

## EFFECTS OF CHRONIC AND HEAVY POLLUTION ON MACRO- AND MEIOBENTHOS OF HAVANA BAY, CUBA.

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### ABSTRACT

Infaunal communities (macro- and meiobenthos) and abiotic environmental factors were sampled at four stations in Havana Bay, Cuba in June, 2006. A comparison of concentration of pollutants with reference values indicated that the bay is heavily polluted. Several kinds of pollutants: hydrocarbons from petroleum, heavy metals, organic matter, and organochlorine pesticides were recorded from sediment and eutrophication was present in water. There were differences in the nature of contamination between the inlets: the most polluted were Marimelena, fundamentally by metals of industrial origin (Cr, Co, Mn, Ni, V), and Atarés mainly by metals of urban origin (Pb and Zn) and organic enrichment. The infaunal communities (macro- and meiobenthos) were strongly depleted in number of taxa and density; and defaunation occurred in the most polluted inlet of the bay. Only pollution-tolerant species of free-living marine nematodes (*Sabatieria pulchra*, *Terschellingia longicaudata*, and *Parodontophora xenotricha*) were present in very low densities in the sediments. The most plausible explanation of these features was the deleterious effects of pollution on infauna. Hydrodynamic regime apparently modules the effects of pollution on nematode assemblages in entrance channel of the Bay by increasing the available microhabitat and/or enhancing process of colonization.

Key words: meiobenthos; macrobenthos; pollution; environmental assessment; ASW, Cuba.

### RESUMEN

Las comunidades de la infauna (macro- and meiobentos) y varios factores abióticos fueron muestreados en cuatro estaciones en la Bahía de La Habana, Cuba, en junio del 2006. Una comparación de concentración de contaminantes con valores de referencia indicó que la bahía está fuertemente contaminada. Varios tipos de contaminantes (hidrocarburos del petróleo, metales pesados y pesticidas organoclorados) fueron detectados en los sedimentos y se registró eutrofización en el agua. Existieron diferencias en la naturaleza de la contaminación entre las ensenadas: las más contaminadas fueron Marimelena, fundamentalmente por metales de origen industrial (Cr, Co, Mn, Ni, V) y Atarés, principalmente por metales de origen urbano (Pb y Zn) y enriquecimiento orgánico. Las comunidades de la infauna estuvieron fuertemente impactadas en el número de taxa y la densidad; y una defaunación casi total ocurrió en la ensenada más impactada. Solo especies de nemátodos marinos de vida libre tolerantes a la contaminación (*Sabatieria pulchra*, *Terschellingia longicaudata*, and *Parodontophora xenotricha*) estuvieron presentes en muy bajas densidades en los sedimentos. La explicación más plausible de estos resultados es los efectos de la contaminación sobre la infauna. El régimen hidrodinámico aparentemente modula los efectos de la contaminación sobre las comunidades de nemátodos en el canal de entrada de la bahía por aumentar la cantidad de micro-hábitat disponible y/o facilitar los procesos de colonización.

Palabras clave: meiobentos; macrobentos; polución; evaluación ambiental; ASW, Cuba.

Marine pollution is a highly concerning phenomenon threatening health and habitat of human-kind (Worm *et al.*, 2006) and causing significant economical losses (Ofiara and Seneca, 2006). Bays and harbours associated to coastal cities are particularly exposed to pollution because of concentration of people, industries and harbourage operations. Often, the effects of pollution

are reinforced by natural features of bays as the fine-grained sediments that tend to bind organic and inorganic pollutants, the collection of run-off from land, the limited circulation of water and the reduced tidal flux (Paggi *et al.*, 2006).

The Havana Bay (Cuba Island), surrounded by Havana City with about 800 000 inhabitants,

receives contaminants from a variety of sources: land-based (e.g. an oil refinery and a power station) and diffuse inputs (e.g. drainage from city, discharges from ships), riverine/stream discharges and atmospheric fallout (Colantonio and Potter, 2006). During the 1980's the basin had been collecting a huge quantity of wastewaters and disposal from previously mentioned industrial and domestic activities, transforming the bay into a heavily polluted marine system (UNDP, 2002). At begin of 1990's, a strong national economical crisis reduced notably the input of contaminants from industrial and harbourage origin (Maal-Bared, 2006). From 2000 onwards, an economical recovery has been occurring and was coupled with protectional environmental initiatives directly related to the bay; those initiatives should show positive environmental impact after year 2007 (UNDP, 2002).

The initiatives on restrain of pollution and the evaluation of their possible success demand the implementation of a program for monitoring pollution. Recently, several research papers have been published concerning to contamination in estuaries and bays by heavy metals (see Fernandez *et al.*, 2007); organic pollutants and hydrocarbons (Muniz *et al.*, 2004) in estuaries or bays. However, only benthic infaunal community studies, together with sediment chemistry and toxicity tests, can determining if contaminants cause pollution (i.e. impacts on resident populations) (Chapman, 2007). Present study does not carry out toxicity tests; but the inclusion of both macrobenthic and meiobenthic communities, in addition to sediment chemistry, would be particularly useful for analysis of the effects of disturbance.

