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ARTICULO ORIGINAL

PERSISTENT IMPACTS ON THE FREE-LIVING NEMATODE ASSEMBLAGES IN HAVANA BAY: COMPARATIVE ANALYSIS BETWEEN 2006 AND 2016.

Impactos persistentes en comunidades de nemátodos de vida libre de la bahía de la Habana: Análisis comparativo entre los años 2006 y 2016.

José Andrés Pérez-García[1*], Diana Marzo-Pérez[1], Jesús Beltrán-González[2], Maickel Armenteros[3*].

[1] Centro de Investigaciones Marinas – Universidad de la Habana. Calle 16, No. 114, entre 1ra y 3ra, Miramar, La Habana, CP 11300, Cuba.

[2] Centro de Investigación y Manejo Ambiental del Transporte. Carretera del Cristo # 3, Regla, CP 11200, Habana, Cuba.

[3] Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Circuito Exterior S/N, Ciudad de México, 04510, México.

[*] Autores para correspondencia:

jose.andres@cim.uh.cu
maickel.armenteros@gmail.com

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ABSTRACT

Havana bay is one of the most polluted semi-enclosed basins in the world due to deleterious influence of urban and industrial effluents originated from Havana city. The sustained impact of contamination for decades have practically defaunated the benthic system affecting the monitoring of the environmental quality. In this study, we compared the free-living nematode assemblages between 2006 and 2016 to assess the changes in the sediment and estimate if the environmental quality has improved in 10 years. Nematodes were collected from four sites in the bay in 2006 and 2016 and identified to species level using the same methodology. Havana bay has improved some environmental variables in 2016 compared with 2006. However, still exist synergistic deleterious effects on the nematofauna of stressors such as hypoxia and toxicity by heavy metals and petroleum hydrocarbons. Nematodes showed extremely impoverished assemblages dominated by two cosmopolitan, detritivores and tolerant species: *Terschellingia longicaudata* and *Sabatieria pulchra*. The synthesis of our results indicates that environmental quality of Havana bay remains precarious and there is a necessity to reformulate the program of mitigation because the reduction of pollution has not significantly improved the benthic communities.

KEY WORDS: *pollution, sediments, semi-enclosed bay, meiobenthos, nematodes.*

RESUMEN

La bahía de la Habana es uno de los sistemas estuarinos más contaminados del mundo debido a la influencia negativa de desechos urbanos e industriales originados en la ciudad de la Habana. El impacto sostenido de la contaminación durante décadas prácticamente ha defaunado el sistema bentónico lo que dificulta el monitoreo de la calidad *ambiental. En este trabajo se comparan las comunidades de nemátodos de vida libre entre los años 2006 y 2016 para evaluar los cambios en los sedimentos y estimar si la calidad ambiental ha mejorado en el lapso de 10 años. Los nemátodos fueron colectados en cuatro sitios en la Bahía en 2006 y 2016 e identificados hasta nivel de especie empleando la misma metodología. La bahía de La Habana ha mejorado algunas variables ambientales en 2016 comparado con 2006. Sin embargo, persisten efectos negativos y sinérgicos sobre la nematofauna como hipoxia y toxicidad por metales pesados e hidrocarburos del petróleo. Los nemátodos tuvieron comunidades empobrecidas y dominadas por dos especies cosmopolitas, detritívoras y tolerantes:* Terschellingia longicaudata *y* Sabatieria pulchra. *La síntesis de la información ambiental indica que la calidad ambiental de la bahía de la Habana continúa siendo precaria y que existe la necesidad de reformular el programa de mitigación dado que la reducción de la contaminación no ha mejorado significativamente a las comunidades bentónicas.*

PALABRAS CLAVE: contaminación, sedimentos, bahía de bolsa, meiobentos, nemátodos.

INTRODUCTION

Bays are among the estuarine systems subjected to greater anthropogenic impact (MacCracken *et al*., 2009). The geographical settings of semi-enclosed bays have favored the historical development of cities and port facilities because physical protection from storms and availability of freshwater supply. However, the semi-enclosed shape limits the seawater circulation between the basin and the open sea increasing the vulnerability of the system because of the stagnation of waters and concentration of pollutants (Snelgrove *et al*., 2009). Even more, the effects of local stressors such as eutrophication, heavy metal, and hydrocarbon contamination can be influenced by global stressors such as seawater acidification and warming (Hewitt *et al.,* 2016; Chollet *et al*., 2017).

Havana bay is one of the most polluted semi-enclosed bays in the world (Armenteros *et al*., 2009a) and the most polluted coastal marine habitat in Cuba (Aguilar *et al*., 2008; Díaz-Asencio *et al*., 2011). Havana city is the most important source of pollutants into the bay because of the discharge of complex effluents (e.g. sewage) from three streams, more than 10 large pluvial drains, and many diffuse sources. Industrial effluents (e.g. hydrocarbons, heavy metals) are originated from a petroleum refinery and a fuel power plant (Villasol & Beltrán, 2004; Colantonio & Potter, 2006). The pollutant load have caused persistent and deleterious effects on the biota of Havana bay resulting in an ecosystem almost defaunated except for some meiobenthic taxa (Herrera-Moreno & Amador-Pérez, 1983; Armenteros *et al*., 2009a). The pollution decreased in Havana bay during the 1990s due to the economic contraction of Cuba that caused closure of many industries and affected the social welfare. Also the 1990s marked a change in the approach to environmental issues in the bay with the implementation of the program for the integrated management of the basin (Villasol & Beltrán, 2004).

