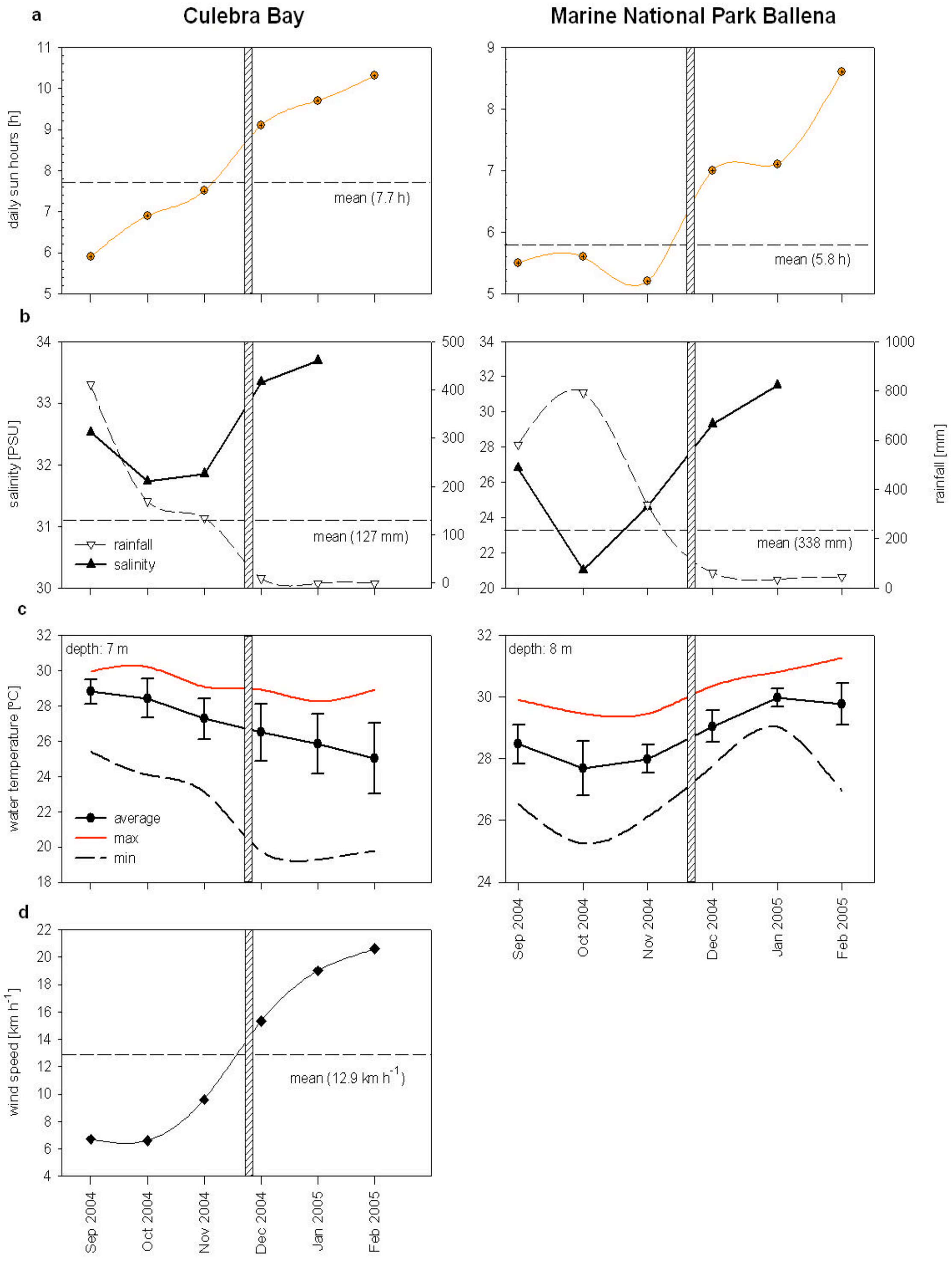


Figure 6. Mean daily sun hours (a), monthly rainfall and surface water salinities (b), sub-surface water temperatures (c) and mean wind speed (d; only Liberia) from September 2004 to February 2005 are shown. Surface water salinities are mean values of the sampling stations (see Fig. 2 and Fig. 4). For the sub surface water temperatures (c), standard deviations, maxima and minima are given. Mean values of the year 2004 are given in dashed horizontal lines. The vertical bars indicate the approximate time of the transition between rainy to dry season.

3.3 Water quality parameters over the study period

Surface and bottom salinities as well as silicate concentrations were used to examine the sources and intensity of fresh water input at both study sites (Tables 1 and 4). Also, changes in the concentration of other dissolved inorganic nutrients, i.e. nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_4^+) and phosphate (PO_4^{3-}) were monitored during the study period (Figs. 7 and 9). Their inter-seasonal comparison is summarized in the Tables 2 and 5. At the same time, the changes in suspended matter (SusMat), total particulate carbon (Ctot), particulate organic carbon (POC) and particulate organic nitrogen (PON) were examined (Figs. 8 and 10) and compared according to rainy and dry season (Tables 3 and 6). Finally, results of Secchi-disk readings in the MNPB are presented (Table 7).

3.3.1 Transition from rainy to dry season in Culebra Bay

During the sampling period from September 2004 to January 2005, surface water salinities of all sampling stations at CB were in the narrow range from 31.6 to 33.8.. Salinities of the surface water samples were lower during the rainy season, but within the same month, they did not differ substantially between the sampling stations (Δ_{max} = 0.8 in November). Deep water salinity was lower at station 3 (depth: 7 m) than at station 2 and 1 in September and October, probably because of better mixing with the surface water layer at station 3, but from November to January, values were not considerably different between the single stations (Δ_{max} = 0.5). The only water sample with a salinity higher than 34 was measured in the deep water sample of station 2 in January.

Silicate concentrations of the surface water samples were peaking in October, reaching up to 3.4 μM. At the coral reef Güiri-Güiri (station 3) values were always higher than or equal to those observed at station 1 and 2, which could be explained by the closer distance to the coast, and thus higher influence of terrestrial run-off or tidal motion. Deep water silicate concentrations were in the range between 0.8 and 2.7 μM during the rainy season and between 1.3 and 4.8 μM during the dry season. The highest concentration (4.8 μM) was determined in the deep water sample of station 2 in January.

Table 1. Seawater salinities (S in PSU) and silicate (Si) concentrations (in μM) measured monthly at three sampling stations at CB, station depths are given with the station numbers, sampling depths are shown (in m).

		Sep		Oct		Nov		Dec		Jan	
Station # (depth)	Sampling depth [m]	**S**	**Si**	**S**	**Si**	**S**	**Si**	**S**	**Si**	**S**	**Si**
1 (47 m)	0	32.6	0.6	31.7	2.1	31.6	1.0	33.3	1.0	33.8	1.4
2 (25 m)	0	32.4	0.5	31.7	2.4	31.7	0.9	-	-	33.5	1.6
3 (8 m)	0	32.6	0.6	31.7	3.4	32.3	2.1	33.3	2.6	33.8	2.9
1 (47 m)	16	33.9	1.1	33.8	1.8	32.2	0.9	-	-	33.8	1.6
2 (25 m)	16	33.7	1.2	33.6	2.1	32.4	1.0	-	-	34.3	4.8
3 (8 m)	7	31.9	0.8	32.2	2.7	32.5	2.6	33.5	2.8	33.9	1.3

For all other dissolved inorganic nutrients, i.e. NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-}, there was no recognizable trend along the single sampling stations, thus monthly concentrations of the individual stations were averaged (Fig. 7). Only in two particular cases there was an evident difference among the sampling stations, which is also the reason for the high standard deviations of some mean values: First, during the dry season, surface water concentrations of ammonia were higher in the surface water of the reef Güiri-Güiri (station 3). Second, in January, bottom concentrations of silicate, NO_3^-, NO_2^- and PO_4^{3-} were elevated only at station 2 (Fig. 7). While the reason for the first case might be due to relatively higher phyto- and zooplankton concentrations and in the surface water layers, it is reasonable that the underwater topography plays an important role in explaining the second case. Station 2 lies further offshore than the shallow station 3 and water samples of station 2 were taken closer to the sea bottom than those from station 1 (Table 1). This water sample was enriched in nutrients (Fig. 7), it had the highest salinity (Table 1) and furthermore it was 1.8 to 2.0°C cooler than those taken from the stations 1 or 3, respectively. The latter suggests that it originated from a cooler and more dense, nutrient enriched water body from the deeper ocean that neither reached until the coral reef (station 3) nor was it to be detected 31 m above the sea bottom at the offshore station 1.

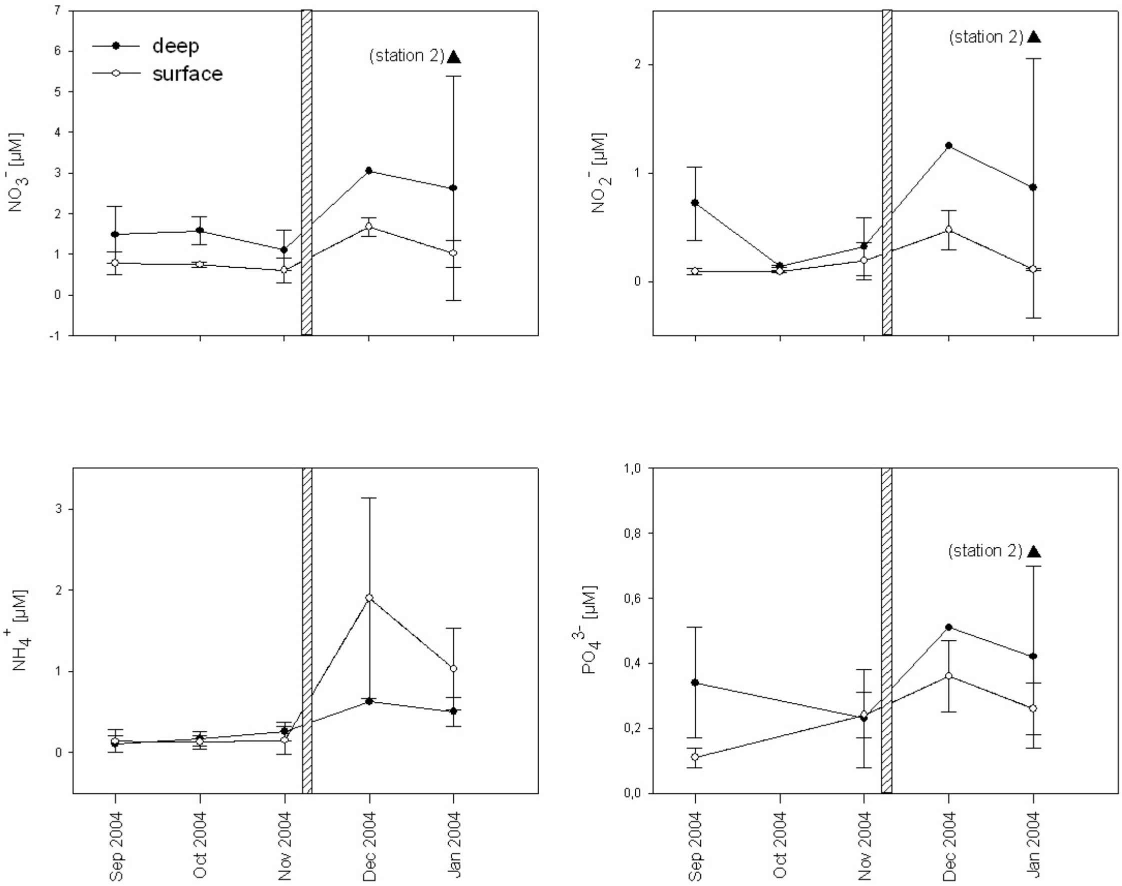


Figure 7. Mean values of dissolved inorganic nutrient concentrations (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}) measured monthly at three sampling stations in front of the coral reef Güiri-Güiri at CB; mean surface water and deep water concentrations (±SD) are shown; vertical bars separate rainy and dry season; triangles show single values of deep water samples of station 2 in January 2005.

