*Serie Oceanológica No. 15, 2016* **ISSN 2072-800x**

# Relative abundance of benthic macroalgae (RAM) as a tool in rapid environmental quality assessment

# *Abundancia relativa de macroalgas bentónicas (RAM) como una herramienta en la evaluación rápida de calidad ambiental*

Arsenio José Areces Mallea[*], Patricia González-Sánchez[*] and Jorge Bacallao Guerra[**]

[*]Instituto de Oceanología, Ministerio de Ciencia, Tecnología y Medio Ambiente, Cuba
[**]Instituto de Cibernética, Matemática y Física, Ministerio de Ciencia, Tecnología y Medio Ambiente, Cuba

## ABSTRACT

A rapid way to assess environmental quality status of marine rocky bottoms is considered, using theoretical abundance of macroalgae in relation to habitat. EDIS, the designed index, was evaluated in two shallow bottom areas along a nutrification gradient in Havana City coast. Bayesian methods showed that empirical distribution of EDIS was roughly normal and its curve shape allows reliable comparisons among habitats with similar composition of macroalgae but with different environmental qualitative grade. EDIS has a smaller intrinsic subjectivity, due to avoid *a priori* qualification of macroalgae taxa. With limited sampling efforts and low calculation requirements, the index generates fairly good representations of dominance within or inter habitats; it is easy to apply and cost-effective.

## RESUMEN

Se analiza una forma rápida de evaluar la calidad ambiental de los fondos marinos rocosos, usando las abundancias teóricas de macroalgas en relación con el hábitat. El índice diseñado, EDIS, fue evaluado a lo largo de un gradiente de nutrificación en dos zonas con fondos someros de la costa de la ciudad de La Habana. Los métodos bayesianos mostraron que la distribución empírica del índice EDIS fue aproximadamente normal, y permite comparaciones confiables entre hábitats con una composición similar de macroalgas pero con diferente grado cualitativo de su calidad ambiental. EDIS tiene una menor subjetividad intrínseca a causa de no calificar *a priori* ningún taxón de macroalgas. Con limitados esfuerzos de muestreo y bajos requerimientos de cálculo, el índice genera representaciones bastante adecuadas de la dominancia dentro o entre hábitats; es fácil de aplicar y es costo-efectivo.

**Keywords:** Rocky shores, macroalgae, environmental quality, Bayesian analysis.
***Palabras clave****: costas rocosas, macroalgas, calidad ambiental, análisis bayesiano.*

## INTRODUCTION

Anthropogenic stress, basically due to enrichment, is strongly associated with reductions in species richness and evenness in marine habitats (Johnston & Roberts, 2009), and very often shifts the community structure towards dominance of opportunistic species (Borowitzka, 1972; Regier & Cowell, 1972), in such a way that highly stressed or disturbed marine environments are inhabited by annual species with high growth rates and reproductive potential; while in undisturbed marine environments, perennial species thrive with low growth rates and reproductive potential (Murray & Littler, 1978; Sousa, 1980; Duarte, 1995; Schramm, 1999). Most macroalgae, as sessile and photo-synthetic organisms, react with great connectivity to the abiotic and biotic aquatic environments that surround them, and represent sensitive bioindicators of their changes. Thus, benthic macrophytes are considered good indicators of water quality (Fairweather, 1990; Gorostiaga & Díez, 1996; Soltan *et al.*, 2001), and recent literature refers to several indices using them as key bioindicators (EEI: Orfanidis *et al*., 2003; CARLIT: Ballesteros *et al*., 2007; BENTHOS: Pinedo *et al*., 2007; RSL: Wells *et al*., 2007; CFR: Juanes *et al*., 2008), particularly in regions like Europe where changes in marine benthic vegetation have been fairly well documented (Schramm & Nienhuis, 1996), and seaweeds have been increasingly considered in coastal management and conservation plans (EEC, 1992, 1994, 2000; Orfanidis *et al*., 2001; Panayotidis *et al*., 2004).

As part of sound concepts in ecology, relative species abundance has been widely used in biodiversity descriptors (Hill, 1973; Tóthmérész, 1995). Although macroalgae have been considered a focal group in shallow marine benthos due to its high biomass, RAM data appear *per se* only surveyed in few sites (Vroom & Page, 2006; Tribollet & Vroom, 2007), and as a tool in environmental or trophic gradient assessment studies, they have been scarcely documented in published literature (Areces, 1997; Soares *et al*., 2010 a, b).

This study aims at showing how RAM data of infralittoral communities can be managed for monitoring environmental quality and for typifying different benthic habitats in a simple and reliable way.

## MATERIALS AND METHODS

### *Working area*

Two sampling stations were chosen and fixed on a rocky bottom in the upper infralittoral zone (1.0-1.5 m depth), close to the seashore. Station S1, protected by a shallow barrier, was positioned 1.3 Km west of Quibu River mouth (82$^{o}$ 27’39,7’’ N and 23$^{o}$5’39,5’’ W) on Havana City coast. Station S2, with similar topographic conditions and less protected, was located closer to Quibu River, at a distance of just 0.45 Km from its mouth (Fig. 1). The survey was done twice at station S1, in winter (January 22, 2010) and summer (August 20, 2011), and only once in summer (August 25, 2011) at station S2. Due to the influence of river discharges, the area shows a remarkable enrichment gradient from the river mouth towards the west, and has been well characterized considering many studies carried out in this region of the Cuban shelf, including hydrochemistry (Lluis 1974; Gómez-Quintero & Areces, 1976; Rodas-Fernández *et al*., 2013; Perigó-Arnaud, 2013), species inventories and ecological characterizations (Vinogradova 1974; Herrera-Moreno & Alcolado 1983; Alcolado & Herrera 1987; Valdés-Muñoz & Garrido 1987; Herrera & Martínez 1987; Lugioyo & Rodríguez 1988; del Valle *et al*., 1992; Rodas-Fernández *et al*., 2013).

