An approach based on nematode descriptors for the ecological quality (EcoQ) classification of the Malaysian coasts

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Abstract Marine free-living nematodes have been employed in ecological studies, and have been proved to be suitable bioindicators of pollution-induced effects on the benthic domain. This study represents the first attempt to use nematode descriptors in order to assign an Ecological Quality (EcoQ) status to the Sarawak coasts, so integrating the methods actually applied by the Department of Environment (DOE). Three nematode parameters including colonizers-persisters percentage, Maturity index (MI), and Shannon diversity index (H') were used as they have been recognized as the best descriptors of the EcoQ status in coastal habitats. The thresholds applied to the nematode parameters of the Sarawak showed mainly a moderate and bad EcoQ status of the study sites, except for the site S5 that revealed a good EcoQ. The sites with the worst EcoQ were characterized by low salinity values that suggests freshwater inflows as a primary source of pollution in these areas. This is confirmed by both H' and MI indices that showed a close positive relation with the salinity. Results obtained in the site S4 may suggest that pollution left "traces" in the nematode assemblages that cannot be detected from other parameters of the water column. This investigation highlights that the analysis of nematode parameters for the ecological assessment could be effectively applied by the environmental policies of the countries in rapid growth. Furthermore, it certainly suggests the need of management and conservation actions in the Sarawak coasts aimed at a more sustainable use of the marine resources to prevent possible biodiversity losses.

Keywords Zoobenthos • Meiofauna • Ecological quality assessment • Anthropogenic impact • Malaysia

Introduction

Coastal seas suffer from several local stresses such as pollution, eutrophication and fishing effects, but also from other phenomena such as global warming and ocean acidification (Doney 2010). Sale et al. (2014) documented a progressive reduction of the ecosystem functionality and resilience leading to loss of essential goods and services for human populations especially in tropical regions that are subject to a rapid industrial development. Malaysia has experienced an industrial, agricultural and tourist remarkable growth in the recent decades that have also resulted in a number of environmental problems (e.g. deforestation, pollution, overfishing and destruction of coral reefs). Protocols of the Environmental Quality Act (EQA, 1974) have been applied by the Department of Environment (DOE), that is the principal institution for environmental protection in Malaysia and aims at the prevention, reduction, and control of pollution, and the enhancement of the ecological quality. EQA is basically a pollution-prevention law, which should prevent water pollution through the prohibition or the limited discharge of some substances unless licensed by the DOE.

The water quality monitoring of Malaysia started in 1978, but in 1985 in the two States of Sabah and Sarawak and only in 1999 in the coastal waters of some islands. Up until 2010, the Interim Marine Water Quality Standard (IMWQS) analysed the concentration of 9 parameters for assessing the ecological status of the Malaysian coasts: *Escherichia coli*, oil and grease, total suspended solids, arsenic, cadmium, chromium, copper, lead and mercury. However, several authors have documented that studies based only on physico-chemical variables (Goodsell et al. 2009; Semprucci et al. 2016) or single indicator organisms such as *E. Coli*, an indicator of fecal contamination (see Boi et al. 2015 for review), may give only a partial description of the ecosystem status. Then some international regulations such as two European Directives (Water Framework Directive, 2000/60/EC and Marine Strategy Framework Directive, 2008/56/EC) recognized the relevance of indices based on benthic invertebrates to effectively document the ecological status of marine areas along with a possible loss of ecological functioning (Van Hoey et al. 2010; Semprucci et al. 2013a, 2014; Borja et al. 2014).

Macrobenthos descriptors (e.g. assemblage structure, indices based on tolerant or sensitive species, presence or absence of indicator species) are currently widely used to detect the effects of anthropogenic impact in water bodies. However, the interest in the use of free-living nematodes features in the marine biomonitoring has been increased in the last years. Nematodes, in fact, are the most abundant and diverse component of the marine soft bottoms and have a recognized role in the marine ecosystem functioning (e.g. Balsamo et al. 2010; Appeltans et al. 2012). Furthermore, their intermediate position in the trophic chains, the direct contact with sediment pollutants and the short biological life cycles make them very good bioindicators for detecting environmental changes (Balsamo et al. 2012; Moens et al. 2013). Moreno et al. (2011) and Semprucci et al. (2014a, 2015b) tested some nematode features (e.g. taxonomic and trophic diversity, life strategies, colonizer-persister classes, Maturity Index) for the Ecological Quality (EcoQ) assessment, in accordance to the European Directive in temperate regions (Mediterranean Sea). Among the parameters studied, Maturity index, colonizer-persister classes and Shannon diversity appeared to be the best descriptors for the monitoring of the marine EcoQ (Semprucci et al. 2015a). The same have been applied in Indian Ocean by Semprucci et al. (2014b) revealing promising results for ecological assessment in tropical regions.

The present investigation has been carried out in front of ten river mouths of Sarawak, which is the largest state of Borneo, located in its northwest side with a total of 2,636,000 inhabitants. This study represents the first attempt to use nematode descriptors to evaluate and assign an Ecological Quality (EcoQ) status to the Sarawak coastal ecosystems. It also aims at offering a new possible tool for evaluating the anthropogenic impact in Malaysian ecosystems and at further calibrating the use of these descriptors in tropical regions.

Materials and methods

Field sampling

Sampling was carried out in 2008 along the coast of Sarawak during the dry season. The sediment samples for the study of the nematode assemblages were collected from subtidal sites located in front of the river mouths of ten estuaries (Fig. 1). Study sites, sampling, experimental design and sample processing techniques are described in detail in Chen et al. (2012). Hereinafter, a brief summary of the methods is reported. The samplings were carried out during the low tides. Sediments were collected using a perspex corer with an inner diameter of 2.5 cm driven to a depth of 5 cm (Tita et al. 2000). Three replicates of sediments were taken in each station and then fixed in 4% formaldehyde solution (according to Danovaro et al. 2004). At each station, an additional sediment sample for grain size analysis was taken, and physico-chemical parameters (i.e. salinity PSU, temperature °C, DO mg l^{-1} and pH) were measured using HORIBA U20-XD multimeter.

