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(Version 5.0)



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# Instructions for the use of the AMBI index software (Version 5.0)

Ángel Borja, Julien Mader, Iñigo Muxika<sup>1</sup>

## Abstract

The AZTI's Marine Biotic Index (AMBI) was developed in 2000. Some years later a software was created for the calculation and made available in the page <http://ambi.azti.es>. Several versions of this freely available software have been created. In this paper we explain the new AMBI 5.0 version, for Windows 7, which has some novelties regarding the import of data for the calculation, the change of species and the process of saving all the information. Moreover, a new species list, with more than 6300 species from the entire world has been included.

## Resumen

El índice AMBI (AZTI's Marine Biotic Index) fue desarrollado en el año 2000. Algunos años más tarde se creó un programa para el cálculo del índice y se creó una página para su descarga (<http://ambi.azti.es>). Desde entonces, varias versiones del programa han visto la luz. Ahora presentamos el nuevo AMBI 5.0, para Windows 7, que incluye varias novedades relacionadas con la importación de los datos, el cambio de las especies y el proceso de guardar la información. Además, se ha añadido una nueva lista de especies, con más de 6300 especies de todo el mundo.

## Introduction

In response to the publication of the paper of Borja *et al.* (2000), on the AZTI's Marine Biotic Index (AMBI), for the assessment of benthic quality, many scientists expressed an interest in the procedure for the calculation of such index.

This pushed us to create a freeware application which would facilitate the work and the extension of this tool. The application was made available at AZTI's web page (<http://ambi.azti.es>). After the creation of this software the use of AMBI has been extended in the Ecological Status assessment, within the Water Framework Directive (WFD) (Borja *et al.*, 2004a) and has been used in many countries worldwide (see the list of references at the end).

As the use of AMBI alone can produce some problems in such assessment, we propose the use of Factorial Analysis, including AMBI, richness and Shannon's diversity, for the WFD ecological assessment (Borja *et al.*, 2004a). Then, Muxika *et al.* (2007) refined the calculation, and the new tool was called 'Multivariate AMBI' or M-AMBI. Hence, we decided to incorporate within the AMBI software the derivation of the

M-AMBI value, which can be used as the Ecological Quality Ratio (EQR) defined by the WFD (see Borja *et al.*, 2004a, for details). Some recommendations on the use of M-AMBI must be consulted in Borja *et al.* (2008a). For additional information on this methodology, you can write to the authors or consult the papers mentioned in the reference list.

Here, we include some instructions to facilitate the creation of your own files, from the last version we have made: the *AMBI 5.0*, for use only under Windows 7. For Windows XP you can use previous AMBI versions. There are some novelties in this version which are being explained here. This version includes also an updated species list (March, 2012) with >6,300 taxa over the world.

## Input file

As in previous *AMBI* versions, this new version needs an Excel Input file (created previously by you), which you select with the "**Browse**" button (see Figure 1). Previously, you can select a custom Work directory (in 'Work Path' button) for browsing there each time you run *AMBI 5.0*.

If you have previously created an AMBI results file (using any of the *AMBI* versions), you can use this file as input for the M-AMBI calculation (see the button 'Go to M-AMBI' on left-top of the Figure 1). If you have not these data you can calculate M-AMBI after the AMBI derivation (see below).

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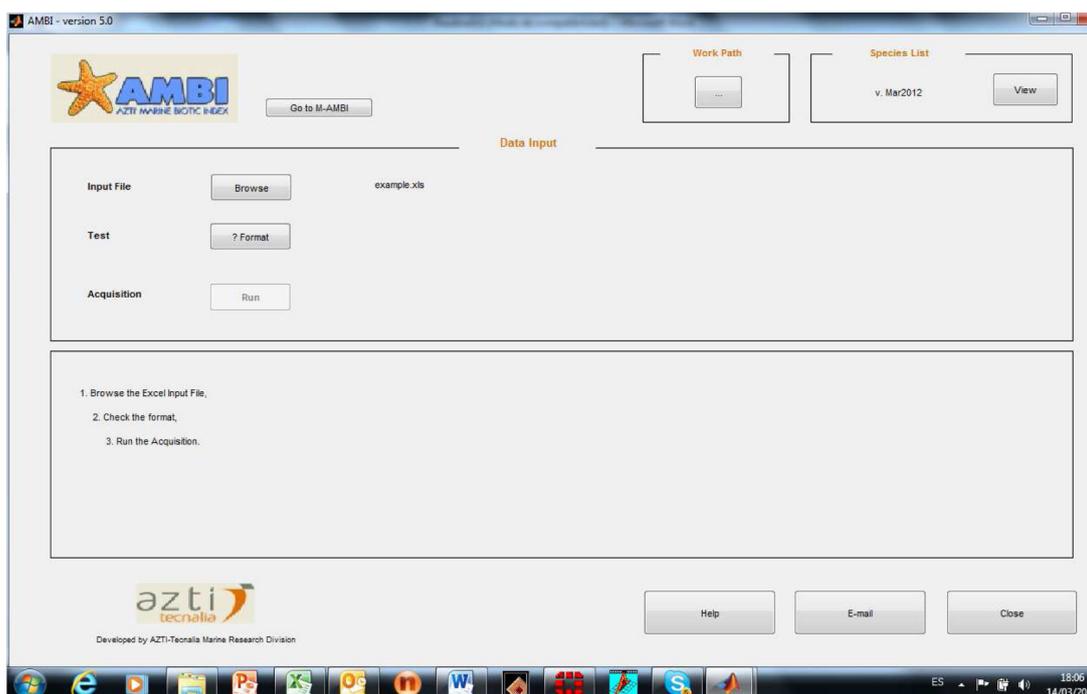


Figure 1. The first screen in *AMBI 5.0*.

For using *AMBI*, now you have **two possibilities of data input**, from an Excel file: (i) as table format (as in previous versions), and (ii) as database format, with 3 or 4 columns, and with or without headings.

## Table format

When creating your Excel file, the two first rows can include respectively the Station names and the Replicate names (the program is suitable to detect different formats in your file, either with or without replicates in your sampling stations). The first column must contain the list of benthic species found in your study (cf. *examples.xls*, in Figure 2).