Biological monitoring is key to assess the environmental quality of any basin but its implementation is limited by the availability of biological taxa susceptible to be measured. For instance, only small metazoans (i.e. meiofauna) can withstand in highly polluted and hypoxic environments such as Havana bay (Armenteros *et al*., 2009a). Within meiofauna, the free-living marine nematodes are the most abundant taxon and usually are well-represented in soft-bottoms of bays (Heip *et al.*, 1985). Additionally, nematodes exhibit biological traits that enhance their use as indicators of environmental quality (Bongers & Ferris, 1999; Kennedy & Jacoby, 1999; Austen & Widdicombe, 2006; Balsamo *et al.*, 2012; Semprucci *et al.*, 2015b): holobenthic life cycle (i.e. no planktonic stage), small body size, high population densities, high species richness, fast generation rates, and differential response to disturbances. Furthermore, nematodes have been recently proposed as an indicator of Ecological Quality Status (EQS) of marine coastal ecosystems (Semprucci *et al.*, 2013; Chen *et al.*, 2017; Bianchelli *et al.*, 2018). The quantitative assessment of the EQS can be achieved by the presence/absence of tolerant species; and by the determination of two nematode-based indexes proposed by Moreno *et al.* (2011): the maturity index (MI) and the index of trophic diversity (1 – IDT).

Havana bay has been subjected to heavy and chronic environmental impacts at least since two centuries ago. However, only three studies have been published in its basin addressing environmental processes in the last 10 years: (i) Diversity and abundance of benthic communities (Armenteros *et al.*, 2009a), (ii) historical reconstruction of sedimentation processes and pollution (Díaz-Asencio *et al.*, 2011), and (iii) fecal contamination (López-Pérez *et al.*, 2013). Other studies have focused on other faunal assemblages in the vicinity of the bay such as corals reefs (Duran *et al.*, 2018) and hydroids (Castellanos-Iglesias *et al.*, 2018). In this contribution, we compared the abiotic setting and meiofauna between 2006 and 2016 in order to assess potential environmental changes. This comparison would provide evidence to evaluate the success of a 15 years environmental management program and the response of nematode assemblages to long-term and heavy pollution in a tropical ecosystem.

MATERIALS AND METHODS

Study site

Havana bay, located in the northwestern region of cuban archipelago, is a semi-enclosed bay with a narrow entrance channel connecting the gulf of Mexico with three inner inlets (Fig. 1). The bay has 5.2 km^2 of area, mean depth of 9 m, and a tidal range of 0.3 m. There is a rather weak estuarine regime because several creeks draining freshwater into the basin mainly during the wet season (May-September). The main water exchange with the ocean is through weak tidal currents and the renovation time is in average around 5–7 days. Pulse events such as hurricanes and cold fronts cause the most important natural physical disturbance on the water column and sediments.

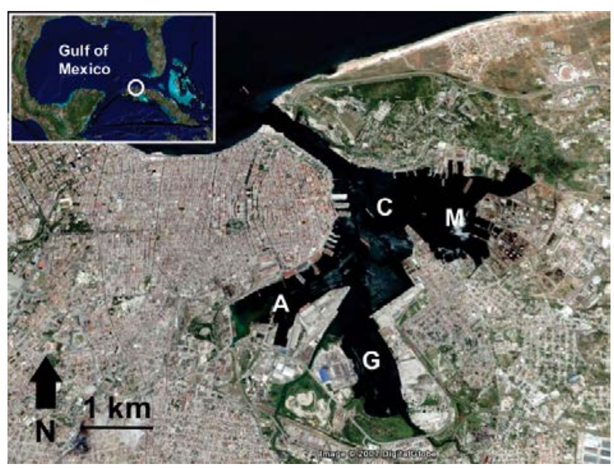

Fig. 1. Satellite photograph of Havana bay indicating the four sampling sites at each of the inlets. C = Centro, M = Marimelena, G = Guasabacoa, and A = Atarés. Note the high level of urbanization surrounding the bay.

Sampling

The sampling was made in four sites within Havana bay: Centro (N 23°21'10", W 82°20'38"), Marimelena (N 23°08'07", W 82°20'08"), Guasabacoa (N 23° 07'23", W 82°20'34"), and Atarés (N 23°07'23", W 82°21'18"). The last three sites correspond to inlets with different nature of impact (e.g. Atarés with eutrophication and fecal material and Marimelena affected with industrial effluents) (Fig. 1). The first sampling was made in July 2006 and the second one in September 2016; both during ebb tide to minimize the influence of adjacent open waters.

Superficial and bottom salinities was measured *in situ* with a handheld probe HANNA (accuracy: 0.01 psu). In addition, salinity data measured in nine months in 2016 (Beltrán *et al.,* 2016) were used in order to analyze the estuarine regime and potential stratification of the ecosystem. Bottom water and sediment samples were collected by duplicated in the four sites using a Van Dorn bottle of 2.5 L and a Van Veen grab respectively; they were stored in 1 L jars and preserved at -4 °C until analyses. The following variables were measured in bottom water: dissolved oxygen (DO), total phosphorus (TP), silicate (SiO_4^-), and total suspended solids. The following variables were measured in sediments: total petroleum hydrocarbons (TH), heavy metals (Fe, Ni, Pb, Cr, Cu, and V), and total organic matter (TOM).

Sediments for meiofauna were collected in July 2006 using a Petersen Grab (0.067 m^2 of effective area) and in September 2016 using a Van Veen Grab (0.037 m^2 of effective area); both sampling devices had a depth of penetration into the sediment of around 10 cm. The collected volume of sediment in each deployment of the grab was defined as a replicate. Three replicates per site were taken in 2006 and three in 2016 resulting in 24 samples (2 years × 4 sites × 3 replicates). Samples were preserved in 1 L jars with formalin 10% v/v sediment and 2–3 drops of Rose of Bengal staining were added.