### *Sampling procedure*

Frequency distribution of macroalgae was calculated by the *Line Point Transect* method (Ambrose, 2002), as presence-absence in 1000 points located on a 10 X 10 m frame set parallel to the coastline and fixed on each station. To assure the position of the frame, a 10-m transect with count points separated 10 cm along the rope was moved from a bottom mark to consecutive positions at 1 m distance each. This way, 20 transects on both stations integrated the data set. Merely conspicuous entities, with height of 0.5 cm or more, were recorded and identified to species level. Crustose coralline macroalgae were seen as a whole group. The nomenclature and authorities of identified taxa is based on manuals and current checklists of Caribbean phycoflora: Taylor, 1960; Littler & Littler, 2000; Wynne, 2011, and were updated consulting *AlgaeBase* (Guiry & Guiry, 2015).

Figure 1. Distribution of stations along the coastline.

*Figura 1. Distribución de las estaciones a lo largo de la costa.*

Despite data arrays were settled on 1000 points in each station, minimum sampling effort to achieve representative RAM estimates was determined using random samples of different number of points and analyzing their trend in cumulative curves of richness and abundance.

*Data analysis*

To measure the resemblance between both sites, the ecological data matrix was studied by Q-mode analysis, using Bray-Curtis coefficient as an association amount, previously standardizing the data into relative abundance by dividing each species count by the total of macroalgae counts in every transect. Nonmetric multidimensional scaling (MDS) was used to represent transects in each station in a map, and similarity percentage analysis (SIMPER), to highlight the contribution of each taxon for differences within groups of transects (Clarke & Gorley, 2006). Both analyses were performed using the PRIMER-E v.6 software (Clarke & Gorley, 2006).

For comparison in a numerical scale, environmental relatedness between sites was in addition assessed using only RAM data, as an index, EDIS, designed as follows:

$$\text{EDIS (Environmental Disturbance)} = \mathbf{C}^{*} \sum_{i=1}^{k} (\text{fexp}-\text{ft})^{2}/\text{ft}$$

Where:

**fexp** is the experimental or observed frequency of species **i**,

**ft** is the theoretical frequency assigned to species **i**, and

**k** is the amount of species involved.

**C** is a constant corresponding to: **C= c/S**, where **c** is the proportion of sampling points covered with macroalgae and **S** the proportion of species registered at the station in relation to all the species included in the analysis. Values of **C** can rank between **1/N** and **k**, when only one species is present with 100% coverage, **N** being the sampling effort or number of points sampled.

EDIS was planned to be used for comparison and its values increase when dissimilarity augments regarding the place considered as reference, but they can be established between 0 and 1, allowing inter-site comparisons. In this case, EDIS calculation has to be replicated at least twice in the studied location or during a given period, and after that, the formula: f(x)= (x-M)/(m-M) should be applied, where **x** is EDIS mean value, **m** is its minimum value and **M** is the maximum value observed in the trial.

Although representatives of *in situ* macroalgae community may be selected with any coverage or relative frequency percentage, only those members with 10% or more were considered in this study. Thus, the effect of rare species, significant in richness or diversity records but not so much for typifying habitats, or in trophic analysis, was neglected. The figures used in the index to state the competitive ability of the selected species to colonize substratum habitat (ft) were obtained by standardizing its particular coverage maximum in a similar habitat considered as a reference site, against the total of coverage maxima of all of the taxa chosen for the study, registered in that reference site.

Maximum coverage of crustose coralline macroalgae and the seven species included in EDIS index are shown in Table 1. These data were obtained from a previous 22-month pilot research concerning temporal variations of macroalgae community at station S1, assumed as reference for shallow and moderately enriched rocky bottoms (González-Sánchez, 2011).

Table 1. Maximum coverage of crustose coralline macroalgae and seven species of macroalgae with 10% or more coverage, obtained from July 2009 to March 2011 at sampling station S1.

*Tabla 1. Datos de cobertura máxima de macroalgas coralinas incrustantes y siete especies de macroalgas con 10% o más de cobertura, obtenidos desde julio de 2009 hasta marzo de 2011 en la estación de muestreo S1.*

| **Taxonomical category** | **PHYLUM** | **TAXA** | **Coverage (%)** |
|---|---|---|---|
| Species | Ochrophyta | ***Dictyopteris delicatula*** | 82,23 |
| “ | Rhodophyta | ***Amphiroa fragilissima*** | 50,07 |
| “ | Ochrophyta | ***Dictyota ciliolata*** | 28,28 |
| Group | Rhodophyta | ***Crustose corallines*** | 21,28 |
| Species | Rhodophyta | ***Hypnea spinella*** | 19,77 |
| “ | Rhodophyta | ***Pterocladiella capillacea*** | 16,6 |
| “ | Rhodophyta | ***Gelidiella acerosa*** | 12,41 |
| “ | Chlorophyta | ***Ulva lactuca*** | 11,61 |

Bayesian statistical inference, which relies on degrees of belief or subjective likelihoods and can allow the annealing of density likelihood functions, was used to simulate EDIS values and its empirical likelihood distribution, taking into account a multinomial model. Dirichlet distribution, with all its parameters fixed at 0.5, was used as the prior distribution, for it is the conjugate of multinomial distribution when it is multiplied by the likelihood function (i.e. it has a similar functional form).

For programming and managing calculations with collected data sets, R environment (R version 2.5.0) was used, distributed under the terms of the GNU General Public License, Version 2, June 1991. The parameters of the multinomial model were calculated in WinBUGS version 1.4 (Spiegelhalter *et al*., 2003) using 4000 iterations after a burn-in of 1 000 iterations. Bootstrap standard errors were applied to calculate 95% confidence intervals (Efron & Tibshirani, 1993).

## RESULTS

In comparison to ultimate values obtained as presence-absence in 1000 sampled points, numerical simulation showed that an effort over 100 or 150 count points were enough to achieve a representative figure of total macroalgal abundance at both stations. Trends became reasonably asymptotic with less than one fifth of the sampled points (Fig. 2A). This situation is not the same concerning number of species per sampling effort (Fig. 2B), due to random effects of rare species. Their influence is obvious and none is well represented with any sample size. On the other hand, the standard deviation (±1SD) of combined samples of similar size registered in both richness curves is also higher than those corresponding to abundance curves. This attribute can just become roughly similar in both stations with no less than 250 count points.