**Warning!!:** The first cell in the station names row (cell A1) and the first cell of the replicate names row (cell A2) **must be empty**. Hence, in Figure 2, the cells A1 and A2 are empty. This is only in case you have replicates. If you have not replicates only cell A1 must be empty.

As abovementioned, the new version of *AMBI 5.0* allows you to use many Excel files, if you have more than 255 samples. You can select now just only one sheet, all sheets from the file (using ‘Select all’), or selecting several sheets, by using Control key (Figure 3), and the software will paste all sheets. **However, you must take into account next warnings:**

- All sheets must have the same structure.
- If you have replicates in one sheet and no replicates in another, you must have the second row empty in the sheet without replicates.
- **All sheets to be pasted must have the same species list in column A (avoid errors, misspellings, spaces (especially after the name of the species, since sometimes the name can be not recognised), etc., the best way is to copy and paste your first column from a sheet to the remainder), ordered in the same way (if not you will get an error saying that you have differences between lists of species in your sheets). The software detects if the number of species is different in the different sheets selected, and gives you an error message.**

|    | A                      | B | C | D | E | F | G | H | I | J | K | L | M | N | O  | P | Q | R | S | T  | U  | V   | W | X  | Y   | Z   | AA  | AB | AC | AD | AE | AF |
|----|------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|----|---|---|---|---|----|----|-----|---|----|-----|-----|-----|----|----|----|----|----|
| 1  |                        | 1 |   | 2 |   | 3 |   | 4 |   | 5 |   | 6 |   | 7 |    | 8 |   | 9 |   | 10 |    | 11  |   |    |     |     |     |    |    |    |    |    |
| 2  |                        | a | b | c | a | b | c | a | b | c | a | b | c | a | b  | c | a | b | c | a  | b  | c   | a | b  | a   | b   | a   | b  | a  | b  |    |    |
| 3  | Abarenicola claparedei |   |   |   |   |   |   |   |   |   |   |   |   |   |    |   |   |   |   |    |    |     |   |    | 1   | 1   |     |    |    |    |    |    |
| 4  | Abra alba              |   |   |   |   |   |   |   |   |   |   |   |   |   |    |   |   |   |   |    |    |     |   |    |     |     | 3   | 1  | 2  |    |    |    |
| 5  | Abra prismatica        |   |   |   |   |   |   |   |   |   |   |   |   |   | 1  |   |   |   |   |    |    |     |   |    |     |     |     |    |    |    |    |    |
| 6  | Abra sp.               |   |   |   |   |   |   |   |   |   |   |   |   |   |    |   |   |   |   |    |    |     |   |    |     | 1   |     | 1  | 2  |    |    |    |
| 7  | Alkmaria romijni       |   |   |   |   |   |   |   |   |   |   |   |   | 2 | 29 |   |   |   |   | 35 | 23 | 180 |   |    |     |     |     |    |    |    |    |    |
| 8  | Astarte sp.            |   |   | 1 |   |   |   |   |   |   |   |   |   |   |    |   |   |   |   |    |    |     |   |    |     |     |     |    |    |    |    |    |
| 9  | Bathyporeia elegans    |   |   | 1 |   |   |   |   |   |   |   |   |   |   |    |   |   |   |   |    |    |     |   |    |     |     |     |    |    |    |    |    |
| 10 | Callianassa tyrrhena   |   |   |   |   | 1 |   |   |   |   |   |   |   |   |    |   |   |   |   |    |    |     |   |    |     |     |     |    |    |    |    |    |
| 11 | Capitella capitata     |   |   |   |   |   |   |   |   |   |   |   |   |   | 1  |   |   |   |   |    |    |     | 1 | 10 | 505 | 518 | 117 | 80 | 58 | 11 |    |    |
| 12 | Carcinus maenas        |   |   |   |   |   |   |   |   |   |   |   |   |   |    |   |   |   |   |    |    |     |   |    |     |     |     |    |    |    |    |    |
| 13 | Caulerella alata       |   |   |   |   | 1 |   |   |   |   |   |   |   |   |    |   |   |   |   |    |    |     |   |    |     |     |     |    |    |    |    |    |
| 14 | Caulerella zetlandica  |   |   |   |   |   |   |   |   |   |   |   |   |   |    |   |   |   |   |    |    |     |   |    |     |     |     |    |    |    |    |    |

Figure 2. The initial Excel file to calculate *AMBI*.