Processing of samples

DO was measured with the Winkler's method (accuracy: 0.01 mg L^{-1}). TP and SiO_4^- were measured after guideline by Grasshoff *et al.* (2002). Total suspended solids were determined by the gravimetric method. TH in sediment were measured using a gas chromatographer (ATI Mattison Genesis Series FTIR; lower detection limit: 0.001 mg L^{-1}) after APHA (1998). Six heavy metals in sediments (i.e. Fe, Ni, Pb, Cr, Cu, and V) were determined by inductively-coupled plasma mass spectrometer (ICP-MS) using digestion with HNO_3/HCl. TOM in sediments were measured by difference of weight after ignition at 550 °C in a furnace for 3 hours. All used reagents were pure for analysis.

All the bulk sediment samples were processed, i.e., no sub-sampling was made. In the laboratory, samples were sieved with 38 µm filtered tap water through 45 and 500 µm mesh sieves in order to retain the meiofauna and macrofauna fractions, respectively. The finest fraction of sediment that passed through the 45 µm mesh sieve was discarded.

The meiofauna was separate from sediment using the flotation technique in high density solution of sucrose (Armenteros *et al.*, 2008). The supernatant containing most of the meiofauna organisms was preserved in formalin 10% with Rose of Bengal stain and remaining sediment discarded. The supernatant was observed under an

Olympus stereomicroscope (maximum magnification: 115X) and all the organisms were identified to higher-level taxon (e.g. nematodes, copepods) and counted. The nematodes were sorted out from the samples and mounted in permanent preparations for microscopy after Vincx (1996). Nematodes were identified to the lowest taxonomic level with the aid of the taxonomic literature: Platt and Warwick (1983; 1988), Schmidt-Rhaesa (2014), Warwick *et al.* (1998) and species descriptions. The taxonomy was corrected using the online database World Register of Marine Species (WoRMS: www.marinespecies.org).

Data analyses

The calculated values were expressed as medians because the relatively low number of observations and the high variability in the data. Salinity measured in 2016 was compared between surface and bottom levels using a Kruskal-Wallis test for each site; the months were used as replicates (N = 9). The rest of the abiotic variables had a single measurement (N = 1) at each site and in each year, so no statistical test for spatial differences could be done. We also refrained to test for temporal differences (i.e. 2006 *versus* 2016) because the low number of observations per year (i.e. N = 4) and the relatively large variation within the group. Therefore, only a qualitative assessment of changes was given for the abiotic data. Data of density and species richness of nematodes had larger replication to compare between 2006 and 2016 (N = 12 per year). This comparison was made using a Permutational Analysis of Variance (PERMANOVA) in the software PRIMER 6.1.15 with the following settings: Euclidian distance as a measure of resemblance and 999 permutations.

The ecological quality status (EQS) of sediments was assessed based on two metrics of nematode assemblages (Moreno *et al*., 2011; Bianchelli *et al*., 2018): (i) maturity index (MI) based on the proportion of colonizer/persister species (i.e. c-p scale) (Bongers, 1990; Bongers *et al*., 1991); and (ii) index of trophic diversity (1–ITD) based on the proportion of trophic groups (i.e. detritivores, epigrowth feeders, predators, and omnivores).

RESULTS

Abiotic factors

Salinity measured in 2016 was significantly ($p < 0.05$) lower at surface than at bottom for the four sites after K-W tests. The medians across the nine months are shown in brackets: Centro (surface 32 vs. bottom 36, Fig. 2A); Marimelena (surface 34 vs. bottom 36, Fig. 2B); Guasabacoa (surface 31 vs. bottom 36, Fig. 2C); and Atarés (surface 20 vs. bottom 36, Fig. 2D).

Dissolved oxygen (DO) at bottom level increased from 2006 (0.99 mg L^{-1}) to 2016 (2.56 mg L^{-1}). Centro had a similar value of DO at both years, but all the three remainder sites increased DO content in 2016 (Fig. 3A). Total phosphorus (TP) at bottom level content increased from 2006 (1.20 µmol L^{-1}) to 2016 (1.76 µmol L^{-1}). There was an increase in TP content in three sites in 2016, but slightly decreasing in Atarés in the same year (Fig. 3B). Silicate content at bottom level decreased from 2006 (5.04 µmol L^{-1}) to 2016 (1.84 µmol L^{-1}). Centro had similar values of silicate at both years, but all the three remaining sites decreased in 2016 (Fig. 3C). The content of total suspended solids (TSS) was higher in 2006 (84.5 mg L^{-1}) than in 2016 (73.5 mg L^{-1}). Centro and Marimelena had a similar value of TSS,