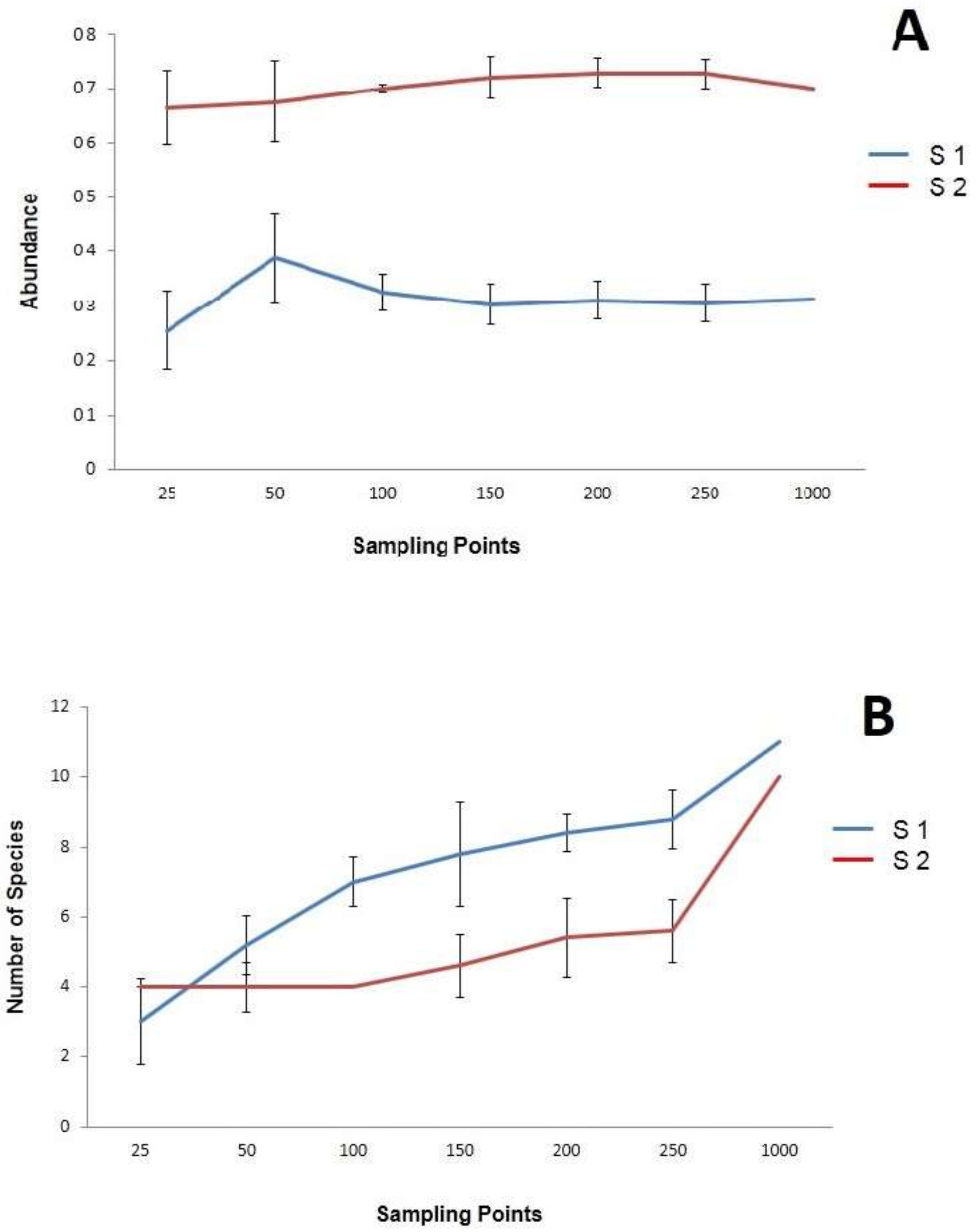

Figure 2. Total macroalgal abundance (A) and number of species (B) in relation to sampling effort. Mean values and standard deviation (±1SD) derived from recombination of five random samples of 25 to 250 count points extracted from 1000 points sampled in summer at stations S1 and S2.

*Figura 2. Abundancia total de macroalgas (A) y número de especies (B) con relación al esfuerzo de muestreo. Valores medios y desviación estándar (±1SD) derivada de la recombinación de cinco muestras aleatorias con 25 hasta 250 puntos de conteo extraídos de 1000 puntos muestreados en verano en las estaciones S1 y S2.*

Affinity of transects in sampled sites are not the same regarding richness and relative abundance of macroalgal species. In summer, as shown in Fig. 3, transects linked to stations S1 (1-10) and S2 (11-20) clearly disaggregate and two main groups can be appreciated within each station, according to the number of species registered. Their relatedness is also unequal. At S1, transects with lesser species are closer among themselves, whereas at S2 those with the higher number of species are found in closest association. SIMPER analysis also shows that all the species included in EDIS index were not only representatives in rocky bottoms of the area, but they also contribute in a significant manner to the relatedness of sites and transects. Accordingly, *Pterocladiella capillacea*, *Ulva fasciata, Amphiroa fragilissima, Halimeda opuntia, Bryopsis ramulosa* and particularly *Dictyopteris delicatula* define affinities between transects (Table 2), and their relative abundance characterize macroalgal community in both sites.