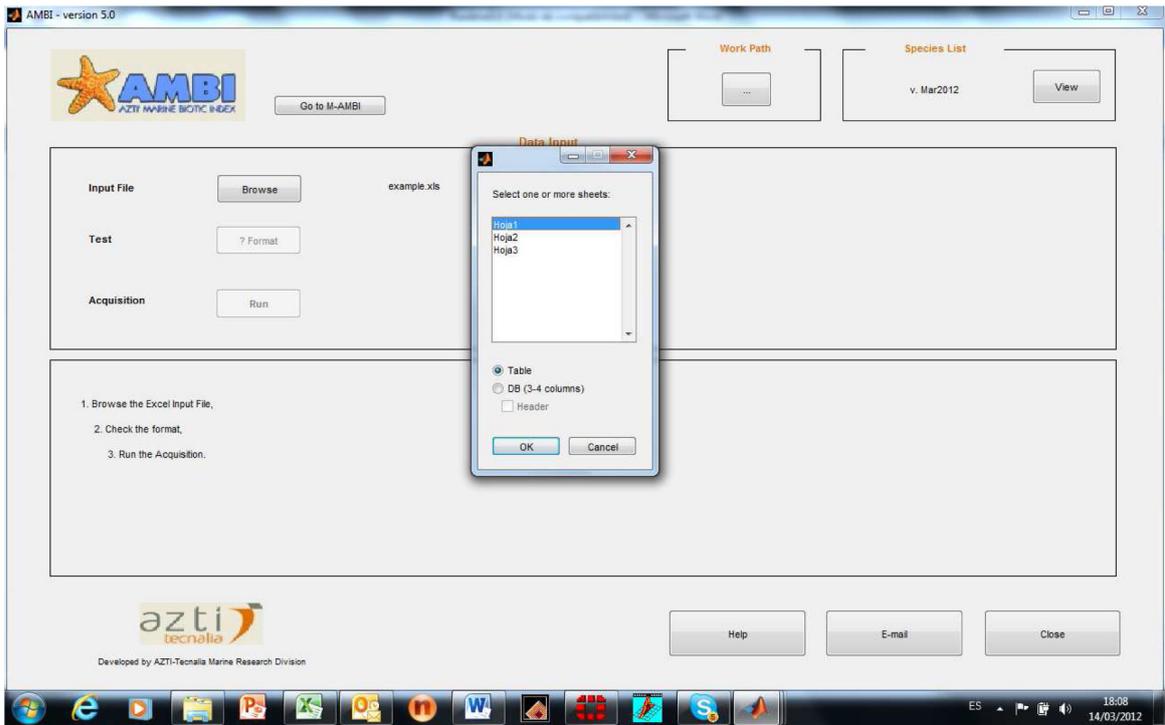


Figure 3. Selection of sheets from the Excel file.

## Database format

Alternatively, you can use also information from a database, exported into an Excel file with 3 (station, species, density) or 4 (stations, replicates, species, density) columns, with or

without headings in the first row (Figure 4). This allows you to include thousands of data in an easier way, avoiding the problem associated to the presence of similar species names in column 1 of several sheets, as in the previous way of importing data. However, you should be aware that it is convenient to get the species names corrected before exporting the information from a database.

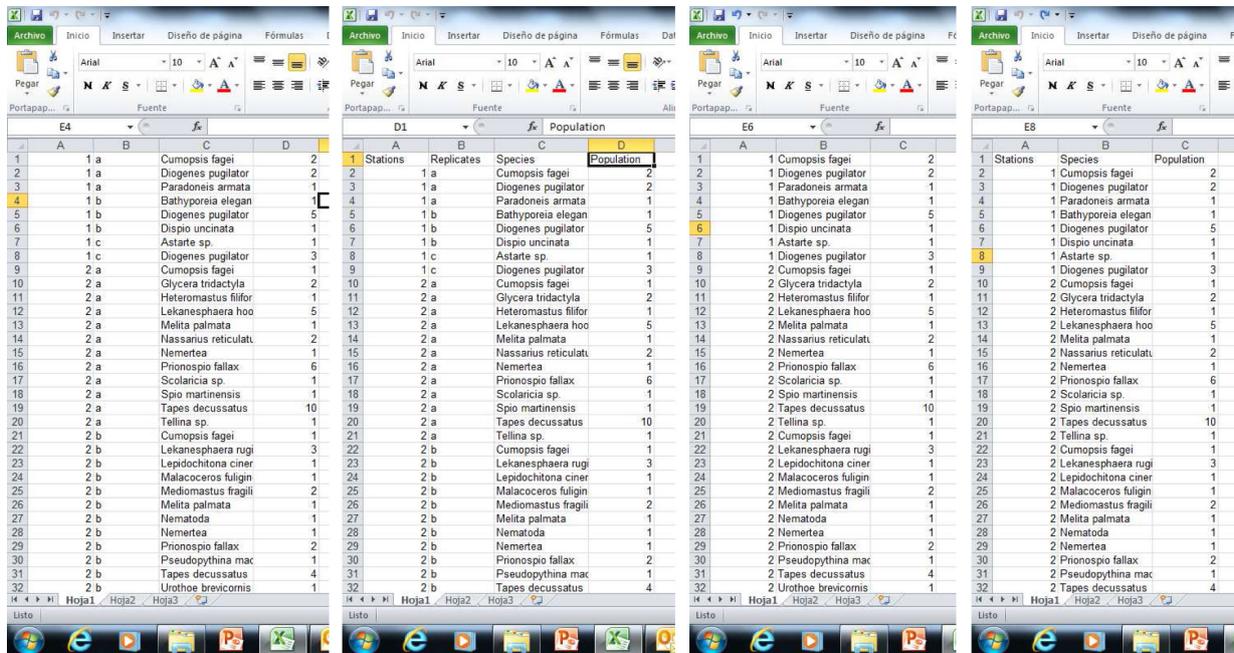


Figure 4. The initial Excel file to calculate AMBI, from a database. Left: an example without headings, and 4 columns (station, replicate, species, density); Middle left: the same, with headings; Middle right: an example without headings, and 3 columns (station, species, density); Right: the same, with headings.

You can select the type of Excel file in which your data are included (Figure 3), as table, database (DB) as 3 or 4 columns, and if the file (in the last cases) has headings or not (in this case you must pencil the 'header' box in Figure 3).

## Testing the information imported

Then, after importing the data (whatever one of the two ways you select), you must test the format of your Excel file input with the “? Format” button (Figure 5). Check the total number of species, the number of stations and, eventually, the number of replicates for each station (in the example you can see that there

are different replicates for each station). If you detect any error, you can cancel the import and correct the Excel input file. In some cases, the error could be in the format of the text. Hence, avoid the use of bold, italics, symbols, etc., in your data file. If you agree with the detected characteristics of the input file, you can press the “OK” Button to enable the import.

## Running the AMBI calculation

After completing the format test, you can Run the acquisition (Figure 6).

