A methodology for applying Taxonomic Sufficiency and benthic biotic indices in two Mediterranean areas.

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Abstract. Biotic indices have been developed to summarise information provided by benthic macroinvertebrates, but their use can require specialized taxonomic expertise as well as a time-consuming operation. Using high taxonomic level in biotic indices reduce sampling processing time but should be considered with caution, since assigning tolerance level to high taxonomic levels may cause uncertainty. A methodology for family level tolerance categorization based on the affinity of each family with disturbed or undisturbed conditions was employed. This family tolerance classification approach was tested in two different areas from Mediterranean Sea affected by sewage discharges. Biotic indices employed at family level responded correctly to sewage presence. However, in areas with different communities among stations and high diversity of species within each family, assigning the same tolerance level to a whole family could imply mistakes. Thus, use of high taxonomic level in biotic indices should be only restricted to areas where homogeneous community is presented and families across sites have similar species composition.

Keywords: Macroinvertebrates; Taxonomy Sufficiency; Biotic index; BENTIX; BOPA; Mediterranean

Introduction.

The high population density in coastal areas has led to many environmental problems and the need of developing effective strategies for their monitoring and evaluation of their ecological status. In order to attain this aim, several tools have been developed, using ecological indicators to supply synoptic information about this status (Salas et al., 2006). One of the components more widely used as indicator in marine environmental assessment is the macrobenthos. Benthic organisms are good ecological indicators because they are relatively sedentary, thus unable to avoid deteriorating water/sediment quality. They have relatively long life-spans, show marked responses to stress depending on their species-specific sensitivity/tolerance levels, and play a vital role in cycling nutrients and materials between the underlying sediment and the overlying water column (Borja et al., 2000; Dauer, 1993; Dauvin et al., 2007; Ferraro and Cole, 1995; Gray et al., 1988).

Several biotic indices have been developed to summarise information provided by the status of benthic communities. These indices are useful tools to communicate with managers because they reduce complex scientific data, integrate different types of information, and produce results that can be easily interpreted in the perspective of water quality management (Chainho et al., 2007; Wilson and Jeffrey, 1994). However, some of these indices require taxonomic classification to species level that can be a labour intensive and time-consuming operation (De Biasi et al., 2003), producing a reduction of the use of macroinvertebrates in monitoring studies due to requirement of specialized taxonomists, sampling and identification costs (Bilyard, 1987; Dauvin et al., 2003; Warwick, 1993).

According to several authors, analyses of higher than species taxonomic level may not produce a substantial loss of discriminatory power for studies of anthropogenic effects on benthic infauna (Ellis, 1985; Ferraro and Cole, 1990). These higher levels (e.g. family) give useful results in multivariate ordinations, since if the abundance and composition of taxa differ in polluted and unpolluted areas, little or no relevant information may be lost by identifying organisms to higher taxonomic level (Gray et al., 1990; Help et al., 1988; Herman and Heip, 1988; Olsgard et al., 1997; Somerfield and Clarke, 1995; Warkick, 1993). However, the taxonomic level of identification may have a great influence on biotic indices, since sensitivity to pollution for the same taxonomic group may differ from one species to another. In this way, biotic indices as BENTIX (Simboura and Zenetos, 2002), AMBI (Borja et al., 2000) or BQI (Rosenberg et al., 2004) were created on the basis of taxonomic identification at the species level, attributing a sensitivity/tolerance level to each individual species. Some studies proposed to develop an adaptation of these indices for use at high taxonomic level (Dimitriou et al., 2012) and two biotic indices have been developed in order to reduce this taxonomic effort solving problems registered from use of high taxonomic levels: BOPA (Dauvin and Ruellet, 2007) and BITS (Mistri and Munari, 2008). BOPA index is restricted to a part of the community, being based on the principle of antagonism between a sensitive group (order Amphipoda) and opportunistic polychaetes (Dauvin and Ruellet, 2007). Whereas BITS is based on the whole community working at family level identification, though it is specific for Italian non-tidal lagoons, where each family is composed by a few number of species (Mistri and Munari, 2008).

BENTIX (Simboura and Zenetos, 2002) has been tested successfully in a variety of Eastern Mediterranean benthic ecosystem subjected to organic pollution, oil spills accidents, mining-impacted ecosystems (Simboura et al., 2005, 2007; Zenetos et al., 2004) and aquaculture (Simboura and Argyrou, 2006). This index classifies benthic species in only two groups, with respect to other indices like AMBI that requires classifying taxa in 5 groups. Therefore, an approach for higher taxonomic levels could be affordable to BENTIX since certain families, for example Capitellidae, have a clear disturbance reaction pattern and this family could be assigned to the "tolerant" group, while on the other hand capitellid species are classified among three different groups by AMBI (GIII, GIV and GV). However, other families could contain species with different tolerance levels, such that they would present different disturbance reaction depending on their specific composition of the studied community or area. To cope with the main inconvenience of assigning ecological groups to these taxa, we used a methodology based on Smith et al (2001) and Pelletier et al. (2010) which allow to determine the degree of affinity of each taxa with disturbed or undisturbed conditions in a specific area. The aim of this paper is to elucidate the advisability of using higher taxonomic level in biotic indices normally using species level information as BENTIX, and in biotic indices that are partly using species information (opportunistic polychaetes) as BOPA, testing their response to sewage pollution in two different areas of the Mediterranean Sea: Iberian Peninsula east coast (Spain, Western Mediterranean) and Saronikos Gulf (Greece, Eastern Mediterranean).

Material and methods.

Studied areas.

The Western Mediterranean area (figure 1) is located off the Iberian Peninsula east coast (NE Spain) where five locations affected by sewage outfalls were analyzed. These outfalls correspond to the villages of Vinaroz (location I), Benicarló (location II), Peñíscola (location III), Alcossebre (location IV) and Torreblanca (location V) (figure 1a). Wastewater is discharged through submarine pipelines at a depth of approximately 15 m. The mean sewage flow reaches 222597 m^3/month; the highest flow was registered in location II (502612 m^3/month) whereas the lowest was registered in location V (43256 m^3/month). Wastewater treatment plants from locations I, II, III and IV utilize only a pre-treatment process, which includes an automated mechanically raked screen, a sand catcher and grease trap. Whereas, secondary treatment, consisting of biological treatment of activated sludge, was implemented in location V. The study area has a constant water depth, homogeneous bottom sediment and is uniformly inhabited by the medium-to-fine sand community of *Spisula subtruncata* (da Costa, 1778). This homogeneous area with several well established pollution spots represents an ideal site for investigating links between macrofaunal assemblages and the effect of contaminants (de-la-Ossa-Carretero et al., 2009, 2010; Del-Pilar-Ruso et al., 2010). At each location, two sites were sampled at three distances from the discharge (0, 200 and 1000 m), following the coastline in order to keep a constant depth. All samples were collected in July during five consecutive years (2004 to 2008). Three Van Veen grab samples (400 cm^2) were obtained at each station for the study of the benthic community; samples were sieved through a 0.5 mm screen and preserved in 10% formalin for further identification at family taxonomic level.