Laboratory analysis

Sediment analysis was carried out according to Bale and Kenny (2005) and Buchanan (1984) and the percentages of sand, silt and clay were calculated. The nematode fauna was obtained according to the methods described in Somerfield et al. (2005). Nematode specimens of each sample were counted and mounted in slides with anhydrous glycerol. Specimens were identified under an Olympus BX 51. The identification to species or putative species level was made using Platt and Warwick's pictorial keys (1983, 1988), Warwick et al. (1998) and NeMys online identification key (Guilini et al., 2016).

Data analysis

Aggregation of nematode data identified at species level to genus level is suggested by Somerfield and Clarke (1995) in order to reduce the region effects. Therefore, the data set published by Chen et al. (2012) was aggregated at genus level before calculating the nematode parameters. Maturity Index (MI), Shannon Index (H'), and colonizer-persister (c-p) classes were chosen as the best nematode descriptors to detect the EcoQ of the Malaysian coastal waters according to Semprucci et al. (2015a). MI (Bongers 1990; Bongers et al. 1991) was calculated as the weighted average of the individual c-p values:

$$MI = \sum \upsilon\ (i) f\ (i)$$

where υ is the c-p value of genus *i* and f (*i*) is the frequency of that genus. Bongers (1990) suggested MI as a semi quantitative measure of the nematode response to an environmental disturbance. Bongers distinguished *r*-strategist species (colonizers or c–p 1), which are more tolerant to environmental variations, and *k*-strategist species (persisters or c–p 5), which are more sensitive. Shannon's diversity index (H', Shannon and Weaver 1949) ($\log_2$ based) was calculated to describe the nematode assemblage at genus level. Among the diversity indices, H' has been selected as candidate for the EcoQ class definition due to its wide application in the field studies (Semprucci et al. 2015a).

Principal component analysis (PCA) was applied on nematode parameters (c–p%, MI, H') and environmental data (salinity, temperature, DO, pH, sand, silt and clay). Prior to statistical analysis, a logarithmic transformation log (1 + ×) was performed to remove the effects of orders of magnitude difference between variables, normalize the data and increase the importance of smaller values (Coccioni et al. 2009). The nematode parameters were used as active variables, while environmental data were projected on the factor-plane as supplementary variables without contributing to the results of the analysis (Semprucci et al. 2014a). PCA may provide a subdivision of the river mouths based on the projection of the cases in the factor plane and an insight of the possible influence of the environmental variables on the nematode attributes using the projection of the variables (STATISTICA v.8 software).

The classification of EcoQ follows the thresholds first proposed by Moreno et al. (2011), and then modified by Semprucci et al. (2014a, b) (Table 1). The final classification was obtained by merging the EcoQ results of all the nematode parameters considered in this study. The results of c–p%, MI and H' were combined by averaging when the values leading to three different EcoQ classes, while the most represented EcoQ class was assigned when two or all the EcoQ classes obtained were the same (Fig. 2).

Results

The data on the environmental parameters are summarized in Table 2. The pH was prevalently acid, with the only exceptions at the sites S3, S8, S9 and S10. The DO values varied between 1.8 mg l^{-1} (S1) and 12.2 mg l^{-1} (S8): the highest values were generally found in the southern part of the study area. The salinity varied from 19.0 PSU (S4) to 32.0 PSU (S10) and also in this case the highest values were found in the southern sites. Temperature ranged from 26.4 (S6) to 30.8 °C (S2) and they were overall higher in the northern sites. The sediments of the study area were mainly represented by the sandy fraction that was dominant in the sites S8 (99%), S6 and S4 (97%), followed by S1 (76%) and S10 (71%). Silt was the second most abundant sediment fraction with values ranging from 1.1% (S8) to 78.0% (S2). Clay fraction was not abundant in the study area, with very low values ranging from 0% (S4 and S8) to 19.0% (S7).

The highest abundances of nematodes were recorded at S10 (412.0±5.7 ind. 10 cm^{-2}), followed by S4 (328.0±56.6 ind. 10 cm^{-2}) and S5 (148.0±50.9 ind. 10 cm^{-2}), while S6 appeared completely defaunated (Table 3). A total of 51 species belonging to 20 genera was recorded. The most abundant genera were *Pseudocella* (20%), *Sabatieria* (16%), *Daptonema* (14%), followed by *Paraodontophora* (6%) and *Terschellingia* (5%). The lowest H' values were revealed at S2 (0.2±0.3) and S9 (0.8±1.1), while the highest ones at S3 and S4 (2.4±0.6 and 2.4±0.0), followed by S8 and S10 (both 2.1). The lowest MI values were recorded at S9 (2.0±0.0) and S4 (2.1±0.0), the highest values at S2 (4.9±0.2) and S5 (4.2±0.1), followed by S8 (3.0±0.4), S1 (2.9±0.1) and S3 (2.7±0.1). As for the c–p classes only c–p 1 (extreme colonizers) was not found. C–p 2 (general colonizers) were generally dominant in the study area. The lowest percentages were found at S5 (24%), the highest ones at S2 and S9 (100% represented by *Pseudolella*, *Parodontophora*, *Daptonema* and *Sabatieria*) followed by the site S4 (90% mainly *Metachromadora* and *Trileptium*), S10 (75% mainly *Sabatieria*, *Hopperia* and *Metachromadora*). Overall, c–p3 was the second dominant c–p class, that however was completely absent at S2 and S9, while was the most abundant class at S7 (53% mainly *Choniolaimus*, *Spirinia* and *Sphaerolaimus*), and S1 (38% mainly *Terschellingia*). C–p 4 (persisters) were poorly represented in the study sites with the highest values at S8 (42%, mainly *Halalaimus* and Dorylaimid species), followed by S3 (26% mainly *Pomponema*), while in the other sites the class was present with percentages lower than 6%. C–p 5 (extreme persisters) were present only in two sites: S5 and S1 (69% and 18% mainly due to *Pseudocella*).

The first two principal components were identified, and together explained 76% of the variance. The first principal component (PC1), explained 52% of the variance and was mainly affected by H' (-0.93), MI (-0.88), c–p3 (-0.81) and c–p2 (-0.80), while the secondary variables were DO (-0.47) and salinity (-0.45) (Fig. 3). PC2 explained 24% of the variance and was mainly influenced by the following primary variables: c–p5 (-0.91) and c–p4 (0.68). The secondary variables that affected the factor plane were temperature (-0.55), pH (0.49), silt and clay percentages (-0.45 and -0.43, respectively) (Fig. 3). PCA analysis carried out on the whole area showing the projection of the cases was illustrated in Fig. 4.