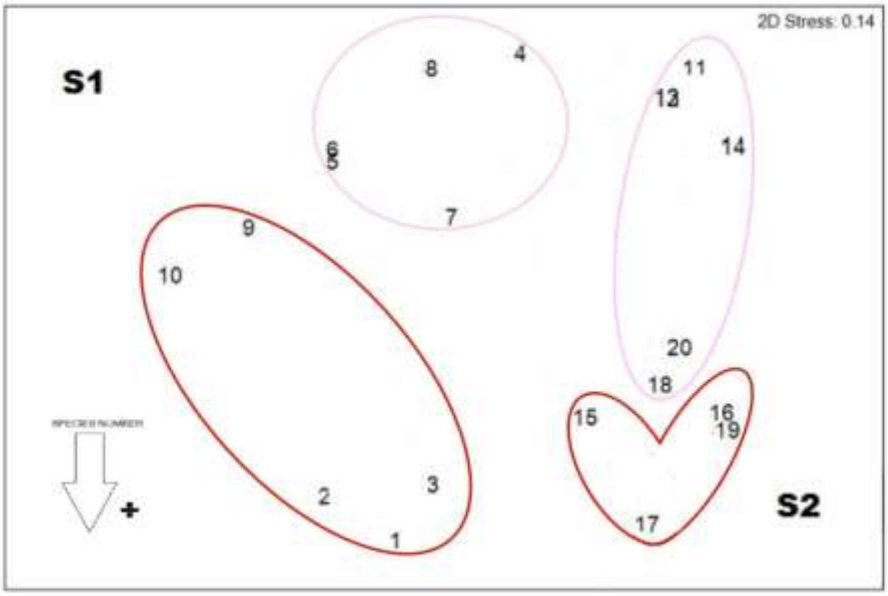

Figure 3. MDS grouping of transects belonging to stations S1 and S2. With red borders (---) groups with the highest number of species. With pink borders (----) groups with reduced number of species.

*Figura 3. Agrupación mediante análisis MDS de transectos pertenecientes a las estaciones S1 y S2. Con bordes rojos (---) los grupos con el mayor número de especies. Con bordes rosados (----) los grupos con un número reducido de especies.*

Table 2. Breakdown into percent contribution of the species present in the data set, average similarity within and average dissimilarity between stations. Values of similarity and dissimilarity are highlighted.

*Tabla 2. Desglose en porciento de contribución de las especies presentes en el conjunto de datos, similitud promedio en cada estación y diferencia promedio entre estaciones. Se resaltan los valores de similitud y disimilitud.*

| **SPECIES** | SIMILARITY WITHIN STATIONS | | DISSIMILARITY BETWEEN STATIONS **50.87** |
|---|---|---|---|
| | S1 **61.04** | S2 **62.22** | |
| *Dictyopteris delicatula* | 55.49% | 76.91% | |
| *Pterocladiella capillacea* | 19.81% | | 18.84% |
| *Ulva lactuca* | 14.68% | | |
| *Amphiroa fragilissima* | | 9.88% | 13.05% |
| *Halimeda opuntia* | | 9.07% | 12.93% |
| *Bryopsis ramulosa* | 4.64% | | 18.84% |
| *Gelidiella acerosa* | | | 5.69% |
| *Hypnea spinella* | | | 6.74% |
| *Laurencia caraibica* | | | 4.61% |

Empirical distribution of EDIS in samples of 4000 index values was approximately Gaussian (Figs. 4, 5 and 6) at both stations, either in winter or summer. Mean value, median, variances and 95 % bootstrap-t confidence intervals are shown in Table 3.

Table 3. Parameters obtained from EDIS index distribution at sampling stations.

*Tabla 3. Parámetros obtenidos de la distribución del índice EDIS en las estaciones de muestreo.*

| **Station** | **Mean** | **Median** | **Variance** | **95 % Bootstrap-t Confidence intervals** |
|---|---|---|---|---|
| **E1-winter** | 0.4862059 | 0.4866128 | 0.001145891 | 0.4220650-0.5539052 |
| **E1-summer** | 0.3402298 | 0.3405953 | 0.0005897024 | 0.2905495-0.3886646 |
| **E2** | 1.3502 | 1.3512 | 0.0025 | 1.254161-1.452809 |

It is easy to perceive that distress is not the same along the coastline. Considering the macroalgal community at station S1 as reference, disturbance (assumed in this case as all the effects related to eutrophication) increases at station S2, near Quibu

River mouth. The confidence intervals of this index at both stations are narrow and non-overlapping. According to them, it is possible to infer that the differences observed in EDIS values can be statistically significant. Moreover, the comparison of summer and winter EDIS values at S1 showed the effect of a reduction in environmental stressors in summer. This situation has been shown in other shallow marine communities (Areces & Martínez, 1992).

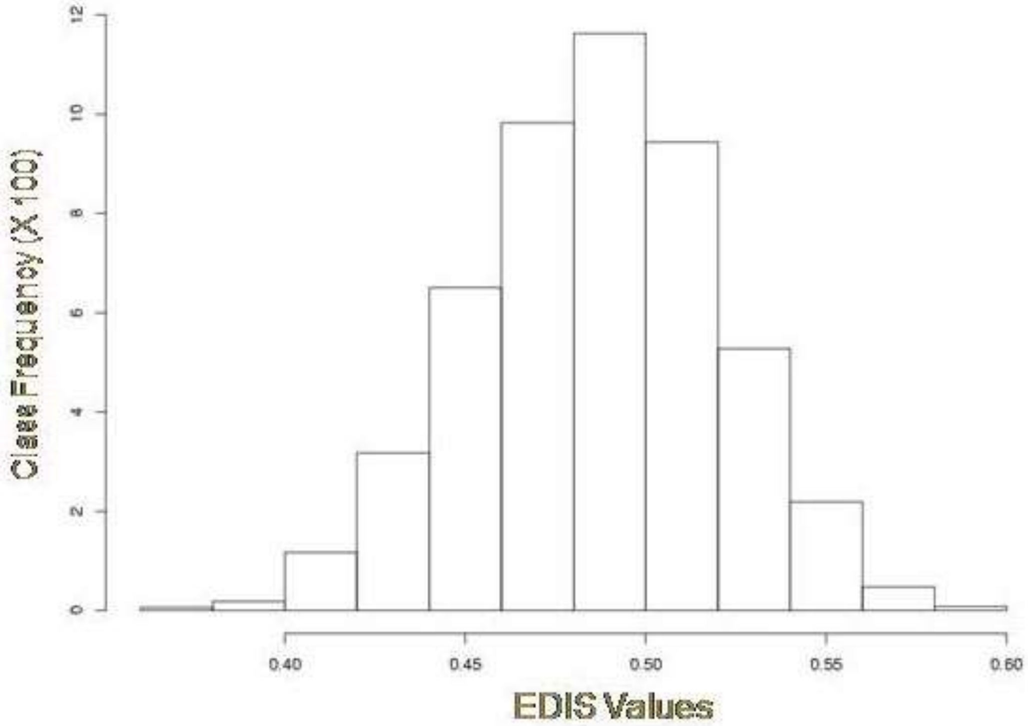

Figure 4. Grouping and associate class frequencies of 4000 values generated by EDIS. Station S1. Winter.