After Fernandez *et al.* (2007), there are only three reports in peer review international journals on distribution of metals in Cuba; two of them (Gonzalez and Torres, 1990; Gonzalez *et al.*, 1999) reported information from sites of discharges or outfalls near Havana Bay, but not properly inside the bay. An additional study by Gelen *et al.* (2005) but not referred to by Fernandez *et al.* (2007), reported heavy metal levels in Havana Bay using nuclear techniques. There are no studies published in widely accessible journals, on organo-chlorine pesticides, polychlorinated biphenyls, and hydrocarbons in Cuban sediments (Fernandez *et al.*, 2007). In addition, the most recent study (Herrera-Moreno and Amador-Pérez, 1983) on benthic communities in Havana Bay was carried out more than 20 years ago. The evaluation and publication of levels of contamination in Havana

Bay and effects of pollution on biota is required for three main reasons: i) the lack of information on pollution with respect to Cuba available for the scientific community; ii) Cuban sites have been reported with the highest values of concentration of metals in sediment in the Caribbean basin (Fernandez *et al.*, 2007); and iii) the recovery of industrial and harbourage activities would again increase the pressure on the urban ecosystem (Colantonio and Potter, 2006), including the Havana Bay.

Two main components of the ecosystem were included in our study: the abiotic environment (including overlying water and sediment) and the benthic faunal communities. The overlying water should be the first indicator of recovery of environmental quality and is a key matrix for the primary production of phytoplankton. The sedimentary matrix retains the contaminants in pore water and as binding to particles and thus constitutes an accuracy target for long term monitoring of contamination. The infaunal communities (macro- and meiobenthos) have been historically used as indicators of environmental quality. For nematode assemblages, the numerically dominant taxon in sediments in Havana Bay, the identification to putative species/genus level was considered suitable for assessment of environmental quality of marine sediments (Somerfield and Clarke, 1995).

In present contribution, we report on the levels of key contaminants for marine environment (e.g. heavy metals, hydrocarbons), and also on the status of benthic communities (meio- and macrobenthic) using high taxonomic resolution for most abundant taxa in sediments (i.e. nematodes) of the Havana Bay. A comparison with biotic and abiotic databases from 20 years ago allows us to estimate the level of recovery of the bay and the usefulness of mitigation/constrain strategies on pollution for the Bay. Hereby, we describe the spatial distribution of the contamination and benthic communities in four stations that characterize the bay; and discuss the deleterious effects of pollution on studied biota.

## MATERIAL AND METHODS

### Study area and sampling design

The Havana Bay (N23°08' and W82°20'), in the south-eastern part of the Gulf of Mexico, NW shelf of Cuba Island, is a semi enclosed bay with 5,2 km<sup>2</sup> area, mean depth of 9 m, and mean tidal ran-

ge of 0.3 m. The bay has three main inlets (Atarés, Guasabacoa and Marimelena) and several creeks drain to the basin (Fig. 1). The type of sediment varies from fine sand in the entrance channel to fluid mud in the inner of inlets; the colour of sediment was black with strong smell to hydrogen sulphide and petroleum, presence of petroleum drops and disposals was common. The main exchange of water of the bay with oceanic waters is due to tidal currents and the time of water renovation is around 5 - 7 days. However, short-duration weather events as tropical storms in summer or cold-fronts in winter can provoke a very high hydro-dynamism with mixture of waters and transport of sediment inside the bay and exchange with oceanic waters (pers. observation).

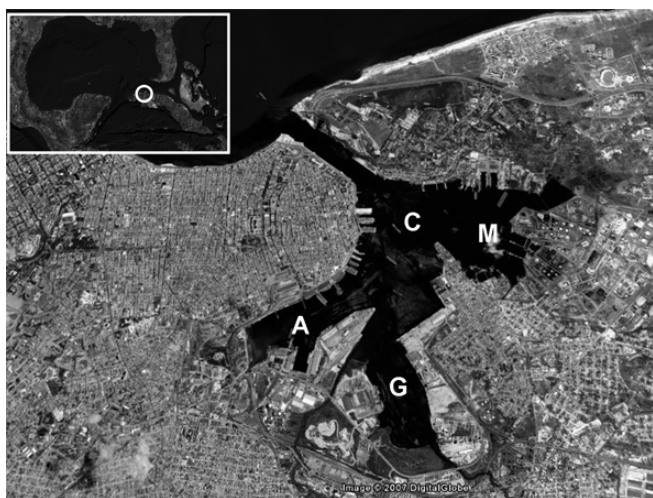


Fig. 1. Study zone (the inset shows the location of the Bay in larger scale); the sampling stations are indicated with types. Labels of stations: C=Centro, A=Atarés, M=Marimelena, and G=Guasabacoa. Note the high level of urbanization around the bay.

