VARIATION OF MACROALGAE BIOMASS IN CIENFUEGOS BAY, CUBA.

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ABSTRACT

The biomass of macroalgae was measured in Cienfuegos Bay to determine the spatial and temporal variations of macroalgae communities. The mean values of biomass ranged from 7.5 (Playa Alegre station in February-01) to 1055.4 gm^{-2} (Junco Sur station in October-00). The morphofunctional group dominant in all stations was the coarse forms, represented by the species *Hypnea spinella* (C. Agardh) Kützing, *Acanthophora spicifera* (M. Vahl) Børgesen, *Gracilaria blodgettii* Harvey, and *Chondrophycus papillosus* (C. Agardh) Garbary & Harper. The north lobule, characterized by more impact of urbanization and industrialization harboured few macroalgae species relative to the site of south lobule. *Hypnea spinella* and *Gracilaria* spp., tolerant species to a wide range of ecological conditions were dominant species at locations of north lobule. Macroalgal diversity was greatest at the station located at south lobule; this station exhibited more oceanic conditions, which is explained by the abundance of *Chondrophycus papillosus*, characteristic of oligotrophic areas. It was observed a remarkable seasonal variation in biomass values at all stations. The highest biomass at north lobule was registered during the rainy period; it could be due to enrichment effects in this period. The highest biomass at the station of south lobule was registered during the dry period, the overgrowth of a filamentous green algae observed in this period could be explained by more transparency and disposition of light at the water in dry season.

Key words: biomass; macroalgae; bays; multidimensional analysis; ASW, Cuba.

RESUMEN

Se estudió la biomasa de macroalgas en la bahía de Cienfuegos con el objetivo de determinar su variación espacio-temporal. Los valores medios de biomasa oscilaron entre 7.5 (Playa Alegre en febrero-01) y 1055 gm^{-2} (Junco Sur en octubre 00). El grupo de las formas carnosas fue el dominante en todas las estaciones de colecta, representado principalmente por las especies *Hypnea spinella* (C. Agardh) Kützing, *Acanthophora spicifera* (M. Vahl) Børgesen, *Gracilaria blodgettii* Harvey, y *Chondrophycus papillosus* (C. Agardh) Garbary & Harper. El lóbulo norte, caracterizado por mayor impacto de la urbanización e industrialización, presentó menor diversidad de macroalgas en comparación con la estación del lóbulo sur. *Hypnea spinella* y *Gracilaria* spp, especies tolerantes a amplios rangos de condiciones ecológicas fueron dominantes en las estaciones del lóbulo norte. En la estación ubicada en el lóbulo sur se registró la mayor diversidad. Esta estación evidencia mejores condiciones oceánicas, lo cual está sustentado por la abundancia de *Chondrophycus papillosus* (C. Agardh) Garbary & Harper, característica de aguas más limpias. Se observó una notable variación temporal en los valores de biomasa en todas las estaciones. En las estaciones del lóbulo norte se registró el mayor valor de biomasa en el período lluvioso, lo cual puede ser debido a una mayor entrada de nutrientes durante ese período. En la estación del lóbulo sur se registró el mayor valor de biomasa en el período de seca, registrándose un sobrecrecimiento de un alga verde filamentosa que al parecer fue estimulado por una mayor transparencia y disposición de luz en el agua en esta época del año.

Palabras clave: biomasa; microalgas; bahías; análisis multidimensional; ASW, Cuba.

Cienfuegos Bay represents the most important natural resource in the Cienfuegos province. Port and marine activities represent one of the principal multiple uses of the bay. The coastal area of the bay is also important for tourism, particularly for watersports, swimming and sport fishing. Other important activities within the bay are associated with industry, agriculture and urban development.

The marine macroalgae of Cienfuegos Bay represent a flora of over 69 species (Moreira *et al.*, 2003). They play important ecological roles, including primary production and provision of habitats that support phytal communities of invertebrates. Populations of the frondose agarophyte *Gracilaria* spp. and carragenophyte *Hypnea spinella* are particularly abundant in

Cienfuegos. They are distributed approximately in fifteen natural banks with a depth between 0.2 and 2 meters (León *et al.*, 2002). Many areas of the bay show total absence of macrophytobenthos.