*Figura 4. Agrupación y frecuencias de clases asociadas a 4000 valores generados por EDIS. Estación S1. Invierno.*

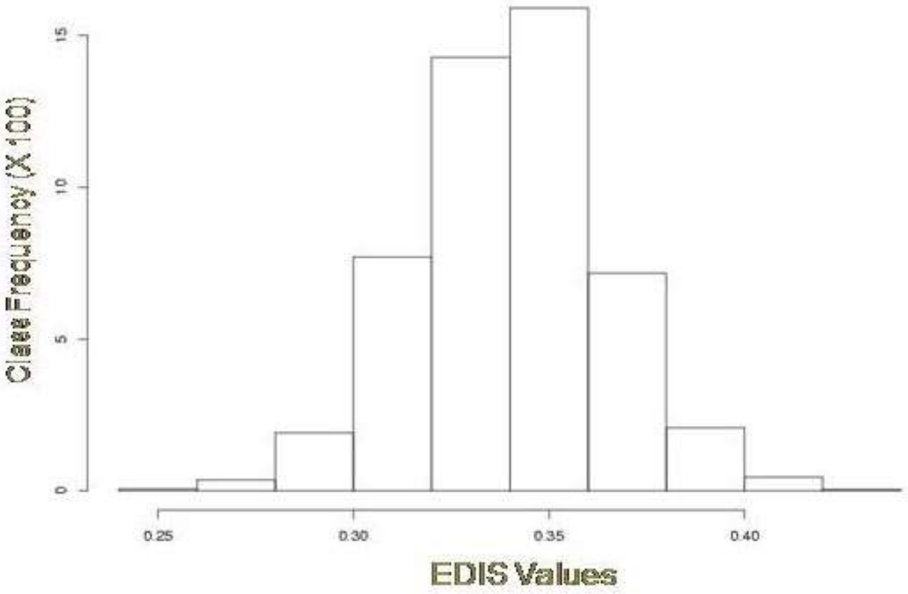

Figure 5. Grouping and associate class frequencies of 4000 values generated by EDIS. Station S1. Summer.

*Figura 5. Agrupación y frecuencias de clases asociadas a 4000 valores generados por EDIS. Estación S1. Verano.*

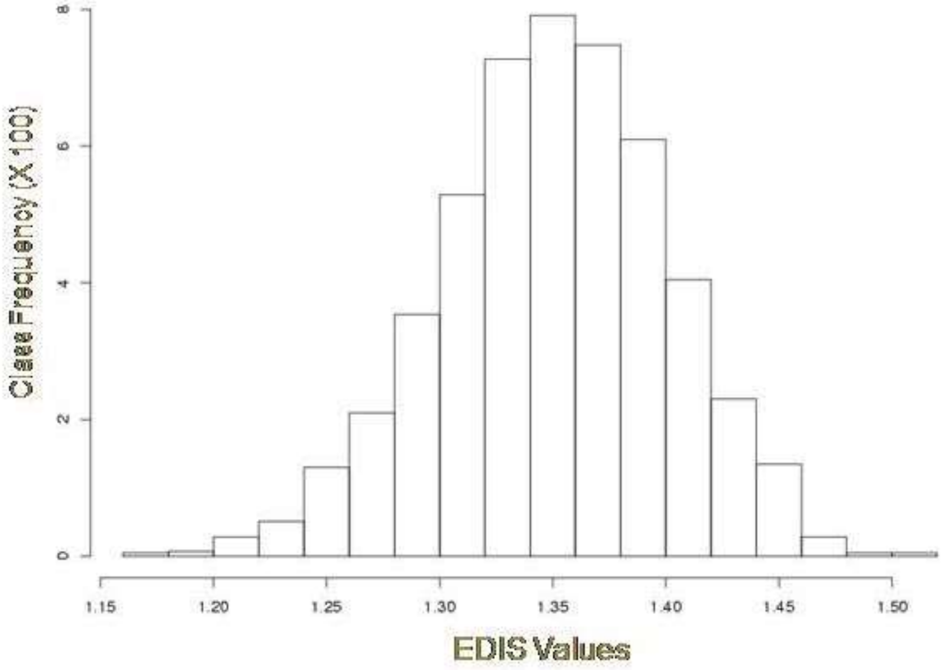

Figure 6. Grouping and associate class frequencies of 4000 values generated by EDIS. Station S2. Summer.

*Figura 6. Agrupación y frecuencias de clases asociadas a 4000 valores generados por EDIS. Estación S2. Verano*.

## DISCUSSION

Up to date, a large number of concepts and numerical techniques (indicator taxa, diversity and biotic indices, multivariate tools) sustain the theoretical ecology background. The approach to ecological quality analysis through biotic indices is an old but still relevant development (Elliott, 1996; Borja *et al*., 2000; Gibson *et al*., 2000; Ballesteros *et al*., 2007). Biotic indices reduce to a single univariate statistic the dimensionality of complex ecological data sets and can be represented just with a numerical figure. Despite this fact, the use and interpretation of indices such as diversity has been subjected to long discussions (Clarke & Warwick, 1994; Jennings & Reynolds, 2000), due to biased results caused by sample size, sampling methodology, and taxonomic skill in the identification of species. Furthermore, all of these indices are generally habitat-type dependent and, consequently, values concerning species and community changes can only be compared if the same methodology and taxonomic expertise levels have been followed (Panayotidis *et al*., 2004). Thus, several studies have found indices that consider taxonomic relatedness and multivariate analyses of community structure to be more sensitive and powerful means of detecting ecological impacts than those considering only diversity and species richness (McRae *et al*., 1998; Schratzberger *et al*., 2000).