Discussion

There are main factors that influence the water quality in Malaysia and that may be harmful to aquatic organisms and public health (Amneera et al. 2013). Accordingly, water quality monitoring may become an important defense tool and nematodes are good candidates as descriptors of the EcoQ because they may mirror the loss of functioning in the marine ecosystems (Danovaro et al. 2008). Furthermore, nematode parameters selected by Moreno have been defined promising for the ecological assessment of marine areas and already applied according to the European Directives (Vanaverbeke et al. 2011; Semprucci et al. 2015a).

When the data of the Sarawak were analysed by PCA, the H', MI, c–p 3 and c–p 2 were contributed to the PC1 that positively correlated to the environmental variables DO and salinity (Fig. 3). The class c–p 5 mainly contributed to PC2 and was positively related to temperature and mud fractions, while the class c–p 4 to pH. Among the ten river mouths, the case projection revealed a separation of the study sites that highlights the worst EcoQ conditions and were all sites located in the positive part of the 1 × 2 factor plane (S6, S2 and S9) (Table 4, Fig. 4). The sites located in the first quadrant of the factor plane appeared represented by the sites with the highest percentage of sensitive species (c–p 5) (S5 and S1), while c–p 3 and c–p 4 appeared the classes that mainly contributed to the subdivision of the remaining sites (Table 2, Figs. 3, 4). The thresholds applied to the nematode attributes showed mainly a generally moderate or bad EcoQ status of the study sites, among which only the site S5 revealed a good EcoQ (Table 4).

In detail, the site S6 was the only one that result completely defaunated. *Daptonema*, *Sabatieria*, *Pseudolella*, *Parodontophora*, *Metachromadora*, *Trileptium* were the c–p 2 genera that mainly contributed to the bad EcoQ status of the sites S2, S4 and S9. Among them, *Daptonema*, *Sabatieria*, *Metachromadora*, *Trileptium* are well-recognized as indicators of stressful conditions (Bongers et al. 1991; Losi et al. 2012; Semprucci et al. 2013b; Santos et al. 2014; Zeppilli et al. 2015; Jouili et al. in press), while scarce information is available for *Parodontophora* and *Pseudolella* that, however, seem colonizer genera (Soetaert et al. 1995; Semprucci et al. 2010). The genus *Pseudocella*, classified by Bongers as an extreme persister belonging to the c-p5 class, gave the main contribution to the high EcoQ revealed S5. According to Bongers et al. (1991) the representatives of this family have generation times of ~1 year and comprise species that are very sensitive to pollutants.

The sites with the worst EcoQ were also characterized by low salinity values, which suggests freshwater inflows as the primary source of pollution in the areas. This is in agreement with previous studies that have recognized a strong influence of the riverine discharges on meiofaunal and nematode assemblages (Danovaro et al., 2000; Semprucci et al., 2010, 2013; Frontalini et al., 2011). In this respect, the indices H' and MI showed a close and positive relation with salinity level confirming the possible negative influence of river inflow on both of them. Salinity and DO seemed to be related to Shannon diversity, even more than the sediment grain size that normally is a primary factor in structuring meiofauna assemblages (Ndaro and Ólafsson1999; Adão et al. 2009).

Moens and Somerfield (2007) argument that the lack of complete empirical knowledge on the life strategies of all marine nematodes may be a limitation on the application of MI. Accordingly, the collection of additional data especially in a large range of geographical regions is fundamental to test the use of MI in marine habitats. Up to now MI was proved to be a good tool for evaluating the anthropogenic impact on Mediterranean coasts (see Semprucci et al. 2015a for details). The consistence of the trends of both H' and MI indices in Sarawak state may be a further confirmation.

An environmental classification using the Malaysian standards was not available in the whole study area (i.e. in the sites S1, S2 and S7) during the sampling year (2008). Thus, an effective comparison of the results based on the nematode parameters and DOE standards in all the sampled sites was not possible, but the presence of some previous data on the area makes possible some useful comparisons and inferences (Table 5). In particular, the EcoQ obtained by nematode indices was consistent with the DOE classification in the 2008 at S3, S5, S6, S8 and S10. Both the methods showed from moderate to poor EcoQ status at S3, S8 and S10, while a good and bad EcoQ at S5 and S6, respectively. In particular, S6 was the only site with completely defaunated sediments and high levels of suspended solids, hydrocarbons and heavy metals as well. As previously explained, the low salinity values detected in most sites with a bad EcoQ suggest a relevant impact of the rivers on the benthos of the front area. Instead, nematode parameters seemed to be more sensitive to pollution than those considered by DOE at S4 and S9 rivers in the 2008. Indeed, nematode descriptors documented a bad EcoQ status in these sites, while DOE standards revealed only a slight pollution by suspended solids. However, it is note of worthy that the site S4 in the previous DOE reports (2004, 2005, 2007) showed phenomena of pollution by suspended solids and total fecal coliform: they may have left "traces" in the nematode assemblages that live in close contact with sediments and interstitial waters and thus are more and longer exposed to pollution than other organisms (Balsamo et al. 2012). In this view, the selection of EcoQ descriptors that may integrate the response of both the biotic and the abiotic components of the marine ecosystem is an important target of the modern ecology and may give a more accurate classification of the water quality (Casazza et al. 2002).

This study documents the first application of nematode parameters for the ecological assessment of the Malaysian sediments, and strongly suggests the need of management and conservation actions in the Sarawak coasts to assure a more sustainable use of the marine resources and preventing possible biodiversity losses. This approach should be recommended in the environmental policies of the countries in rapid growth since it provides reliable data on different and complex aspects of the coastal ecosystems, and should be adopted by environmental agencies world-wide as a synthetic and direct measure of the EcoQ status.