The Eastern Mediterranean area is located in Saronikos Gulf, surrounding the Athens metropolitan area (figure 1b). The gulf receives the effluents of the central sewage outfall of Athens through a deep underwater outlet situated on Psittalia Island, at the inner part of Saronikos Gulf, discharging through two 1870 m long submarine pipelines and at a depth of approximately 65 m the treated urban sewage effluents. The Psyttalia Waste Water Treatment Plant-WWTP (http://www.eydap.gr/) is the main wastewater treatment plant in the greater Athens area, receiving an average wastewater flow of approximately 730000 m^3/d. It has been in operation since 1994, through a stage-wise construction that involved three phases: Phase A (Primary treatment) completed in 1994, Phase B (advanced Secondary biological treatment) , using activated sludge processes was completed in 2004 and Phase C (Tertiary treatment), comprising the Sludge Thermal Drying Unit completed in 2007. Nowadays wastewater treatment achieves suspended solids and organic load reduction by about 93% and total nitrogen reduction by about 80% in comparison with influent loads. At the time of the study period (2000-2004) only the primary treatment was functioning, while the secondary had been just implemented.

The effects of the Psittalia sea outfalls on the ecosystem of Saronikos Gulf have been monitored regularly by the Hellenic Centre for Marine Research (HCMR) from 1986 (Krasakopoulou et al., 2010; Siokou-Fragou et al., 2004) and up to date.

Samples were collected from a network of 19 stations in the inner Saronikos Gulf, extending for the Psittalia outfall station (S7) and at an increasing distance for the point source of the effluents (figure 1B). Fifteen stations were visited in May, December 2000 and April 2002 and 19 stations in May 2003 and February 2004. At each station two replicate benthic samples were collected with a Box Corer benthic sampler of 1000 cm^2 sampling surface and sieved through a 1 mm sized mesh. Samples were preserved in a 4% buffered formalin solution and the fauna were sorted and identified to the species level, except from broken or unidentifiable specimens.

Family tolerance classification.

The BENTIX index (Simboura and Zenetos, 2002) was designed to fit the Mediterranean benthic ecosystem. It is based on the concept of indicator groups and uses the relative contribution of tolerant (GT) and sensitive species (GS) in the fauna weighted analogously to derive a single formula

$$BENTIX= [(6) (\%GS) + (2) (\%GT)]/100$$

where the numerical factor ‘6’ is assigned to the sensitive species group GS and the factor ‘2’ to the tolerant species groups GT (http://bentix.ath.hcmr.gr/).

In order to cluster families in these groups, a method based on Pelletier et al. (2010) and Smith et al. (2001) was employed.

Firstly, stations from the two areas were selected based on previous studies (de-la-Ossa-Carretero et al. 2009; Simboura et al., 2005; Simboura and Reizopoulou, 2008) to clearly reflect a well defined bipolar situation with one end corresponding to disturbed ones and the other end to undisturbed conditions. In the Iberian Peninsula east coast (figure 1), stations closest to outfalls from locations where wastewater treatment plants utilise only a pre-treatment process (locations I, II, III and IV) were classified as disturbed, whereas stations at 1000 meters to these outfalls were considered in undisturbed condition. In Saronikos Gulf, apart from the distance from the outfall another important factor, the hydrology and dispersion pattern of the sewage plume was also taken into account. Moreover, the magnitude of the pollutant load depends also on the amount and quality of the sewage flow and they were critical factors for the selection of the distance to the outfall for designing “disturbed” and “undisturbed” stations. Thus, stations up to 5000 m from Psittalia island (S7, S3, S8, S39Z, S26, marked in the map as black circles) were classified as disturbed or affected by Psittalia. Station S8 located at around 8000 m from Psittalia island was selected among the “disturbed” stations as being located in the south-western direction of the gulf. Stations at a distance greater than 5000 m from the outfall and located at the eastern side of the gulf (S11A, S11B, S16, S16A, S16B, S26B, marked in the map as white circles) were considered undisturbed. Another group of stations (marked in the map of figure 1 as grey circles), namely S11, S26A, S46Z and S13 was characterised as in intermediate condition or “influenced” by Psittalia outfalls, their distance being greater than 5000 m.

Besides samples not classified as disturbed or undisturbed conditions, the last sampling year of both areas was excluded of the available dataset for this classification process, in such a way that around 50% of the sampled stations were selected to develop the analysis leaving the other 50% to validate results obtained when this classification of family tolerance level is applied in BENTIX index (table 1).Principal Component Analysis was used to ordinate these selected disturbed and undisturbed stations based on family composition. These stations were scaled, projecting them in only one pollution vector from undisturbed to disturbed stations, obtaining a score for each station referred with this axis. The pollution vector was established based on the position of all the stations looking for the vector that divide in the best way between disturbed and undisturbed stations. This pollution score was calculated using the following equation (Pelletier et al., 2010):

$$\text{Pollution score} = \text{SCORE 1} \times \cos(\alpha) + \text{SCORE 2} \times \sin(\alpha)$$

where SCORE 1 and 2 are the scores for Principal Components and α is the arctangent of the pollution vector slope. This projected score was rescaled from 0 to 100 in order to aid in interpretation.

The position of families *i* (*pi*) on this pollution vector defined in the ordination space was computed as (Smith et al., 2001):

where a_{ij} is the abundance of families *i* in sample *j, t* is the total of samples *j* in vector score, and g_j is the pollution projected score on the ordination gradient for sample *j.*

According to this position, BENTIX groups were assigned to each family. In this way, sensitive group (GS) was assigned to families whose position corresponded to pollution score of undisturbed stations; meanwhile tolerant group (GT) was assigned to families whose position corresponded to pollution score of disturbed stations. Boundary score between tolerant and sensitive group was established based on mean between 90th and 10th percentile of pollution score of undisturbed and disturbed stations. Families occurring in less than 4 stations were considered rare and they were not grouped but were assigned to group 0 that in the BENTIX software correspond to the non assigned taxa. Because of the low abundance of rare families, there were no cases where the BENTIX result was classified by the software as a low confidence assessment.

Once these groups were assigned to each family at different areas, BENTIX index was calculated using family abundance matrix (hereafter BENTIX_{fam}) of stations excluded from this family tolerance classification to validate the results of the method.

Comparison of results according to taxonomic level analysis and between biotic indices (BOPA and BENTIX).

Agreement and correlation between BENTIX and BOPA indices for both identification levels, species and family, was analysed. BOPA index was calculated according to Dauvin and Ruellet (2007) using the modified thresholds presented in de-la-Ossa-Carretero and Dauvin (2010) for Ecological Status classification. For species level identification, we used the list of opportunistic polychaetes genera and species given by Dauvin and Ruellet (2007), whereas for family level identification we used Capitellidae, Spionidae, Cirratulidae and Dorvilleidae since they are usually accepted as tolerant and most of the species of Dauvin and Ruellet (2007) list belong to these families (Hilbig, 1995; Karakassis et al, 2000; Pearson and Rosenberg, 1978).