Differences between the deep and surface water samples indicate a vertical stratification of the water column, which is usually not expected during upwelling events. However, the observations made here and also measurements of sub-surface water temperatures (see section 3.2) indicate that the region is not permanently influenced by upwelling waters during the dry season, but rather by increased frequencies of wind driven upwelling events (Jiménez 2001). In fact, wind speeds were low during the morning hours, the time when the water sampling took place, which might be the explanation for the vertical stratification that was observed here.

One limitation of this study is that data could be taken only on a monthly basis. Nevertheless, due to the narrow range of seawater salinities and low silicate concentrations over the entire study period, the role of terrestrial runoff is likely to be only marginal. In order to examine the differences between rainy and dry season, the dissolved inorganic nutrient concentrations were averaged for each depth and season

(Table 2). The ratios between dry and rainy seasonal were in the range of 1.57 to 9.8, indicating elevated nutrient concentrations during the dry season. Similar differences have also been reported by D'Croz and Robertson (1997) in the Gulf of Panama, only that their mean, i.e. surface and deep water, NO_3^- + NO_2^- concentrations for the dry (1.03 ± 0.05 μM) and rainy season (0.27 ± 0.01 μM) were lower than those shown in this study. The PO_4^{3-} concentrations were in the same range during the dry season, but approximately twice as high during rainy season. Thus, qualitatively, the results are similar, while quantitative differences could have resulted from unequal regional characteristics or methodological discrepancies. In January 1997, Mueller-Parker and Cortés (2001) conducted a water quality survey in CB and compared to the concentrations of this study (Table 2), their mean (surface and deep water) concentrations of NO_3^- (0.91 ± 0.87 μM) and NO_2^- (0.08 ± 0.09 μM) were lower, but NH_4^+ (1.93 ± 1.96 μM) higher than in this study. However, the mean DIN and PO_4^{3-} concentrations are in the same range as the surface concentrations of this study, indicating that on average, the dissolved inorganic nutrient concentrations were higher in 2004/2005 than in January 1997. It is important to note that during their survey in January 1997, water temperatures were 28ºC, almost 2ºC higher than those measured in January 2005, indicating that the influence of upwelling waters might have been stronger in the present study. Nevertheless, apart from NO_2^-, all mean concentrations are well in the range of the minimum and maximum concentrations observed in 1997.

Table 2. Comparison of dissolved inorganic nutrient concentrations (±SD) between rainy season (September – November 2004) and dry season (December 2004 – January 2005) at CB; for each season, values of all depth and sampling stations were averaged; ratios between dry season (DS) and rainy season (RS) are given with p-values (Mann-Whitney's Rank Sum Test); DIN=dissolved inorganic nitrogen (nitrate+nitrite+ammonia).

	Rainy season	Dry season	DS:RS	p-value
Nitrate [μM]				
Surface	0.71 ± 0.22	1.28 ± 0.44	1.81	**<0.05**
Deep	1.39 ± 0.51	2.73 ± 2.27	1.97	0.41
Nitrite [μM]				
Surface	0.12 ± 0.10	0.25 ± 0.22	2.04	0.08
Deep	0.39 ± 0.34	0.95 ± 1.00	2.43	0.50
Ammonia [μM]				
Surface	0.14 ± 0.12	1.38 ± 0.86	9.8	**<0.05**
Deep	0.18 ± 0.11	0.53 ± 0.16	2.9	**<0.01**
DIN [μM]				
Surface	0.97 ± 0.20	2.91 ± 1.26	3.00	**<0.001**
Deep	1.96 ± 0.66	4.21 ± 3.32	2.15	0.26
Phosphate [μM]				
Surface	0.18 ± 0.09	0.30 ± 0.10	1.71	0.08
Deep	0.28 ± 0.15	0.44 ± 0.24	1.57	0.35

Since the values for suspended matter (SusMat), total particulate carbon (Ctot), particulate organic carbon and nitrogen (POC and PON) were similar at the stations 1 and 2, they were averaged (Fig. 8). Comparing the offshore stations 1 and 2 with the shallower reef station 3, the major differences were observed in September. While at station 3 all parameter had the highest value in September, at station 1 and 2 values were lower than in October. However, no consistent pattern can be recognized during the course of time, but still, mean concentrations of all these parameter were higher during the rainy season (Table 3). Statistical significance was only detected for the surface C:N-ratio ($p<0.05$) of the surface water (Table 3), while it is important to mention that statistical power was low (n=4-9). Based on these observations, sub-surface water visibility can be expected to be marginally higher during the beginning of the dry season, although the concentration of particulate organic matter is likely to increase with time due to higher nutrient availability and solar irradiance as it was observed in the Gulf of Panama by D'Croz and Robertson (1997).

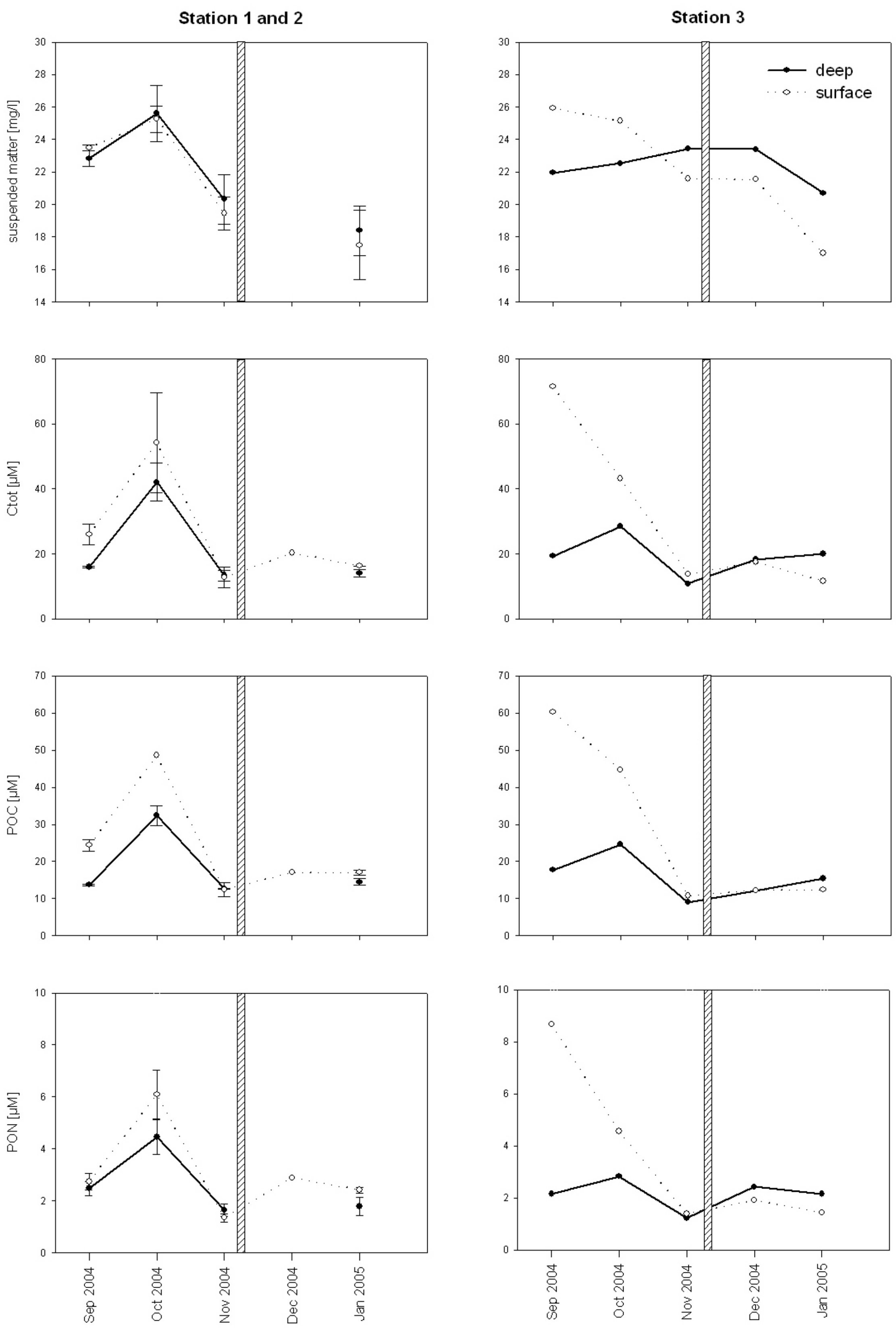


Figure 8. Mean concentrations of suspended matter, total particulate carbon (Ctot), particulate organic carbon (POC) and particulate organic nitrogen (PON) at CB. Values of station 1 and 2 were averaged (±SD); surface water and deep water concentrations are shown.

Table 3. Comparison of suspended matter (SusMat), total particulate carbon (Ctot), particulate organic carbon (POC), particulate organic nitrogen (PON) and the atomic C:N-ratios between rainy season and dry season at CB; for each depth and season, values of all stations were averaged (±SD); ratios between rainy season (DS) and dry season (RS) are given with p-values (Mann-Whitney's Rank Sum Test).

	Rainy season	Dry season	RS:DS	p-value
SusMat [mg/L]				
surface	23.22 ± 2.56	19.45 ± 3.21	1.19	0.060
deep	22.83 ± 2.09	20.21 ± 2.54	1.13	0.148
Ctot [μM]				
surface	34.91 ± 22.37	16.37 ± 3.11	2.13	0.240
deep	22.37 ± 12.44	16.62 ± 3.11	1.35	0.825
POC [μM]				
surface	29.75 ± 19.04	15.14 ± 2.62	1.97	0.354
deep	18.77 ± 8.89	14.15 ± 1.46	1.33	0.825
PON [μM]				
surface	3.89 ± 2.64	2.21 ± 0.56	1.76	0.364
deep	2.59 ± 1.20	2.03 ± 0.37	1.28	0.604
C:N				
surface	8.72 ± 0.93	7.02 ± 1.02	1.24	**<0.05**
deep	7.31 ± 1.19	7.20 ± 1.67	1.02	1.060

3.3.2 Transition from rainy to dry season in the Marine National Park Ballena

In the MNPB, surface and deep water salinities covered a wide range of values in the course of the sampling period (surface: 19.1 – 31.6, deep: 26.4 - 34.1). Since neither surface salinities nor silicate concentrations of station 2 and 3, differed essentially from each other, the averaged values were compared with those of station 1, the closest to the river mouth (Table. 4). Surface salinities at station 1 were always lower than at station 2 and 3 (hereafter station 2-3), but differences between the station 1 and station 2-3 were higher during the rainy than during the dry season. Silicate concentrations in the proximity to the river mouth Boca Coronado were always higher than at the station 2-3. However, at station 1, silicate concentration maintained at a level of over 29 μM until December, before it dropped to lower levels only in January, while at station 2-3, a reduction from high (>29 μM) to low concentrations (<13 μM) could be observed already in December. In January, silicate concentrations were finally below 3.3 μM at all stations.