Several ecological studies of macrophytes in the world (Powell *et al.*, 1989; Zieman *et al.*, 1989, De Casabianca *et al.*, 1997), and in Cuba (Martínez-Daranas *et al.*, 1996; Perdomo, 1998) use the biomass as impact indicator (*e. g.* eutrophication) on the macrophytes communities. In marine or lagoon environments, eutrophication generally causes a decrease in specific diversity of benthic macrophyte communities and monospecific communities with opportunistic and nitrophilous species such as *Ulva* spp., *Cladophora* spp. and *Gracilaria* spp. prevailing (De Casabianca *et al.*, 1997; Peckol and Rivers, 1996).

The purpose of this study is to analyze spatial variations of macroalgae communities (biomass) and morphofunctional groups during different seasons along bay.

MATERIALS AND METHODS

Study area

Cienfuegos Bay is located in 22º 9' N and 80º 27' W, southern central region of Cuba (Fig. 1). The bay has an area of 90 km² and an average depth of 14 m. The topography of the bay is simple and the most important feature is a narrow and shallow wall (average depth 1 m) that divides the basin into two lobules. Several rivers flow towards the bay, forming a complex estuary. The main rivers are Damují, Salado, Arimao and Caonao. These rivers are polluted by several land based sources of pollution. Other less extensive river basins are also present, such as El Ingles, Las Calabazas and Manacas creeks, and these all contribute with additional significant levels of pollutants to the bay. These land-based sources of pollutants include nutrients (from agricultural and domestic sources), chemicals (industrial and agricultural), suspended sediment, etc. The north lobule receives the major part of wastewater's discharges from Cienfuegos city, Damují and Salado rivers; El Ingles, Las Calabazas and Manacas creeks. The south lobule suffers a minor anthropogenic disturbance, principally from Caonao and Arimao rivers (Villasol *et al.*, 1990).

Weather in the study area is divided in two seasons: dry (November-April) and rainy (May - October) season. The annual mean temperature is 24.7°C, the highest monthly temperature occurs in rainy season, 27.0°C in June, and the lowest occurs in dry season, 21.6°C in January. The annual mean rainfall is 1376.5 mm; 1078.7 mm fall in rainy season and 297.8 mm in dry season. The rainiest month is June (214.7 mm) and December (32.6 mm) is the driest.

The bay has a marked vertical stratification caused by runoff from land and low tidal mixture. In rainy season (May–October) the mean values of surface salinity are low (16 - 20‰), but the bottom water keeps higher values similar to ocean waters. In dry season, salinity has values between 30 and 32‰ in along the water column (Seisdedo and Muñoz, 2005).

Sampling design and processing of samples

Nineteen collections (samples) of macroalgae were undertaken in five months (July-2000, October-2000, November-2000, February-2001 and July-2001) in four stations (Table 1) in shallow waters (less than 2 m in each station). La Milpa station is out of industrial and urban area and could be considered a control site. Prácticos station could not be sampled in November-00.

Table 1. Sampling stations in Cienfuegos Bay.

Station	Latitude (°N)	Longitude (°W)	Substrate
La Milpa	22°04.43´	80°27.40´	rocky/sandy
Junco Sur	22°07.40´	80°26.10´	sandy/rocky with rubbles
Prácticos	22°08.31´	80°27.70´	rubbles predominant
Playa Alegre	22°07.42´	80°26.50´	sandy/muddy

In each station, two parallel transect lines were located across the sampling area (Dodge *et al.*, 1982) (in order to cover the range of environmental conditions within the station). Along each transect, a variable number of quadrants (replicates) were located at an equal distance. The criterion followed was to take more quadrants in stations with a higher number of species (grossly assessed by visual inspection of the collected material in the field). Each quadrant has 0.24 m² of area and all macroalgae inside were carefully collected in plastic bags.

The organisms were preserved in buffered formalin 5% with salt water. The macroalgae were identified below stereo and microscope according to Taylor (1960) and Littler and Littler (2000). The algae were put on filter paper to eliminate excess water and weighted (gross weight) in a balance (accuracy: 0.1 g).