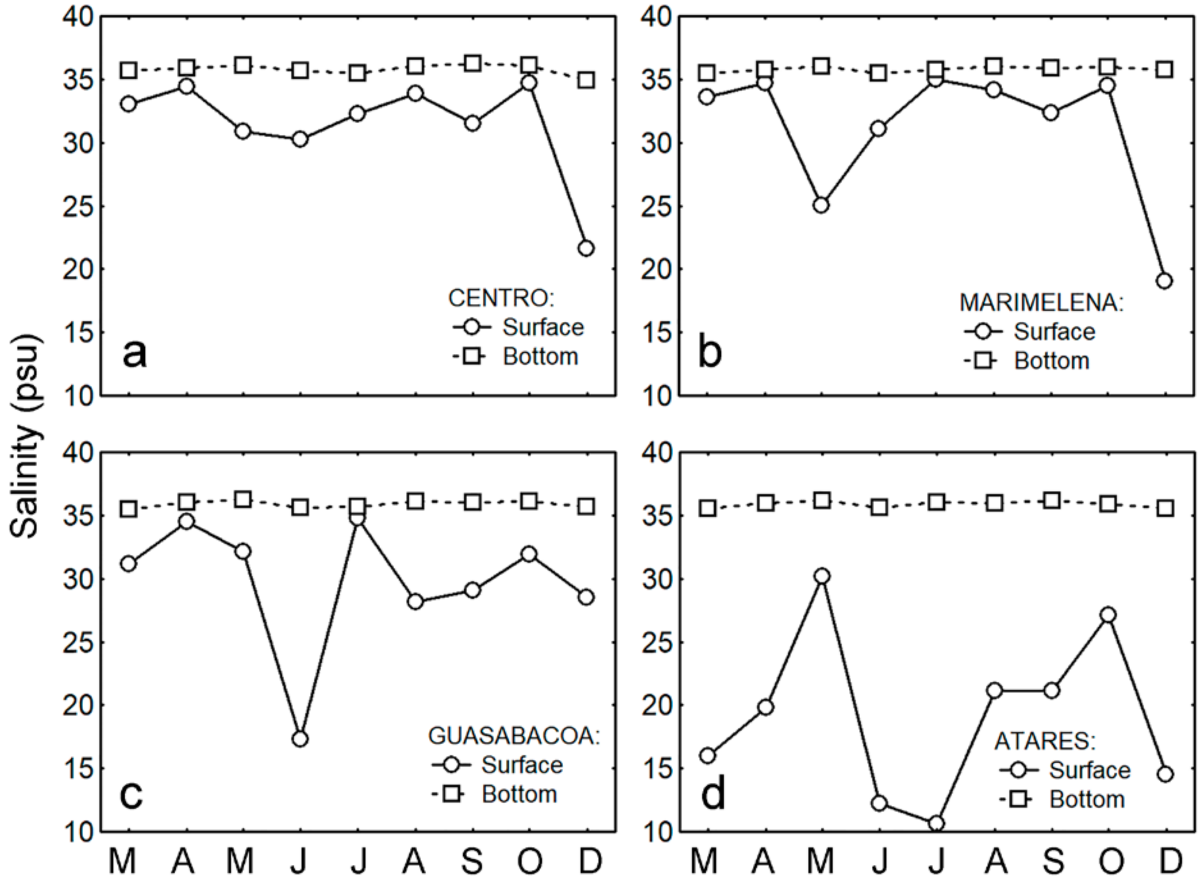

Fig. 2. Salinity in surface and bottom levels at four sampling sites in Havana bay through nine months in 2016. **a.** Centro. **b.** Marimelena. **c.** Guasabacoa. **d.** Atarés.

but Guasabacoa and Atarés decreased in 2016 (Fig. 3D).

Total organic matter (TOM) in sediments decreased from 2006 (23%) to 2016 (11%). TOM decreased in three sites but slightly increased in one (Marimelena) (Fig. 4A). Total petroleum hydrocarbons (TH) in sediment also decreased from 2006 (1334 mg kg^{-1}) to 2016 (1031 mg kg^{-1}); the decreasing occurred in all sites (Fig. 4B).

Iron and nickel contents were higher in 2006 (2.5% and 73 mg kg^{-1} respectively) than in 2016 (1.0% and 18 mg kg^{-1}, respectively); both heavy metals decreased in the four sites (Fig. 5A and B). Lead content decreased from 2006 (209 mg kg^{-1}) to 2016 (61 mg kg^{-1}); Pb decreased in three sites but was slightly higher only in Marimelena (Fig. 5C). Chromium content also decreased from 2006 (67 mg kg^{-1}) to 2016 (25 mg kg^{-1}); Cr content decreased in three sites but increased in Atarés (Fig. 5D). Copper content also decrease from 2006 (129 mg kg^{-1}) to 2016 (117 mg kg^{-1}); Cu content decreased in Centro and Guasabacoa, but increased in Marimelena and Atarés (Fig. 5E). Vanadium content was roughly the same at both years: 87 mg kg^{-1}. However, there were different trends per site in Vanadium content from 2006 to 2016: decreased in