In tropical oligotrophic areas nutrient/herbivorous models such as the RDM (Littler & Littler, 1984; Littler *et al*., 2006) encourage the use of RAM data to assess benthic ecosystem shifts and resilience losses at a community scale. A primary mechanism in diversity decrease is the elimination of sensitive species as a result of exposure to contaminants and the subsequent monopolization of resources by tolerant species. Yet, if nutrient availability is a limiting factor, an increase of nutrient pulses may cause greater primary production, greater resource heterogeneity and, as a final consequence, enhanced species diversity (Hall *et al*., 2000; Matthews *et al*., 2005). Nutrient enrichment can also trigger the die-off of dominant species, leaving space available to opportunistic species. Accordingly, improved algal diversity has been found in some intertidal reefs receiving significant inputs of nutrients like phosphorus (Abou-Aisha *et al*., 1995). All these facts acting together may sustain Pearson and Rosenberg paradigm that states three progressive steps when benthic communities are subject to improvements in habitat quality: abundance increases, species diversity increases, and dominant species change from pollution-tolerant to pollution-sensitive (Pearson & Rosenberg, 1978).

Pollution has never been associated with the complete exclusion of life forms in a location, and often 50–70% of species are able to tolerate the contaminant load (Johnston & Roberts, 2009). Besides, several opportunistic species can also exist in pristine ecosystems, by adequate seasonal timing to take full advantage of environmental resources (Orfanidis *et al*., 2003). Hence, the selection of intolerant species and particularly pollution-tolerant or opportunistic ones (recognized as potential bioindicators of impacted systems; Johnston & Roberts, 2009) are of great interest but a complex task. Without knowledge of ecophysiological responses and natural variability limits, selection of disturbance-sensitive taxa based on intuitive criteria can be misleading. In this case, probably a better approach may be to combine frequency information in a qualifying index using as reference those RAM data seen in “healthy” or “representative habitats”. Also, if sound information about biogeographic distribution of opportunistic or bioindicators species is available, results can be enhanced in a reliable way when frequency data are weighed by expertise judgments.

This approach using merely RAM data can be simple and robust, but compels to categorize *a priori* habitat types and working scales. In fact, Panayotidis *et al*., (2004) states that if reference taxa, their abundance levels and their variation ranges for each habitat type are previously elucidated, low-budget monitoring programs based on presence-absence or relative abundance of indicator taxa with respect to the total observed flora can be easily achieved. Unless key species can be easily recognized in the field, presence can be also circumscribed at genus level. It has been demonstrated that sampling at genus level saves substantial time with a negligible decrease in resolution. Actually, seaweed abundance measurements using genus-level identification compared with species-level identification resulted in a 97% alike rank order of similarity relationships between samples (Bates *et al*., 2007).

Bayesian models are suitable for problems with multiple thresholds and can account for spatial or temporal correlation (Beckage *et al*., 2007). As shown in the present paper, when extensive data sets are lacking, these models also make easier to forecast empirical distribution of any biotic index, facilitating the perception about real differences during statistical inference when they are used for comparisons.

Like PRC (Pardal *et al*., 2004), the proposed RAM-based method makes use of less-disturbed or polluted areas as reference sites, but in such a way that they may be extended to any stressed location, providing a powerful tool for environmental quality assessment. Its versatility can be applied also for classificatory supervision in habitat mapping. To achieve both goals, a fairly simple working schedule should be put into practice:

a) Define habitat types and working scales.
b) Identity the focal species or groups in these habitats. A good choice may be to select the minimum number of hierarchical categories with maximized coverage or relative frequencies necessary to reach 50% or more of the total macroalgal stratum, when the analysis concerns quality assessment, or no less than 70% for spatial relatedness.
c) Establish their relative frequencies or coverage ranges and select theoretical frequencies of all focal species or groups considered.
d) Consider that a fixed scale of the index is better during within-habitat comparisons (in this case replication is necessary), and that the inclusion in the index of species with very low theoretical frequency (<0.03) can be misleading.

## CONCLUSIONS

1. EDIS, suggested as a routine method for environmental quality assessment, is almost immediate, easy to apply and cost-effective. The required knowledge is reduced to the recognition of focal species or genus of macrophytes in each habitat type and their associate frequencies.
2. EDIS protocol is suited to forecast, with less subjectivity, succession trends and environmental changes due to resilience loss and climate changes, and can be applied also in habitat mapping and biodiversity studies.
3. With sampling effort of 200 count points, a rather good representation of total coverage or abundance of those species with 3% coverage or higher, at least in shallow rocky bottoms, can be achieved.

## REFERENCES

Abou-Aisha, K. M., Kobbia, I. A., Elabyad, M. S., Shabana, E. F. and Schanz, F. 1995. Impact of phosphorous loadings on macro-algal communities in the Red Sea coast of Egypt. *Water, Air & Soil Pollution*, 83: 285–297.

Alcolado, P. M., y Herrera, A.1987. Efectos de la contaminación sobre las comunidades de esponjas en el litoral de La Habana, Cuba. *Rep. Invest.,* (Cuba), 68: 17 p.

Ambrose, R. F. 2002. Transects, quadrats, and other sampling units. In: Murray, SN, Ambrose, RF, Dethier, MN, editors. *Methods for performing monitoring, impact, and ecological studies on rocky shores*. Coastal Research Center, Marine Science Institute, University of California, Santa Barbara, California, pp. 98-122.

Areces, A. J. y Martínez, J. C. 1992. Dinámica, estacionalidad y efectos de la epifauna colonizadora sobre el cultivo de la agarófita *Bryothamnion triquetrum* (Gmelin) Howe. *Avicennia* (Cuba), 0: 43-59.

Areces, A. J. 1997. La ficoflora intermareal como bioindicadora de calidad ambiental. Estudio de caso: el litoral habanero. En: *Sustentabilidad de la biodiversidad, un problema actual. Bases científico-técnicas, Teorizaciones y Proyecciones.* K. Alveal y T. Antezana (eds.), Univ. de Concepción, Concepción, Chile, p. 569-589.

Ballesteros, E., Torras, X., Pinedo, S., García, M., Mangialajo, L. and Torres de, M. 2007. A new methodology based on littoral community cartography dominated by macroalgae for the implementation of the European Water Framework Directive. *Mar. Poll. Bull*., 55: 172–180.