Weighted Kappa analysis (Cohen, 1960; Landis and Kosch, 1977) was used to evaluate the agreement, employing the methodology proposed by Borja et al. (2007). The equivalence table from Monserud and Leemans (1992) was used to establish the level of agreement of the two indices. In addition, since the importance of misclassification is not the same between close categories (e.g., between high and good, or poor and bad) as between distant categories (e.g., between high and moderate, or high and bad), we chose to apply Fleiss–Cohen weights (Fleiss and Cohen, 1973). The Pearson correlation coefficient was calculated between ecological quality ratios (EQR) of each index and identification level. Full comparison was only carried out in Eastern Mediterranean data, since data at species level was not available from the Iberian Peninsula east coast.

Results.

Western Mediterranean.

PCA analysis showed a clear stratification between stations closer to outfall and stations sited at 1000 m distance from these outfalls. Pollution vector was established from stations sited at 1000 m to station sited at outfall corresponding to location II (figure 2).

Pollution scores assigned to each location showed a range of values from 0 to 23.19 at undisturbed stations (stations at 1000 m distance from outfalls), and from 21.31 to 100 at disturbed stations (stations closest to outfalls) (figure 3). Boundary score between disturbed and undisturbed stations was established as 23.39. Based on this boundary and p_i of each family, GS was assigned to 41 of the 132 families identified, GT was assigned to 49 families and 42 families were not grouped due to their low occurrence.

BENTIX$_{fam}$ assigned always high and good status to stations at 200 and 1000 m to outfalls and the station closest to the outfall of station V (the one with the best water treatment). Meanwhile, stations closest to outfall of location II were evaluated as moderate or poor status, and stations closest to outfalls of locations I, III and IV were evaluated as moderate status. The same classification pattern was obtained through all sampling years, including year 2008 which was excluded in family tolerance classification process (figure 4).

Eastern Mediterranean.

PCA analysis showed a clear stratification of stations considered disturbed and undisturbed. Pollution vector was established from undisturbed station 16B to station sited at Psittalia outfall S7 (figure 5).

Pollution scores assigned to each location showed a range of values, from 0 to 22.21 at undisturbed stations, and from 24.62 to 100 at stations closer to outfalls. Score value of 22.69 was established as boundary between scores of undisturbed and disturbed stations (figure 6). Based on this boundary and p_i, GS was assigned to 52 of the 194 families identified, GT was assigned to 42 and 100 families were not scored due to their low occurrence.

All the stations close to Psittalia outfall characterized in the methodological approach as "disturbed" were classified as moderate, poor or bad status by BENTIX$_{fam}$, including samples of the last sampling campaign which was excluded of the family tolerance classification process. According to BENTIX based on the species level these stations were characterized as poor or moderate.. Regarding to stations sited in the influence area of the outfall (characterized in the methodological approach as "influenced"), moderate status was commonly assigned to S11 and S26A (as also with the BENTIX species assessment), whereas S13 and S46Z were classified as poor and one campaign of station S11 obtained a good status. Finally, good and high status was assigned to most of the stations considered undisturbed in the methodological approach, including the last sampling campaign, with the exception of some samples of S16 and S16A that were classified in moderate status (figure 7). When using BENTIX species level these "undisturbed" stations were characterized as mostly good, except station S16A that was classified as moderate.

Comparison of BENTIX and BOPA at family and species level identification.

Regarding to Saronikos Gulf, most of the comparisons showed a strong Pearson correlation, the highest value was obtained between values of BOPA with BOPA at family level (0.99), and BENTIX and BENTIX$_{fam}$ (0.88), whereas the lowest value was obtained between BENTIX$_{fam}$

and BOPA at family level, though the correlation was also strong (table 3). Kappa analysis showed a “good” agreement between most of the other comparisons with the exception of an “almost perfect” agreement between BOPA at different level identification and of “moderate” agreement between BENTIX and $BENTIX_{fam}$. Similar Pearson correlation and Kappa analysis values were obtained in Western Mediterranean area when $BENTIX_{fam}$ and BOPA at family level were compared (Pearson correlation: 0.74; Kappa value: 0.56(G)).

With respect to percentage of each Ecological Status derived from the different taxonomic levels in Saronikos Gulf area, $BENTIX_{fam}$ tended to classify a higher percentage of high and poor status than using BENTIX or BOPA, whereas in Iberian Peninsula east coast area $BENTIX_{fam}$ assigned a higher percentage of moderate and poor status with respect to BOPA at family level that classified a higher percentage of high status (figure 8). However, BENTIX at species level was the strictest option, since it produced the lowest percentage of acceptable conditions, good or high status, and the highest percentage of moderate status. On the other hand BOPA index gave similar results at different taxonomic levels, obtaining a high percentage of good and moderate status and showing a certain overestimation of the Ecological Status compared to the BENTIX.

Discussion.

Analyses of benthic community at high taxonomic levels have previously given useful results in multivariate ordinations for studies of anthropogenic effects on benthic infauna (de-la-Ossa-Carretero et al., 2011; Del-Pilar-Ruso et al. 2007; Gray et al., 1990; Olsgard et al., 1997; Somerfield and Clarke, 1995). Some authors also stressed that higher levels are more appropriate to reflect a contamination gradient than analyses based on species abundances, which are likely to be complicated by natural environmental heterogeneity (Olsgard et al., 1997; Warkick, 1988). However, it could be difficult to apply Taxonomic Sufficiency concept with indices like BENTIX (in which various species are divided into two groups according to their sensitivity to organic pollution) since many macrobenthic invertebrate congeners could have different pollution tolerances and species-level identification could be essential (Resh and Unzlcker, 1975).

In order to apply BENTIX index at family level identification, grouping families according to their sensitivity has to be a reliable process. Then, using high taxonomic levels with biotic indices could be a possibility but it should only be considered in specific and well studied areas with certain conditions. Areas with homogeneous bottom sediment and uniform benthic communities could allow more accuracy in this family level tolerance categorization, since species diversity and hence variability of degree of disturbance tolerance within each family is lower than in other areas where a variety of substrata and more diverse communities were detected. Saronikos Gulf presents different types of habitats (Simboura and Zenetos, 2002) and high diversity of species within each family is expected, while the Iberian Peninsula east coast analyzed area is characterized by a homogeneous substrate colonized by medium-to-fine sand community of *Spisula subtruncata* (de-la-Ossa-Carretero et al., 2009). Patchiness of Eastern Mediterranean studied area maybe the reason why a higher percentage of rare families occurring occasionally in some stations were not able to be grouped with respect to Western Mediterranean area, where Taxonomic Sufficiency was previously employed with good results using multivariate analysis (de-la-Ossa-Carretero et al., 2011; Del-Pilar-Ruso et al. 2010).