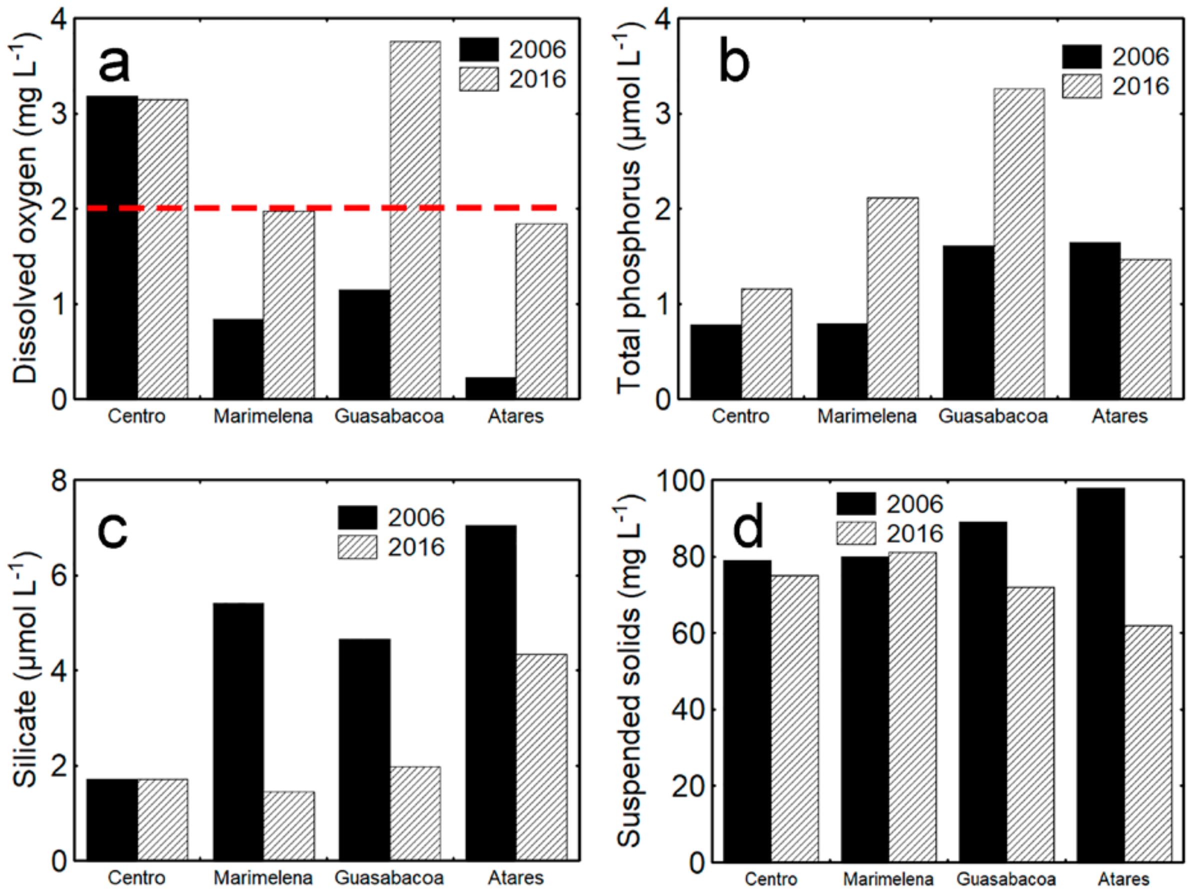

Fig. 3. Variables of water column of four stations in Havana bay in 2006 and 2016. **a.** Dissolved oxygen. **b.** Total phosphorus. **c.** Silicate. **d.** Total suspended solids. Red dashed line indicates threshold of hypoxia (2 mg L^{-1}).

Centro and Marimelena, but increased in Guasabacoa and Atarés (Fig. 5F). In summary, the content of Fe, Ni, Pb, and Cr showed clear depletion from 2006 to 2016; meanwhile Cu and V increased in some sites in 2016 compared to 2006.

Nematode assemblages

We recorded 454 nematodes belonging to 25 species, 21 genera, seven families, and four orders. Other meiofauna and macrofauna were essentially absent from the samples. The best represented nematode order was Monhysterida. Density of nematodes did not significantly change between 2006 and 2016 (PERMANOVA, p = 0.42). Centro had a low density of nematode and the other sites were essentially defaunated (Fig. 6A). Species richness did not show significantly changes between 2006 and 2016 (PERMANOVA, p = 0.84). Again, Centro had low values of richness in both years, and Marimelena and Guasabacoa very low values in 2006; the other sites were almost defaunated (Fig. 6B).

The taxonomic composition of the nematode assemblage indicated an impoverished assemblage dominated by few species. Namely, only two species contributed to the 71% of the total abundance: *Terschellingia*

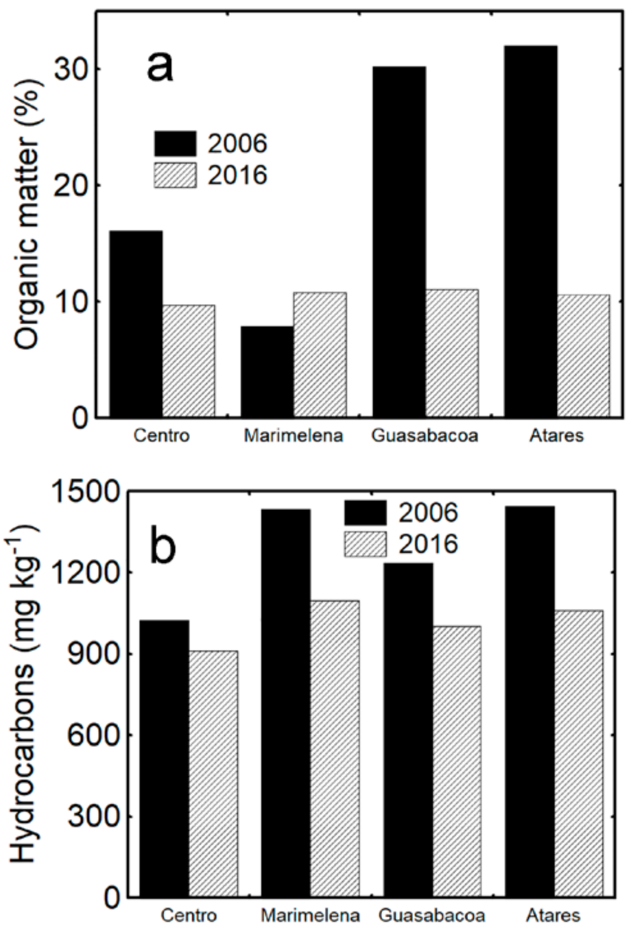

Fig. 4. Organic content in sediments of four stations in Havana bay in 2006 and 2016. **a.** Particulate organic matter. **b.** Total petroleum hydrocarbons.

longicaudata (36%) and *Sabatieria pulchra* (35%) (Table 1). Other two species of *Terschellingia* and one species of *Sabatieria* were also reported. In 2016, the sites Guasabacoa and Atarés were defaunated. An ordination of the samples by multidimensional scaling did not indicate any clustering of samples regarding the years of collection or sites (Supplementary material S1).