Bates, C. R., Tobin, M. L., Scott, G., and Thompson, R. 2007. Weighting the cost and benefits of reduced sampling resolution in biomonitoring studies: perspectives from the temperate rocky intertidal. *Biol. Conser*., 137: 617-625.

Beckage, B., Joseph, L., Belisle, P., Wolfson, D. B. and Platt, W. J. 2007. Bayesian change-point analyses in ecology. *New Phytologist*, 174: 456-467.

Borja, A., Franco, J. and Pérez, V. 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Mar. Poll. Bull*., 40: 1100–1114.

Borowitzka, M. A. 1972. Intertidal algal species diversity and the effects of pollution. *Australian Journal of Marine and Freshwater Research*, 25: 73-84.

Clarke, K. and Gorely, R. 2006. Primer 6. Plymouth, UK: Plymouth Marine Laboratory.

Clarke, K. R. and Warwick, R. M. 1994. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation. *Plymouth Marine Laboratory*, UK: 144 pp.

del Valle, R., Espinosa, J., y Castillo del, N. 1992. Efectos de la contaminación sobre las comunidades de moluscos del litoral rocoso al W de la Bahía de La Habana. *Rep. Invest.,* (Cuba), 5:8 p.

Duarte, C. M. 1995. Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia*, 41: 87–112.

EEC, 1992. Council Directive for the Habitats, 92/43/EEC, *Off. J. Eur. Communities*. 22/7/1992.

EEC, 1994. Proposal for a Council Directive on the ecological quality of water. 94/C 222/06, *Off. J. Eur. Communities*, 10/8/94.

EEC, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Off. J. Eur. Communities*, 43: 1–72.

Efron, B. and Tibshirani, R. 1993. *An introduction to the bootstrap*. Chapman & Hall Inc., Nueva York, 436 pp.

Elliott, M.1996. The derivation and values of ecological quality standards and objectives. *Mar. Poll. Bull*., 32: 762–763.

Fairweather, P. G. 1990. Sewage and the biota on seashores: assessment of impact in relation to natural variability. *Environmental Monitoring and Assessment,* 14: 197–210.

Gibson, G. R., Bowman, M. L., Gerritsen, J. and Snyder, B.D. 2000. *Estuarine and Coastal Marine Waters: Bioassessment and Biocriteria Technical Guidance*. EPA 822-B-00-024. U.S. Environmental Protection Agency, Office of Water, Washington, D C.

Gómez-Quintero, M. J. y Areces, A. J. 1976. Algunas consideraciones hidroquímicas sobre el río Quibú y áreas litorales limítrofes. Inédito, *Arch. Cient. Inst. Oceanol.,* Cuba, 19 pp.

González-Sánchez, P. 2011. *Variaciones temporales del macrofitobentos en un pavimento rocoso somero al oeste de La Habana, Cuba*. Inédito, Tesis en opción al título de Licenciado en Ciencias Biológicas, Centro de Investigaciones Marinas, Universidad de La Habana, 38 pp.

Gorostiaga, J. M. and Díez, I. 1996. Changes in the sublittoral benthic marine macroalgae in the polluted area of Abra de Bilbao and proximal coast (northern Spain). *Marine Ecology Progress Series*, 130: 157–167.

Guiry M. D. and G. M. Guiry. 2015. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org (last accessed 25/05/2015)

Hall, S. J., Gray, S. A. & Hammett, Z. L. 2000. Biodiversity–productivity relations: an experimental evaluation of mechanisms. *Oecologia*, 122: 545–555.

Herrera-Moreno, A., y Alcolado, P. M. 1983. Efectos de la contaminación sobre las comunidades de gorgonáceos al Oeste de la Bahía de La Habana. *Cienc.. Biol.* (Cuba), 10:69–85.

Herrera, A., Martínez, N. 1987. Efectos de la contaminación sobre las comunidades de corales escleractíneos al oeste de la bahía de La Habana. *Rep. Investig*. (Cuba), 62:29.

Hill, M. O. 1973. Diversity and Evenness: A Unifying Notation and Its Consequences. *Ecology*, 54(2): 427-432.

Jennings, S. and Reynolds, J. D. 2000. Impacts of fishing on diversity: from pattern to process. In: M. J. Kaiser, S. De Groot (eds), *Effects of Fishing on Non-Target Species and Habitats*. Blackwell Science, Oxford, pp. 235–250.

Johnston, E. L. and Roberts D. A. 2009. Contaminants reduce the richness and evenness of marine communities: A review and meta-analysis. *Environ. Poll.,* 157: 1745–1752.

Juanes, J. A., Guinda, X., Puente, A. and Revilla, J. A. 2008. Macroalgae, a suitable indicator of the ecological status of coastal rocky communities in the NE Atlantic. *Ecological Indicators*, 8(4): 351-359.

Littler, D. S. and Littler, M. M. 2000. *Caribbean Reef Plants*. OffShore Graphics, Inc., Washington, D.C., 542 pp.

Littler, M. M., Littler, D. S. and Brooks, B. L. 2006. Harmful algae on tropical coral reefs: Bottom-up eutrophication and top-down herbivory. *Harmful Algae*, 5: 565–585.

Littler, M. M. and Littler, D. S. 1984. Models of tropical reef biogenesis: the contribution of algae. In: F.E. Round, D.J. Chapman, (eds), *Progress in Phycological Research*, Vol. 3. Biopress, Bristol, pp. 323–364.

Lluis, M. 1974. Estudios hidrológicos de un área semicerrada y de las aguas adyacentes en la costa Norte de la provincia de La Habana. *Serie Oceanol*. (Cuba),19:5–45.

Lugioyo, M. y Rodríguez, F.1988. Aislamiento e identificación de bacterias del sedimento de la costa Norte de C. de La Habana, Cuba. *Rep. Investig*. (Cuba), 4: 9 p.

Matthews, A., Grenfell, H. R., Hayward, B. W. and Horrocks, M. 2005. Foraminiferal record of sewage outfall impacts on the inner Manukau Harbour, Auckland, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 39: 193–215.

McRae, G., Camp, D. K., Lyons, W. G. and Dix, T. L. 1998. Relating benthic infaunal community structure to environmental variables in estuaries using nonmetric multidimensional scaling and similarity analysis. *Environmental Monitoring and Assessment*, 51: 233–246.