Four sampling stations were located inside the bay and sampled on June 2006; three of them at each inlet and a fourth at the central area (Centro) (Fig. 1). At each station, three sampling units (SU) were taken at random for determination of the structure of communities of macro- and meiobenthos. A SU consisted of an extraction of sediment using a Petersen grab (Rigosha Co. Japan); this grab is recognized as a suitable device for quantitative sampling in muddy bottoms in shallow waters (Eleftheriou and Moore, 2005). The main features of the used grab were the followings: surficial area of 0.067 m<sup>2</sup>; depth of penetration into sediment around 10 cm; and two 40 µm gauze-covered windows in the upper part.

A single measurement of abiotic variables was carried out at each station. A Van Veen grab (surficial area: 0.005 m<sup>2</sup>; depth of penetration into sediment around 6 cm) was used for taking samples of sediments for the analysis of grain size, organic content and heavy metals concentration. An oceanographic bottle Van Dorn of 4 L was used to collect samples of water in surface for analyses of dissolved oxygen and nutrients; the sampling of water was done during ebb tide to avoid the influence of oceanic waters.

### Processing of samples

Previous information about depleted density of benthic fauna in Havana Bay led us to process of whole samples (i.e. not sub-sampling was carried-out) in order to obtain the maximum of animals possible per SU. The samples of sediment for faunal communities were fixed with 8% formalin within plastic bags. In the laboratory, samples were sieved using 500 and 45 µm mesh sieves; the material retained on the sieves was considered as macro- and meiobenthos respectively. Separation of macrobenthos from sediment was done by manual pickup of animals under stereomicroscope (Zeiss MB-9, 16 - 25 x). Extraction of meiobenthos from sediment was done in high density sugar solution (crystals of commercial sugar dissolved in water, 1.16 g cm<sup>-3</sup>); the efficiency of sorting of this method in our laboratory is higher than 90% (unpublished data). Sorted organisms were preserved in vials with 4% formalin and stained with 1% alcoholic eosin. After one week, at least, organisms (both macrobenthic and meiobenthic) were identified to high taxonomic level (e.g. polychaetes, copepods) and counted. Nematodes were the only organisms identified to species/genus and following protocol was performed. Briefly, nematodes were picked out, and subjected to three successive steps of inclusion in mixtures of ethanol - glycerol until they remained in pure glycerol. They were mounted inside paraffin rings on glass slides, covered with glass cover slides and paraffin slowly melted in order to seal the montage. The identification to putative genus/species was carried out under Olympus CX 31 microscope at maximum magnification (1 000 X) with the use of the pictorial keys of Platt and Warwick (1983; 1988), Warwick *et al.* (1998) and NeMys database (Deprez *et al.*, 2005).

The dissolved oxygen was measured by the Winkler's method (accuracy: 0.01 mg L<sup>-1</sup>) and the salinity using a digital salinometer (Tsrumi Seiki;

accuracy: 0.1 psu). The determination of the concentration of the different types of nutrients in water: nitrate ( $\text{NO}_3$ ), nitrite ( $\text{NO}_2$ ), ammonium ( $\text{NH}_4$ ), phosphate ( $\text{PO}_4$ ), total phosphorus (Pt) and silicate ( $\text{SiO}_4$ ) was carried out after the guidelines of Grasshoff *et al.* (1999). Total suspended solids were determined by the gravimetric method. The concentration of total hydrocarbons in sediment was measured with gas chromatography (ATI Mattison Genesis Series FTIR; lower detection limit:  $0.001 \text{ mg L}^{-1}$ ) after APHA's guideline (APHA 1998). The samples for heavy metals in sediments were digested with  $\text{HNO}_3/\text{HCl}$  and concentration was determined by inductively-coupled plasma mass spectrometer (ICP-MS). The content of organic matter in sediment was measured by difference of weight after ignition at  $550^\circ\text{C}$  in a furnace for 3 hours. The percentage of silt plus clay of sediment was determined by the gravimetric method using a shaker (Retsch AS 200) and  $63 \mu\text{m}$  test sieves as limit between sand and silt/clay fraction. All used reagents were pure for analysis.

### Data analysis

The very low number of organisms of macrobenthos and meiobenthos recorded from the samples induced us to express the densities as animals  $0.1 \text{ m}^{-1}$  (a close approximation to real area of sampling device:  $0.067 \text{ m}^2$ ). Nematodes were grouped by trophic guilds after the classification of Wieser (1953). The maturity index of nematode assemblages (Bongers *et al.*, 1991) was calculated for each sample, as well as the average per station. Where the c-p value was unknown for genus/-species (e.g. *Acanthonchus*, *Nannolaimoides*) the value corresponding with the family was used.

Data were analyzed by univariate and multivariate techniques, using software PRIMER 5.2.9 (Clarke and Warwick, 2001). Analyses of variance (ANOVA) were performed on univariate measures of community (i.e. number and density of taxa or operational taxonomic units). The presence of outlier values and the homogeneity of variance were checked using diagnosis graphics (i.e. variance versus mean, residuals versus mean), and where data did not fulfill assumptions of parametric statistic they were transformed as logarithm + 1. Results of analyses with untransformed and transformed data are presented and compared. Where results of both analyses were similar, graphics were built on untransformed data for easier interpretation. Coefficients of correlation product-moment of Pearson were calculated between variables looking for trends across stations. Permutation-based tests

(ANOSIM) were applied looking for differences in multivariate community structure across stations. Numerical ordinations of the samples, on basis of similarity matrices, were represented in 2-d plots using non-metrical multidimensional scaling (nmMDS). The similarity matrices were calculated using the index of similarity of Bray-Curtis; data were not transformed due to relative low number of animals per sample (i.e. dominance never exceeded two orders of magnitude).