The deep water samples showed a different pattern regarding salinity and silicate concentrations. There was a steady decrease in salinity from station 1 to station 3 during the rainy months (September – November), which was most likely caused by the decreasing distance to the surface water layers and thus, probably due to a better mixing of the water layers. Finally in the dry months, the differences between the stations were as low as 0.2. Better mixing with the surface layers is also believed to explain the increase in deep water silicate concentrations from station 1 to 3 in the months of the rainy season. During this period, silicate concentrations at all stations ranged from 9.2 to 22 μM, whereas during the dry months December and January, concentrations were below 3.1 μM.

Table 4. Seawater salinities (S in PSU) and silicate (Si) concentrations (in μM) measured monthly at three stations in the MNPB. Surface salinity (Sal in PSU) and silicate (Si) concentrations (in μM) of station 2 and 3 were averaged (2-3) and the difference between station 1 and station 2-3 (Δ1 vs. 2-3) is shown bold faced. Values below the suggested minimum (25 PSU) for coral reef development (Coles and Jokiel 1992) are in *red*.

		Sep		Oct		Nov		Dec		Jan	
Station # (depth)	Sampling depth [m]	**S**	**Si**	**S**	**Si**	**S**	**Si**	**S**	**Si**	**S**	**Si**
1 (20 m)	0	25.5	40.6	*19.1*	48.4	*21.6*	36.4	28.6	29.2	31.4	3.3
2+3*(8;6 m)	0	28.1	30.0	*22.9*	35.4	27.7	29.1	30.0	11.7	31.6	2.2
Δ1 vs. 2-3	**0**	**-2.6**	**10.6**	**-3.8**	**13.0**	**-6.1**	**7.3**	**-1.4**	**17.5**	**-0.2**	**1.1**
1 (20 m)	16	34.1	9.2	30.8	10.6	30.4	9.3	32.0	1.5	31.9	2.2
2 (8 m)	7	33.3	10.2	28.0	11.1	28.3	15.0	31.8	3.1	31.7	2.0
3 (6 m)	5	30.5	14.9	26.4	18.1	27.7	22.1	31.8	2.4	31.7	1.4

*ΔS_{max} between station 2 and 3 = 0.7; ΔSi_{max} between station 2 and 3 = 2.7

Surface and deep water concentrations of NO_3^-, NO_2^- and NH_4^+, together: dissolved inorganic nitrogen (DIN), and PO_4^{3-} concentrations of all sampling stations are shown in Figure 9. During the rainy season, both, in the surface as well as in the deep water samples, a negative trend along the distance from station 1 to station 3 could be observed for DIN. This trend could also be observed for PO_4^{3-} concentrations in the surface water samples of September. During the dry season, DIN and PO_4^{3-} concentrations were reduced to 74 and 21 % in the surface water samples and to 34 and 15 % in the deep water samples, respectively (Table 5). These observations underline the importance (or negative impact) of the river mouth Boca Coronado as a source for dissolved inorganic nutrients during the rainy season.

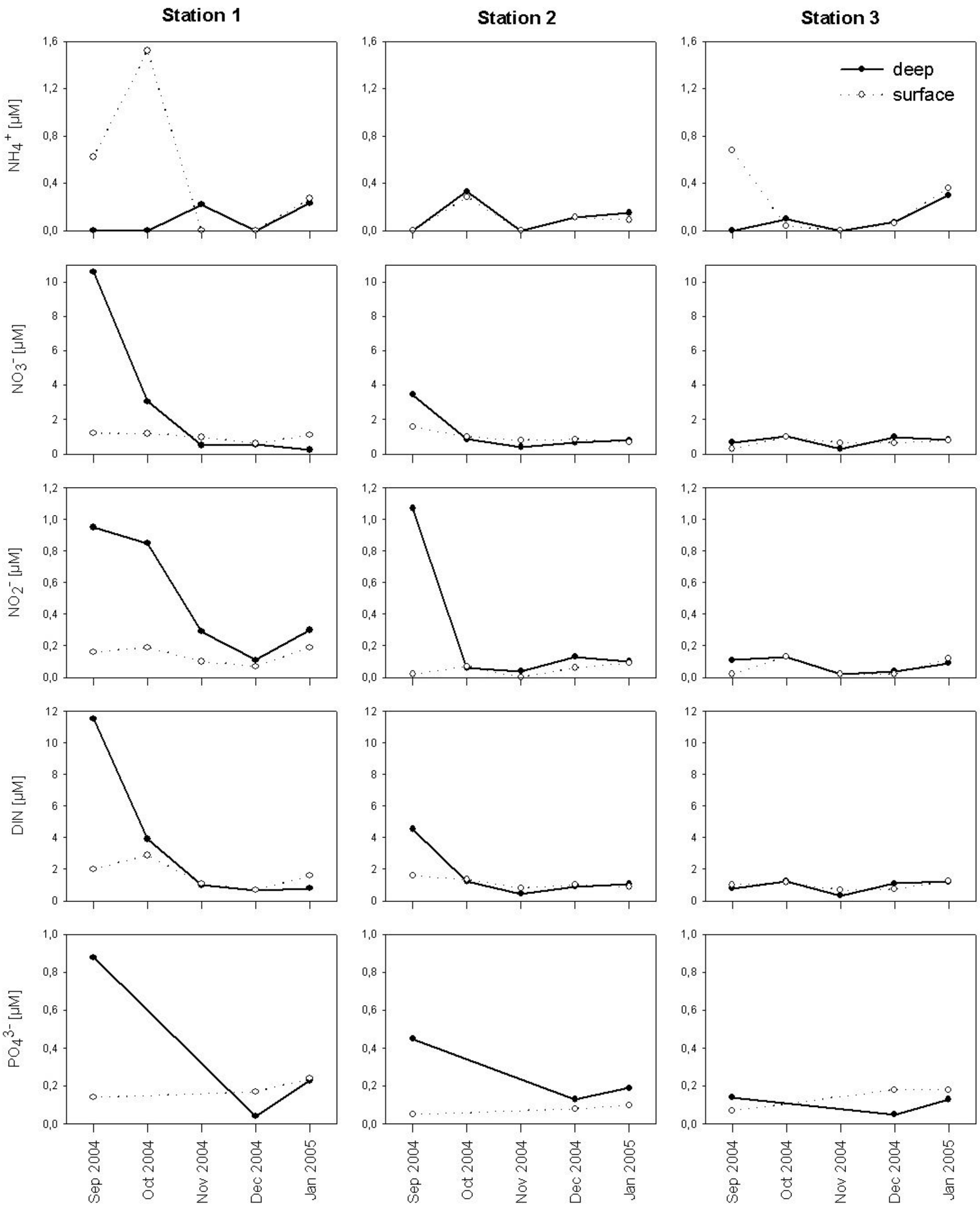


Figure 9. Mean values of dissolved inorganic nutrient concentrations (NO_3^-, NO_2^-, NH_4^+, DIN and PO_4^{3-}) measured monthly at three stations parallel to the coastline of the MNPB; surface water and deep water concentrations are shown.

Table 5. Comparison of dissolved inorganic nutrient concentrations between rainy and dry season in the MNPB; for each depth and season, values of all stations were averaged (±SD), DIN=dissolved inorganic nitrogen (nitrate+nitrite+ammonia).

	Rainy season	Dry season	DS:RS
Nitrate [μM]			
surface	0.96 ± 0.36	0.78 ± 0.19	0.81
deep	2.31 ± 3.31	0.68 ± 0.26	0.29
Nitrite [μM]			
surface	0.08 ± 0.07	0.09 ± 0.06	1.16
deep	0.39 ± 0.44	0.13 ± 0.09	0.33
Ammonia [μM]			
surface	0.35 ± 0.52	0.15 ± 0.14	0.43
deep	0.07 ± 0.12	0.14 ± 0.11	1.98
DIN [μM]			
surface	1.39 ± 0.69	1.02 ± 0.34	0.74
deep	2.78 ± 3.61	0.95 ± 0.20	0.34
Phosphate [μM]			
surface	0.74 ± 0.72	0.16 ± 0.06	0.21
Deep	0.87 ± 0.52	0.13 ± 0.07	0.15

For the parameters: Ctot, POC and PON, no consistent similarity or trend could be observed when the individual stations were compared with each other (Fig. 10). All parameters had their maximum during one of the rainy months, before they decreased towards the dry season.

Statistical tests to determine the differences between rainy and dry season could not be applied on the data sets of the MNPB, since the differences among the individual sampling stations were too high to pool all surface or deep water samples, as it was done for CB. Performing a statistical test for each station was not reasonable, since the number of samples was to low to obtain a reliable result.