$BENTIX_{fam}$ showed a response to the presence of pollution due to sewage outfalls in both areas since the lowest values were obtained in stations adjacent to outfalls. Focusing in last sampling year, whose dataset was excluded in the family classification process,, in Iberian Peninsula east coast area the stations classified as the worst status correspond with those closest to the outfall characterized by the highest flow of sewage effluents in which only pre-treatment is operational, location II. Meanwhile, stations near the location V sewage outfall obtained the highest values of $BENTIX_{fam}$, since this is the location with the lowest flow and the only one where secondary treatment takes place. In Saronikos Gulf, the lowest index values were obtained in stations closest to Psittalia outfall, but though the highest values were obtained in undisturbed stations (S11B and S26B), other undisturbed stations sited far away from the outfall (S16 and S16A) obtained low $BENTIX_{fam}$ values and were classified in moderate status during the last sampling campaign. As discussed above, in areas with high diversity of benthic communities and benthic habitats, the probability of diversification of species' response to disturbance within the same family increases. In the case in which in an area are found species of the same family with different responses to disturbance and those species are dominant in the community, the use of indices based on Taxonomic Sufficiency would be inappropriate for pollution monitoring (Dauvin et al., 2003; Mistri and Munari, 2008).

Results obtained with $BENTIX_{fam}$ were similar to those obtained with BOPA index (de-la-Ossa-Carretero et al., 2009) showing a good agreement between both indices in the two areas. However, $BENTIX_{fam}$ Ecological Status classification was more severe in stations close to outfalls with low sewage flow only pre-treated, in such a way that stations classified as good status by BOPA, were considered as moderate during some sampling campaigns when $BENTIX_{fam}$ was employed. BOPA index only takes into account amphipods and opportunistic polychaetes, whereas BENTIX considers the whole community. E.g., some families of bivalves are scored in this work as tolerant or opportunistic taxa, as Lucinidae or Tellinidae. Considering these families would allow the detection of changes in benthos that are not considered using BOPA index.

On the other hand, BOPA clusters all amphipods, except the genus *Jassa* Leach (Ischyroceridae), in the same group; however, in order to calculate $BENTIX_{fam}$, some amphipods families were assigned to tolerant group following the proposed PCA methodology. Although amphipods are generally more sensitive to polluted sediments than other benthic organisms (Arvai et al., 2002; Cesar et al., 2004; Dauvin and Ruellet, 2009; Rand and Petrocelli, 1985; Riba et al., 2004), not all species showed the same level of sensitivity. In this way, though most amphipod species were classified in the sensitive group by AMBI and BENTIX classifications, some amphipod species were recorded as tolerant. E.g., certain species of Corophiidae and Ampeliscidae have been reported as well adapted to environmental stress (Ingole et al., 2009; Lowe and Thompson, 1997). Both families were scored in different way for each studied area for $BENTIX_{fam}$ calculation (table 2). In this way, we can find discrepancies between amphipod species classification if we check the BENTIX and AMBI lists of scores. Adapting family level classification to BQI, Dimitriou et al., (2012) obtained low sensitivity values for both families, however some species presented higher sensitivity values indicating that occur in areas with no or minor disturbance. Discrepancies in tolerance among different species within the same genera could produce misclassifications (Rosenberg et al. 2004), Using BQI-family (Dimitriou et al., 2012), families with a few opportunistic species will tend to have sensitivity values close to that of their "opportunistic" members when these species will be very abundant in the disturbed stations of the data set. The family tolerance classification process presented in this paper would produce the same assumption. However, when BQI-family or BENTIX-family will be applied, the results of this inconsistency would not involve a misclassification (Dimitriou et al., 2012) since the higher abundances of these families would correspond to an increase of the abundance of their "opportunistic" members. Whereas in the case the "sensitive" members dominated, the family classification process would cluster the family in the sensitive group. These cases force to run this classification process in each area before applying the index based on family taxonomic level.

Other families also showed discrepancies among both areas when tolerance level is assigned (table 2). These discrepancies could be due to the differences of specific composition among studied communities or areas. Among crustaceans, cumaceans were classified in a different way; their species composition is depth-dependent and highly related to parameters as granulometry, grain size or organic content (Corbera and Cardell, 1995), parameters that vary among both areas. Among polychaetes several families showed discrepancies, e.g. Dorvilleidae was classified as tolerant for western Mediterranean sea because of the dominance of opportunistic *Ophryotrocha* genus, whereas this family was classified as sensitive in Saronikos Gulf where it was dominated by *Protodorvillea kefersteini* (McIntosh, 1869) and *Schistomeringos rudolphii* (delle Chiaje, 1828), species that though are classified as disturbance-indifferent species in AMBI and as tolerant by BENTIX, their abundance increased in undisturbed stations from Saronikos Gulf that were classified as good by BENTIX where a certain level of tolerant species may occur. Other families, despite only containing one species also showed discrepancies, as *Branchiostoma lanceolatum* (Pallas, 1774) family Branchiostomidae, species that despite being considered sensitive it was classified in tolerant group in Iberian Peninsula east coast where a high interannual variation of this species was previously reported (de-la-Ossa-Carretero et al. 2011).

Classification of species or families along a sensitivity-tolerance continuum is thus a very difficult task and still a matter of debate (Grémare et al., 2009; Labrune et al.; 2006). The need of revision of the ecological classification of species, taking into account also different environmental conditions and spatio-temporal variability, was highlighted by several authors (de-la-Ossa-Carretero et al., 2010; Ruellet and Dauvin, 2007; Tataranni and Lardicci, 2010), this revisions could help to discover general patterns at higher taxonomic level and check when this generalization could be adopted or should be avoided as in the case where natural variability could produce mistakes in the assignment of the tolerance level.

In this sense, previous studies only considered analyses at high taxonomic level as an acceptable procedure in environmental monitoring and pollution assessment programmes (Ferraro and Cole, 1995; Vanderklift et al., 1996; Warwick, 1988) due to the fact that it produces a significant reduction in cost without an important loss of information. E.g. in the case of Saronikos Gulf a total of 616 species that belong to 194 families were identified, taking into account that the savings associated are related to the number of categories where specimens must be placed (Ferraro and Cole, 1995; Thompson et al., 2003), using family taxonomic level could reduce the sampling processing time by an estimated 68% in Saronikos Gulf. This saving was higher than in other areas (Ferraro and Cole, 1995; Thompson et al., 2003), since there were a higher percentage of polytypic families. Eastern Mediterranean is an oligotrophic, euhaline and microtidal environment, where the benthic fauna is usually very diverse and evenly distributed with no one species naturally dominating over 10% and naturally a high number of species are evenly distributed over the benthic population (Simboura and Reizopoulou, 2008).