## RESULTS

### Abiotic variables

The concentration of nutrients in the water column ( $\text{NO}_3$ ;  $\text{NO}_2$ ;  $\text{NH}_4$ ;  $\text{PO}_4$ , Pt and  $\text{SiO}_4$ ) showed a large variability across stations, with coefficients of variation (CV) between 29 and 95 %. The values of dissolved oxygen, salinity and total suspended solids had a lower spatial variation (CV = 10, 5 and 12% respectively). An intermediate value of the coefficient of variation was 22% for hydrocarbons in the water. The values of dissolved oxygen were higher than  $5 \text{ mg L}^{-1}$  at any station; indicating well oxygenated surface waters (Table 1). The station Centro showed lowest values for all nutrients; though presented the highest values of total suspended solids (Table 1). Peak values of ammonium were recorded in stations Atarés and Marimelena ( $14$  and  $16 \mu\text{mol L}^{-1}$  respectively) (Table 1). There were no clear trends between nutrients after an analysis of correlation, nor for other abiotic variables in the water column (e.g. dissolved oxygen, salinity and total suspended solid).

The distribution across stations of total hydrocarbon in sediment was relatively homogeneous (CV = 19%). The other variables showed a marked variations across stations, with silt + clay content and concentration of Ni having larger fluctuations (CV = 72 and 79% respectively) (Table 2). Marimelena station showed the highest concentration of some heavy metals (Co, Cr, Mn, Ni, V and Fe) in their sediments; Atarés had the highest concentration for the metals Pb and Zn (Table 2).

Trends in abiotic data should be interpreted with caution due to the low number of observations ( $n = 4$ ) for each variable. However, several apparent trends hatch out from the data; firstly, a high correlation ( $r > 0.9$ ) was found between the percent of silt + clay in the sediment and the organic matter content. The heavy metals, in general, showed a high correlation among them ( $r > 0.9$ );

Table 1. Abiotic variables measured in the water column (surface level) at four stations in Havana Bay, Cuba. DO = dissolved oxygen; TP = total phosphorus; TSS = total suspended solids; TH = total hydrocarbons. DO, TSS, and TH in  $\text{mg l}^{-1}$ , all nutrients in  $\mu\text{mol l}^{-1}$ .

|            | DO  | Salinity | NO <sub>3</sub> | NO <sub>2</sub> | NH <sub>4</sub> | PO <sub>4</sub> | TP   | SiO <sub>4</sub> | TSS | TH   |
|------------|-----|----------|-----------------|-----------------|-----------------|-----------------|------|------------------|-----|------|
| Centro     | 6.9 | 30.4     | 2.14            | 0.27            | 1.44            | 0.07            | 1.02 | 7.2              | 80  | 0.18 |
| Atarés     | 5.8 | 31.9     | 0.19            | 0.51            | 13.51           | 0.83            | 1.82 | 14.9             | 72  | 0.21 |
| Marimelena | 7.5 | 29.5     | 1.81            | 0.62            | 16.42           | 0.32            | 1.14 | 13.4             | 77  | 0.27 |
| Guasabacoa | 6.8 | 28.4     | 0.78            | 0.48            | 6.35            | 1.58            | 1.75 | 21.1             | 61  | 0.17 |

Table 2. Abiotic variables measured in the sediments at four stations in Havana Bay, Cuba. OM = organic matter; S/C = silt + clay; TH = total hydrocarbons. OM, S/C, and Fe in %, heavy metals in  $\mu\text{g g}^{-1}$  dry weight sediment.

|            | OM   | S/C | TH   | Co   | Cr  | Cu  | Mn  | Ni  | Pb  | V   | Zn  | Fe   |
|------------|------|-----|------|------|-----|-----|-----|-----|-----|-----|-----|------|
| Centro     | 16.1 | 18  | 1025 | 6.1  | 71  | 181 | 273 | 73  | 244 | 67  | 617 | 2.24 |
| Atarés     | 32.0 | 51  | 1446 | 7.1  | 62  | 138 | 263 | 73  | 271 | 87  | 765 | 1.85 |
| Marimelena | 7.9  | 15  | 1434 | 20.0 | 151 | 119 | 578 | 229 | 55  | 116 | 212 | 3.79 |
| Guasabacoa | 30.3 | 66  | 1234 | 11.0 | 62  | 107 | 413 | 58  | 174 | 87  | 382 | 2.78 |

the metals: Co, Cr, Mn, Ni and Fe had a positive relationship between them. The other two metals (Pb and Zn) showed high correlation between them, but negative correlation with the previously mentioned group.