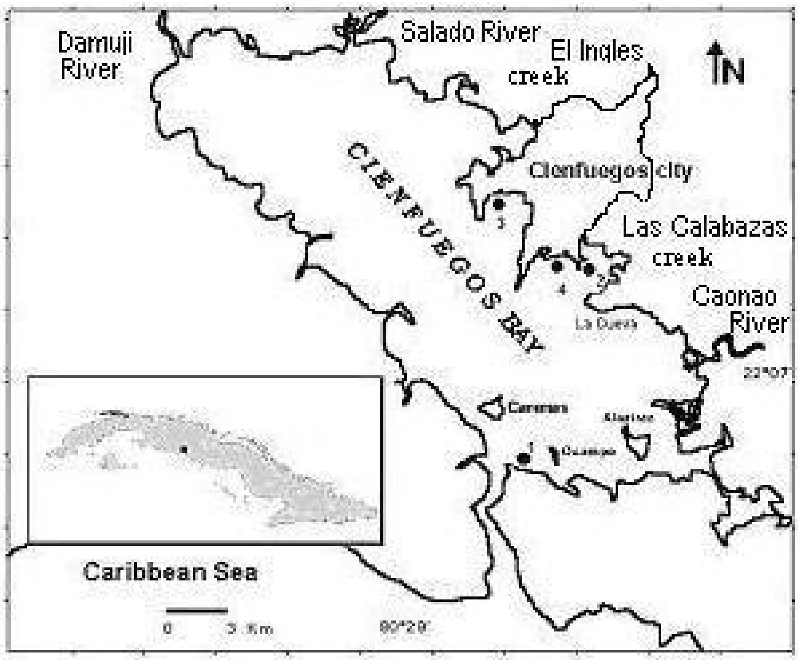

Fig. 1. Location of sampling stations in Cienfuegos Bay.

Data analysis

Univariate measures: The software Statistica 5.0 (Statsoft, 1995), was used for graphical and univariate analysis. The macroalgae species were clustered in five morphofunctional groups (after Litter and Litter, 1980): a) sheet, b) filamentous, c) coarsely branched, d) thick leathery and e) jointed calcareous. The total biomass of macroalgae was tested by two-way ANOVA for differences relating to the factors: a) month (five levels) and b) station (four levels). A single combination of treatments (Prácticos station – November-00 month) was not sampled; it causes an incomplete design with a lack of efficiency in the assessment of components of variance. The data were transformed (using Taylor´s power law after Elliot, 1971) until a point of non-correlation between mean/variance. The transformation used was $(data)^T$ with $T = 0.09$ (equivalent to classical logarithm transformation). An ANOVA test, with low or null correlation mean/variance should be consistent enough to moderate violations of parametric assumptions (*e. g.* non-normality, heterocedasticity) (Zar, 1996).

Species accumulation curves were used to assess the adequacy of sample size (Elliot, 1971), allowing an unbiased evaluation of biodiversity. Shannon diversity index (H´) (logarithms base e) was calculated (based on biomass data) using Jackknife procedure in order to obtain mean and dispersion values for H´. A two-way ANOVA test was carried out to test differences between months and stations for the case of H´. The values were not transformed because the correlation mean/ variance was null ($r = 0.01$).

The software PRIMER 5.2.9 (Clarke and Warwick, 2001) was used for multivariate analysis of communities. Triangular matrices were computed based on similarities between every pair of samples, using the Bray-Curtis coefficient and transformed data (in order to reduce the contribution of dominant species in the similarity matrix). A nmMDS (non-metrical multidimensional scaling) distribution was applied to examine the separation of samples based on the community structure (*e. g.* biomass of each specie in each sample). The similarity matrix was computed with the biomass average for each combination (*e. g.* all replicates from station-month combination were averaged). This procedure shows “averaged” communities and reduces the point´s jam in the plot. A two-way ANOSIM hypothesis test (factors: station and month) was carried out to calculate differences in the macroalgae assemblages between months and stations, according to the biomass of all species (transformed data). This time, the similarity matrix was computed on biomass data of each replicate, with a sufficient number of permutations. The routine SIMPER was run to look for the species that contributed more to average similarity of groups of samples.

RESULTS

Univariate measures of community structure.

The total number of collected species of macroalgae was 33: 18 Rhodophyceae, 5 Phaeophyceae and 10 Chlorophyceae (Table 2). The mean values of total biomass ranged from 7.5 (Playa Alegre station in February-01) to 1055.4 g/m^2 (Junco Sur station in October-00) (Fig 2). There are no clear patterns of biomass relating to month or station.

Table 2. Lists of species.