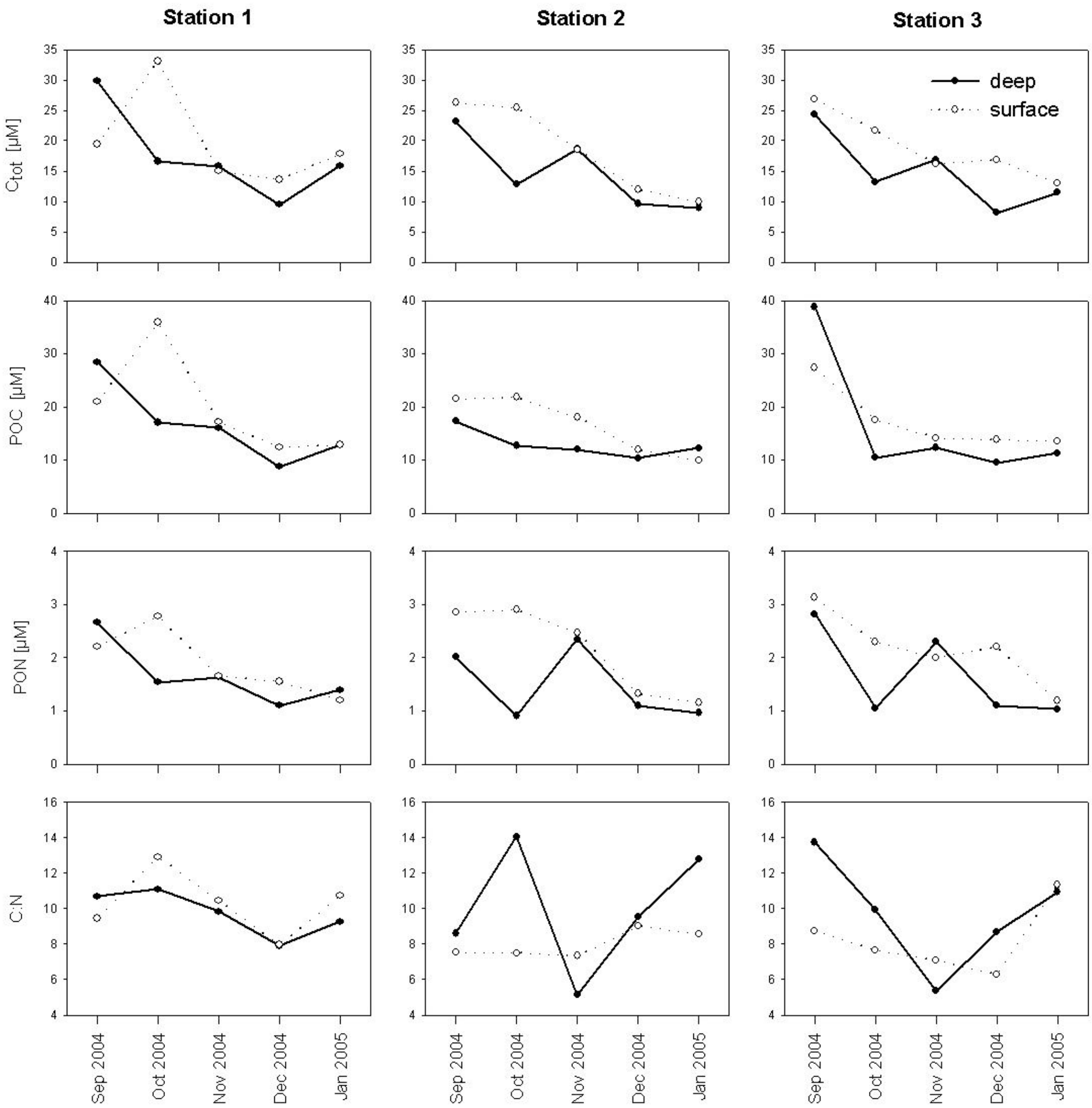


Figure 10. Monthly concentrations of suspended matter (SusMat), total particulate carbon (Ctot), particulate organic carbon (POC) and particulate organic nitrogen (PON) and C:N ratios at three sampling stations in the MNPB.

Table 6. Comparison of total particulate carbon (Ctot), particulate organic carbon (POC), particulate organic nitrogen (PON) and the atomic C:N-ratios between rainy season and dry season in the MNPB; for each season, values of all stations were averaged (±SD); ratios between rainy season (RS) and dry season (DS) are shown.

	Rainy season	Dry season	RS:DS
Ctot [μM]			
surface	22.48 ± 5.87	13.84 ± 2.98	1.62
deep	19.01 ± 5.64	10.59 ± 2.82	1.8
POC [μM]			
surface	21.58 ± 6.54	12.38 ± 1.43	1.74
deep	18.33 ± 9.34	10.83 ± 1.59	1.69
PON [μM]			
surface	2.47 ± 0.48	1.44 ± 0.40	1.72
deep	1.92 ± 0.68	1.11 ± 0.15	1.73
C:N			
surface	8.21 ± 1.20	8.97 ± 2.06	0.92
deep	9.82 ± 3.15	10.42 ± 1.84	0.94

In general, low temperatures, low salinity and high nutrient discharges represent restrictive conditions for coral reef development (Glynn *et al.* 1983). The data presented here demonstrate that low salinities and high silicate concentrations during the rainy season were related to the impact of terrestrial runoff from the river mouth Boca Coronado, since silicate concentrations were inversely proportional to salinity, which is typical when rivers transport dissolved silicate from their basins into the sea. The influence of this impact on the coral reef Tres Hermanas lasted probably until December 2004. Also other dissolved inorganic nutrient concentrations and particulate organic matter (POM) concentrations were elevated during the rainy season. However, during the rainy season, a negative trend from the river mouth to the coral reef could only be observed for DIN and PO_4^{3-} concentrations, but not for POM. The lowest surface water salinity of 22.9 at the coral reef Tres Hermanas was measured during the rainy season (October 2004) and implies that corals, at least those which were exposed to the surface water layers, suffered temporary from low salinity stress, since this value is below the suggested lower limit (approx. 25) for corals (Coles and Jokiel 1992). Also at greater depth, corals experienced salinities that were close this limit (Table 4). Another important feature for the development of coral reefs is low turbidity of the water column, in order to maintain the photosynthetic carbon flow, i.e. the fixation of inorganic carbon by zooxanthellae (Muscatine 1990), and light-enhanced calcification (Barnes and Chalker 1990). Monthly Secchi-disk readings at the sampling stations showed that during the rainy season, visibility was extremely low (Table 7). In fact, the average sedimentation rate was 6.5 times higher (data not shown) during the rainy months of this study (September - November 2004) than it was during the dry months, i.e. December 2004 – January 2005 (Alvarado *et al*. 2005), thus low visibility represents another source of stress for corals in the MNPB during this period.

Table 7. Monthly Secchi-disk readings (in m) at the water sample stations in the MNPB.

Month	Station 1	Station 2	Station 3	Mean
Sep	1	3	3	2.3
Oct	0.2	1.2	0.5	0.6
Nov	6	4	4.5	4.8
Dec	6.5	9.0*	7.0*	7.5*
Jan	5.5	9.0*	7.0*	7.2*

* Secchi-disk reached the bottom

3.3.3 Regional and seasonal comparison of water quality parameters

In the present study, the seasonal variability of environmental parameters during the transition from rainy to dry season has been characterized by providing a set of monthly data. Although the sampling was limited to a period of five months, some important data on water quality parameters during the transition from rainy to dry season have been presented in the previous sections (summarized in Table 8). The interregional comparison supports the assumption that the number of major contributors to a regional difference of environmental parameters between CB and the MNPB can be reduced to a number of two: the influence of wind-induced seasonal upwelling in the region of CB and terrestrial runoff in the MNPB.

In general, nutrient concentrations are very low in tropical reef waters except in regions, where upwelling or terrestrial runoff is important (D'Elia and Wiebe 1990). In the present study, both exceptions are given: CB is influenced by seasonal upwelling of cold waters during the dry season and the MNPB by high amounts of terrestrial runoff during the rainy season.

In the MNPB, it could be identified that terrestrial runoff from the river mouth Boca Coronado, associated to high rainfall during the rainy season, was a major source of dissolved inorganic nutrients during the period from September to November 2004. Due to the absence of wind-driven upwelling of nutrient-enriched waters and the knowledge of the seasonal rainfall pattern, it is reasonable that this assumption will also prove to be true on a yearly scale. Other consequences of the high river discharge included reduced seawater salinities that were close to the lower limit for corals. Combined with reports of high sedimentation rates (Alvarado et al. 2005), which is congruent with the observation of high turbidity within the water column (Table 7), these factors represent stressful conditions and could be limiting factors for the development of coral reefs and communities in the MNPB. During the dry season, the impact of fresh water input became negligible. As a consequence, seawater salinity returned to 31.4 and 31.9 in January 2005, and the underwater visibility increased substantially. Furthermore, water temperatures were observed to increase, while wave motion was lower due to the absence of strong winds and offshore swells, altogether representing more favorable conditions for coral reef health.

Different to the conditions in the MNPB, terrestrial runoff played only a minor role in CB, which was reflected by relatively constant salinities and low silicate concentrations during the rainy season. During the dry season, strong offshore winds seem to have increased the incidences of upwelling which caused water temperatures to decrease, while the dissolved inorganic nutrient concentrations increased. This suggests that seasonal upwelling of cool waters from the deeper ocean was the major source of dissolved inorganic nutrients, as it has been shown for other areas where seasonal upwelling occurs (D'Croz and Robertson 1997). In this context it is important to mention that cool water temperature has been reported to reduce zooxanthellae densities (Saxby et al. 2003), representing probably the most important stress factor for the coral reefs of CB. The amount of suspended particulate matter was decreasing from September 2004 to January 2005, although it is likely to increase again in the course of the dry season, when nutrient concentrations and solar radiation are expected to be high, and thus providing favorable conditions for enhanced primary production.

Table 8. Seasonal and regional comparison of environmental parameters and major features of the regional characteristics of CB and the MNPB; RS = rainy season, DS = dry season, POM = particulate organic matter.

Major features	Culebra Bay	Marine National Park Ballena
Terrestrial runoff	low during RS	high during RS
Wind-induced upwelling	present during DS	absent throughout the year
Observations:	Seasonal comparison	
Salinity	no major variation	very low during RS
Turbidity	marginally lower in the DS	very high in the RS; low in the DS
Water temperature	lower in the DS	higher in the DS
DIN and PO_4^{3-} concentrations	higher in the DS	higher in the RS
POM concentrations	marginally higher during RS	higher during RS
Major source of nutrients	upwelling during DS	terrestrial runoff during RS

3.4 Seasonal variations in zooxanthellae density and chlorophyll concentrations

For both study sites zooxanthellae densities and chlorophyll *a* and *c* concentrations (per area and per cell) were determined during the period from September 2004 to January 2005 (Figs. 11 and 12). The determination of monthly mean densities of zooxanthellae at CB revealed a significant (ANOVA; $p<0.05$) reduction in January compared to the period from October to December (Fig. 11a). Chlorophyll *a* (chl *a*) concentrations per area increased from September to October ($p<0.05$), but then decreased again from November 2004 to January 2005 ($p<0.05$), the month when the chl *a* concentration was lowest (Fig. 11b). The chl *a* concentration per zooxanthellae was not different among the rainy months (September – November), but was reduced ($p<0.05$) in December 2004 and January 2005 (Fig. 11b). Unlike for chl *a*, the chlorophyll *c* (chl *c*) content per area was already reduced in December and chl *c* concentrations per cell were, similar to chl *a* per cell, significantly higher ($p<0.05$) during the rainy season than during the dry season (Fig. 11c). Since the decrease in chlorophyll concentrations was lower for chl *a* than for chl *c* during the course of time, the ratio chl *a:c* was significantly ($p<0.05$) increased in the dry season compared to the rainy season (Fig. 11e). Finally, the total chlorophyll content (chl *a+c*) per area and per cell was higher during the rainy season than in December or January ($p<0.05$) and the total chlorophyll concentration per area was significantly higher ($p<0.05$) in December than in January (Fig. 11d).

In September 2004, the differences in zooxanthellae density and chlorophyll concentrations between the different depth zones (shallow, intermediate, deep) of the coral reef Güiri-Güiri were also examined (Table 9). Chl *a* contents per area as well as per cell were significantly higher ($p<0.05$) in the deeper zone compared to the intermediate zone, but no significant differences could be observed for any other parameter.