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References

Adão H, Alves AS, Patrício J, Neto JM, Costa MJ, Marques JC (2009) Spatial distribution of subtidal nematode communities along the salinity gradient in southern European estuaries. Acta Oecol 35: 287-300

Amneera WA, Najib NW, Yusof SR, Ragunathan S (2013) Water Quality Index of Perlis River, Malaysia. Int J Civil & Environ Eng 13: 55-59

Appeltans W, Ahyong ST, Anderson G et al (2012) The magnitude of global marine species diversity. Curr Biol 22: 2189-2202

Armenteros M, Ruiz-Abierno A, Fernández-Garce R, Pérez-García JA, Díaz-Asencio L, Vincx M, Decraemer W (2009) Biodiversity patterns of free-living marine nematodes in a tropical bay: Cienfuegos, Caribbean Sea. Estuar Coast Shelf Sci 85: 179-189

Bale AJ, Kenny AJ (2005) Sediment analysis and seabed characterization. In: Eleftheriou A & Mclntyre A (eds) Methods for the study of marine benthos, 3rd Edn. Blackwell Science Ltd, Oxford, pp 43-81

Balsamo M, Albertelli G, Ceccherelli VU et al (2010) Meiofauna of the Adriatic Sea: current state of knowledge and future perspectives. Chem Ecol 26(1): 45-63

Balsamo M, Semprucci F, Frontalini F, Coccioni R (2012) Meiofauna as a tool for marine ecosystem biomonitoring. In: Cruzado A (ed) Marine ecosystems. InTech Publisher, pp 77-104

Boi P, Amalfitano S, Manti A, Semprucci F, Sisti D, Rocchi MB, Balsamo M, Papa S (in press) Strategies for water quality assessment: multiparametric analysis of the bacterial community in river waters. River Res Appl DOI: 10.1002/rra.2872

Bongers T (1990) The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14-19

Bongers T, Alkemade R, Yeates GW (1991) Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the Maturity Index. Mar Ecol Prog Ser 76: 135-142

Buchnan JB (1984) Sediment analysis. In: Holme NA & Mclntyre AD (eds) Methods for the study of marine benthos. Blackwell Science Ltd, Oxford, pp 41–65

Casazza G, Silvestri C, Spada E (2002) The use of bio-indicators for quality assessments of the marine environment: example from the Mediterranean Sea. J Coast Conserv 8: 147-156

Chen CA, Mohd Long S, Mohd Rosli N (2012) Spatial distribution of tropical estuarine nematode communities in Sarawak, Malaysia (Borneo). Raffles Bull Zool 60: 173-181

Coccioni R, Frontalini F, Marsili A, Mana D (2009) Benthic foraminifera and trace element distribution: a case-study from the heavily polluted lagoon of Venice (Italy). Mar Pollut Bull 56: 257-267

Danovaro R, Gambi C, Dell'Anno A, Corinaldesi C, Fraschetti S, Vanreusel A, Vincx M, Gooday AJ (2008) Exponential decline of deep-sea ecosystem functioning linked to benthic biodiversity loss. Curr Biol 18: 1-8

Danovaro R, Gambi C, Manini E, Fabiano M (2000) Meiofauna response to a dynamic river plume front. Mar Biol 137: 359-370

Danovaro R, Gambi C, Mirto S, Sandulli R, Ceccherelli VU (2004) Meiofauna. Biol Mar Mediterr 11: 55–97

Doney SC (2010) The growing human footprint on coastal and open-ocean biogeochemistry. Sci 328: 1512-1516

Franco MA, Steyaert M, Cabral HN, Tenreiro R, Chambel L, Vincx M, Costa MJ, Vanaverbeke J (2008) Impact of discards of beam trawl fishing on the nematode community from the Tagus estuary (Portugal). Mar Pollut Bull 56: 1728-1736

Frontalini F, Semprucci F, Coccioni R, Balsamo M, Bittoni P, Covazzi-Harriague A (2011) On the quantitative distribution and community structure of the meio and macrofaunal communities in the coastal area of the Central Adriatic Sea (Italy). Environ Monit Assess 180: 325-344

Goodsell PJ, Underwood AJ, Chapman MG (2009) Evidence necessary for taxa to be reliable indicators of environmental conditions or impacts. Mar Pollut Bull 58: 323-331

Guilini K, Bezerra TN, Eisendle-Flöckner U et al (2016) NeMys: world database of free-living marine nematodes. http://nemys.ugent.be. Accessed 13 October 2016

Jouili S, Essid N, Semprucci F, Boufahja F, Nasri A, Beyrem H (in press) Environmental quality assessment of El Bibane lagoon (Tunisia) using taxonomical and functional diversity of meiofauna and nematodes. J Mar Biol Assoc U.K. ID JMBA-05-16-OA-0149

Losi V, Montefalcone M, Mariapaola Moreno M, Giovannetti E, Gaozza L, Grondona M, Albertelli G (2012) Nematodes as indicators of environmental quality in seagrass (Posidonia oceanica) meadows of the NW Mediterranean Sea. Adv Oceanogr Limnol 3: 69-91

Moens T, Somerfield P (2007) Marine vs terrestrial nematodes as bioindicators: different approaches to very similar questions? 1st International Symposium on Nematodes as Environmental Bioindicators. Heriot Watt University, Edinburgh, UK, 11-13 June 2007

Moens T, Braeckman U, Derycke S et al (2013) Ecology of free-living marine nematodes. In: Schmidt-Rhaesa A (ed) Handbook of zoology: Gastrotricha, Cycloneuralia and Gnathifera, vol. 2: Nematoda. De Gruyter, Berlin, Germany, pp 109-152

Moreno M., Semprucci F, Vezzulli L, Balsamo M, Fabiano M, Albertelli G (2011) The use of nematodes in assessing ecological quality status in the Mediterranean coastal ecosystems. Ecol Indicators 11: 328-336

Ndaro SGM, Ólafsson E (1999) Soft-bottom fauna with emphasis on nematode assemblage structure in a tropical intertidal lagoon in Zanzibar, eastern Africa: I. spatial variability. Hydrobiologia 405: 133-148

Platt HM, Warwick RM (1983) Free-living marine nematodes, Part I. British Enoplids. Cambridge University Press, Cambridge

Platt HM, Warwick RM (1988). Free-living marine nematodes, Part II. British Chromadorids. Cambridge University Press, Cambridge

Sale PF, Agardy T, Ainsworth CH et al (2014) Transforming management of tropical coastal seas to cope with challenges of the 21st century. Mar Pollut Bull 85: 8-23

Santos J, Gomes R, Vasconcellos R, Silva D, Araújo F (2014) Effects of morphodynamics and across-shore physical gradients on benthic macroinfauna on two sandy beaches in south-eastern Brazil. J Mar Biol Assoc U.K. 94(4): 671-680. doi:10.1017/S0025315414000010