Rhodophyceae
Champia parvula (C. Agardh) Harvey
Chondria leptacremon (Melvill) De Toni
Ceramium sp.
Acanthophora spicifera (M. Vahl) Børgesen
Digenea simplex(Wulfen) C. Agardh
Chondrophycus papillosus (C. Agardh) Garbary & Harper
Spyridia filamentosa (Wulfen) Harvey
Agardhiella subulata (C. Agardh) Kraft & M. J. Wynne
Gracilaria blodgettii Harvey
Gracilaria cervicornis (Turner) J. Agardh
Gracilaria damaecornis J. Agardh
Gracilaria mammillaris (Montagne) M.A. Howe
Grateloupia filicina (J.V. Lamouroux) C. Agardh
Gelidium pusillum (Stackhouse) Le Jolis
Gelidiella setacea(J. Feldmann) J. Feldmann & G. Hamel
Hypnea musciformis(Wulfen) J.V. Lamouroux
Hypnea spinella(C. Agardh) Kützing
Jania capillacea Harvey
Phaeophyceae
Ectocarpus sp.
Sphacelaria novae-hollandiae Sonder
Dictyota cervicornis Kützing
Padina sanctae-crucis Børgesen
Rosenvingea intricada (J.Agardh) Børgesen
Chlorophyceae
Cladophora glomerata (Linnaeus) Kützing
Cladophora sp.
Chaetomorpha linum (F. Müller) Kützing
Chaetomorpha minima Collins & Hervey
Enteromorpha flexuosa Wulfen
Ulva fasciata Delile
Ulva lactuca Linnaeus
Caulerpa sertularioides (Gmelin) M.A. Howe
Codium decorticatum (Woodward) M.A. Howe
Acetabularia crenulata J.V. Lamouroux0

The two-way ANOVA on biomass data (all summed species) indicates significant differences (d.f. = 11; F=3.31; p<0.001) in the interaction month/station. There is notable temporal variation of biomass in all stations. Junco Sur station in rainy and dry months (October, November and February) shows the largest values of total biomass (Fig. 2). February has the highest values of biomass in La Milpa and Junco Sur stations, but also the lowest values at Prácticos and Playa Alegre stations.

The morphofunctional group dominant in all stations was the coarsely branched, mostly represented by genera *Hypnea*, *Acanthophora, Gracilaria* and *Chondrophycus*. The second position in a rank of dominance is shared by sheet and filamentous groups.The cumulative curves show asymptotic trends in all sampled stations and months (Fig. 3). This suggests the sample size is sufficient to assess the diversity and number of species in the communities. The values of Shannon index show great variation between months and stations respect to diversity (Fig 4). The two-way ANOVA test indicates significant differences in H´s values between months (d.f. = 4; F = 2.81; p=0.026) and stations (d.f. = 3; F = 14.1; p<0.001) but failure in detect differences in the interaction (d.f. = 11; F = 1.66; p=0.084).

The station with the highest diversity was La Milpa, with a number of species (range: 16–22) higher than any other station. The Junco Sur station showed the lowest values of diversity and number of species (Fig. 4). The temporal variation of H indicates that October and November are the months with the highest diversity values. In the Prácticos station this statement is not robust because the H´s value is absent in November.

Multivariate measures of community structure

For log-transformed data, the nmMDS distribution plot shows a clear separation of samples from different stations (Fig. 5). The samples from La Milpa and Playa Alegre stations have a distinctive community structure forming separate sample groups. But, Prácticos and Junco Sur stations can not be distinguished. Although the samples from Prácticos station are closer each other than Junco Sur, indicating less variability in the structure of community.