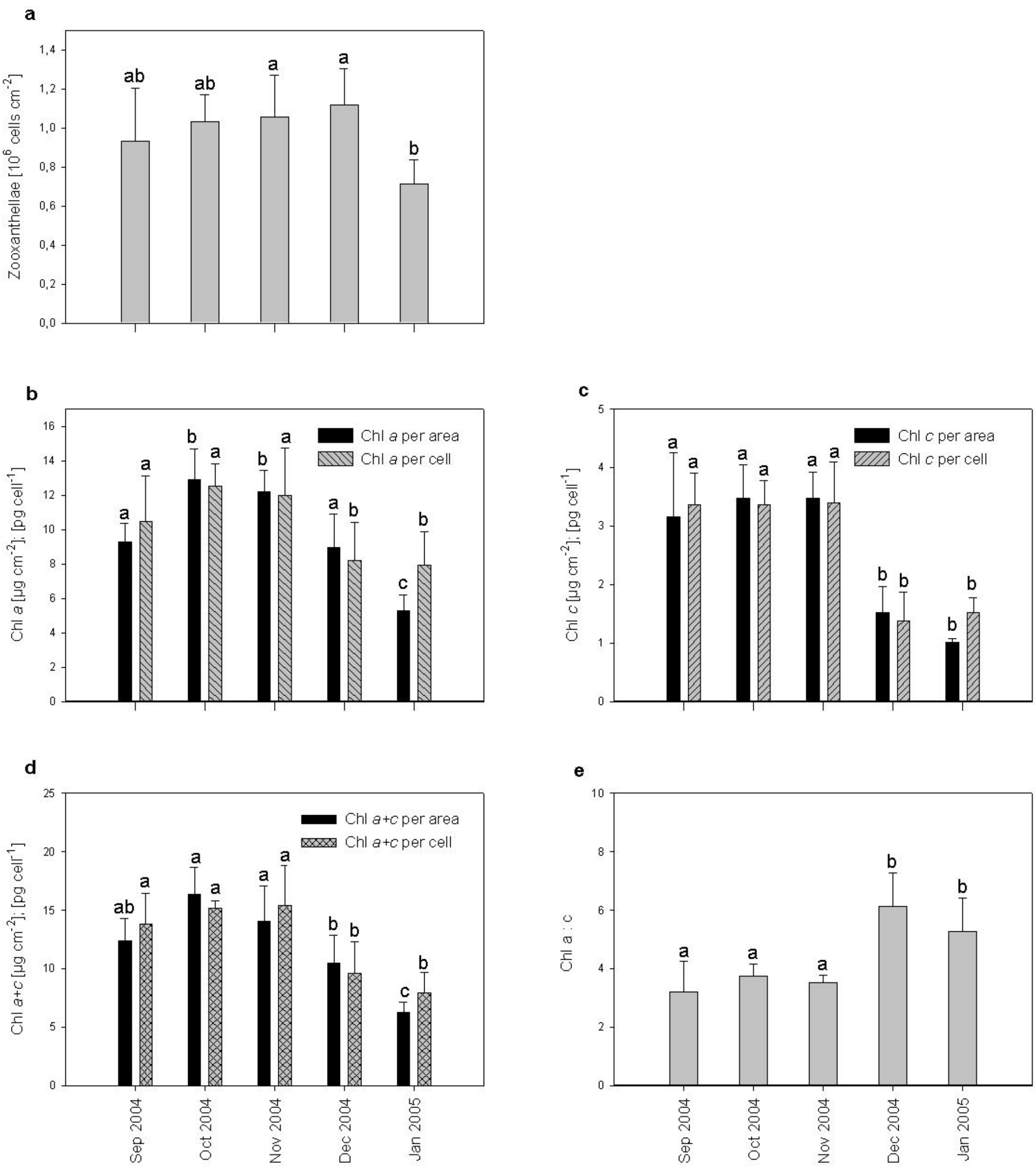


Figure 11. Monthly mean zooxanthellae density (a), chlorophyll (Chl) *a* (b) and Chl *c* (*c*) concentrations per area and per cell, Chl *a* and *c* (Chl *a+c*) concentrations per area and cell (d) and the ratio of Chl *a* and *c* (Chl *a:c*) (e) were determined from samples taken in the coral reef Güiri-Güiri at CB. [abc]means with different letters are significantly different at a level of $p<0.05$.

Table 9. Comparison of mean (±SD) zooxanthellae densitiy (ZD) and mean (±SD) chlorophyll (Chl) concentrations between the different depth zones of the coral reef Güiri-Güiri in Culebra Bay in September 2004.

	Shallow	Intermediate	Deep
ZD [10^5 cells cm^{-1}]	9.80 ± 1.96	9.33 ± 2.71	8.42 ± 2.46
Chl *a* [$\mu g/cm^2$]	11.46 ± 2.39[ab]	9.26 ± 1.08[a]	11.96 ± 1.55[b]
Chl *a* [pg/cell]	11.75 ± 1.33[ab]	10.48 ± 2.65[a]	15.05 ± 3.77[b]
Chl *c* [$\mu g/cm^2$]	2.92 ± 0.98	3.16 ± 1.10	2.77 ± 0.33
Chl *c* [pg/cell]	3.02 ± 0.92	3.37 ± 0.54	3.48 ± 0.87
Chl *a+c* [$\mu g/cm^2$]	14.39 ± 3.12	12.42 ± 1.87	14.73 ± 1.67
Chl *a+c* [pg/cell]	14.76 ± 2.11	13.85 ± 2.62	18.53 ± 4.52
Chl *a:c*	4.12 ± 0.96	3.21 ± 1.05	4.36 ± 0.70

[ab] means with different letters are significantly different at $p < 0.05$.

In the MNPB, chlorophyll concentrations showed a similar pattern over the course of time as it was observed at CB (Figs. 12b – e), but the one for zooxanthellae densities was different. In September and October, zooxanthellae density was significantly lower (ANOVA; Tukey: $p<0.05$) than in December and January, while the density in November was not significantly different from any of the other months (Fig. 12a). All parameters determined for chlorophyll concentrations, i.e. chl *a*, *c* and *a+c* concentrations per area and per cell were significantly higher during the rainy season than during the dry season ($p<0.05$), except for chl *a* per area. The decrease in chlorophyll content was higher for chlorophyll *c* than for chlorophyll *a*, thus chlorophyll *a:c* ratios were higher in the dry season than they were in the time from September to November.

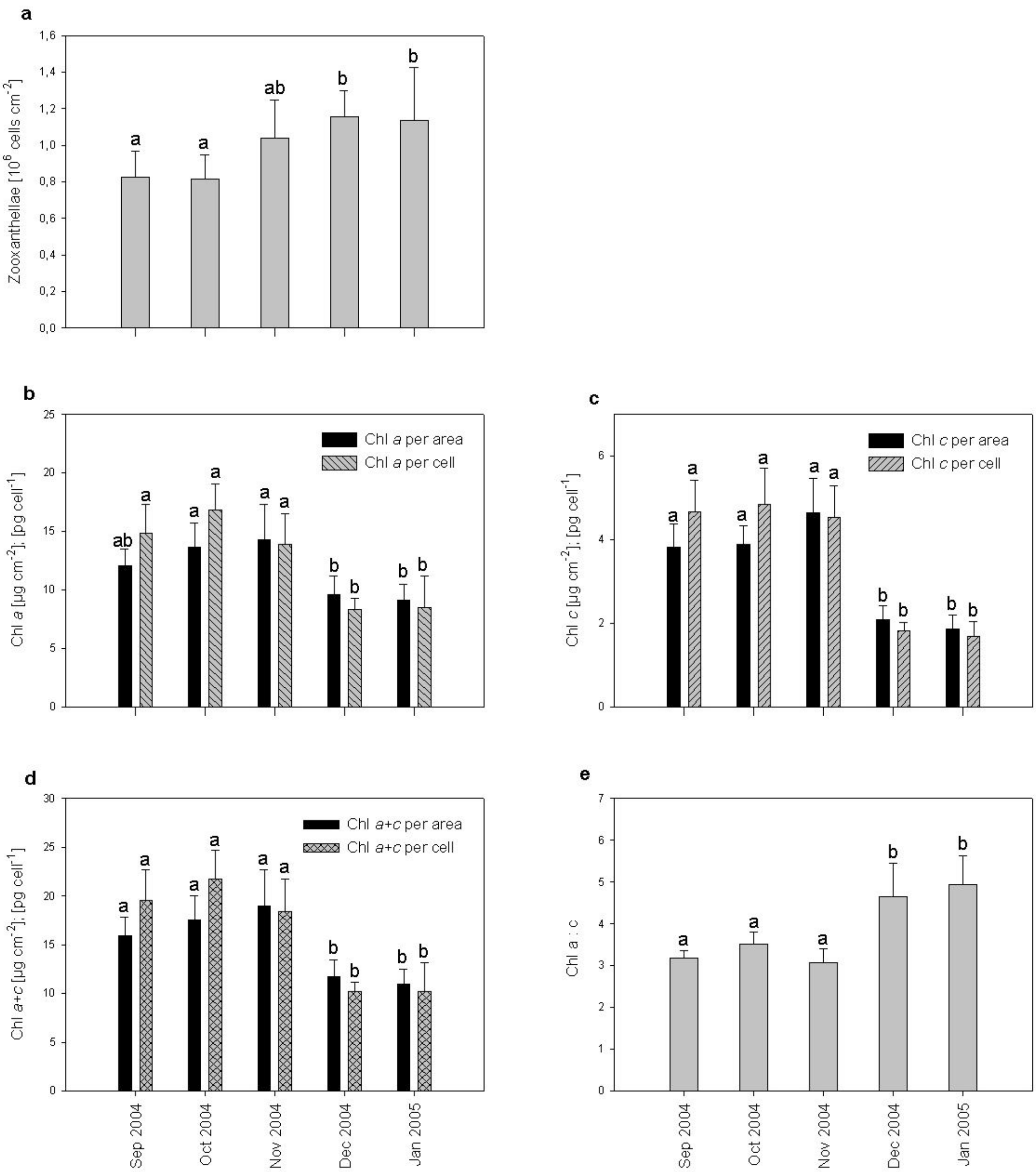


Figure 12. Monthly mean zooxanthellae density (a), chlorophyll (Chl) *a* (b) and Chl *c* (*c*) concentrations per area and per cell, Chl *a* and *c* (Chl *a+c*) concentrations per area and per cell (d) and the ratio of Chl *a* and *c* (Chl *a:c*) (e) were determined from samples taken in the coral reef Tres Hermanas in the MNPB. [abc]means with different letters are significantly different at a level of $p<0.05$.

Table 10. Seasonal comparison of zooxanthellae densities (ZD), chlorophyll (Chl) concentrations (Chl *a*, Chl *c*, Chl *a+c*) and *a*:*c*-ratios (± SD) at CB and in the MNPB; *$p<0.05$, ** $p<0.001$ (t-test).