Semprucci F, Balsamo M, Sandulli R (2016) Assessment of the Ecological quality (EcoQ) of the Venice lagoon using the structure and biodiversity of the meiofaunal assemblages. Ecol Indic 67C: 451-457. doi: 10.1016/j.ecolind.2016.03.014

Semprucci F, Boi P, Manti A, Covazzi Harriague A, Rocchi M, Colantoni P, Papa S, Balsamo, M (2010) Benthic communities along a littoral of the Central Adriatic Sea (Italy). Helgoland Mar Res 64: 101-115

Semprucci F, Balsamo M, Frontalini F (2014a) The nematode assemblage of a coastal lagoon (Lake Varano, Southern Italy): ecology and biodiversity patterns. Sci Mar 78: 579-588

Semprucci F, Colantoni P, Sbrocca C, Baldelli G, Balsamo M (2014b) Spatial patterns of distribution of meiofaunal and nematode assemblages in the Huvadhoo lagoon (Maldives, Indian Ocean). J Mar Biol Assoc U.K. 94: 1377-1385

Semprucci F, Frontalini F, Covazzi-Harriague A, Coccioni R, Balsamo M (2013) Meio- and macrofauna in the marine area of the Monte St. Bartolo Natural Park (Central Adriatic Sea, Italy). Sci Mar 77: 189-199

Semprucci F, Losi V, Moreno M (2015a) A review of Italian research on free-living marine nematodes and the future perspectives in their use as Ecological Indicators (EcoInd). Mediterr Mar Sci 16: 352-365

Semprucci F, Frontalini F, Sbrocca C et al (2015b) Meiobenthos and free-living nematodes as indicators at the time of the Water Framework Directive, a study case in the Adriatic Sea. Environ Monit Assess 187: 251. doi: 10.1007/s10661-015-4493-7

Semprucci F, Moreno M, Sbrocca S, Rocchi M, Albertelli G, Balsamo M (2013b) The nematode assemblage as a tool for the assessment of marine ecological quality status: a case-study in the Central Adriatic Sea. Mediterr Mar Sci 14: 48-57

Shannon CE, Weaver W (1949) The mathematical theory of communication. University of Illinois, Urbana, Illinois.

Soetaert K, Vincx M, Wittoeck J, Tulkens M (1995) Meiobenthic distribution and nematode community structure in five European estuaries. Hydrobiologia 311: 185-206

Somerfield PJ, Clarke KR (1995) Taxonomic levels, in marine community studies, revisited. Mar Ecol Prog Ser 127: 113-119

Somerfield PJ, Warwick RM, Moens T (2005) Meiofauna techniques. In: Eleftheriou A & McIntyre A (eds) Methods for the study of marine benthos, 3rd Edition. Blackwell Science Ltd, Oxford, pp 269-272

Steyaert M, Deprez T, Raes M et al (2005) Electronic key to the free-living marine nematodes. http://nemys.ugent.be. Accessed 13 October 2016

Tita G, Desrosiers G, Vincx M (2000) New type of hand-held corer for meiofaunal sampling and vertical profile investigation: a comparative study. J Mar Biol Assoc U.K. 80: 171-172

Vanaverbeke J, Merckx B, Degraer S, Vincx M (2011) Sediment-related distribution patterns of nematodes and macrofauna: two sides of the benthic coin? Mar Environ Res 71: 31-40

Warwick RM, Platt HM, Somerfield PJ (1998). Free-living nematodes (Part III) Monhysterids. In: Barnes RSK & Crothers JH (eds) Synopsis of British fauna, No. 53. Shewsbury, Field Studies Council, 296 pp

Zeppilli D, Sarrazin J, Leduc D et al (2015) Is the meiofauna a good indicator for climate change and anthropogenic impacts? Mar Biodiv 45: 505-535. doi: 10.1007/s12526-015-0359-z

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Figure 1

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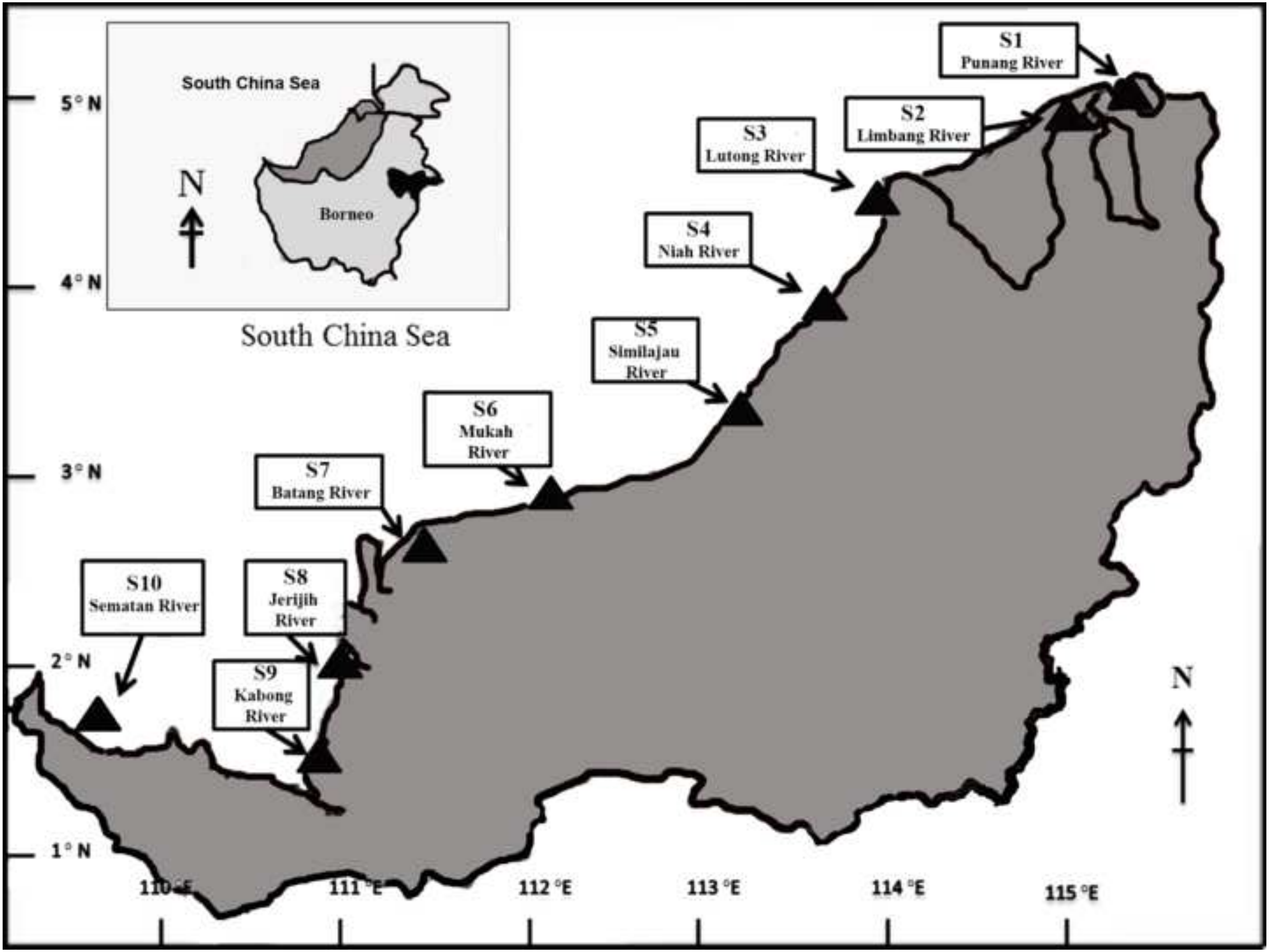