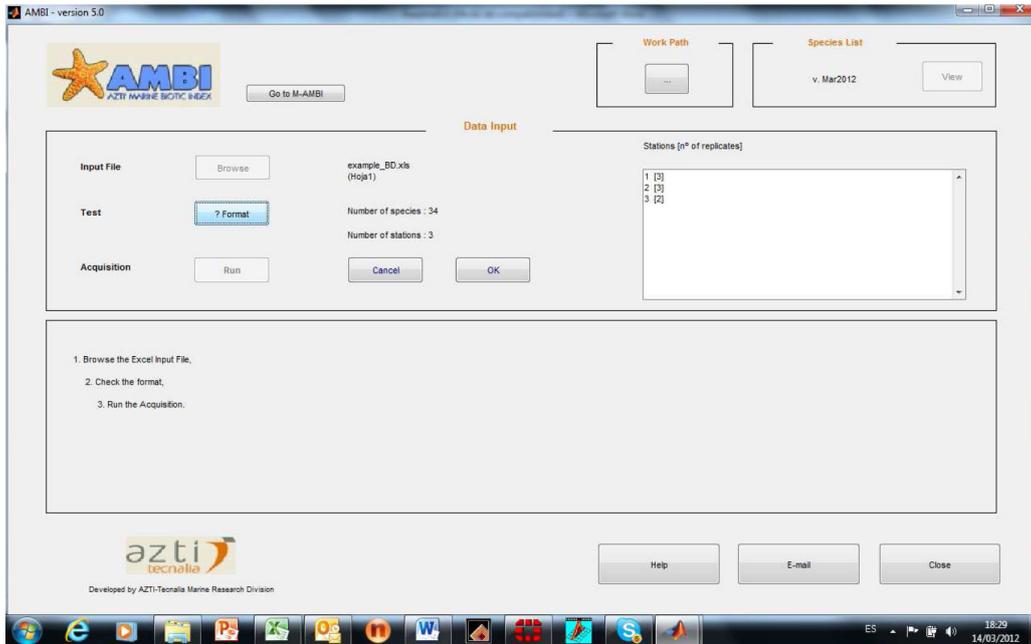


Figure 5. Testing the format of your Excel file.

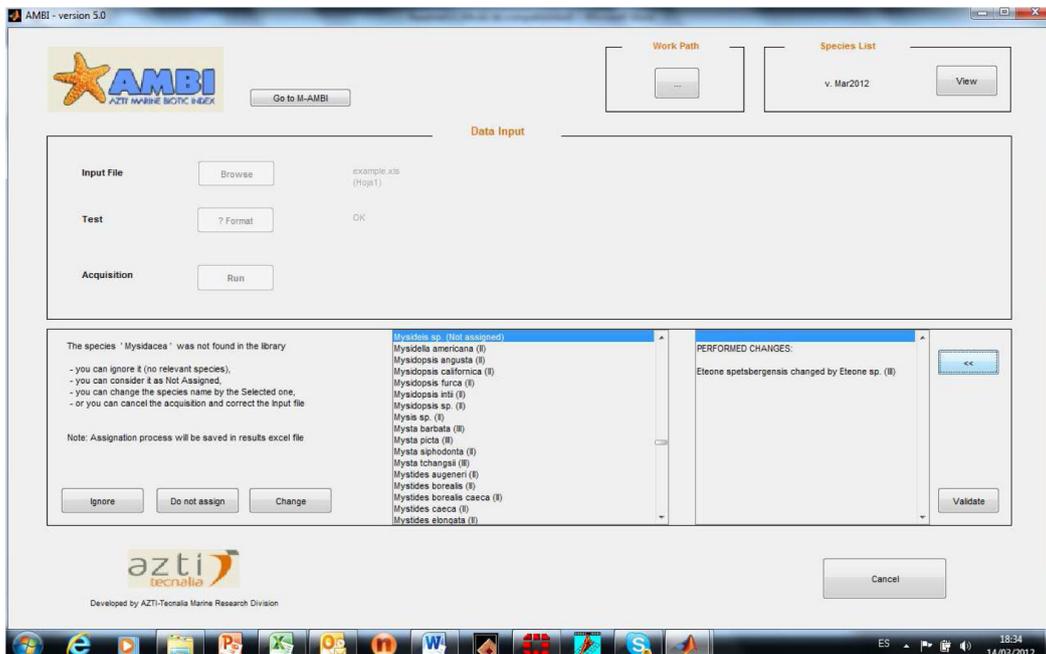


Figure 6. Reference Species List and Assigning the Ecological Group and testing the names of the species.

The software will automatically assign the Ecological Group. The assignation is based on the Species List that *AMBI 5.0* is using as reference. It can be visualized by clicking the “View” button (Figure 6). You can download from <http://ambi.azti.es> the last list of taxa updated by scientists from AZTI (you need to check it regularly, because some changes can occur, after recommendations from different experts, and consultation of ERMS database and others, for spelling taxonomical names). In some cases, **you should have to check the name of the species** (Figure 6): when the sentence “the species was not found” appears, this taxa is not in the species list because either/or: (a) the species is not included; (b) your species name does not coincide with our species’ name (some changes should be checked: y, h, b, v, etc.); and (c) our species name is wrong; etc.

Compared to previous versions, now a new window is opened on the right part, showing you the changes you have made (Figure 6). If you have changed the species in a wrong way you can unselect (<<) the species and try again, at any time during the running process. When you have finalized the process a ‘Validation’ button appears (Figure 6). You can revise your changes and, if everything is correct, you can validate the changes and the calculation occurs.

For each of the “unknown” species, you have four options (see Figure 6):

- To ignore this species considering that it is not relevant in the AMBI calculation (see our Guidelines in Borja and Muxika, 2005), with “Ignore” button (the taxa will be removed from the analysis),
- To consider the species as ‘Not assigned’ to an Ecological Group, with “Do Not Assign” button (the taxa will be incorporated, but not assigned),
- To change the name of the species by one of the taxa

selected in the reference list (e.g., if the name is not correct or change by another close taxa present in the list), with “Change” button,

- To cancel the acquisition for correcting the Input file, with “Cancel” button.

All this new assignation process for “unknown species” will be saved in an excel file (\*-res.xls) located in the Input File directory, in the sheet ‘Changes’ (this is also a new issue in this version, since in previous ones the list of changes was saved separately). This allows you to repeat the process in the future, giving you the copy of all changes.

Also in the reference list, some species has not an Ecological Group assigned (it appears as “Not assigned”). We do not know the group: if you have any knowledge about its ecological behavior or characteristics, you can communicate it to us: [aborja@azti.es](mailto:aborja@azti.es). Other Ecological Groups appear as I, II, III, IV and V, equivalent to those groups shown in Borja *et al.* (2000).

The software calculates the percentage of each group on each sample (%I, %II, etc.), the percentage of ‘Not Assigned’ species (%n.a.), the AMBI, the BI, and offers the classification of the pollution or disturbance in the sample (Figure 7).