The proportion of good-colonizer nematodes (c-p 2) decreased from 60% in 2006 to 36% in 2016. The proportion of moderately colonizer nematodes (c-p 3) increased in 2016 (63%) respect to 2006 (32%). No nematodes with c-p 4 were found during the sampling. The maturity index negligibly increased from 2.2 in 2006 to 2.6 in 2016. The index of trophic diversity was largely the same in 2006 and 2016 (0.50 vs. 0.52 respectively).

DISCUSSION

In this study, we have gathered abiotic and biotic indicators of environmental quality from Havana bay in an interval of ten years (i.e. 2006 and 2016). A previous report revealed high concentrations of organic and inorganic pollutants in bay and consequently severely impacted benthic communities (Armenteros *et al.*, 2009a). A comparison of such indicators seems pertinent to evaluate the success of the program of management focused on improving the environmental quality of the bay. Also, from an ecological point of view, it is relevant to known how the nematode populations respond to long-term environmental impacts.

Havana bay functions as an estuary with salinity-driven stratification that persisted throughout the year but intensified during the wet season. Bottom levels of full marine salinity (i.e. 35 psu) during all the year 2016 suggested a stable stratification of water column and reduced mix with surface water despite drops of salinity because rainfalls. This stratification promotes the seawater stagnation within the bay, limits the bottom water exchange with the adjacent open ocean (Florida Straits), and consequently reducing the dilution of contaminants and replenishment of oxygen. The natural estuarine regime reinforces the effects of contamination in the basin

Table 1. Density of nematodes (individuals 10 cm^{-2}) in four sites (three replicates per site summed) of Havana Bay in 2006 and 2006. Species are ordered by contribution to total abundance (last column). Dashes indicate absence.

	Centro		Marimelena		Guasabacoa		Atares		% total
Species	**2006**	**2016**	**2006**	**2016**	**2006**	**2016**	**2006**	**2016**	
Terschellingia longicaudata	0.53	3.29	0.039	-	-	-	-	-	36.2
Sabatieria pulchra	0.92	1.86	0.38	0.054	0.074	-	0.015	-	35.3
Terschellingia communis	0.02	0.59	-	0.027	0.125	-	-	-	7.2
Daptonema spp.	0.06	0.19	-	-	0.018	-	-	-	2.7
Terschellingia gourbaultae	-	0.19	0.02	0.027	0.015	-	-	-	2.5
Parodontophora xenotricha	0.15	-	-	-	-	-	0.015	-	2.3
Leptolaimus acicula	-	0.13	-	-	-	-	-	-	1.9
Metadesmolaimus sp.	-	0.13	-	-	-	-	-	-	1.6
Sabatieria breviseta	0.069	-	0.061	-	-	-	-	-	1.1
Theristus spp.	0.030	-	0.079	-	-	-	-	-	1.1
Pomponema clavicaudatum	-	0.11	-	-	-	-	-	-	1.1
Promonhystera faber	-	0.11	-	-	-	-	-	-	1.1
Dorylaimopsis sp.	0.057	0.027	-	-	-	-	-	-	0.9
Cienfuegia cachoi	-	0.027	-	0.054	-	-	-	-	0.9
Leptolaimus papilliger	-	0.081	-	-	-	-	-	-	0.7
Pseudoterschellingia ibarrae	-	-	0.039	-	0.039	-	-	-	0.7
Aponema torosus	-	0.054	-	-	-	-	-	-	0.5
Acanthonchus cobbi	0.034	-	-	-	-	-	-	-	0.5
Metachromadora sp.	0.015	-	-	-	0.018	-	-	-	0.4
Comesa warwicki	-	0.027	-	-	-	-	-	-	0.3
Longicyatholaimus sp.	-	0.027	-	-	-	-	-	-	0.2
Megadesmolaimus sp.	-	0.027	-	-	-	-	-	-	0.2
Metacyatholaimus chabaudi	-	0.027	-	-	-	-	-	-	0.2
Nannolaimoides sp.	0.02	-	-	-	-	-	-	-	0.2
Sigmophoranema sp.	0.015	-	-	-	-	-	-	-	0.2
Total	1.93	0.62	0.29	0.023	6.91	0.16	-	-	

without obvious contribution from this natural condition to the recovery. Thus, mitigation measures concerning human development seem to be the only affordable strategy to improve the environmental quality.

The concentrations of dissolved oxygen improved from 2006 to 2016 in the three formerly more depleted sites (Marimelena, Guasabacoa, and Atarés); however, hypoxia (DO < 2 mg L^{-1}) still occurred in Marimelena and Atarés. Hypoxia is one of the main environmental problems affecting estuaries around the world (Kodama & Horiguchi, 2011) and semi-enclosed bays in the tropics are more susceptible to be affected because warming reduce the oxygen dissolution in the seawater. The mechanism creating hypoxia in semi-enclosed bays is inextricably linked to the eutrophication and oxidation of accumulated organic matter (Fennel *et al.*, 2009; Rabalais & Gilbert, 2009).