	CB		**MNPB**	
	RS	DS	RS	DS
ZD [10^5 cells cm^{-2}]	10.12 ± 2.14	9.29 ± 2.60	9.13 ± 1.96	11.48 ± 2.08*
Chl *a* [$\mu g/cm^2$]	11.45 ± 2.01	7.54 ± 2.45**	13.41 ± 2.45	9.40 ± 1.43**
Chl *a* [pg/cell]	11.65 ± 2.45	8.10 ± 2.05**	14.99 ± 2.66	8.41 ± 1.81**
Chl *c* [$\mu g/cm^2$]	3.38 ± 0.72	1.32 ± 0.43**	4.18 ± 0.74	2.00 ± 0.34**
Chl *c* [pg/cell]	3.38 ± 0.55	1.44 ± 0.41**	4.67 ± 0.76	1.76 ± 0.27**
Chl *a+c* [$\mu g/cm^2$]	14.14 ± 2.85	8.87 ± 2.84**	17.59 ± 3.11	11.40 ± 1.64**
Chl *a+c* [pg/cell]	14.85 ± 2.65	8.96 ± 2.43**	19.66 ± 3.33	10.17 ± 2.00**
Chl *a:c*	3.48 ± 0.65	5.81 ± 1.18**	3.22 ± 0.33	4.77 ± 0.75**

In the present study, several environmental parameters have been monitored in the natural environment of an upwelling and a non-upwelling region in the eastern Pacific over a five months period from September 2004 to January 2005. This period covered the transition from rainy to dry season with the aim to better understand the influence of changing environmental conditions on the zooxanthellae density and algal chlorophyll concentrations of the reef building, massive coral *Pavona clavus*. Other studies dealing with the effect of seasonality on zooxanthellae densities have been conducted in the higher latitude study sites Hawaii (Stimson 1997), Mauritius (Fagoonee *et al.* 1999), the Bahamas (Fitt *et al.* 2000), and Thailand (Brown *et al.* 1999), closer to the equator. Nevertheless, none of these study sites is located in areas, where seasonal upwelling or high terrestrial runoff dominates the seasonal dynamic of nutrients (and water temperatures) as it is the case for the present study.

Zooxanthellae densities

Zooxanthellae densities in *P. clavus* from the eastern Pacific (Gulf of Panama) have previously been reported in experimental bleaching experiments (Hueerkamp *et al.* 2001) or field observations related to the 1997/98 El Niño event (Glynn *et al.* 2001), but this is the first study on seasonal and regional variations over a longer time period in Central America. In the present study, mean monthly zooxanthellae densities in *P. clavus* at the study sites CB and MNPB ranged from 0.7 to $1.2 \cdot 10^6$ cells cm^{-2} which is about an order of magnitude lower than it has been reported by Hueerkamp *et al.* (2001), but within the same range as reported by Glynn *et al.* (2001). Since zooxanthellae densities for many other species of reef-building corals are shown to range from 0.5 to $5 \cdot 10^6$ cells per cm^2 (Drew 1972; Glynn 1996), differences to the reports by Hueerkamp *et al.* (2001) are probably due to methodological differences.

There is some evidence from field studies as well as experimental studies that increasing temperature is negatively correlated with zooxanthellae densities (Glynn and D'Croz 1990; Brown *et al.* 1999; Fitt *et al.* 2000). Similarly, positive correlations between nutrient concentrations and zooxanthellae densities were reported from field (Stimson 1997; Fagoonee *et al.* 1999) as well as manipulative studies (Muscatine *et al.* 1989). At the first glance, the results of this study seem to contradict these observations, since in both regions, zooxanthellae densities were positively correlated to temperature. However, Saxby *et al*. (2003) observed a significant reduction of zooxanthellae densities in the coral *Montipora digitata* after their exposure to 14ºC (26ºC=ambient condition) under natural light conditions. Also Schlöder and D'Croz (2004) observed decreased densities in *P. lobata* at lower (23.8ºC) than ambient (29.1ºC) temperatures, also under increased nutrient concentrations. This seems to be the best explanation for the situation observed in CB, where zooxanthellae densities were decreased in January 2005, when water temperatures were lower due to increased frequencies of cold, nutrient-enriched water fronts.

At MNPB, other factors than seawater temperature seem to be more important for the density of zooxanthellae, which appears plausible since oceanographic conditions are largely depending on the effect of terrestrial runoff. Extremely low seawater salinities and high turbidity within the water column during the rainy season seem to be the major stress factors for corals and the reason for reduced zooxanthellae densities. In fact, studies by Marcus and Thorhaug (1981), working with *Porites porites* and *Porites compressa*, observed a salinity tolerance of these species between 25 and 37, while at 20 PSU, sub-lethal stress signs, such as mucus production and partial to total bleaching were observed. Also, decreased zooxanthellae densities due to reduced light availability have been observed in studies by McCloskey and Muscatine (1984) as well as Gattuso (1985), studying the effects of depth on the Red Sea coral *Stylophora pistillata*. These authors suggested that this was probably an adaptive mechanism to reduce the effect of self-shading, when pigment concentrations were increased (see also Table 10). Furthermore, it is known that high turbidity limits the light-driven carbon fixation process by zooxanthellae and in that way the scope for cell proliferation (Muscatine 1990). Consequently, the increment in zooxanthellae densities towards the dry season is believed to be due to a relaxation of the stressful conditions that prevail in the MNPB during the rainy season.

Chlorophyll concentrations

Despite the often contradictory statements concerning the dependence of zooxanthellae density on the level of irradiance (reviewed in Stimson 1997), it is generally accepted that chlorophyll *a* and *c* concentrations increase with decreasing irradiance levels (e.g. Dustan 1979; Titlyanov *et al*. 1980; Falkowski and Dubinsky

1981) or increasing depth, respectively (McCloskey and Muscatine 1984). This is congruent with the results of this study, since corals in the deep zone of the coral reef Güiri-Güiri had a lower chl *a* content per area as well as per cell (Table 9). Furthermore, chlorophyll contents were lower during the dry season than during the rainy season, as it was observed at CB and in the MNPB. In both regions, solar radiation is higher during the dry season (Fig. 6a) and the underwater turbidity is lower in the MNPB during this period of the year (Table 7).

The role of nutrients

Corals and their symbionts are known to recycle nutrients very efficiently, however significant losses to the open ocean and sediment must occur and thus sources of nutrients have to be considered (reviewed in Erez 1990). The different sources for nutrients include dissolved inorganic and organic nutrients as well as suspended particulate matter, either by direct uptake, as discussed for zooplankton feeding by corals (Johannes *et al.* 1970), or after digestion by filter-feeders or fish (Erez 1990). The feeding on suspended particulate matter (SPM) and its nutritional value were recently studied by Anthony (1999).

Zooxanthellae growth rates and densities have been shown to increase by experimental addition of ammonia (Muscatine *et al.* 1989; Hoegh-Guldberg 1994), however, the treatment concentrations of 20 μM in these studies were much higher than the levels of ammonia observed in this study (Tables 2 and 5). While a positive correlation between zooxanthellae density and nitrate concentration has been proposed in field studies by Stimson (1997) and Fagoonee *et al.* (1999), this relation was not observed in the present study, since at both study sites zooxanthellae densities were lower in the seasons with higher nutrient concentrations (Table 10). In fact, the nutrient concentrations at CB determined in this study were even higher compared with other upwelling areas like the Gulf of Panama (see section 3.2.1), and furthermore, it is likely that SPM added an supplementary source for nutrients for the corals and their symbionts in the MNPB during the rainy season.

Although the explanations given in the previous sections are believed to explain best the reduced zooxanthellae densities that were observed during January 2005 in CB and during the rainy season in the MNPB, increased heterotrophic feeding on zooplankton or SPM might have played an additional role. In fact, in the upwelling season at CB, the tentacles of *P. clavus* were observed to be extended also during the daytime, while during the rainy season, this was only observed at night (Bezy, pers. comm.). It seems probable that growth of the zooxanthellae population was inhibited by different stress factors as it was discussed above, but zooxanthellae densities may also have been actively reduced by their coral hosts, because energy may have been supplied in surplus due to additional heterotrophic feeding. Therefore, if during the dry season heterotrophic feeding was a supplementary energy source for *P. clavus*, this could also explain the higher growth rates that have been observed in CB and the Gulf of Panama, both upwelling regions, compared to the Gulf of Chiquirí, which is a non-upwelling region (Wellington and Glynn 1983; Jiménez and Cortés 2003).

3.5 Seasonal variations in inorganic phosphorus concentrations in the skeleton of *Pavona clavus*

Three coral core slabs were analyzed for their acid-available, hereafter inorganic, phosphorus (P_{inorg}) content (in ppm). Starting from the sampling dates, 30th and 31st of January 2000, the P_{inorg} concentrations were determined with a sub-seasonal resolution, i.e. four samples per year, back to 1990 (core X2 and X3) and 1988 (core X6), respectively (Fig. 13b). Table 11 presents the mean inorganic phosphorus concentrations for all sampling years, which were divided into four sub-seasons.

The chronology of the skeletal banding was based on the assumption that the production of a high density (HD) band was initiated every year at the beginning of the rainy season, although it is likely that there was a certain time lag between the seasonal transition and the beginning of the HD band production, possibly due to acclimatory processes of corals and their symbionts. This is consistent with studies by Wellington and Glynn (1983), who studied the onset and duration of HD and low density (LD) formation using X-radiographs of *P. clavus* and *Pavona gigantea* from the Gulf of Panama and the Gulf of Chiquirí. They collected samples at four different times of the year and observed that irrespective of the species or sample site, the onset of the HD band formation was around mid-June (± one or two weeks). Since one set of their samples originated from another upwelling area of the eastern Pacific, the Gulf of Panama, where oceanographic and meteorological conditions are comparable to those at CB, their timing of the HD band formation was adopted. The only modification was a time-shift of one month, since the period of high insolation has been reported to be from January to May in the Gulf of Panama (Wellington and Glynn 1983), while the months with the highest insolation at CB are from December to April (see section 3.1).

Analysis of the tissue layer (Fig. 13a), consisting of two sub-seasonal samples, resulted in elevated P_{inorg} concentrations with high standard deviations (19.86 ± 12.57 ppm and 7.08 ± 5.12 ppm, respectively), which was probably due to additional acid-available phosphorus from dried tissue residuals within these samples. Similar observations have been reported from studies by Dodge *et al.* (1984). Here, these values are presented in Table 11, but they were omitted in the illustration of the P_{inorg} concentrations (Fig. 13b) as well as excluded for the statistical analysis. In the remaining samples, mean P_{inorg} concentrations ranged between 2.34 ± 0.41 ppm and 5.57 ppm (Table 11). Apart from some time-shifted deviations among the individual colonies, a synchronous pattern of alternating peaks and lows was observed in the analyzed cores (Fig. 13b). These deviations are believed to be caused by a combination of inter-colonial differences and the methodology of the coral skeleton sampling. Inter-colonial variation might have been caused by a number of physical and biological factors the individual colonies were exposed to. Physical influences possibly included: unequal accessibility to nutrients and light or differences in temperature and/or water currents, simply owing to its individual position and surrounding environment within the coral reef community. Corals might also have experienced different biological influences, such as: bites of corallivore fishes, invasion of the skeletal matrix by boring organisms or suffering from coral diseases. Similarly, differential susceptibility to coral bleaching might have promoted inter-colonial differences. Apart from the possibility of differential physical and biological influences, an important methodological aspect has to be considered. One meteorological year, i.e. from one HD band to the next one, was divided into four equidistant sub-samples (Fig. 13a). If the growth rate was constant over time, then each sub-sample would represent a quarter year, but there is strong evidence from the literature that growth rates are higher during LD than during HD band formation (Wellington and Glynn 1983). For this reason, the dating of the sub-seasonal samples

(Table 11) is only an approximate estimate, which probably differs somewhat from year to year, thus adding a methodological component to the explanation of inter-colonial differences. This simplification of the sampling methodology was necessary, since there were no recognizable sharp lines, but rather irregular interceptions of narrow LD bands within the HD bands, that would have allowed consistent separation of the two band types. Nevertheless, despite the limited time resolution, the assignment of the sub-seasonal samples to either the beginning or the end of a rainy season (or a dry season, respectively), can be expected to be correct. According to the foregoing, individual sub-seasons were assigned to the following months: May to July (RS 1 19xx), August to October (RS 2 19xx), November to January (DS 19xx/19xx+1) and February to April (DS 19xx+1). A comparison between these four sub-seasons revealed that P_{inorg} concentrations were highest during the end of the dry season ($p<0.05$) and significantly higher ($p<0.05$) at the beginning than at the end of the rainy season (Table 11).