However, these analyses should be addressed with caution, taking into account that assigning tolerance level to high taxonomic levels can pose a certain degree of uncertainty in areas where high species diversity is expected. This could be the reason why using the Taxonomic Sufficiency concept could imply misclassifications in these areas, where patchiness of benthic communities has been described and high diversity within each family is expected, since assigning score to each family implies presuming that all species within the family have similar response. In this way, BITS (Mistri and Munari, 2008) were limited to non-tidal Italian lagoons, whose conditions favour the presence of few species in very high densities. In fact, despite using species level classifications Smith et al (2001) stratified Southern Californian Shelf in different depth zones before employing Benthic Response Index and Pelletier et al. (2010) categorized Virginian province into different benthic habitats before identifying estuarine macroinvertebrate pollution indicator species. Thus, employing a method as described in this work to assign tolerance groups to families should be only restricted in areas where a homogeneous community is presented and families have similar species composition.

Following these recommendations, the proposed methodology provides a tentative tool for applying Taxonomic Sufficiency concept with biotic indices as BENTIX with a sufficient degree of confidence, since this approach produces a good classification of areas affected by sewage disposals and allowed saving a significant sampling processing cost and time. In fact, the cost/benefit ratio (CB_L) (Karakassis and Hatziyanni, 2000; Muñiz and Pires-Vanin, 2005; Soares-Gomes et al., 2012) for using BENTIX at family level instead of species level in Saronikos Gulf was 0.17, indicating a weak loss of information. However, it is important to take into account that depending in the area this approach could entail problems. Since, the presence of congeners with different pollution tolerances, especially in areas with different types of habitats and high percentage of polytypic families, could pose a problem when it comes to assigning a tolerance group to each family. This problem could produce mistakes when Ecological Status should be established, in such a way that a correct knowledge of the specific composition of the studied areas would allowed to predict these errors.

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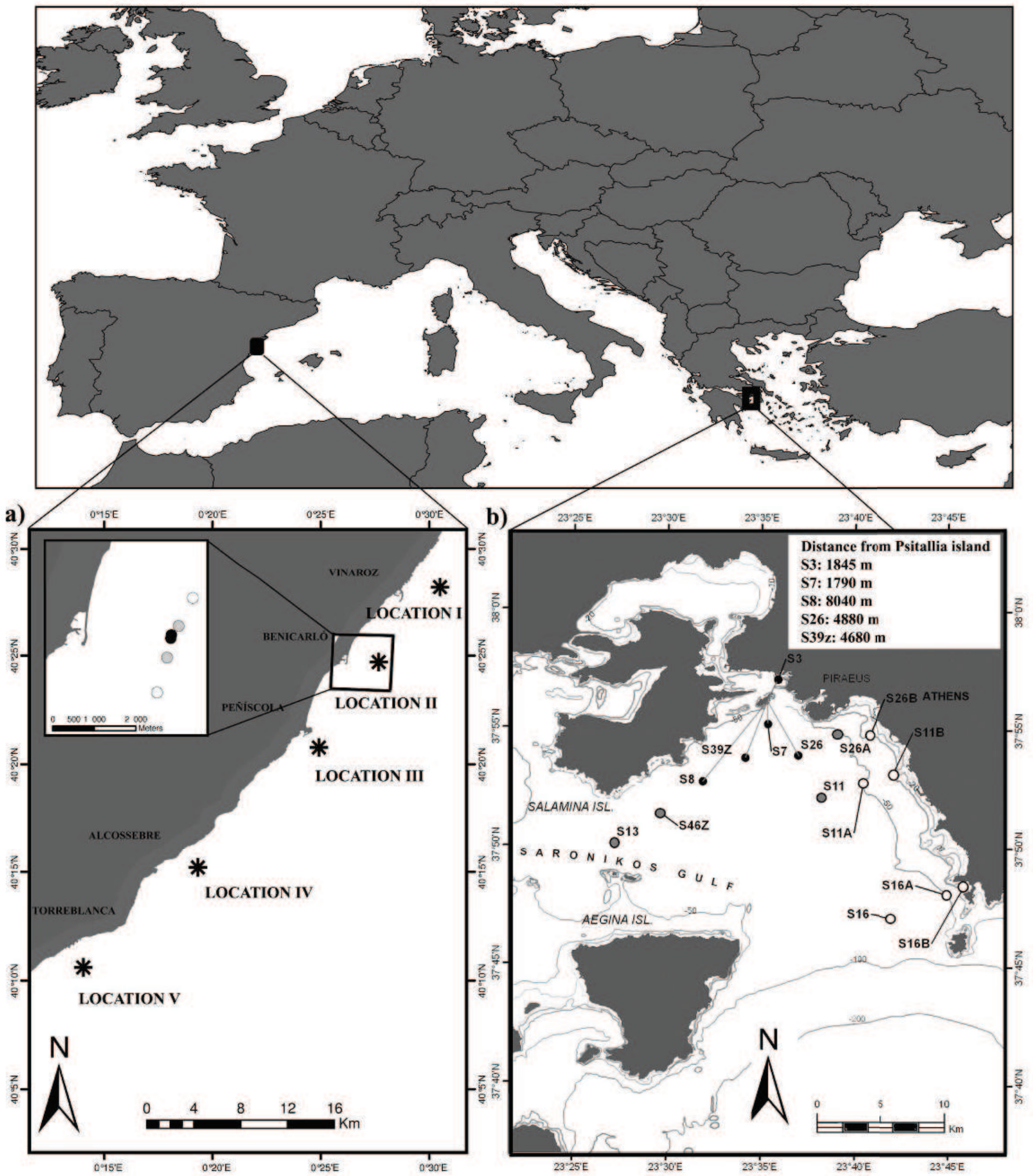

Figure 1. Study areas. a) Iberian Peninsula east coast. Example of sampling stations in location II (colour of circle is related to distance to outfall (black: 0 m, grey: 200 m and white: 1000 m to outfall). b) Saronikos Gulf (colours of circles indicated level of impact from Psittalia outfall: black: disturbed stations; grey: influenced stations; white: undisturbed stations).