### Macrobenthic communities

Six taxa were collected from the macrobenthic samples (i.e. organisms retained in 500  $\mu\text{m}$  sieve) in Havana Bay: Polychaeta, Nematoda, Amphipoda, Nemertinea, Oligochaeta and Copepoda. The mean number of taxa ( $\pm$  SD) was  $2.0 \pm 1.6$ ; and the range: 0 – 5 taxa (Fig. 2). There were no significant differences in number of taxa among stations upon ANOVA test ( $p = 0.22$ ) with untransformed data; similar result existed for log-transformed data. However, the statistical power of ANOVA test was low (0.3) after a post hoc analysis.

The mean density ( $\pm$  SD) of macrobenthos was  $32.6 \pm 94.0$  animals  $0.1 \text{ m}^{-2}$ ; with a range of: 0 – 330.6 animals  $0.1 \text{ m}^{-2}$  (Fig. 2). Polychaetes were the most abundant taxon ( $46 \pm 45\%$  of total density), followed by oligochaetes and nemertines. The two latter taxa showed high spatial variation, i.e. oligochaetes appeared in only one replicate (153 animals  $0.1 \text{ m}^{-2}$ ) from the 12 replicates taken in the entire bay. This huge variability prevented the detection of significant differences in density (statistical power: 0.3) among stations after ANOVA test ( $p = 0.46$ ).

A multivariate permutation test (ANOSIM) showed significant differences among multivariate structure of macrobenthic communities ( $R = 0.46$ ;  $p=0.006$ ; 999 permutations); pairwise test between pair of stations could not be applied due to the low number of replicates (i.e. possible permutations). The ordination plot showed that Centro and Guasabacoa stations seem to have different community structure and relative high similarity among replicates from a single station (Fig. 3). Macrobenthic communities from Atarés and Marimelena had very high dispersion in the plot (i.e. high variability among replicates from a single station) (Fig. 3).

### Meiobenthic communities

Five taxa were recorded from the meiobenthic samples in Havana Bay: Nematoda, Polychaeta, Amphipoda, Copepoda and Oligochaeta. The mean number of taxa ( $\pm$  SD) was  $3.1 \pm 1.1$ , and the range: 3 – 5 taxa (Fig. 2). There were no differences in the mean number of taxa among stations after ANOVA ( $p = 0.15$ ); the same non-significant result was obtained with log-transformed data ( $p = 0.08$ ). A post hoc analysis indicated very low statistical power (0.3) in the ANOVA; obviously the very high variability across stations and the relatively low number of replicates ( $n = 3$  by station) prevent to detect differences.

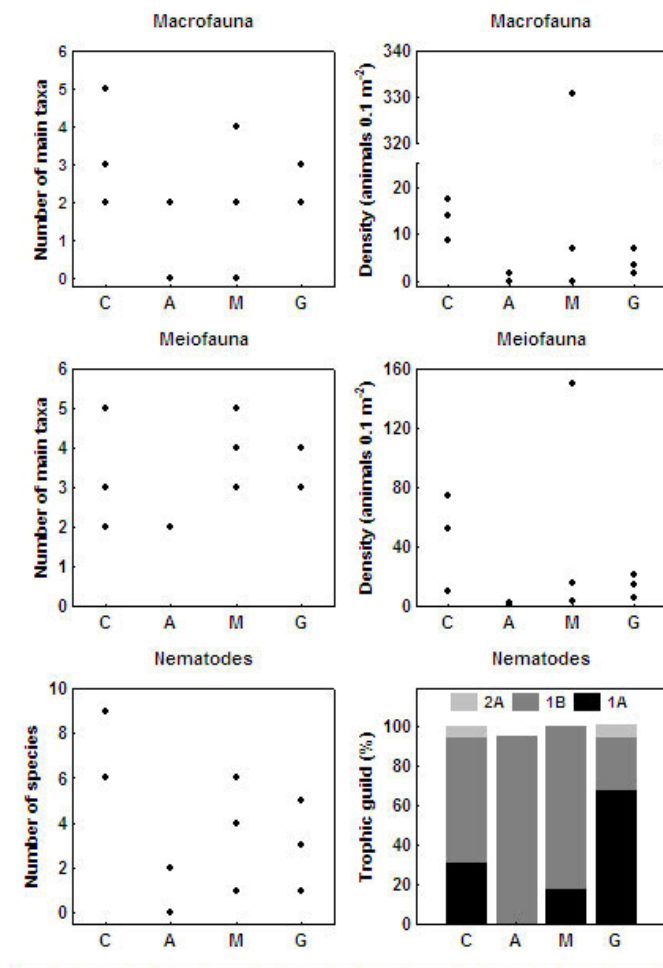


Fig. 2. Univariate measures of macro-, meiobenthic and nematode assemblages at four stations from Havana Bay ( $n=3$  for each station). Labels of stations: C=Centro, A=Atarés, M=Marimelena, and G=Guasabacoa. Trophic guilds: 1A = selective deposit feeder, 1B = non-selective deposit feeder, and 2A = epistrate feeder.