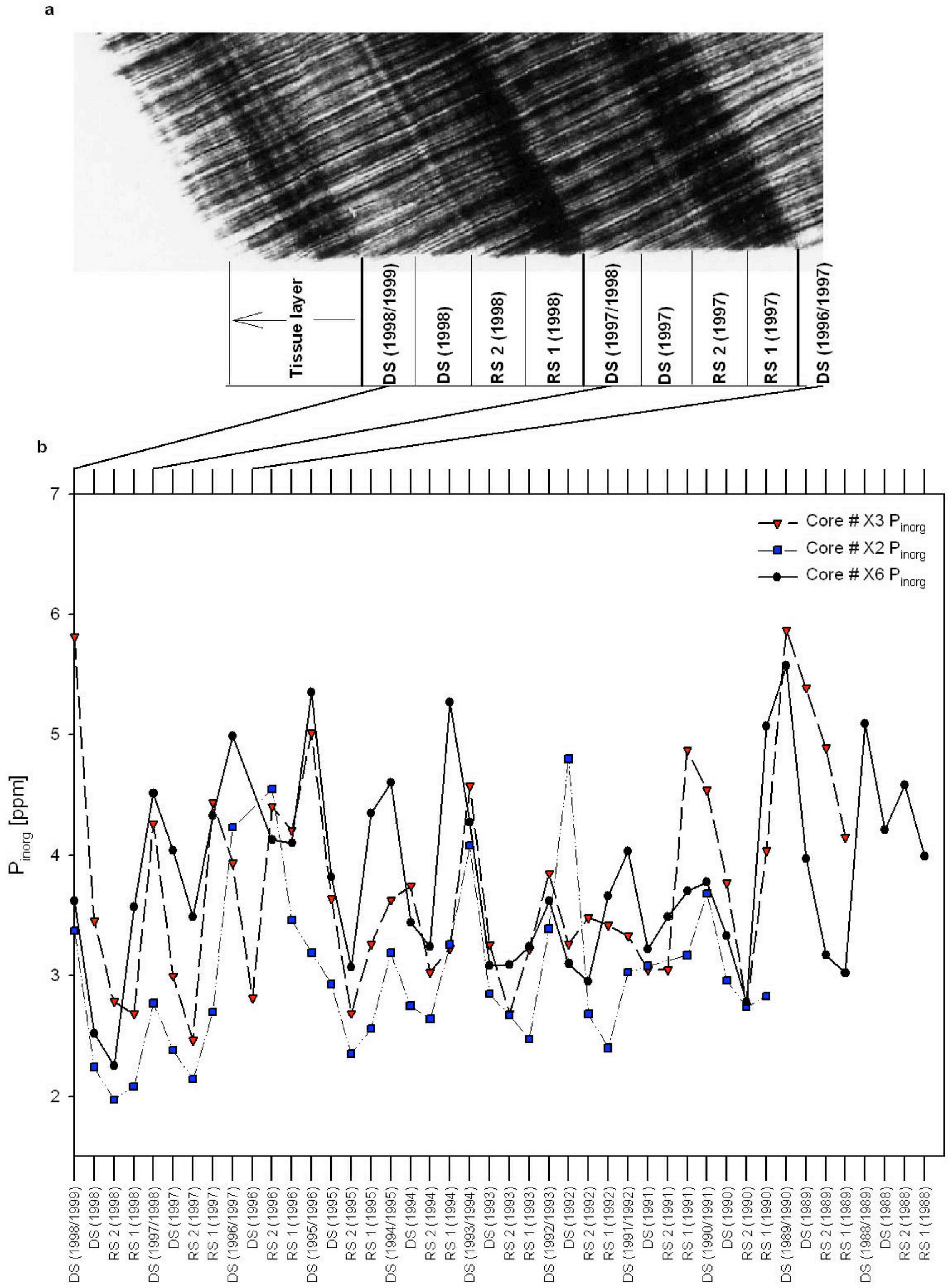


Figure 13. Exemplary design of the sub-seasonal sampling of coral powder along a coral core slab is shown (a) above the graph showing the variations in inorganic phosphorus concentrations (in ppm) in three coral core samples (b) which were taken from the intermediate zone of the coral reef Güiri-Güiri at CB; abbreviations for dry season (DS) and rainy season (RS) are used together with a year index (19xx) to indicate the estimated time of coral skeleton formation represented by the analyzed samples.

Table 11. Mean inorganic phosphorus concentrations (in ppm ±SD) in three coral core slabs of *P. clavus* with a quarter yearly (= 4 sub-seasons) resolution and annual means (±SD); DS = dry season, RS = rainy season; approximate (~) time of each sub-season is given in the first line.

Time (19xx)	n	~Feb – Apr DS (19xx+1)		~Nov – Jan DS (19xx/19xx+1)		~Aug – Oct RS 2 (19xx)		~May – Jul RS 1 (19xx)		Mean**	
1999*	3					19.86	± 12.57	7.08	± 5.12		
1998	3	4.27	± 1.35	2.74	± 0.64	2.34	± 0.41	2.78	± 0.75	3.03	± 0.64
1997	3	3.85	± 0.94	3.14	± 0.84	2.70	± 0.70	3.82	± 0.98	3.38	± 0.81
1996	3	4.39	± 0.54	2.81	(n=1)	4.36	± 0.21	3.92	± 0.40	3.84	(n=1)
1995	3	4.52	± 1.16	3.46	± 0.47	2.70	± 0.36	3.39	± 0.90	3.52	± 0.70
1994	3	3.81	± 0.72	3.31	± 0.51	2.97	± 0.30	3.92	± 1.17	3.50	± 0.59
1993	3	4.31	± 0.25	3.06	± 0.21	2.81	± 0.24	2.97	± 0.44	3.29	± 0.24
1992	3	3.62	± 0.23	3.18	± 0.11	3.04	± 0.40	3.16	± 0.67	3.42	± 0.12
1991	3	3.46	± 0.51	3.11	± 0.09	3.27	± 0.31	3.91	± 0.87	3.43	± 0.29
1990	3	4.00	± 0.47	3.35	± 0.41	2.76	± 0.02	3.98	± 1.12	3.52	± 0.41
1989	1	5.57		3.97		4.17		3.02		3.93	
1988	1	5.09		4.21		4.58		3.99		4.47	
mean		4.02[a]	± 0.37	3.13[bc]	± 0.24	2.99[b]	± 0.57	3.54[c]	± 0.47	3.57	± 0.38

[abc] means (1998-1990) with different letters are significantly different (SNK: $p<0.05$)
* values from layers that probably contain tissue residuals
** values are means of meteorological years (May (19xx) – Apr (19xx+1))

The skeletal matrix of reef-building corals is mainly composed of biomineralized aragonite, the metastable polymorph of calcium carbonate, and small amounts of organic material that plays a major role in the process of skeleton formation (Barnes and Chalker 1990). It has been suggested that calcification includes two major steps: secretion of organic material and formation of crystal nucleation sites, i.e. production of an organic matrix which is then used as a template for extracellular calcification (Goreau and Hayes 1977). A model has been proposed by Highsmith (1979), suggesting that high light levels and temperatures between approximately 23.7 and 28.5°C represent conditions during which the production of organic matrix is high relative to extracellular calcification. Accordingly, increased crystal nucleation, the high energy step during the process of calcification, is believed to be responsible for the formation of LD bands. On the other hand, when matrix production is low relative to extracellular calcification, precipitation preferentially takes place on already existing crystals and in this way the formation of a HD band is promoted. This model supports the idea that calcification rates are increased as a result of photosynthesis, also known by the term: light-enhanced calcification (reviewed by Barnes and Chalker 1990). While in Highsmith's model temperature is the most important factor to determine the type of band to be formed, Wellington and Glynn (1983) have proposed that variations in light levels and possibly reallocation of energy from growth to reproduction may play a more important role than the influence of temperature.

In the literature, there is experimental evidence that apatite, a complex form of calcium phosphate, can be formed on surfaces of aragonite (De Kanel and Morse 1978), but only two studies have documented the content of phosphorus in corals in their natural environment. One study was conducted in the context of environmental phosphorus levels that were associated to the impact of sewage and other phosphorus pollution episodes (Dodge *et al.* 1984) and the other in the context of land use features and associated effluent runoff (Kumarsingh *et al.* 1998). Dodge *et al.* (1984), working in the Caribbean Sea with *Montastrea annularis* from St. Crox and Curacao as well as *Diploria strigosa* from Bermuda, determined mean annual P_{inorg} concentrations in the range from 2.08 ± 0.60 to 4.06 ± 0.49 ppm in corals from the "pollution-free" and 3.62 ± 1.54 to 5.26 ± 1.13 ppm from the "polluted" sites. Also working with *Montastrea annularis*, Kumarsingh *et al.* (1998) reported mean annual concentrations from 1.11 ± 0.60 to 2.11 ± 0.67 ppm for corals from Tobago, West Indies. The mean annual concentrations of the present study (Table 11) do not show a trend over time and the mean value of the years from 1988 to 1998 (3.57 ± 0.38 ppm) implies that the waters of the reef Güiri-Güiri at CB are either "pollution-free" or marginally "polluted" in terms of phosphorus pollution. However, the term "pollution" has to be interpreted with caution, since a major source of phosphorus is associated to upwelling waters during the dry season, apart from the possible influence by runoff of land-based effluents.

Based on results of the present study and supported by long-term studies in the upwelling area of the Gulf of Panama by D'Croz and Robertson (1997), it seems reasonable to assume that at CB, dissolved inorganic phosphate concentrations are also elevated due to seasonal upwelling during the dry season. Furthermore, meteorological data show that the period of high insolation is from December to April (Fig. 14). The reproductive period of *P.clavus* has been observed to be around August – September (Bezy, in prep.). A superimposition of these factors shows that P_{inorg} concentrations in the coral skeleton generally follow the pattern of daily sun hours (Fig. 14). The highest P_{inorg} concentrations are observed during the end of the dry season (DS 2), when light levels are known to be highest and seawater phosphate concentrations supposed to be elevated. The lowest P_{inorg} concentrations are observed at the end of the rainy season (RS 2), when light levels are low and seawater phosphate concentrations reduced (Fig. 14). This is also the reproductive period of *P. clavus*. These observations strongly support that light enhanced calcification, and probably the reallocation of energy from growth to reproduction are not only the main factors to determine the growth rates, and thus the banding patterns of corals (Highsmith 1979; Wellington and Glynn 1983), but also the incorporation of P_{inorg} into the coral skeleton. While the exact mode of phosphorus incorporation is still unknown, it has been proposed that organic matrix production, and thus the probability for crystal nucleation, is favoured by high light levels and exogenously derived sources of nutrients, but reduced during reproductive periods of corals (Wellington and Glynn 1983). Together with the results of this study, it can be postulated that increased availability of seawater phosphate and organic matrix production promotes the nucleation of apatite and in this way the incorporation of phosphorus into coral skeletons.