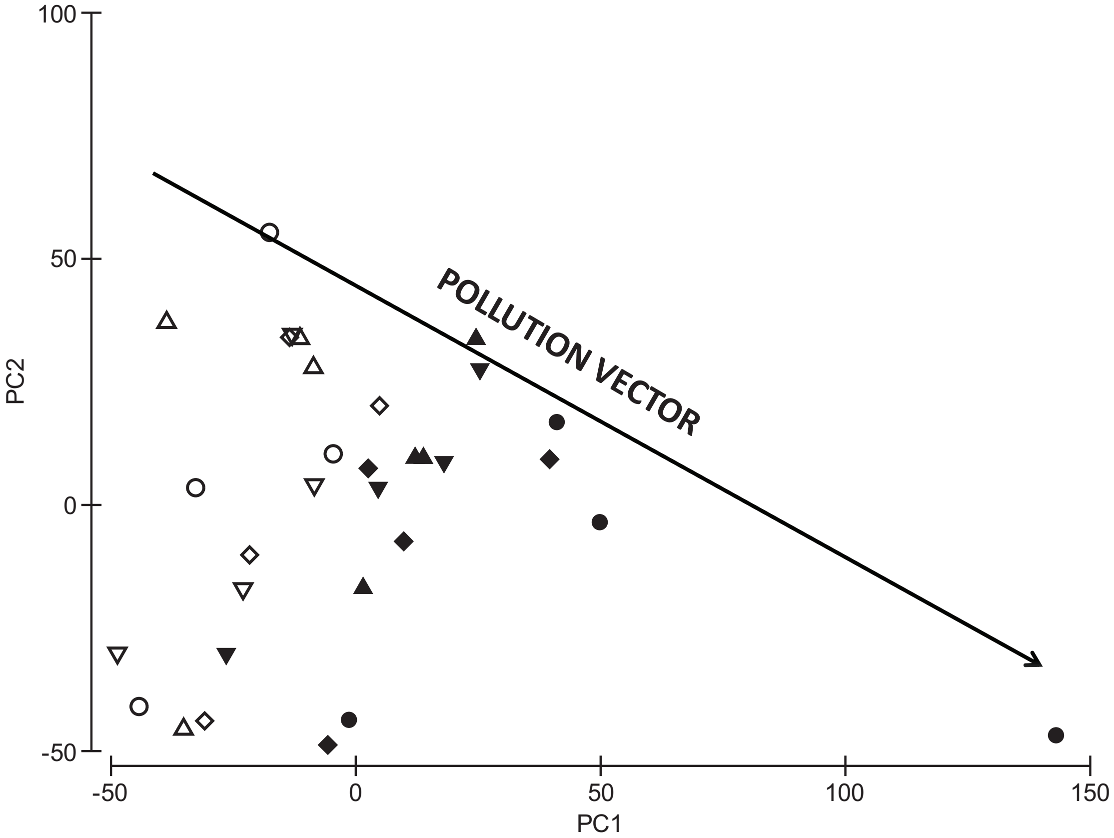

Figure 2. Principal Components Analysis (PCA) of stations from Iberian Peninsula east coast, adjacent and at 1000 m to the sewage outfalls . Percentage of variation explained, PC1: 37.1 %, PC2: 19.5%. Differentiating location (I: ▼, II: ●, III: ♦, IV: ▲) and distance to the outfall (black: 0 m and white: 1000 m). Vector indicated pollution direction to the most disturbed station.

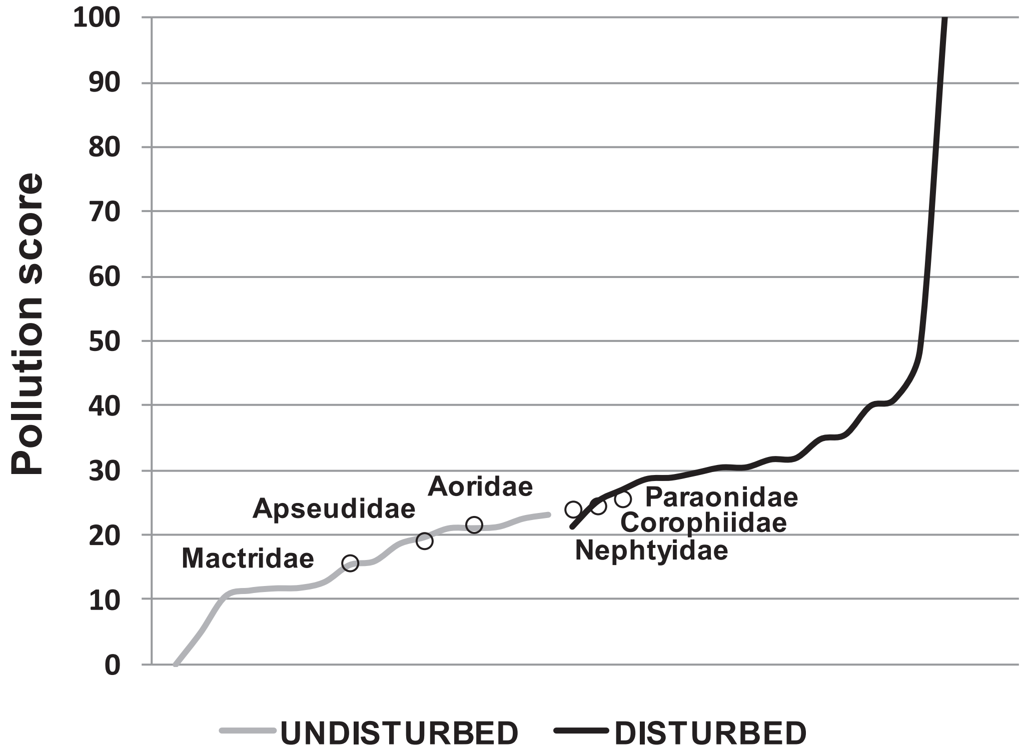

Figure 3. Pollution scores of disturbed (black line) and undisturbed stations (grey line) of Iberian Peninsula east coast and position (p_i) of the most abundant families.

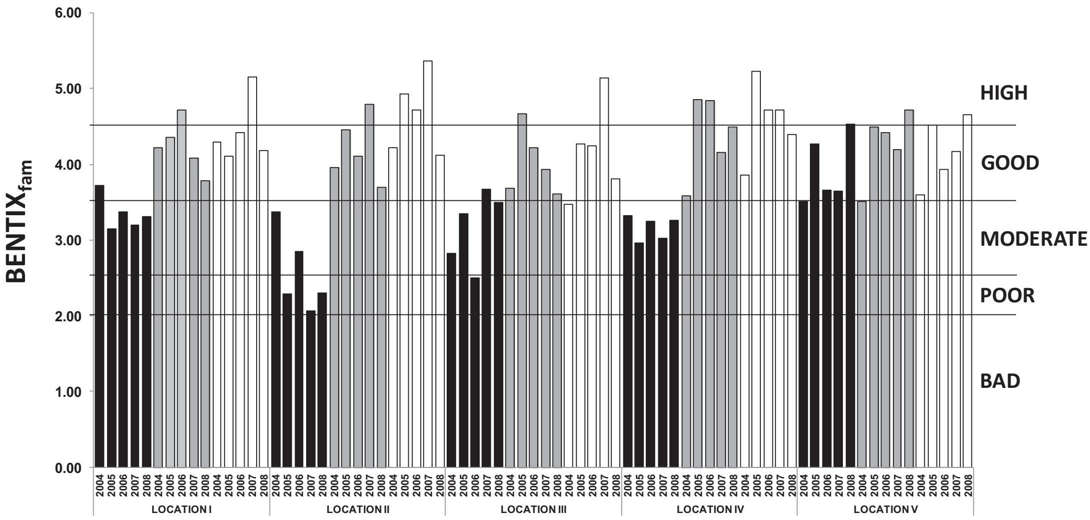

Figure 4. BENTIXfam values for Iberian Peninsula east coast. Colour of bars differentiates distance to the outfall (black: 0 m, grey: 200 m and white: 1000 m).