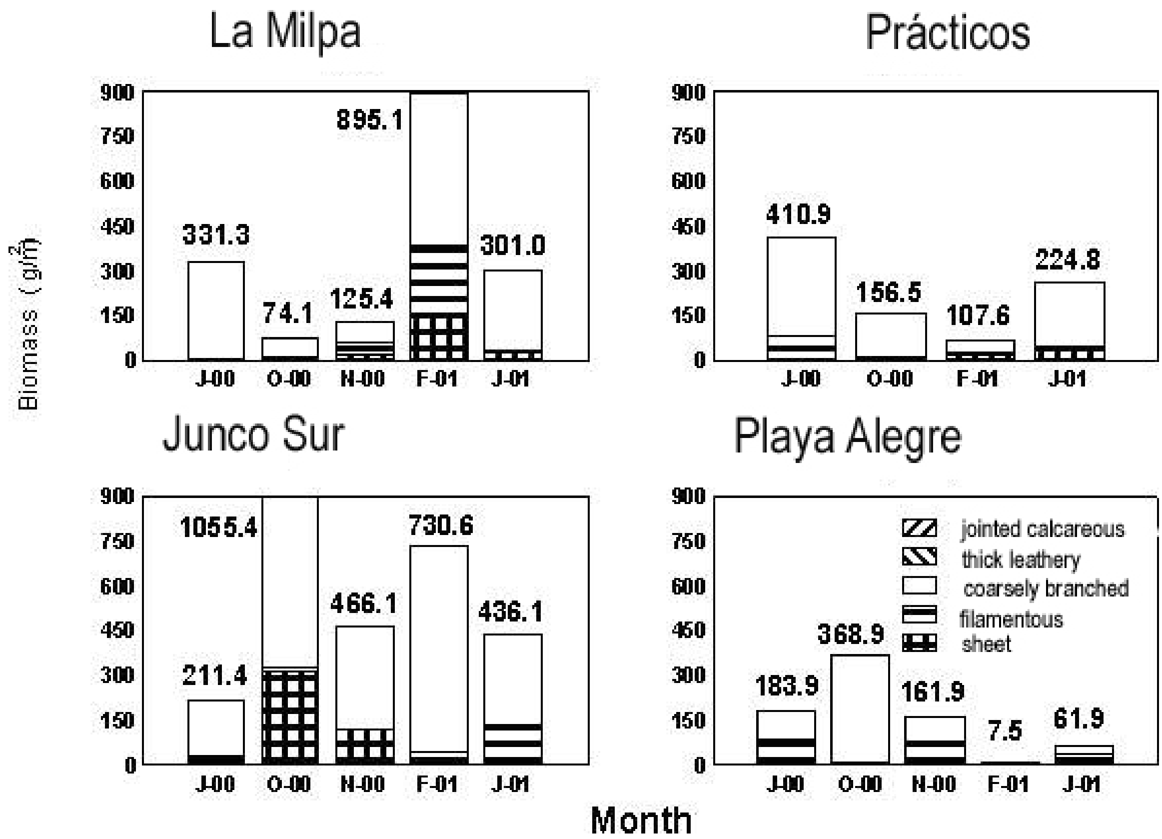

Fig. 2. Biomass of morphofunctional groups of macroalgae (g m^{-2}) in four sampled stations and in five (four for Prácticos) months. The values of total biomass (all groups) are showed upper the bars.

A comparison using a two-way crossed ANOSIM test, based on species biomass, revealed significant differences between stations (999 permutations; global R=0.50; p=0.001) and months (999 permutations; global R = 0.29; p=0,001). The result of global comparisons supports the graphical separation between stations in the nmMDS plot.

In the pairwise tests, all differences between pairs of stations (range R values = 0.31 – 0.68) and pairs of months (range R values = 0.22–0.53) are significant at p=0.001; the number of permutations are sufficiently large (999) in all comparisons. This indicates notable differences in biomass macroalgae community between five sampled months (averaged across all stations) and between stations (averaged across all months).

The average similarity of all samples within a group (*e. g.* averaging all values of similarities from quadrants within a station) could be decomposed into the contributions from each species; this gives species which are typical of a station. This analysis shows three pairs of typical species in each station (Table 3). *Hypnea spinella* is not a good discriminator because it appears as an important contributor in three of the sampled stations.

Table 3. Species that contributes in 50 % (or more) to average similarity of each station (inside parenthesis the value of similarity). See text for details.

Especie	La Milpa (35.4)	Prácticos (34.8)	Junco Sur (36.9)	Playa Alegre (32.6)
Chondrophycus papillosus	*			
Acanthophora spicifera	*			
Gracilaria damaecomis		*		
Hypnea spinella		*	*	*
Gracilaria blodgetti			*	