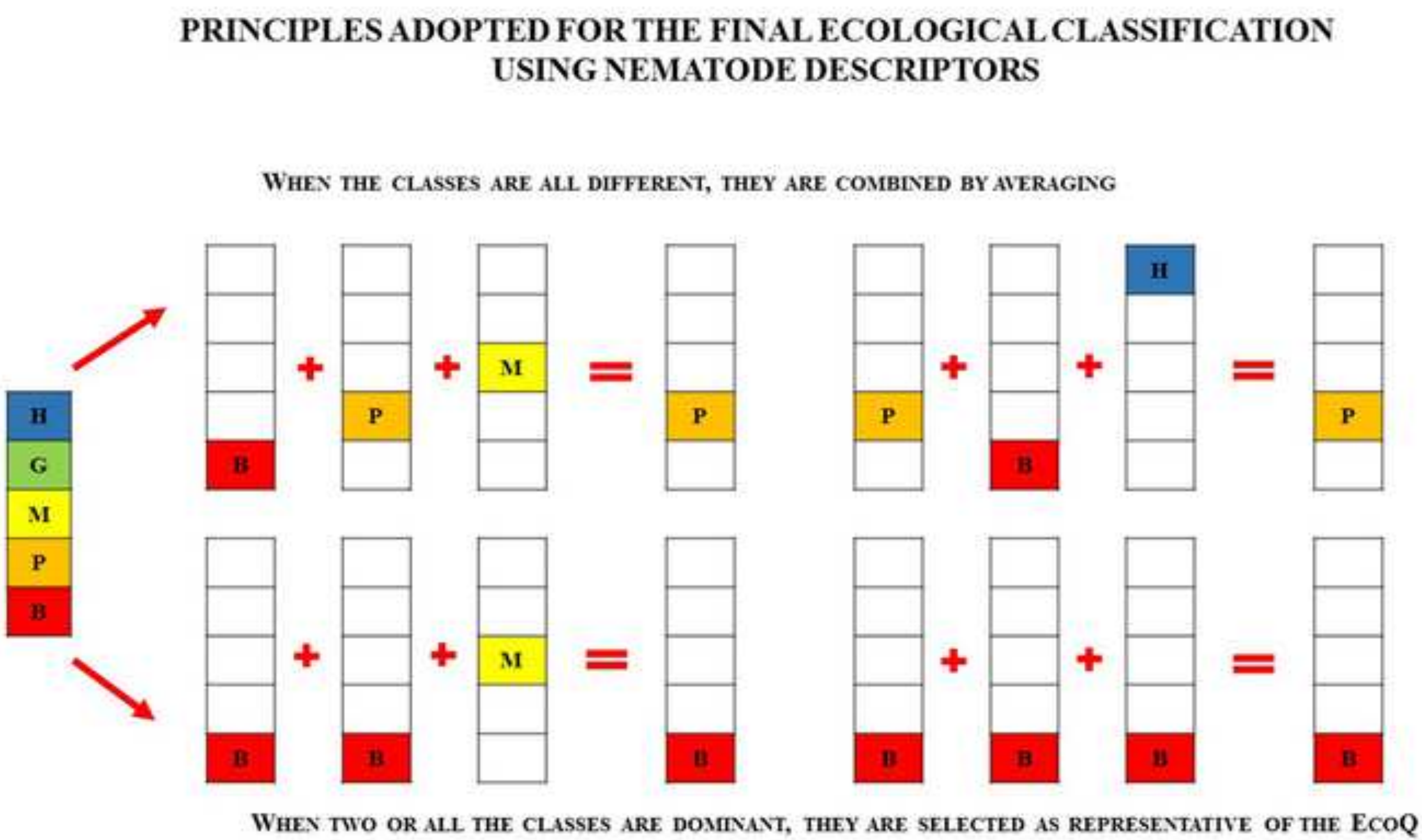
PRINCIPLES ADOPTED FOR THE FINAL ECOLOGICAL CLASSIFICATION USING NEMATODE DESCRIPTORS
WHEN THE CLASSES ARE ALL DIFFERENT, THEY ARE COMBINED BY AVERAGING
H
G
M
P
B
WHEN TWO OR ALL THE CLASSES ARE DOMINANT, THEY ARE SELECTED AS REPRESENTATIVE OF THE ECOQ