In this screen, you can visualize detailed results and a histogram for the highlighted station, or, by means of the Control or Shift keys, you can select a group of stations, for space/time distribution representation or for the corresponding histogram (Figure 7). In the Station Details Screen (Figure 8), you can identify the data for each of the replicates (jumping from the stations, on the top left or bottom part, and replicates, on the top right); the number of taxa; the total number of individuals of the sample; the number of individuals used in the calculation; and the percentage of “Not Assigned” species.

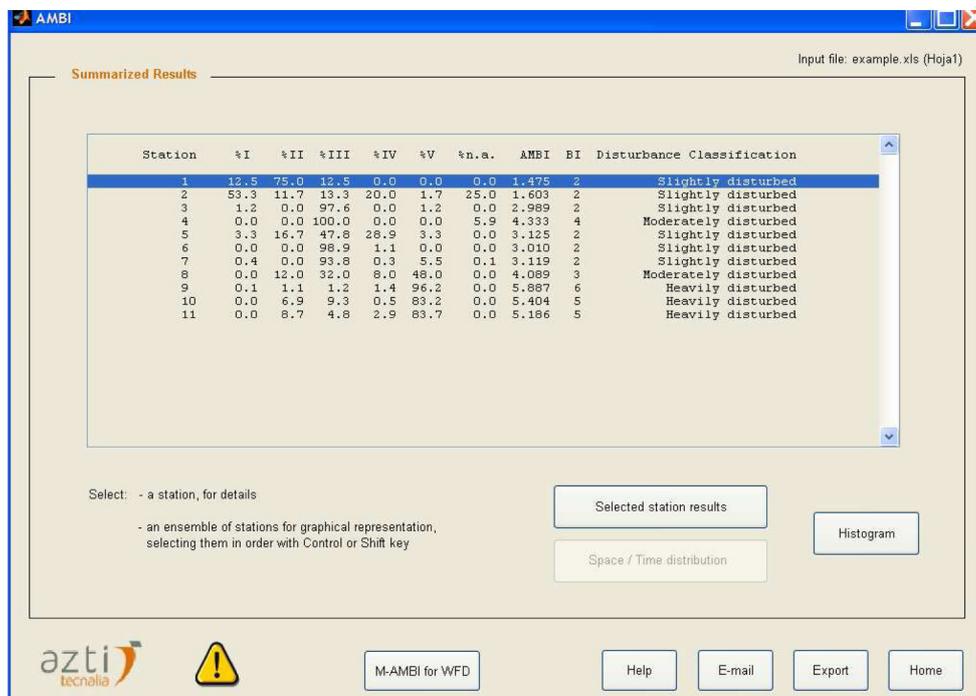


Figure 7. Results of the AMBI and pollution/disturbance classification, by sampling station.

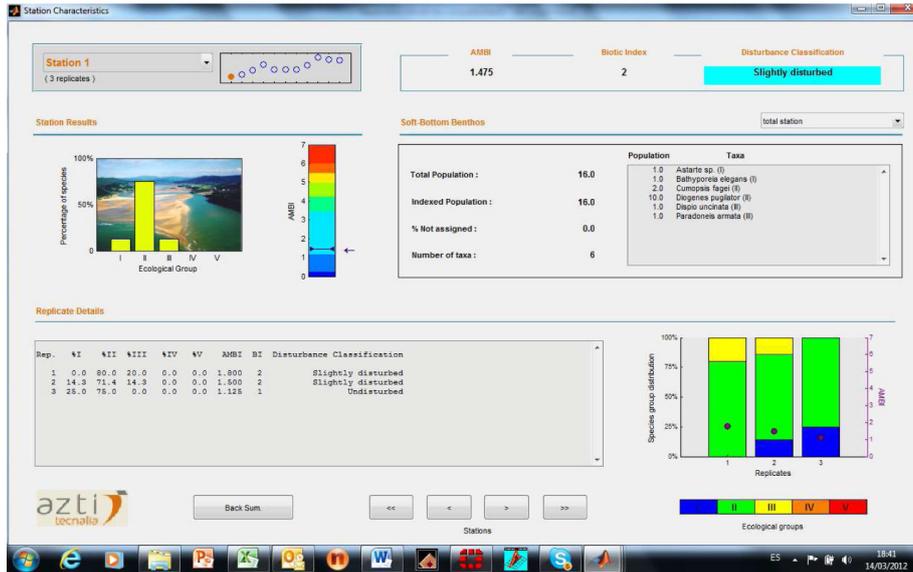


Figure 8. Results from a particular sampling station.

A warning message about results validity (see our Guidelines) should appear if, for some stations:

- The percentage of no assigned individuals is higher than 20%
- The (not null) number of species is less than 3
- The (not null) number of individuals is less than 6

The value of the AMBI index is very useful, in order to study the evolution of a site after an impact (or a water treatment plan, for example), or to detect gradients in a spatial or temporal sense. If the later is the objective, you can select some of the stations (following the gradient, from the source of impact) in the “Results” screen, using the “Control Key” (Figure 7). This

approach gives you a graphical representation (Space / Time Distribution) of the mean AMBI, along the gradient, including the standard deviation (if you have some replicates) (Figure 9).

Alternatively, you can select the results from some or all stations (as shown in Figure 7) and draw a histogram, with the results of each replicate and station (Figure 10). You can “Save” the graphical representations, as image, in PNG, JPEG or TIFF format (Figures 9 and 10). These figures can be save and used in your reports.

You can use the button “Export” (Figure 7), to save your results in an Excel file (\*-res.xls) which contains all results by replicate