Murray, S. and Littler, M. M. 1978. Patterns of algal succession in a perturbated marine intertidal community. *J. Phycol*., 14: 506–512.

Orfanidis, S., Panayotidis, P. and Stamatis, N. 2001. Ecological evaluation of transitionnal and coastal waters: a marine benthic macrophytes-based model. *Mediterranean Mar. Res*. 2 (2), 45–65.

Orfanidis, S., Panayotidis, P. and Stamatis, N. 2003. An insight to the ecological evaluation index (EEI). *Ecological Indicators*, 3: 27–33.

Panayotidis, P., Montesanto, B. and Orfanidis, S. 2004. Use of low-budget monitoring of macroalgae to implement the European Water Framework Directive. *J. Appl. Phycol.,* 16: 49-59.

Pardal, M. A., Cardoso, P. G., Sousa, J. P., Marques, J. C. and Raffaelli, D. 2004. Assessing environmental quality: a novel approach. *Mar. Ecol. Prog. Ser.,* 267: 1–8.

Pearson, T. H. and Rosenberg, R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanogr. Mar. Biol. Ann. Rev*., 16: 229-311.

Perigó-Arnaud, E. 2013. Evaluación de la Calidad Ambiental de la Zona Costera entre las desembocaduras de los ríos Almendares y Santa Ana (Litoral Oeste del Municipio Playa, La Habana). Inédito, *Arch. Cient. Inst. Oceanol.,* Cuba, 24 pp., 10 Figs., 16 Tbls.

Pinedo, S. García, M., Satta, M.P., de Torres, M. and Ballesteros, E. 2007. Rocky-shore communities as indicators of water quality: A case study in the Northwestern Mediterranean. *Mar. Poll. Bull*., 55: 126-135.

Regier, H. A. and Corwell, E. B. 1972. Applications of ecosystem theory, succession, diversity, stability, stress and conservation. *Biological Conservation*, 4 (2): 83-88.

Rodas-Fernández, L., Cerdeira, S., Areces, A. J., Montalvo, J. F., Loza, S., del Valle, R. y Niévares, A. 2013. *Resultado 1. Biología Marina & Resultado 3. Hidroquímica y Contaminación Marina* En Informe Final del Proyecto Calidad Ambiental de la Zona Costera al Oeste de Ciudad de la Habana (CACHA). Inédito, *Arch. Cient. Inst. Oceanol.,* Cuba, p.11-33.

Schramm, W. 1999. Factors influencing seaweed responses to eutrophication: some results from EU-project EUMAC. *J. Appl. Phycol*., 11: 69–78.

Schramm, W., and Nienhuis, P.H. (Editors). 1996. *Marine benthic vegetation: recent changes and the effects of eutrophication*. Springer-Verlag, Berlin, Germany.

Schratzberger, M., Gee, J. M., Rees, H. L., Boyd, S. E. and Wall, C. M. 2000. The structure and taxonomic composition of sublittoral meiofauna assemblages as an indicator of the status of marine environments. *Jour. Mar. Biol. Ass. United Kingdom*, 80: 969–980.

Soares, L. P., Reis, T. N. V., Bernardi, J., Barros, N. C. G., Minei, C. C., Areces, A. J., Guimaräes, S. M. P. B. e Fujii, M. T. 2010. Macroalgas marinhas bentónicas como indicadoras da qualidade ambiental na praias do litoral norte do estado de São Paulo, Brasil. *AOCEANO*, 02351-02353.

Soares, L. P., Reis, T. N. V., Barros, N. C. G., Araujo, P. G., Silva, I. B., Vasconcelos, E. R. T. P. P., Concentino, A. L. M., Areces, A. J. e Fujii, M. T. 2010. Índice de qualidade ambiental utilizando macroalgas como bioindicadoras nas praias de Boa Viagem, Suape e Tamandaré, Pernanbuco, Brasil. *AOCEANO*, 02986-02988.

Soltan, D., Verlaque, M., Boudouresque, C. F. and Francour, P. 2001. Changes in macroalgal communities in the vicinity of the Mediterranean sewage outfall after the setting up of a treatment plant. *Mar. Poll. Bull*., 42, 59–70.

Sousa, W. P.1980. The responses of a community to disturbance: the importance of successional age and species' life histories. *Oecologia*, 45: 72–81.

Spiegelhalter, D., Thomas, A., Best, N. and Lunn, D. 2003. *WinBUGS version 1.4 User Manual*. http://www.mrc-bsu.cam.ac.uk/bug (last accessed: 06/ 2011)

Taylor, W. R. 1960. *Marine Algae of the Eastern Tropical and Subtropical Coasts of Americas*. The University of Michigan Press, Ann Harbor, 870 pp.

Tribollet, A. D. and Vroom, P. E. 2007. Temporal and spatial comparison of the relative abundance of macroalgae across the Mariana Archipelago between 2003 and 2005. *Phycologia,* 46 (2): 187–197.

Tóthmérész, B. 1995. Comparison of different methods for diversity ordering. *J. Veg. Sci.,* 6: 283-290.

Valdés-Muñoz, E. y Garrido, O. H. 1987. Distribución de los peces en un arrecife costero del litoral habanero. *Rep. Invest.* (Cuba), 61:16 p.

Vinogradova, L. A. 1974. Ciclo anual del desarrollo de la micro y macroflora en un área semicerrada y en aguas adyacentes de la plataforma Cubana. *Serie Oceanol*. (Cuba), 19:47–60.

Vroom, P. S. and Page, K. N. 2006. Relative abundance of macroalgae (RAM) on Northwestern Hawaiian Island reefs. *Atoll Res. Bull.,* 543: 533–548.

Wells, E., Wilkinson, M., Wood, P. and Scanlan, C. 2007. The use of macroalgal species richness and composition on intertidal rocky seashores in the assessment of ecological quality under the European Water Framework Directive. *Mar. Poll. Bull*., 55, 151-161.

Wynne, M. J. 2011. A checklist of benthic marine algae of the tropical and subtropical western Atlantic: third revision. *Nova Hedwigia,* 140: 166 p.