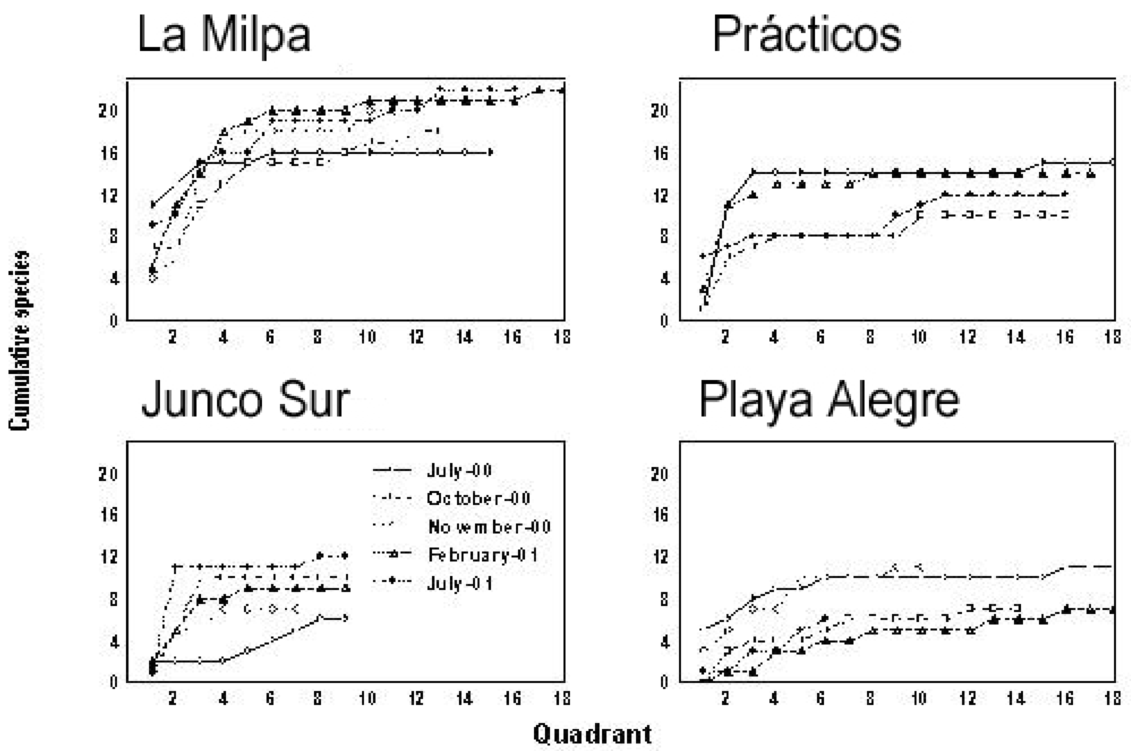

Fig. 3. Species accumulation curves in four sampling stations and in five months (four for Prácticos station). The number of quadrants in Playa Alegre July-00 and Prácticos July-00 are 21 and 19 respectively.

The averaged values of similarity are low (see values in parenthesis in Table 3), reflecting the variability within each station, derived from month pooling. The analysis of contributions to similarities within months (all stations pooled) shows a single species (*Hypnea spinella*) as major contributor. This indicates that *H. spinella* is the unique specie in the study that do not has marked change in biomass along sampled months.

DISCUSSION

Most of macroalgae representative genera found in this study are opportunistic coarse forms as *Hypnea*, *Acanthophora*, *Gracilaria*, *Ulva*, *Enteromorpha*, *Cladophora*, *Ectocarpus*, distinctive of coastal areas with environmental disturbances, such as changes in the nutrients and salinity concentration, both observed in Cienfuegos Bay due to seasonal influence (Seisdedo and Muñoz, 2005). The genus *Hypnea*, especially *H. musciformis* and *Acanthophora* has displaced to the native species in Ohau Bay, Hawai. In this bay, *H. musciformis* forms enormous tangled masses on dead reefs and is considered the most disruptive among the introduced species; *Acanthophora spicifera* is the species of more proliferation and constitutes a serious problem in this bay (Russell, 1992). In China, *A. spicifera* is considered invasive specie on macroalgal cultures (Trono, 1981).

Gracilaria genus is able to resist changes of salinity and temperature, high concentrations of nutrients and the burrowing by siltation of suspended particles (Lapointe, 1989; Santelices and Doty, 1989; Peckol and Rivers, 1996; De Casabianca *et a*l, 1997). During the present study, *Gracilaria* spp. was most abundant at two stations of north lobule (Junco Sur and Prácticos) of the bay, which is the most affected by siltation (Alonso *et al.*, 2001) and by the sewage drainages from Cienfuegos City.

Macroalgal biomass and diversity was lower in Playa Alegre station, this could be related to predominance of sandy-muddy substrate in this station. The seagrass *Halodule wrightii* Ascherson was the dominant submerged macrophyte in this station. *H. wrightii* is a pioneering specie, typical of soft bottoms, which occurs under disturbance conditions (Zieman *et al.*, 1989; Hemminga and Duarte, 2000). The presence of *H.*