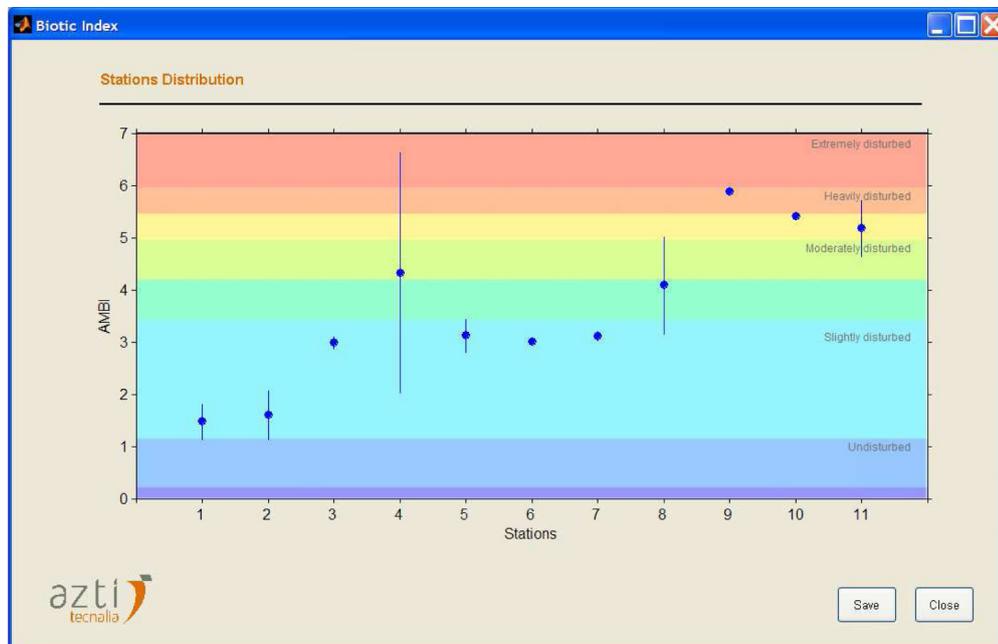


Figure 9. Results from a group of sampling stations.

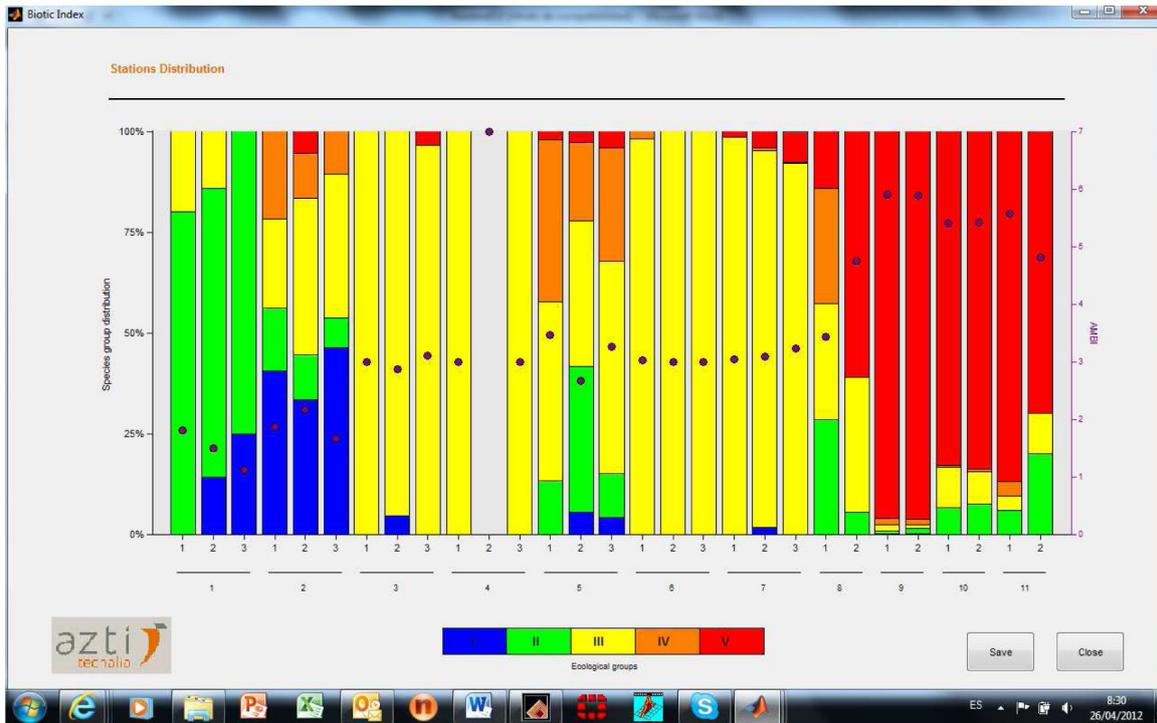


Figure 10. Histogram showing the results from a group of sampling stations.

in the first sheet, and a station summary (calculated on the basis of all replicates) in the second one, together with the changes in species assignment in the third sheet. In *AMBI 4.0* version these results were exported on the basis of a station per column. However, in *AMBI 4.1 and 5.0*, taking into account the possibility of paste several data sheets, the results are exported on the basis of a station per row. The second sheet includes also data of Shannon's diversity ( $\log_2$ ) and richness (number of taxa included in the analysis: remember that those taxa 'ignored' are removed from the analysis and they do not account for richness), which can be used as input data, together with AMBI, in the M-AMBI calculation (see below).

In this version of the software, both sheets show data in

vertical rather than in horizontal (in previous versions), this allows to show data for more than 255 samples. **Warning!** If you have used previous versions of AMBI and you want to use old results, contained in the \*-res.xls file, to calculate M-AMBI, you need to change the orientation from horizontal to vertical.

## Running M-AMBI calculation

Now, if you need to calculate the EQR (the M-AMBI value) and the ecological status, for the WFD, you have different ways:

- If you are calculating AMBI you can use now the button "M-AMBI for WFD", on the left part of the screen (Figures 7 and 11).

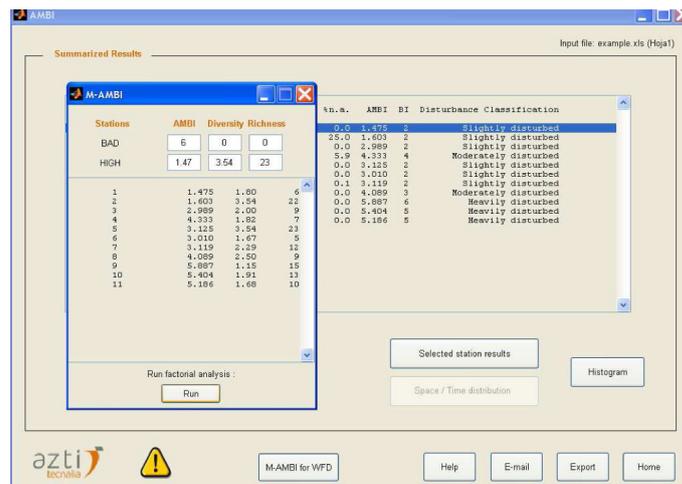


Figure 11. Results of the AMBI, diversity and richness values, by sampling station, together with the selection of 'High' and 'Bad' reference conditions, for the M-AMBI calculation.