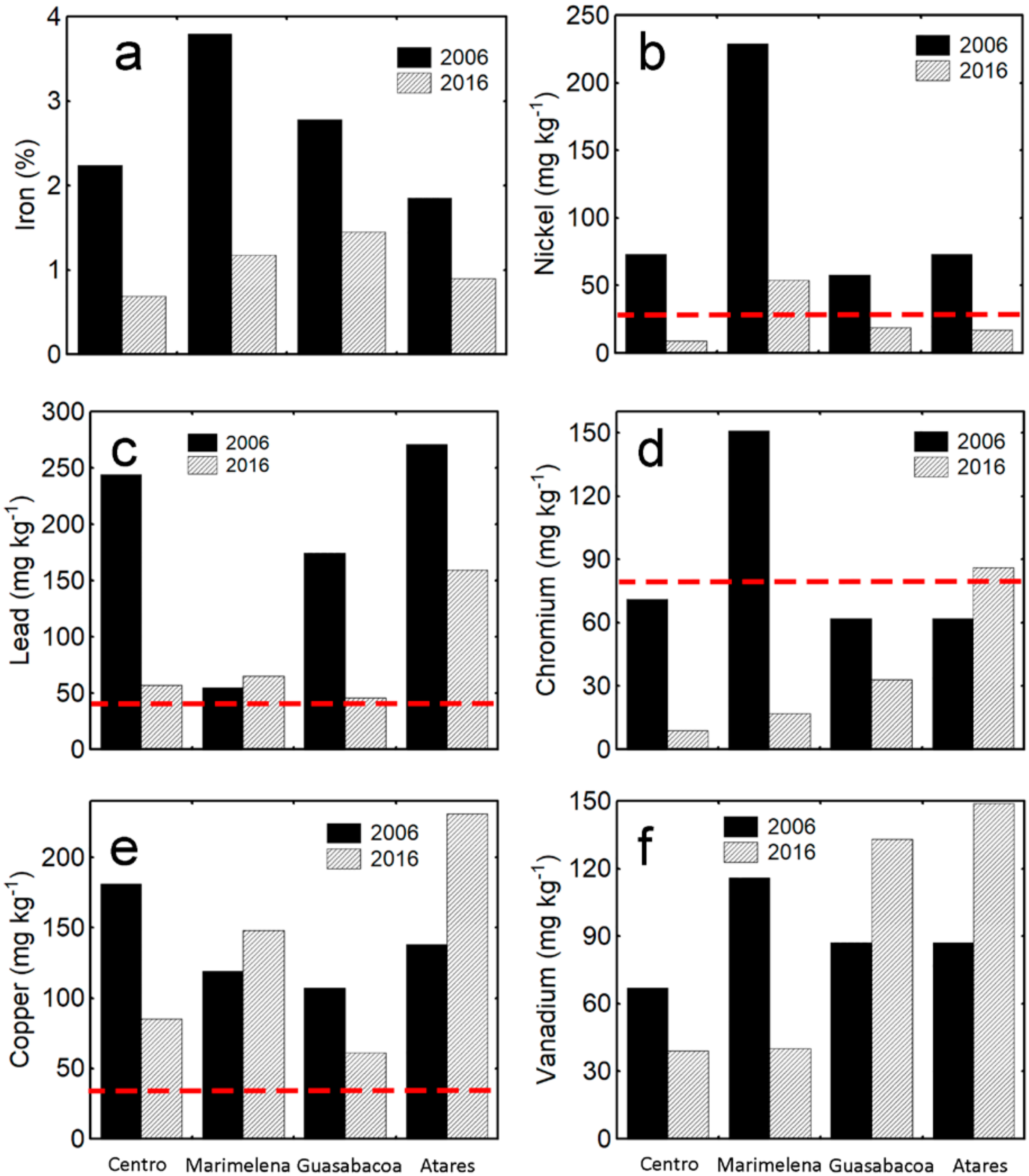

Fig. 5. Heavy metal content in sediments of four stations in Havana bay in 2006 and 2016. **a.** Iron. **b.** Nickel. **c.** Lead. **d.** Chromium. **e.** Copper. **f.** Vanadium. Red dashed line indicates the effects range low after Buchman (1999).

In Havana bay, a considerable fraction of organic content in sediments seemed due to direct sewage discharges from creeks and drains. Measurements of coprostanol and saturated sterols indicated that the sediments of Havana bay

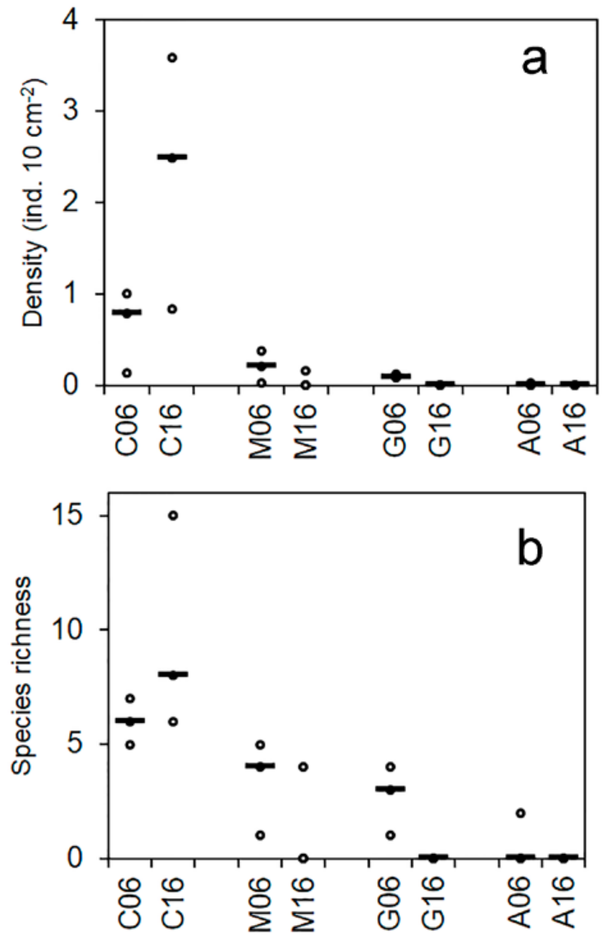

Fig. 6. Nematode assemblages in four sampling sites in Havana bay in 2006 and 2016. **a.** Density. **b.** Species richness. C = Centro, M = Marimelena, G = Guasabacoa, and A = Atares.

were contaminated both by "fresh" fecal material and by sewage discharged without prior treatment (Beltrán *et al.*, 2016; Martins *et al.*, 2018). Atarés continued to be the most affected inlet because the high level of eutrophication and fecal contamination originated from populated towns in Havana city (e.g. Centro Habana and Old Havana) and some industries (Gelen *et al.*, 2005; López-Pérez *et al.*, 2013). Oxygen depletion was reinforced by the limited vertical mixing of water column and has also been also recorded in Cienfuegos bay (South-central region of Cuba) (Díaz-Asencio *et al.*, 2016).