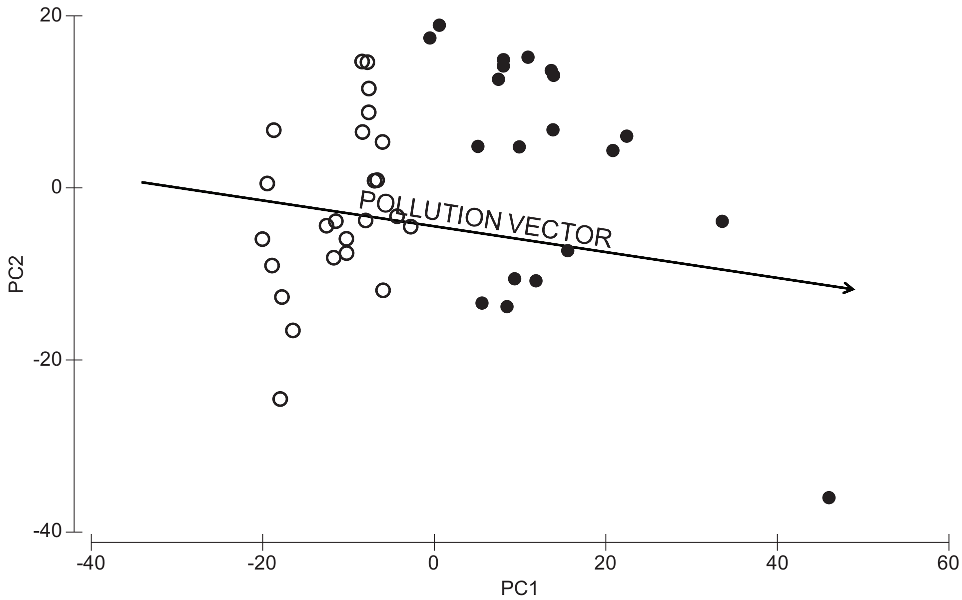

Figure 5. Principal Components Analysis (PCA) of stations of Saronikos Gulf. Differentiating between disturbed stations (black circles) and undisturbed (white circles). Percentage of variation explained, PC1: 29.6 %, PC2: 19.7%. Vector indicated pollution direction to the most disturbed station.

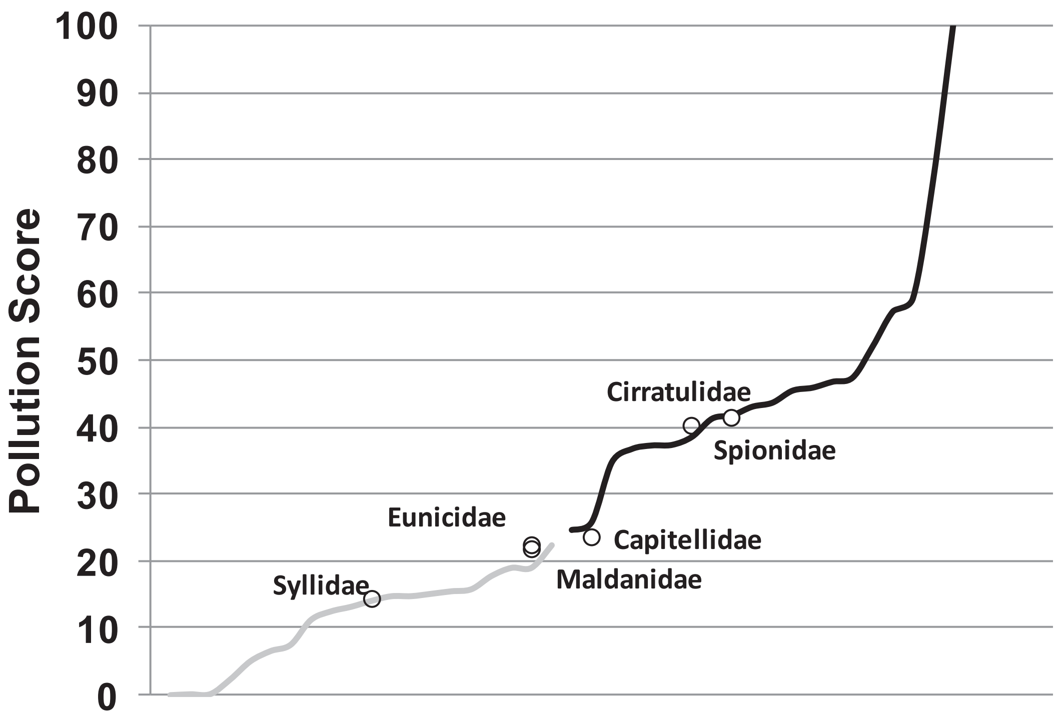

Figure 6. Pollution scores of disturbed (black line) and undisturbed stations (grey line) of Saronikos Gulf and position (p_i) of most abundant families.

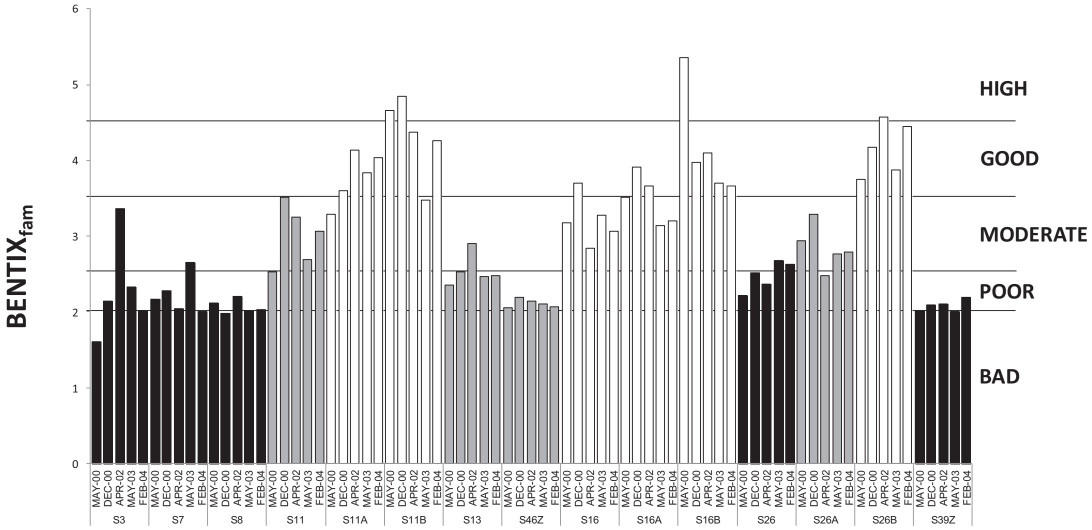

Figure 7. BENTIX$_{fam}$ for Saronikos Gulf data. Colours of bars indicated level of impact from Psittalia outfall: Black: disturbed stations; grey: influenced stations; white: undisturbed stations.