- If you have calculated AMBI previously and you have saved the results into a ‘\*-res.xls’ file, you can start the software and use the button ‘Go to M-AMBI’ in the initial screen (Figure 1).
- If you have your own AMBI, diversity and richness data, and you want to derive M-AMBI, you must include your data in a file, using the same format than in ‘\*-res.xls’ (data should be included in the second sheet and in the same column cells than in this file). Then use the button ‘Go to M-AMBI’ in the initial screen (Figure 1).
- The three ways will show you a screen with the values of AMBI, richness (number of species) and Shannon’s diversity (Figure 11).

The software provides you ‘Bad’ and ‘High’ reference conditions by default (see Borja *et al.* (2004a), and Muxika *et al.* (2007a), for the WFD terminology). As ‘Bad’ conditions the values are always 6 for AMBI and 0 for diversity and richness. For ‘High’ conditions the software selects the lowest AMBI value and the highest diversity and richness values. However, the user can modify these values (bad and high) if some reference conditions have been defined, within the WFD, for the studied area (see Muxika *et al.* (2007a) or Borja *et al.* (2008b) for methodology in determining reference conditions). Remember that, following the WFD, each typology within an eco-region must have its own reference conditions. Hence, although the software provides you these reference conditions by default, you must get your contrasted reference conditions for an accurate ecological status assessment (in Muxika *et al.* (2007a) you can see the reference conditions used in the Basque Country. Some of them can be used in the same typologies, within the Northeast Atlantic eco-region). The stations from different typologies must be analyzed in different datasets, because they could have different reference conditions (see Borja *et al.*, 2012).

When running the software, it uses richness, diversity and

AMBI as variables. Then, the variables are standardized, by subtracting the mean and dividing by the standard deviation. The Factorial Analysis uses principal components analysis as extraction method, using the correlation matrix and extracting 3 factors. The software uses the Varimax rotation, the regression as method for calculating the factor scores, and missing values are excluded using listwise. The factor scores (X, Y, Z) are the new coordinates of each sampling station in the new factor space (Figure 12). These coordinates are used in deriving the EQR or M-AMBI value (Figure 12). If you are using other software to calculate the EQRs, you must be aware that each software may use different algorithms for the Factor Analysis, providing different results (see Borja *et al.*, 2008a).

Remember that, in the Factor Analysis the values can change slightly when adding new data from new stations, this is why we recommend using at least 50 samples in the calculation (see Borja *et al.*, 2008a). Following the WFD, the EQR ranges between 0 (bad status) and 1 (high status). Depending on the reference conditions, M-AMBI values can be lower than 0 or higher than 1 (see Borja *et al.*, 2004a; Muxika *et al.*, 2007).

The AMBI 4.0 software used as default the boundaries intercalibrated within the European benthic intercalibration exercise (Borja *et al.*, 2007). In AMBI 4.1 and 5.0 versions we have included as boundaries those updated in the European intercalibration exercise (these values are now 0.77, for the limit between High and Good status; 0.53, for the limit between Good and Moderate; 0.39, for the limit between Moderate and Poor; and 0.20, for the limit between Poor and Bad status). Hence, you must use these when comparing for the Northeast Atlantic. Then, the corresponding ecological status is derived for each of the sampling locations (Figure 12).

These boundaries can be changed, if necessary, by using the ‘Boundaries’ button (Figures 12 and 13). The default boundaries can be recovered after pressing the ‘Default values’ button (Figure 13).

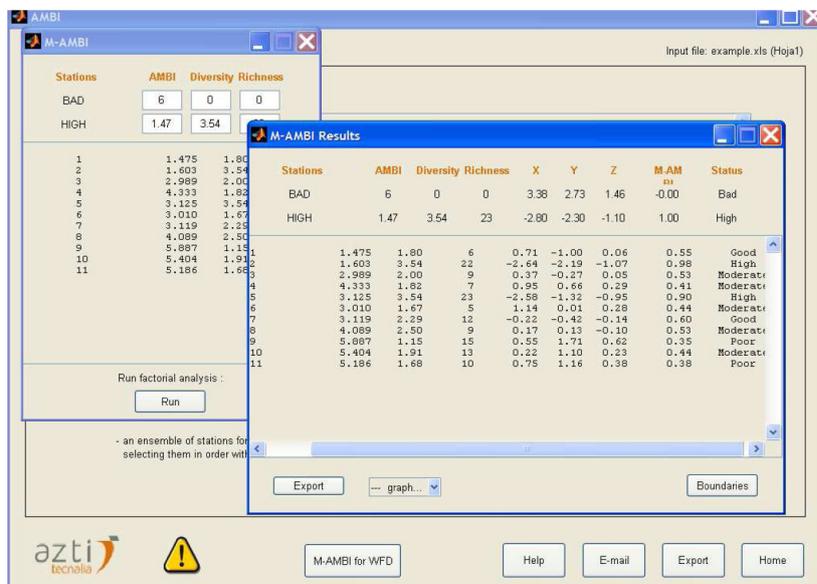


Figure 12. Results of the factor scores (X, Y, Z), M-AMBI, and the corresponding ecological status.

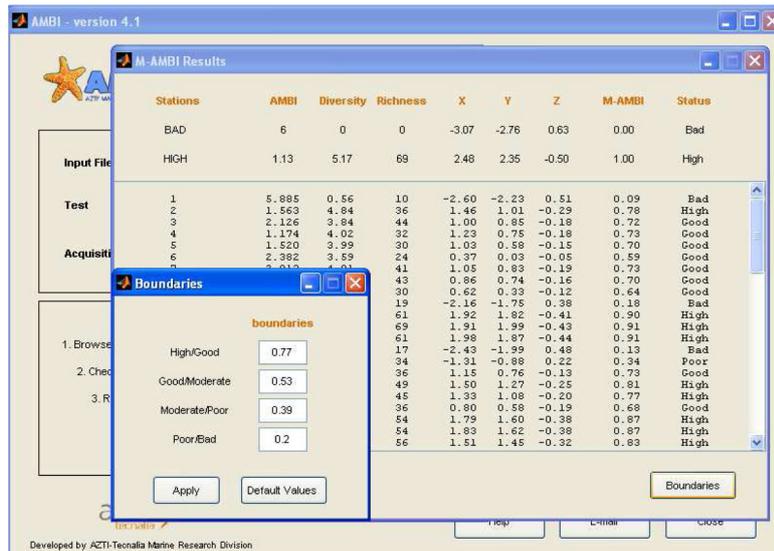


Figure 13. Modification of boundaries between the different ecological statuses.