Figure 3

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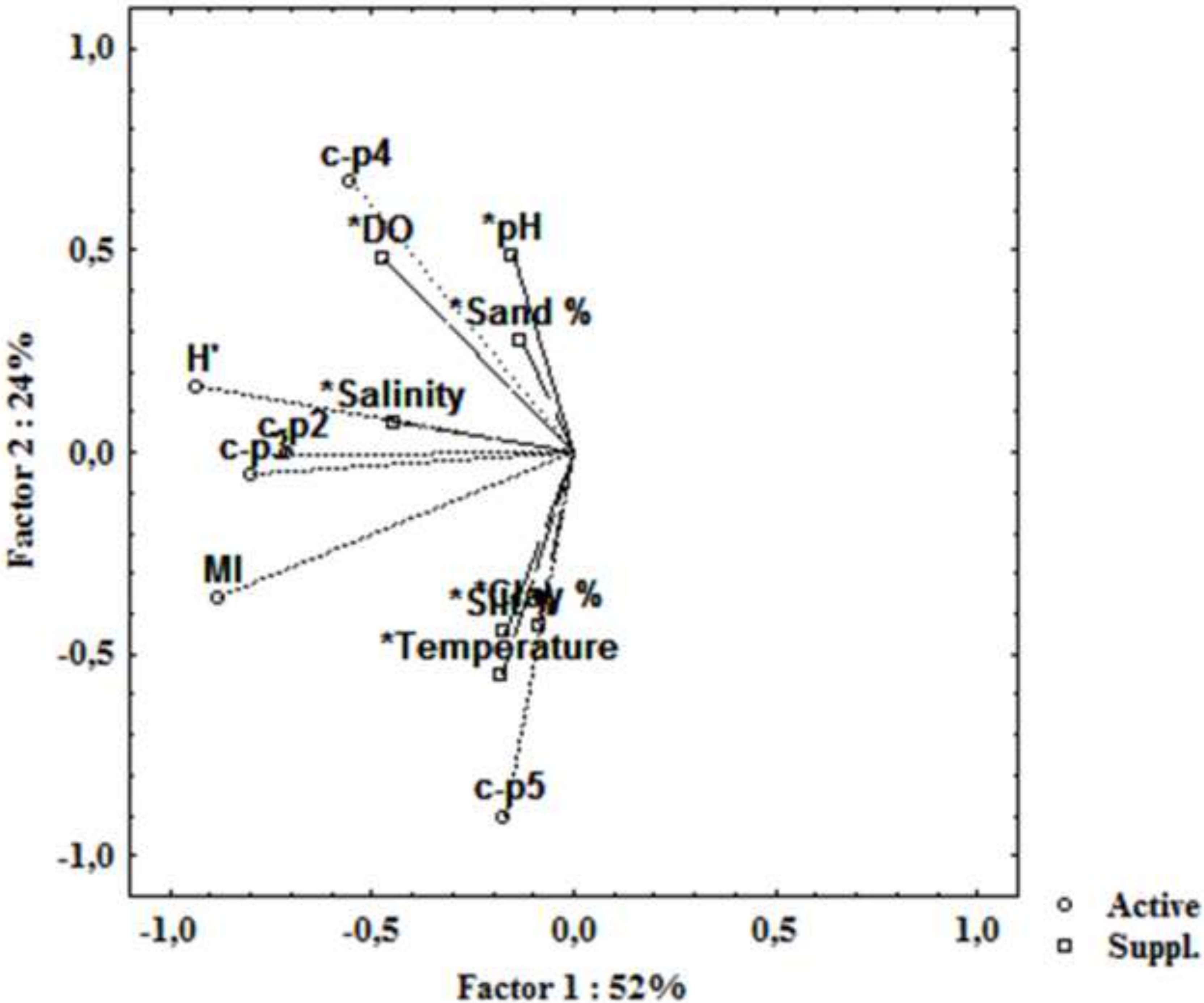

Figure 4

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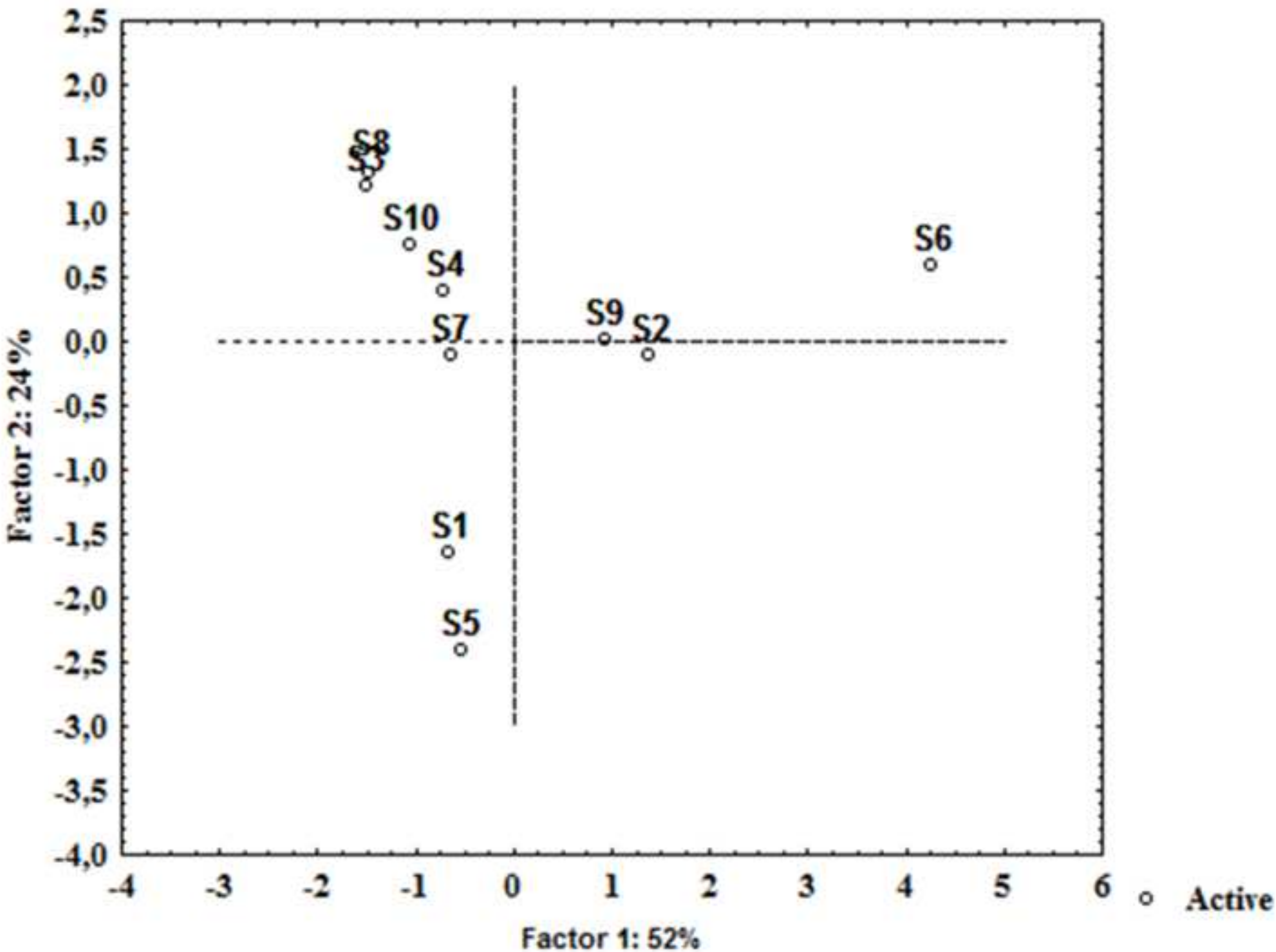