The density of meiobenthos ranged from 1 to 150 animals  $0.1 \text{ m}^{-2}$  (equivalent to  $0.1 - 15$  animals  $10 \text{ cm}^{-2}$ ); with a mean value ( $\pm \text{SD}$ ) of  $29.4 \pm 44.2$  animals  $0.1 \text{ m}^{-2}$  for whole bay (Fig. 2). Nematodes were the most abundant group ( $59 \pm 39\%$  of total density), followed by copepods ( $16 \pm 22\%$ ) and polychaetes ( $9 \pm 24\%$ ). The rank of dominance of taxa was different in Guasabacoa, with copepods reaching 45% of the total meiobenthos (nematodes 52%). No differences were detected in untransformed density of meiobenthos among stations after ANOVA test ( $p = 0.43$ ); transformed data revealed a similar pattern. An analysis of power indicated very low statistical power (0.2) in ANOVA

test, due to previously mentioned features of the data. A huge variability was associated with the Marimelena station (Fig. 2) due to aggregation of 128 juveniles of polychaetes in one of the replicates; this event was not observed in any other of the 11 replicates.

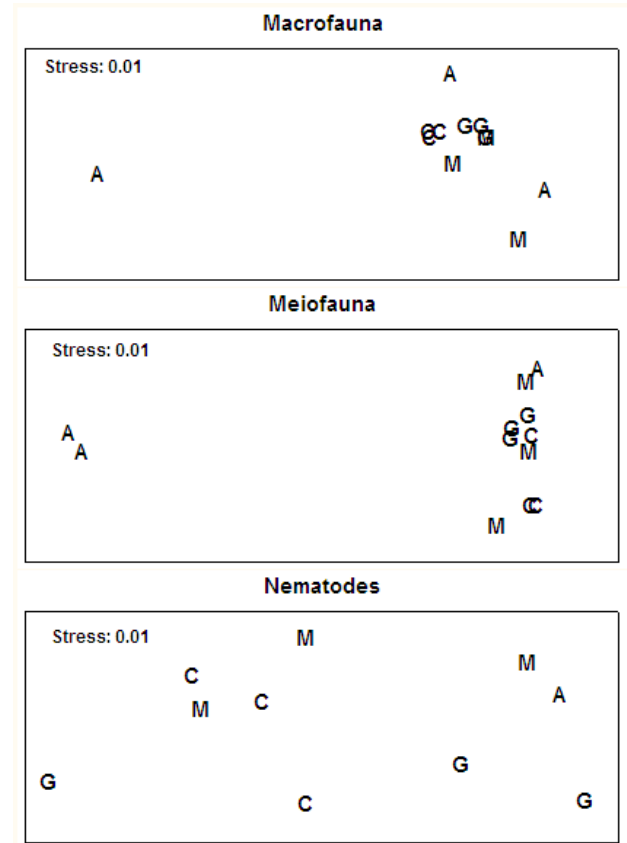


Fig. 3. Multidimensional ordination plots of samples from Havana Bay on untransformed data of macro-, meiobenthic and nematodes assemblages. Labels of stations: C = Centro, A = Atarés, M = Marimelena, and G = Guasabacoa.

The permutation test on multivariate structure of meiobenthic communities showed significant differences among stations ( $R = 0.34$ ;  $p = 0.01$ ; 999 permutations). Interpretation of ordination plot based on meiobenthos data resembled those for macrobenthos (Fig. 3). The stations Centro and Guasabacoa had relatively high similarity among replicates and different composition of meiobenthic communities; the stations Atarés and Marimelena showed very high dispersion in the plot, indicating high variability in the structure of their communities (Fig. 3).

## Nematode assemblages

A total of 15 species and 12 genera of free living marine nematodes were collected from sediments from Havana Bay (Table 3). There was a strong ecological dominance, with 68% of the total abundance constitute by only two species *Sabatieria pulchra* de Man 1907 and *Terschellingia longicaudata* de Man 1906. An analysis of variance showed significant differences among stations ( $p = 0.02$ ) in the number of species of nematodes. The highest average of number of species occurred at Centro (mean  $\pm$  SD:  $6 \pm 1$ ), the lowest at Atarés ( $1 \pm 1$ ) (Fig. 2).

The relative abundance of trophic guilds showed a dominance of deposit feeder nematodes (groups 1A and 1B); the guild epistrate feeder (2A) only appears in low abundance in stations Centro and Guasabacoa (Fig. 2). The guild omnivores/ predators (2B) were not recorded from the Havana bay. The ranking of the average maturity index (MI) of nematode assemblages for each station indicated a gradient (in brackets the MI values): Atarés (2.00) < Marimelena (2.11) < Centro (2.41) < Guasabacoa (2.75).