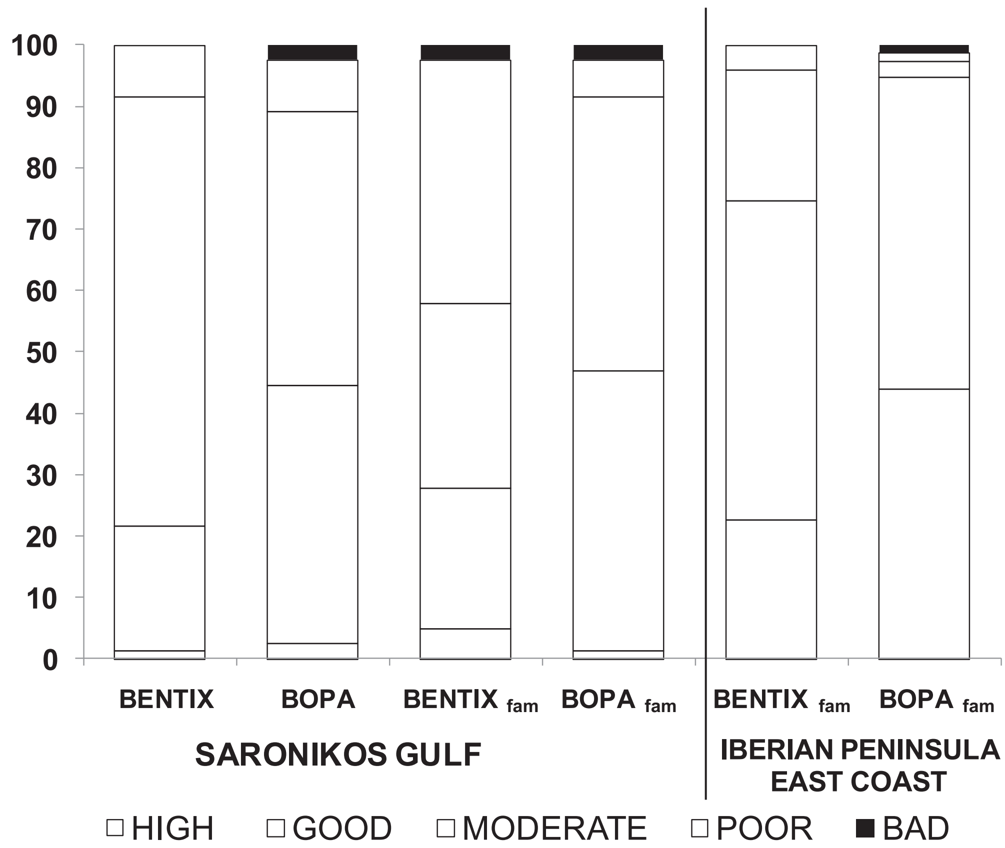

Figure 8. Percentage of each Ecological Status derived from BENTIX and BOPA at different taxonomic levels (species and family).

Table 1. Number and description of samples of each area included and excluded in family tolerance classification process.

			Number of averaged samples and description
Iberian Peninsula East coast			
	Total	75	5 locations x 3 distances x 5 years
	Stations included	32	4 locations x 1 distance (0 m.) x 4 years
			4 locations x 1 distance (1000 m.) x 4 years
	Stations excluded	43	4 locations x 1 distance (0 m.) x 1 year
			4 locations x 1 distance (1000 m.) x 1 year
			4 locations x 1 distance (200 m.) x 4 years
			1 location x 3 distances x 5 years
Saronikos Gulf			
	Total	83	15 stations x 5 years
			4 stations x 5 years
	Stations included	44	5 disturbed stations x 4 years
			6 undisturbed stations x 4 years
	Stations excluded	39	5 disturbed stations x 1year
			6 undisturbed stations x 1 year
			4 stations x 5 years
			4 intermediate stations x 2 years

Table 2. Position (pi) and group assigned to abundant families, GS (group of sensitives) or GT (group of tolerants).

		Iberian Peninsula East coast		**Saronikos Gulf**	
Taxa		pi	GROUP	pi	GROUP
Amphipoda	Ampeliscidae	24.24	GT	21.16	GS
	Aoridae	21.71	GS	19.95	GS
	Caprellidae	23.13	GS	12.99	GS
	Corophiidae	24.59	GT	17.39	GS
	Leucothoidae	20.48	GS	20.13	GS
	Lysianassidae	27.46	GT	16.80	GS
	Oedicerotidae	21.58	GS	13.96	GS
	Photidae	21.23	GS	0.00	0
	Pontoporeiidae	18.20	GS		
	Urothoidae	20.06	GS	11.91	GS
Cumacea	Bodotriidae	23.75	GT	16.09	GS
Decapoda	Paguridae	29.95	GT		
Tanaidacea	Apseudidae	19.20	GS	13.45	GS
Bivalvia	Lucinidae	36.67	GT	31.26	GT
	Mactridae	15.77	GS		
	Veneridae	19.48	GS	23.18	GT
	Thyasiridae			53.21	GT
Cephalochordata	Branchiostomidae	27.37	GT	1.06	GS
Ophiuroidea	Amphiuridae	20.16	GS	18.43	GS
Polychaeta	Ampharetidae	21.12	GS	27.51	GT
	Capitellidae	32.43	GT	22.92	GT
	Cirratulidae	21.46	GS	41.44	GT
	Dorvilleidae	74.48	GT	14.96	GS
	Eunicidae	20.92	GS	21.63	GS
	Glyceridae	26.66	GT	31.87	GT
	Hesionidae	28.69	GT	23.56	GT
	Lumbrineridae	23.32	GS	23.84	GT
	Magelonidae	25.26	GT	16.21	-
	Maldanidae	22.52	GS	22.48	GS
	Nephtyidae	24.08	GT	24.12	GT
	Nereididae	28.76	GT	17.46	GS
	Onuphidae	23.48	GT	15.57	GS
	Orbiniidae	23.65	GT	12.24	GS
	Paraonidae	25.69	GT	30.68	GT
	Phyllodocidae	25.46	GT	26.89	GT
	Sabellidae	21.16	GS	17.08	GS
	Syllidae	24.92	GT	14.47	GS
	Spionidae	27.34	GT	40.21	GT
Sipuncula		21.91	GS	13.96	GS
Nemertea		23.63	GT	33.47	GT
Nematoda		23.93	GT	9.49	GS

Table 3. Kappa values with agreement levels in parentheses (lower left) for the Ecological Status classification and Pearson coefficient values (upper right) for Saronikos Gulf data. (Level of agreement: AP–'Almost perfect'; G–'Good'; M–'Moderate';').

		BENTIX		**BOPA**	
		Species	Families	Species	Families
BENTIX	Species	-	0.88	0.85	0.84
	Families	0.50 (M)	-	0.81	0.8
BOPA	Species	0.56 (G)	0.56(G)	-	0.99
	Families	0.58(G)	0.55(G)	0.92(AP)	-