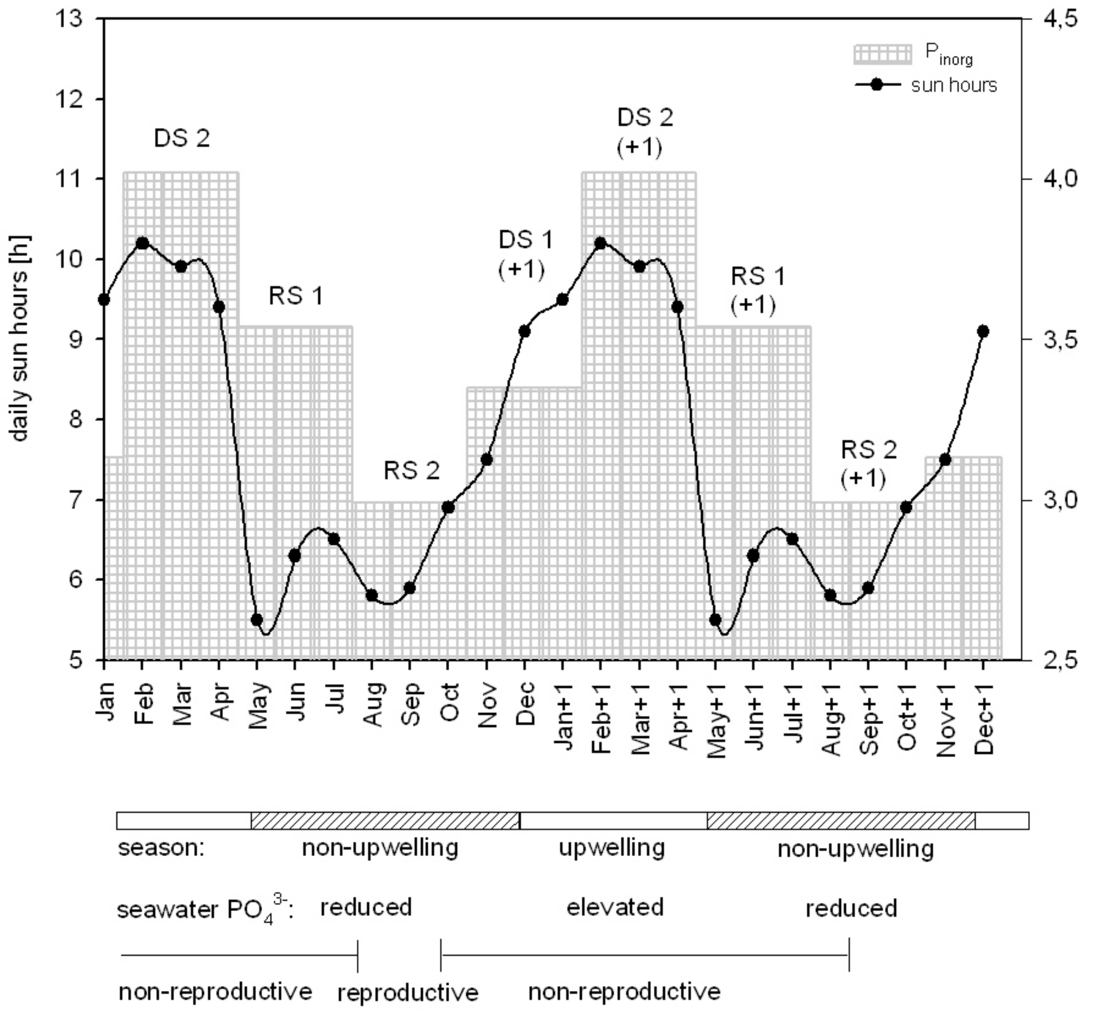


Figure 14. Variations in inorganic phosphorus concentrations in corals (in ppm) and mean monthly sun hours per day (in h) throughout two years. For the first year (Jan - Dec) data of daily sun hours of 2004 and the mean sub-seasonal (1989 – 1999) inorganic phosphorus concentrations (according to Table 11) are shown. For the second year (Jan +1 - Dec +1), the data of the first year are repeated. Shaded and non-shaded bars indicate the approximate duration of the upwelling season and the periods of elevated and reduced seawater phosphate levels. The estimated reproductive period of *P. clavus* is also shown.

4. CONCLUSIONS

The results of this study suggest that the density of zooxanthellae in *P. clavus* responds to seasonal variations of environmental factors. It has been hypothesized that zooxanthellae densities are lowest at the end of the season with highest seawater temperatures but increase shortly after temperatures decrease again. According to the presented results, such generalized hypotheses have to be rejected if regional oceanographic characteristics like seasonal upwelling or strong influence of terrestrial runoff become important features. Furthermore, it was shown that environmental differences of regions only 225 km apart can result in dissimilar patterns of seasonal variation in zooxanthellae densities. Different to the zooxanthellae densities, the seasonal variation in chlorophyll concentrations was observed to be similar at the two environmentally distinct regions CB and MNPB. It was suggested that the best, possibly the only correlate to chlorophyll concentrations is a negative one with ambient light levels.

Another aspect of seasonality was shown in the form of varying P_{inorg} signals within the skeleton of a reef-building coral. To my knowledge, these are the first results to reveal variations in the phosphorus signal in a coral skeleton on a sub-seasonal resolution. It is suggested here that elevated light levels and seawater phosphorus concentrations represent favourable conditions for the incorporation of phosphorus incorporation, while allocation of energy from growth to reproduction and low light levels seem to decrease the rate of this process. Increased organic matrix production, and thus higher nucleation rates of apatite, concomitant with elevated seawater phosphate concentrations is suggested as a mode for the incorporation of P_{inorg} into the coral skeleton. However, even though it is tempting to conclude that the levels of P_{inorg} recorded in coral skeletons reflect ambient seawater phosphate levels, conclusive proofs remain to be established.

For both, zooxanthellae densities and chlorophyll concentrations, a monitoring over an entire year using several coral species could provide supportive data and scope for testing and generalizing the results presented here. Regarding the analysis of P_{inorg} contents in corals, a comparative study by using *P. clavus* cores from a non-upwelling region (e.g. Caño Island), would be useful to test this hypothesis while it could additionally provide answers to the role of water temperature.

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APPENDIX A

Location of sampling stations (GPS data)

Table A1. Location and depth (in m) of the sampling stations at Culebra Bay (CB) and the Marine National Park Ballena (MNPB).

CB	Latitude	Longitude	Station depth
Station 1	10°37'09.7''N	85°42'12.4''W	47
Station 2	10°37'11.9''N	85°41'37.8''W	25
Station 3	10°36'50.4''N	85°41'25.4''W	8
MNPB			
Station 1	09°04'53.3''N	83°41'13.7''W	20
Station 2	09°06'15.8''N	83°42'23.1''W	8
Station 3	09°06'39.8''N	83°42'40.7''W	6

APPENDIX B

Meteorological data

Table B1. Data from the meteorological station at Liberia, including mean air temperature (in ºC), total rainfall (in mm), mean sun hours (in h/d) and wind speed (in km/h). These data are copyright Instituto Meteorologico Nacional, Costa Rica.

Year	Month	Mean air temperature (ºC)	Total rainfall (mm)	Mean sun hours (h d^{-1})	Wind speed (km h^{-1})
2004	1	27.2	0	9.5	16.8
	2	27.5	3	10.2	18.5
	3	29.4	0	9.9	27.0
	4	28.9	0	9.4	14.4
	5	28.9	210	5.5	12.7
	6	28.1	154	6.3	10.0
	7	26.9	159	6.5	8.9
	8	27.8	278	5.8	8.4
	9	26.6	411	5.9	6.7
	10	26.9	169	6.9	6.6
	11	26.6	135	7.5	9.6
	12	27.5	10	9.1	15.3
2005	1	27.4	0	9.7	19.0
	2	27.1	0	10.3	20.6

Table B2. Data from the meteorological station at Damas, including mean air temperature (in ºC), total rainfall (in mm) and mean sun hours (in h/d). These data are copyright Instituto Meteorológico Nacional, Costa Rica.

Year	Month	Mean air temperatures (ºC)	Total rainfall (mm)	Mean sun hours (hrs/day)
2004	1	25.4	53	6.2
	2	25.5	27	8.4
	3	25.8	3	9.0
	4	28.1	167	6.6
	5	27.5	723	3.1
	6	27.3	351	4.3
	7	26.7	363	4.2
	8	27.0	589	4.1
	9	26.5	582	5.5
	10	26.4	793	5.6
	11	26.6	337	5.2
	12	26.8	62	7.0
2005	1	26.6	35	7.1
	2	27.4	45	8.6

APPENDIX C

Sub-surface water temperatures

Table C1. Monthly mean, maximum (max) and minimum (min) sub-surface water temperatures (in ºC ± SD) recorded at 7 m depth in the coral reef Güiri-Güiri, Culebra Bay. These data were provided by courtesy of Dr. J. Cortés and Dr. C. Jiménez.

Month	max	min	mean
Sep 04	29.96	25.41	28.81 ± 0.70
Okt 04	30.21	24.07	28.43 ± 1.09
Nov 04	29.08	23.13	27.26 ± 1.15
Dez 04	28.91	19.76	26.50 ± 1.63
Jan 05	28.24	19.27	25.84 ± 1.70
Feb 05	28.91	19.76	25.02 ± 2.03

Table C2. Monthly mean, maximum (max) and minimum (min) sub-surface water temperatures (in ºC ± SD) recorded at 8 m depth in the coral reef Tres Hermanas, Marine National Park Ballena. These data were provided by courtesy of Lic. J.J. Alvarado.

Month	max	min	mean
Sep 04	29.92	26.54	28.47 ± 0.62
Okt 04	29.46	25.25	27.70 ± 0.88
Nov 04	29.46	26.12	27.99 ± 0.46
Dez 04	30.36	27.77	29.05 ± 0.51
Jan 05	30.81	29.04	29.98 ± 0.29
Feb 05	31.27	26.96	29.77 ± 0.67

APPENDIX D

CD directory: Raw data and statistical analyses

Data file 1: Thesis – Raw data and Statistics – CB.xls

- Raw data of zooxanthellae densities and statistical analysis
- Raw data of chlorophyll concentrations for all month and statistical analyses
- Raw data of zooxanthellae densities and chlorophyll concentrations at different depth (September 2004) and statistical analyses
- Statistical tests for differences between rainy and dry season
- Raw data of dissolved inorganic nutrients and statistical tests for differences between rainy and dry season
- Raw data of particulate nutrients and statistical tests for differences between rainy and dry season

Data file 2: Thesis – Raw data and Statistics – MNPB.xls

- Raw data of zooxanthellae densities and chlorophyll concentrations and summary of statistical analyses
- Statistical tests for differences between rainy and dry season
- Raw data of dissolved inorganic nutrients
- Raw data of particulate nutrients

Data file 3: Thesis –Phosphorus analysis.xls

- Raw data of inorganic phosphorus concentrations in three coral cores of *Pavona clavus*
- Statistical analysis for differences between sub-seasons