A permutation test showed significant differences among stations ( $R=0.41$ ;  $p=0.001$ ; 999 permutations) based on multivariate structure of nematode assemblages. The ordination plot did not show any clear trend across samples (Fig. 3). However, two replicates with zero nematodes from station Atarés were eliminated from the plot since upon their inclusion, the remainder points (samples) collapsed in a same point. An analysis of the contribution of each species to the total similarity of its station indicated that presence of *Terschellingia longicaudata* and *T. communis* accounted for most of the similarity at stations Centro and Guasabacoa respectively; *Sabatieria pulchra* and *Theristus* sp. explained > 90% of similarity at station Marimelena. The absence of nematodes in two out of three replicates at Atarés prevented the calculation of the average similarity among replicates in this station. These samples without nematodes, appeared almost completely defaunated with respect to macrobenthos and meiobenthos; only two and single amphipods respectively occurred.

Table 3. Number of nematodes in the samples (three replicates per station; grab's area = 0.067 m<sup>2</sup>) in Havana Bay. Blank = 0.

| Specie/genus                        | Centro |    |   | Atarés |  |  | Marimelena |   |    | Guasabacoa |   | Total | %  |
|-------------------------------------|--------|----|---|--------|--|--|------------|---|----|------------|---|-------|----|
| <i>Sabatieria pulchra</i>           | 43     | 18 | 1 | 1      |  |  | 2          | 8 | 15 | 3          | 2 | 93    | 48 |
| <i>Terschellingia longicaudata</i>  | 2      | 29 | 4 |        |  |  |            |   | 3  |            |   | 38    | 20 |
| <i>Parodontophora xenotricha</i>    | 9      |    | 1 | 1      |  |  |            |   |    |            |   | 11    | 6  |
| <i>Terschellingia communis</i>      |        | 1  |   |        |  |  |            |   |    | 6          | 2 | 10    | 5  |
| <i>Sabatieria breviseta</i>         | 5      |    |   |        |  |  | 3          | 1 |    |            |   | 9     | 5  |
| <i>Theristus</i> sp.                |        |    |   |        |  |  | 1          | 4 |    |            |   | 5     | 3  |
| <i>Daptonema</i> sp.                | 4      |    |   |        |  |  |            |   |    | 1          |   | 5     | 3  |
| <i>Pseudoterschellingia ibarrae</i> |        |    |   |        |  |  |            | 2 |    |            | 3 | 5     | 3  |
| <i>Dorylaimopsis</i> sp.            |        | 3  | 1 |        |  |  |            |   |    |            |   | 4     | 2  |
| <i>Terschellingia goubaultae</i>    |        |    |   |        |  |  | 1          |   |    |            | 1 | 2     | 1  |
| <i>Acanthonchus cobbi</i>           | 2      |    |   |        |  |  |            |   |    |            |   | 2     | 1  |
| <i>Trichotheristus</i> sp.          | 2      |    |   |        |  |  |            |   |    |            |   | 2     | 1  |
| <i>Metachromadora</i> sp.           |        |    | 1 |        |  |  |            |   |    | 1          |   | 2     | 1  |
| <i>Nannolaimoides</i> sp.           |        | 1  |   |        |  |  |            |   |    |            |   | 1     | 1  |
| <i>Sigmophoranema</i> sp.           |        |    | 1 |        |  |  |            |   |    |            |   | 1     | 1  |

The very high variability in biotic data and the low number of observations for abiotic variables did not allow obtaining reliable trends (correlations) across stations between community measures and abiotic variables.

## DISCUSSION

The comparison with reference values (Long *et al.*, 1995; Buchman, 1999) of the levels of contaminants (heavy metals and hydrocarbons) recorded in present study indicated that sediments in Havana Bay are strongly polluted; and that there are high probabilities that they cause adverse effects on biota. Comparisons with regional reports from Latin America and Wider Caribbean (Muniz *et*

*al.*, 2004; Fernandez *et al.*, 2007 and references herein) rank the Havana Bay as one of the most heavily polluted sites in the region. Recent records of organochlorines pesticides (e.g. DDT, dieldrin, endrin, heptachlor) in the bay (unpublished data) reinforce those conditions of heavy pollution in sediments and imply serious risks to human health. The levels of nutrients in overlying water were high; particularly the phosphorus in overlying water could cause chronic effects on biota (Buchman, 1999). However, the relative high concentration of dissolved oxygen ( $> 6 \text{ mg L}^{-1}$ ) in surface water, in comparison with historical records in the Bay (unpublished data), suggests a recovery of environmental quality in overlying water. This would be the first indication that the program of mitigation of contamination in Havana Bay is yielding successful results. However, Atarés still showed low values ( $< 3 \text{ mg L}^{-1}$ ) in bottom water (unpublished data) suggesting that it is the most organically enriched site in the bay. The large differences in concentration of nutrients in underlying water among stations (e.g. among inlets) should be explained by: i) the main sources of nutrients being land-based (e.g. creeks and industries) and ii) the reduced circulation of mass of water within the bay. The scarce circulation of water appears to reinforce the effects of pollution inside the inlets by avoiding the dilution of contaminants with oceanic waters through entrance channel.