The content of petroleum hydrocarbons has been reduced from 2006 to 2016 as indicated by our results. The most plausible explanation is the reduction of the input of hydrocarbons as consequence of the implementation of the program of management that includes technological improvements at the oil refinery and more strict environmental regulations in transportation and harboring. The reported concentration of hydrocarbons in Havana bay still were high and likely had adverse effects on the benthic fauna because are higher than reference eco-toxicological thresholds (Long *et al.*, 1995; Buchman, 1999; Hagopian-Schlekat *et al.*, 2001). Historically, Marimelena has been severely affected by industrial effluents coming from a petroleum refinery, but technological improvements have reduced the environmental burden on this inlet.

The concentrations of heavy metals has decreased in Havana bay from 2006 to 2016 for most of the sites according to our results. However, the sediment concentrations of nickel, lead, and copper remained near or higher than the values reported as potentially harmful (Long *et al.*, 1995; Buchman, 1999). After reduction of heavy metal content in Marimelena inlet, subjected to most heavy industrial contamination, it is Atarés the most compromised inlet with increased amounts of copper and chromium in 2016. This increase may be caused by transport of heavy metals previously immobilized in deep sediments to the surface due to physical reworking.

Density and species richness of nematodes were consistently low and statistically non-significant between the two sampled years. The reported values were lower than those reported in other estuarine areas considered as strongly impacted (e.g., Armenteros *et al.*, 2009b; Bianchelli *et al.*, 2018; Gao & Liu, 2018). Defaunation occurred in two of the four sites in the bay and even other meiofauna (e.g. copepods, ostracods) were absent. This indicated the persistence during the last 10 years of environmental stressors in Havana bay. The highest abundance and species richness at Centro could be explained by the proximity to the entrance channel enhancing the water exchange with the open ocean. In this semi-enclosed bay, water exchange would enhance the faunal recruitment from outside, oxygen replenishing, and larger heterogeneity of grain size that in turn increased the structural complexity of sediment (Armenteros *et al.*, 2009a).

Taxonomic composition of nematode assemblages was largely the same in 2006 and 2016. Assemblages were dominated by cosmopolitan species highly tolerant to pollution such as *Sabatieria pulchra* and *Terschellingia longicaudata* (Moreno *et al.*, 2011; Nanajkar *et al.*, 2011). Many of other nematodes species occurred in Havana bay have been reported as opportunistic and tolerant species to a variety of disturbances (Moreno *et al.*, 2011; Moens *et al.*, 2014; Semprucci *et al.*, 2016).

The occurrence only of colonizer species (c-p 2 and 3) indicate assemblages tolerant to disturbance and able to rapidly increase the abundance in temporary and/or local favorable conditions (Chen *et al.*, 2017). Trophic structure was also severely distorted with most of the nematodes being detritivores (e.g. *S. pulchra* and *T. longicaudata*). These species are tolerant to large organic loads but also ingest contaminated particles that increase their exposure to pollutants. In particular, the overlapped effects of hydrocarbons and heavy metals on nematode assemblages could be very deleterious causing a significant reduction in abundance (Losi *et al.*, 2013; Monteiro *et al.*, 2018). The trophic guild of epigrowth feeders could also be affected by the turbidity associated with large amount of suspended solids that block the sunlight and affects the development of benthic microalgae.

The functional diversity and trophic structure of nematode assemblages have been successfully used to evaluate the ecological quality status (EQS) of impacted ecosystems (Moreno *et al.*, 2011; Bianchelli *et al.*, 2018). EQS in Havana bay can be defined as precarious based on the synthesis of nematode metrics: maturity index, trophic diversity index, colonizer-persister percentage, and occurrence of sensitive/tolerant genera. Similar results have been reported for harbors affected by discharges of wastewater, petroleum hydrocarbons, and heavy metals (Moreno *et al.*, 2011; Semprucci *et al.*, 2013; Bianchelli *et al.*, 2018).

Each of the above examined stressors was able to negatively affect the nematode assemblages depleting abundance and diversity. However, hypoxia likely had the greatest deleterious influence on nematodes even in the presence of other stressors (Díaz & Rosenberg, 1995; Trannum *et al.*, 2004; Yoshino *et al.*, 2010). The organic load indirectly contributes to hypoxia through the oxygen consumption during the oxidation but also physically limits the amount of interstitial space available for the meiofauna (Papadopoulou *et al.*, 1998). Synergistically, hydrocarbons could affect nematode assemblages both directly and indirectly by contributing to deoxygenation and reducing interstitial space (Semprucci *et al.*, 2015a).

In summary, Havana bay has improved some environmental variables in 2016 compared with 2006. However, still exist synergistic deleterious effects of stressors such as hypoxia and toxicity by heavy metals and petroleum hydrocarbons on the benthic fauna. Nematodes were strongly affected by the pollution resulting in extremely impoverished assemblages with very few species, and dominance of cosmopolitan, detritivores and tolerant species such as *Terschellingia longicaudata* and *Sabatieria pulchra*. The synthesis of the environmental information gathered in this contribution indicated that the ecological quality status of Havana bay was precarious and the necessity to reformulate the program of mitigation because the actual reduction of pollutants did not provide a significant improvement to the benthic faunal communities.

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