Table 1 Thresholds for nematode descriptors in accordance to Semprucci et al. (2014a,b)

Nematode attributes	EcoQ Status				
	High	Good	Moderate	Poor	Bad
Maturity Index (MI)	MI > 2.8	2.8 ≤ MI < 2.6	2.6 ≤ MI< 2.4	2.4 ≤ MI < 2.2	MI ≤ 2.2
Colonizers (from c-p1 to c-p2)	0-20%	20-40%	40-60%	60-80%	80-100%
Moderate colonizers and persisters (from c-p3 to c-p5)	100-80%	80-60%	60-40%	40-20%	20-0%
Shannom Index (H')	H' > 4.5	3.5 < H' < 4.5	2.5 < H' < 3.5	1 < H' < 2.5	0 < H' < 1

Table 2 Physico-chemical parameters of the water column and sediment fraction percentages measured during the sampling at each river mouth

Site features	Punang	Limbang	Lutong	Niah	Similajau	Mukah	Batang	Jerijih	Kabong	Sematan
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Geographical coordinates	N 04° 53.017’ E 115° 20.264’	N 04° 50.195’ E 115° 00.153’	N 04° 28.086’ E 113° 59.788’	N 03° 58.636’ E 113° 42.549’	N 03° 30.954’ E 113° 18.110’	N 02° 54.598’ E 112° 05.390’	N 02° 31.106’ E 111° 23.632’	N 02° 08.437' E 111° 11.340'	N 01° 47.486’ E 111° 06.492’	N 01° 47.760' E 109°47.292’
pH	6.45	6.6	7.0	6.9	6.9	6.9	6.8	7.3	7.6	7.7
DO (mg l^{-1})	1.84	5.2	6.9	5.0	6.6	3.3	4.9	12.2	4.1	11.9
Temperature (C°)	30	30.8	27.0	27.7	30.5	26.4	26.6	29.9	28.8	27.3
Salinity (PSU)	23	20.3	26.0	19.0	28.0	20.3	21.0	30.0	28.5	32.0
Sand %	75	3.3	52.4	97.4	27.3	97.3	16.3	98.6	34.8	70.6
Silt %	20	78.4	33.1	2.6	54.2	1.4	64.9	1.1	57.7	24.7
Clay %	4.9	18.3	14.5	0.0	18.4	1.2	18.8	0.3	7.5	4.7

Table 3 Nematode faunal parameters calculated on the assemblages of each river mouth

Faunal parameters	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
c-p1	0%	0%	0%	0%	0%	defaunated	0%	0%	0%	0%
c-p2	45%	100%	55%	90%	24%	defaunated	47%	42%	100%	75%
c-p3	38%	0%	19%	9%	7%	defaunated	53%	17%	0%	19%
c-p4	0%	0%	26%	1%	0%	defaunated	0%	42%	0%	6%
c-p5	18%	0%	0%	0%	69%	defaunated	0%	0%	0%	0%
MI	2.9±0.1	2.0±0.0	2.7±0.1	2.1±0.0	4.2±0.1	defaunated	2.5±0.2	3.0±0.4	2.0±0.0	2.3±0.2
H'	1.2±0.1	0.2±0.3	2.4±0.6	2.4±0.0	1.4±0.3	defaunated	1.6±0.1	2.1±0.2	0.8±1.1	2.1±0.6

Table 4 Ecological classification of the study sites in agreement with the nematode descriptors

Nematode descriptors	Site									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
c-p %	56	0	45	10	76	defaunated	53	59	0	25
MI	2.9	2.0	2.7	2.1	4.2	defaunated	2.5	3.0	2.0	2.3
H'	1.2	0.2	2.4	2.4	1.4	defaunated	1.6	2.1	0.8	2.1
EcoQ	moderate EcoQ	bad EcoQ	moderate EcoQ	bad EcoQ	good EcoQ	bad EcoQ	moderate EcoQ	moderate EcoQ	bad EcoQ	poor EcoQ

Table 5 Environmental status of the study sites in the period 2004-2008 using the DOE National Water Quality Standards for Malaysia (N/A = data not available)

Years	Data	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
2004	Organic matter	Slightly pollutted	Slightly pollutted	Polluted	Slightly pollutted	Slightly pollutted	Slightly pollutted	Slightly pollutted	Slightly pollutted	N/A	N/A
	Suspended solid	Polluted	Polluted	Polluted	Polluted	Polluted	Polluted	Heavily polluted	Heavily polluted	N/A	N/A
	Total fecal coliform	Heavily polluted	Heavily polluted	Heavily polluted	Heavily polluted	Polluted	Heavily polluted	Heavily polluted	Heavily polluted	N/A	N/A
	Trace elements	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005	Organic matter	Clean	Clean	Polluted	Clean	Clean	Clean	Clean	Slightly pollutted	N/A	N/A
	Suspended solid	Polluted	Polluted	Clean	Clean	Clean	Polluted	Polluted	Polluted	N/A	N/A
	Total fecal coliform	Heavily polluted	Heavily polluted	Heavily polluted	Heavily polluted	Polluted	Polluted	Heavily polluted	Heavily polluted	N/A	N/A
	Trace elements	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2007	Environ. Quality	N/A	N/A	Polluted	Slightly pollutted	Clean	N/A	N/A	N/A	N/A	N/A
2008	Environ. Quality	N/A	N/A	Slightly pollutted by hydrocarbon and suspended solids; polluted by bacteria, trace elements	Slightly pollutted	Clean	Polluted by suspended solids, hydrocarbon, trace elements	N/A	Slightly pollutted by hydrocarbon	Slightly pollutted by suspended solids	Slightly pollutted by domestic sewage and hydrocarbon