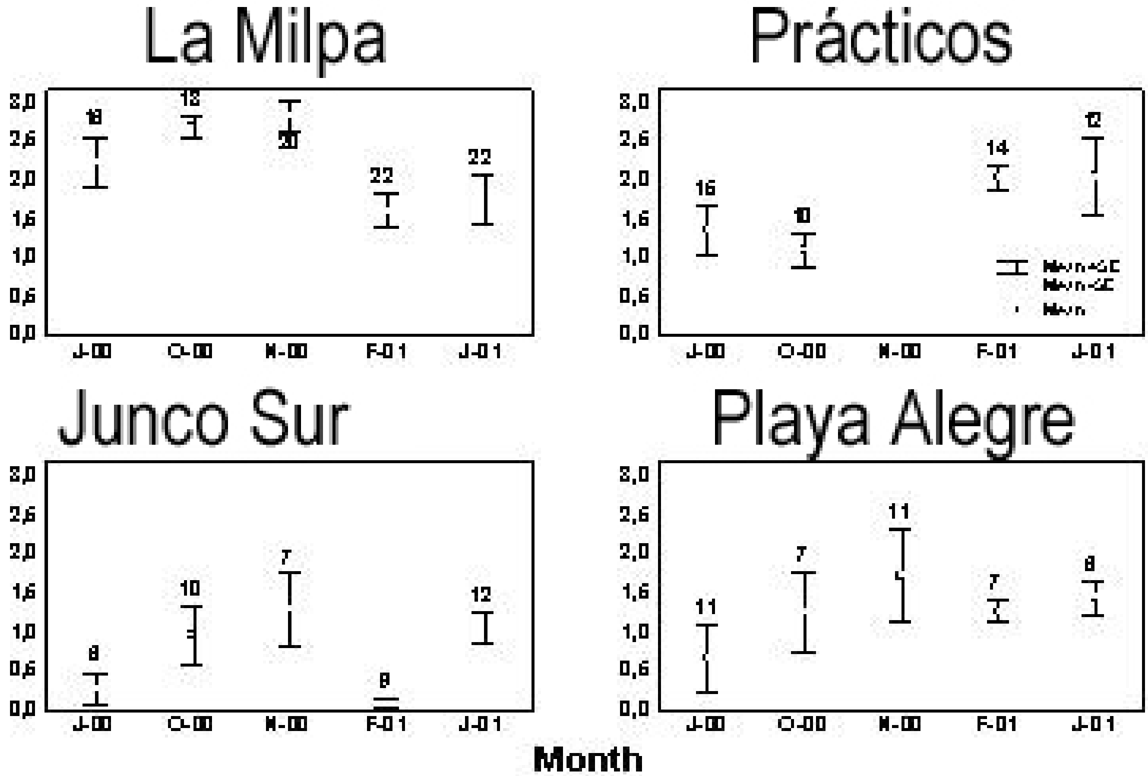

Fig. 4. Mean and standard error (SE) of Shannon diversity index in four stations and five (four for Prácticos station) months. The averaged number of species are showed above the whiskers.

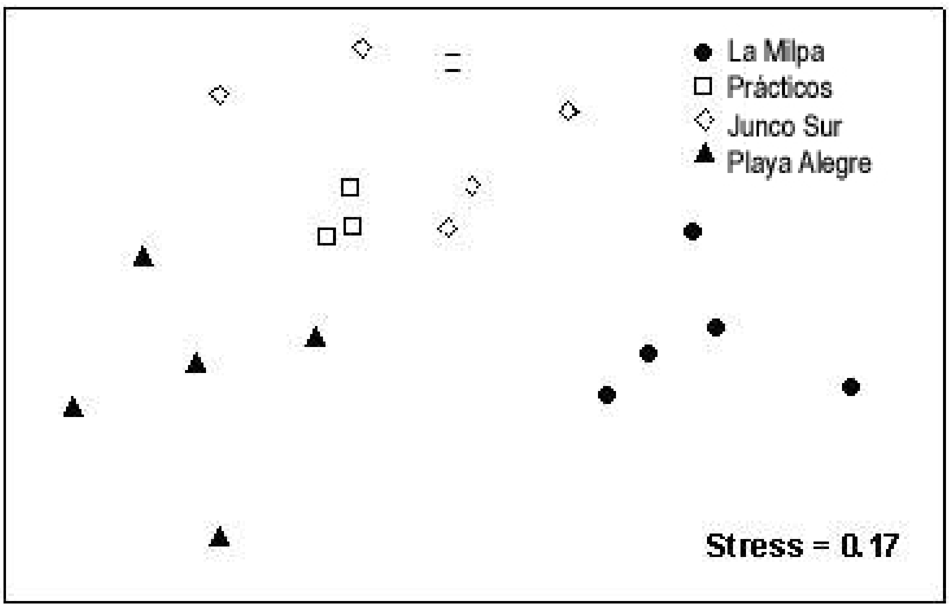

Fig. 5. mnMDS distribution plot of samples from four stations, based on log-transformed biomass of species. Each point represents an "averaged" community (all replicates from station/month combination were averaged).

wrightii instead of *Thalassia testudinum* Banks ex König could be due to the ability of H. *wrightii* to colonize and inhabit areas with extreme ranges in abiotic variables such as salinity, which has seasonal influence in this bay (Seisdedo y Muñoz, 2005). Also, the deposition of sediments has been increased, principally in the north lobule in the last years (Alonso *et al.*, 2001), making favourable the settlement of the rhizophitic *H. wrightii*. Macroalgal biomass was dominated in this station by species of a free-drifting mode of life, growing on *H. wrightii*, as *Hypnea spinella*, *Ectocarpus* sp. and *Chaetomorpha minima*.