Marked differences in levels of contamination exist among the three inlets. The sediments from Marimelena station had the highest levels of contaminants from industrial origin (hydrocarbons, Co, Cr, Mn, Ni, V, and Fe) due to presence of two main industries in its basin: a power station and an oil refinery (Gelen *et al.*, 2005). The Atarés station showed the highest contamination from urban origin (e.g. phosphorus in water, Zn and Pb in sediments) due to the presence of dense settlements of human populations in its basin. The high correlation between concentrations of Zn and Pb has been reported for urbanised and polluted areas (Muniz *et al.*, 2004). Hydrodynamic regime (mainly the tidal currents) largely determines the sedimentary characteristics of an area (Hall, 1994); and is the probable cause of the highest content of suspended solids in water and lowest values of silt/clay in sediments at Centro station. The aforementioned station is located in front of the entrance channel of the bay, where the strongest tidal flows occur between inner bay and oceanic waters (unpublished data).

All univariate measures of benthic community (except number of nematode species) failed for the detection of differences among stations due to low statistical power (less than 0.4 in almost performed tests). This reinforces the importance of multivariate approach for description of heavily stressed communities characterized by high variability in their structure and low number of counted individuals. The increased variability (particularly in Atarés and Marimelena stations) of community structure would be a symptom per se of environmental stress on biota (Warwick and Clarke, 1993).

The infaunal communities (macrobenthos and meiobenthos) in Havana Bay were strongly depleted both in density of individuals and in number of taxa; the simplest explanation is the deleterious effects of pollution. Only pollution-tolerant taxa as polychaetes and nematodes (Paggi *et al.*, 2006) occurred in relative high frequency in the samples. A comparison of values of density of macrobenthos with data from other bays subjected to several types of pollution (e.g. Flemer *et al.*, 1999; Guerra-García *et al.*, 2003; Hatje *et al.*, 2008) indicates almost defaunation of these communities in Havana Bay.

Regarding to meiobenthos, two main features characterized their communities in Havana Bay: the lowest values of density in comparison with studies carried out in comparable bays (e.g. Dalto *et al.*, 2006; Moreno *et al.*, 2008); and the dominance of two nematode species (*Sabatieria pulchra* and *Terschellingia longicaudata*) recognized as tolerant to pollution in the literature (e.g. Millward and Grant, 1995; Lampadariou *et al.*, 1997; Schratzberger *et al.*, 2000; 2006; Buchholz and Lampadariou, 2002; Gyedu-Ababioa and Baird, 2006; Steyaert *et al.*, 2007). These features suggest the existence of chronic and heavy pollution with strong deleterious effects on benthic communities.

Effects of pollution occurred on benthic communities at four studied stations; however, there were differences in the intensity of anthropogenic impact among stations. The interpretation of the univariate and multivariate analysis of community structure suggested that Atarés and Marimelena showed the stronger effects of pollution on their benthic communities. The benthic communities from Centro and Guasabacoa appeared to be less impacted. The significant increase of number of species of nematodes at Centro could be explained by a more intense hydrodynamic regime that enhances: i) a coarser

grain size and more availability of microhabitat (Ndaro and Ólafsson, 1999; Steyaert *et al.*, 1999); and ii) the recruitment into sediments (Bell and Sherman, 1980; Palmer, 1988; Commiato and Tita, 2002). Unfortunately, the determination of specific importance of each of aforementioned process in shaped nematodes assemblages is not possible in present study. Anyway, we remarked the importance of hydrodynamic regime (i.e. boundary layer flow and sediment transport after Hall, 1994; Snelgrove and Butman, 1994) in determining the structure of community even in conditions of heavy pollution.

A comparison of our data with a study carried out in Havana Bay more than 20 years ago (Herrera-Moreno & Amador-Pérez, 1983) should be interpreted with caution since different sampling devices and mesh size of sieves were used in both studies. The general results from Herrera-Moreno and Amador-Pérez (1983) indicated marked depletion of infaunal communities in whole bay (with high frequency of defaunated samples of sediment) particularly Atarés inlet had the worse environmental conditions and almost total absence of infaunal communities. Estimates of our study, in comparison with 20 years ago, showed a higher number and density of taxa in the bay (particularly in reference to macrobenthos). However, it is not possible to state if the cause is a response of infauna from polluted sites to the increase in concentration of dissolved oxygen in water (Guerra-García and García-Gómez, 2005) or the use of different mesh sieves in the processing of samples. As we hope, a recovery of diversity of infaunal communities should cause notable and direct effects on function of the ecosystem (Ieno *et al.*, 2006; Norling *et al.*, 2007), including significant effect scale on oxygen dynamics at basin wide scale (Waldbusser *et al.*, 2004). Anyway, taking in account the very high levels of contaminants cumulated currently in sediments of Havana Bay, a reduction of the level of contamination would take over 1 to 2 decades after contaminant sources be reduced (Bothner *et al.*, 1998).

In summary, the Havana Bay maintained high levels of pollution with deleterious effects on benthic communities; the most obvious were the almost defaunation of sediments and the dominance of pollution-tolerant species of free-living marine nematodes. Atarés and Marimelena inlets had the worse environmental quality; and hydrodynamic regime would enhance the nematode assemblages at Centro station.

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