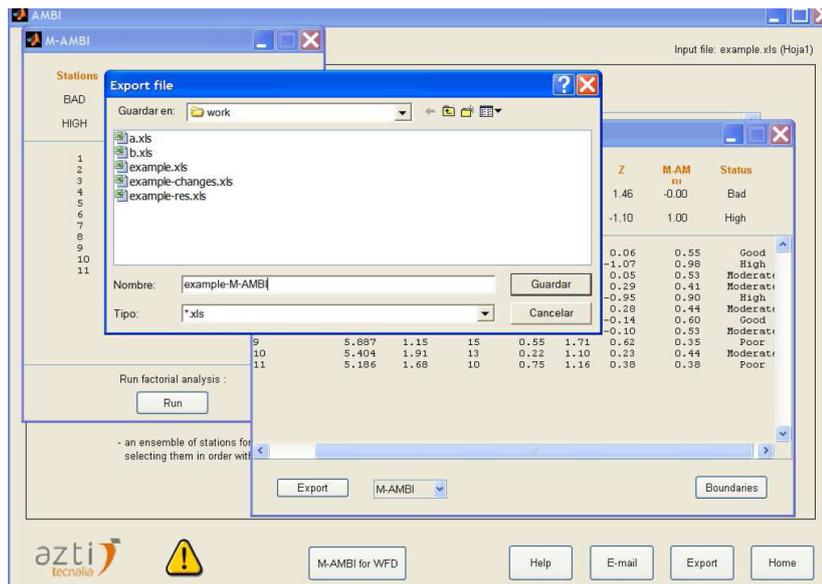


Figure 14. Results exportation to an Excel file.

These results can be exported, by using the 'Export' button (Figure 12). The software creates a new Excel file, which uses the original name of the file plus 'M-AMBI' (Figure 14).

You can use these data to make your own figures; however, the software provides you some default figures (see the button 'graph', in Figure 12). There are four different choices: M-AMBI histograms and three different plots of the factor scores (XY, XZ and YZ, including the position of bad and high status values). The M-AMBI histograms show the values for each of the stations, together with the selected boundaries and ecological status (Figure 15). By modifying the boundaries you can get different figures. Moreover, these figures show the status in the colors of the WFD.

The figures can be exported as png, jpg or tiff, using the button 'save'.

You can consult further developments in the 'References' section, at the end of this document. The guidelines for the use of this tool can be consulted in Borja and Muxika (2005b).

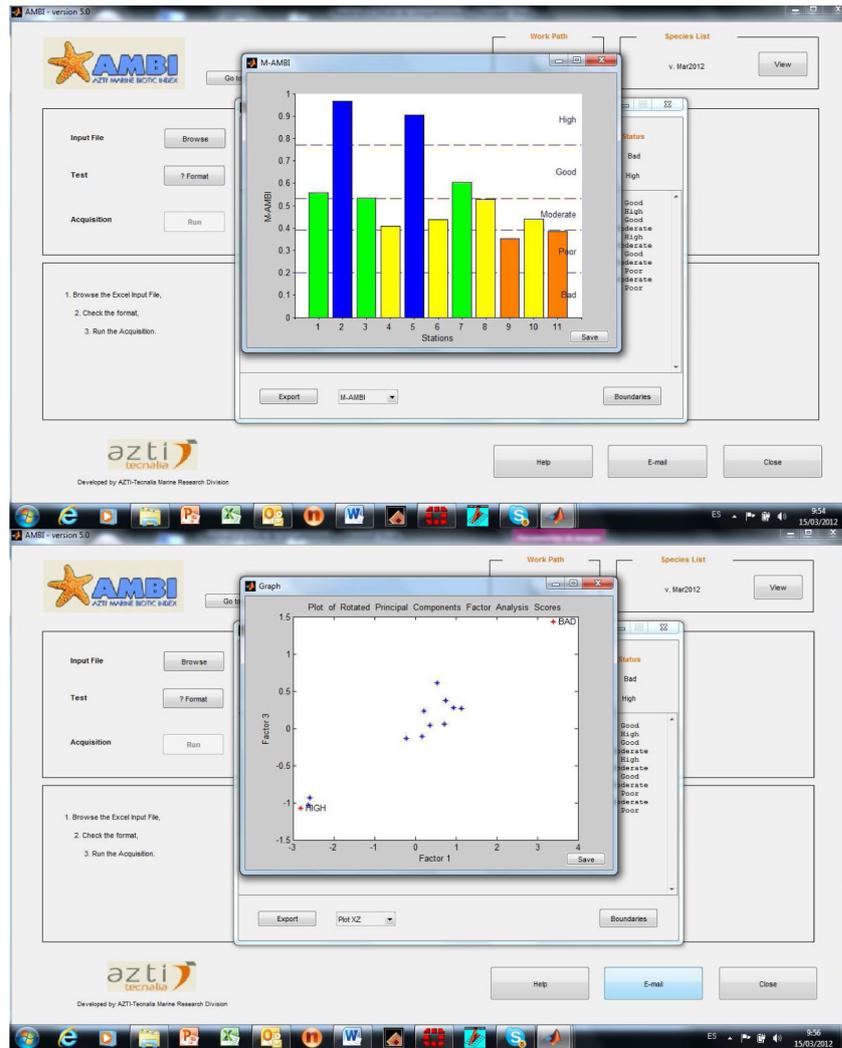


Figure 15. Examples of two M-AMBI plots. Top: M-AMBI histogram; Down: XZ plot.

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