Macroalgal biomass was higher at Junco Sur and La Milpa, two stations with different characteristics. Junco Sur exhibited the lowest diversity and La Milpa the highest. The tolerant species *Hypnea spinella* and *Gracilaria blodgettii* were dominant species in Junco Sur station; this behaviour could be due to the influence of the indirect impact of urban sewage from Las Calabazas creek. La Milpa, the most diverse station located near to adjacent waters of the bay, exhibited relative oceanic conditions; this is supported by the relative abundance of oligotrophic *Chondrophycus papillosus*, present only in this station, which has more disponibility of rocky substratum; similar results were recorded by Cabrera *et al.*, (2005) comparing the control site with areas marked by nutrients enrichment and lack of substrate for fixing in Nuevitas Bay.

There was an outstanding seasonal change in the macroalgal biomass peak that is associated with a change in the dominant specie in the sampling stations. In La Milpa station, macroalgal biomass was higher in February, resulting in an overgrowth of the opportunistic green macroalgae *Cladophora glomerata*. Other macroalgae as *Acanthophora spicifera*, *Chondrophycus papillosus*, and *Ulva fasciata*, were also abundant. This distinctive seasonal pattern in macroalgal biomass peak in La Milpa station is consistent with previous studies of green macroalgal biomass. Martins *et al* (2001) recorded that biomass of filamentous green macroalgae *Enteromorpha* sp. increased in an estuary during winter or dry season. During rainy season, when seasonally maximum nutrient input occurs, biomass decline because off the light extinction coefficient is increased by high turbidity of the water column, thus contributing to decrease the amount of light available for photosynthesis, the input of fresh water to the system also contributes to N-enrichment of water and increase N: P ratios and this suggest that primary production is P-limited (Valiela, 1995). In contrast to this observation, the macroalgal biomass in the north lobule increased during summer (rainy period), when there is a maximum input of nutrients. As it was mentioned above, biomass peak in these stations corresponded to opportunistic rhodophytes as *Hypnea spinella* and *Gracilaria* spp. Additionally, there was no strong evidence of massive bloom of filamentous macroalgae in the bay as occurs in many eutrophized bays and lagoons (Peckol and Rivers, 1996; Valiela *et al.*, 1997).

The peak of biomass at La Milpa station could be associated to the hydrodynamic characteristics of the bay, particularly the circulation pattern and the hydraulic residence time. It is known that the pattern of circulation of waters in the south lobule makes move counterclockwise carrying the interior waters toward the exit channel. It is also known that the hydraulic time of residence in the Bay of Cienfuegos reaches approximately 30 days in the winter station (November-April), and both characteristics can explain the transport of nutrients from Junco Sur to La Milpa station and the peak of biomass registered in this station.

It is interesting to note that the biomass of agarophyte *Gracilaria blodgettii* showed an overall decline in Prácticos station (north lobule) as compared to the results of León *et al.* (2002) in the 90's. *G. blodgettii* was displaced by *G. damaecornis* and by the invasive *Hypnea spinella*. Closure of a Fertilizer Factory, located in the north lobule could be associated with the reduction of $NH4^{+}$ in the bay (Fig 6) and with the decline of *G. blodgettii* in this station. Experiments comparing the effectiveness of ammonia *versus* other nutrients on biomass production of *Gracilaria* spp. have shown that this genus responds more strongly to ammonia than nitrate and phosphorous (Glenn *et al.*, 1999).

CONCLUSIONS

- Macroalgal biomass of Cienfuegos Bay is dominated by opportunistic coarse forms, typical of disturbed areas, such as *Hypnea spinella*, *Acanthophora spicifera* and *Gracilaria blodgettii*.

- Much of spatial-temporal variation in the biomass of macroalgae was related to characteristics of substratum, climatic variations or seasonal influence, hydrodynamic characteristics of the bay and the effect of polluted habitats.

- The principal agarophyte of the bay *Gracilaria blodgettii* showed an overall decline, this could be explained to the reduction of $NH4^+$ in the ecosystem.

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