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Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery

DELIVERABLE

# Deliverable 4.3-4: Manuscript on indicators for hard bottom substrata

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## Content

## Non-technical summary

Bayesian models were built for predicting the status of macroalgae, macrofauna, and macroalgae+macrofauna, within hard-bottom substrata of the Basque coast (Bay of Biscay, north Atlantic), at different shore levels. However, these models were not useful when applied to data from Portugal and Denmark, for validation. The model classification was too assertive, with small differences in the classification between stations in each country. This is usually desirable because it shows a classification with low uncertainty. However, in this specific domain it is a disadvantage since the spatial heterogeneity must be observed in the classification of different stations in an area. Bayesian models can be useful if a large data set is available, including different levels of quality, within a gradient of human pressure.

# 1 Introduction

Most of the studies dealing with responses of macroinvertebrates to human pressures in marine waters have been undertaken in soft-bottom substrata (Borja et al., 2011. Probably, this is because tools assessing the ecological status in marine waters are more developed in soft rather than in hard-bottom substrata (see a list for the European Water Framework Directive (WFD, 2000/60/EC) in Borja et al., 2009).

Therefore, the responses of hard-bottom biota to a plethora of pressures have been studied word-wide. The studied pressures include sewage/outfall/nutrient enrichment (e.g., Archambault et al., 2001; Arévalo et al., 2007; Axelrad et al., 1981), chemical pollutants/oil spills (e.g., Addison et al., 2008; Lewis et al., 1982), thermal pollution (e.g., Langford, 1990), or hydrological changes (e.g., Alongi et al., 2004). In general, studies on hard-bottom substrata, include both macroalgae and macrofauna (Littler and Murray, 1975; Nelson, 1982; Bishop et al., 2002; Crowe et al., 2000; Kraufvelin, 2007; etc.).

This previous research has identified indicators or metrics that can response to those pressures. These include, among others, changes in: presence of ephemeral green algae; presence of perennial, canopy-forming seaweeds; similarity of the species structure/composition with reference sites; total number of species (alpha-diversity); number of herbivore species; in stress-tolerant taxa; opportunistic taxa; engineering species; functional-form groups; dominance of species; variability in community parameters (among replicates); highly resistant, thionitrophilic or opportunistic species; nitrofilus macroalgae; Rhodophyta/Phaeophyta mean Ratio Index; sensitivity level; filter feeders (mussel, barnacle); detritivores; herbivores; micrograzers; ephemeral green algae/bilvalves ratio; vegetation/filter feeders ratio; average taxonomic distinctness; settlement of barnacles; and growth of filter feeders.

The research carried out has allowed developing methodologies and indices that allow evaluating the ecological status of benthic communities, especially within the WFD context. These include: CAR-LIT (Ballesteros et al., 2007b); BENTHOS (Pinedo et al., 2007); Ecological Evaluation Index (EEI) (Orfanidis et al., 2001; Orfanidis et al., 2003); Ecological Evaluation Index continuous (EEI-c) (Orfanidis et al., 2011); Rhodophyta/phaeophyta mean ratio index (Cormaci et al., 1985); TWo-stage INdex (TWIN) (Marchini and Occhipinti-Ambrogi, 2007); Reduced species list (RSL) index (Wells et al., 2007); Quality of rocky bottoms (CFR) index (Juanes et al., 2008); Benthic Index for Rocky Shore (BIRS) (Orlando-Bonaca et al., 2012) or Rocky Intertidal Community Quality Index (RICQI) (Diez et al., 2012).

However, most of these indices/methodologies are based upon the macroalgae, without taking into account macrofaunal communities. This is probably because some of these indices/methodologies have been proposed within the WFD, and this legislation takes into account each biological quality element of the aquatic system separately (i.e. phytoplankton,macroalgae, macrobenthos, etc.). Hence, few attempts have been made to develop an index for assessing the quality of hard substratum fauna (see Hiscock et al., 2005). Ecological knowledge of rocky invertebrate assemblages is capable of detecting environmental effects; its validity as a marine ecosystem indicator is extensively acknowledged (Clarke and Warwick, 1994; Hiscock et al., 2005; Hiscock and Tyler-Walters, 2006; Rogers and Greenaway, 2005). In spite of this observation, gathering the evidence necessary to distinguish between various degrees of community alteration, to establish an ecological status classification compliant with the WFD, is a somewhat challenging task. In this way, the simultaneous use of both flora and fauna may be more appropriate, in determining the ecological status of hard substrata; this is due to the low number of invertebrate taxa, correlated with a disturbance gradient (Hiscock et al., 2005; Goodsell et al., 2009).

It has been discussed about the requisites to develop a methodology to assess water quality within the WFD context, or in a more general context. As an example, Orfanidis (2007) argues that it is need: (i) experimental science using quantitative data for precision and accuracy; (ii) recognition of spatial and temporal heterogeneity of communities, stress–stressor relationship; (iii) predictive modelling; and (iv) sound ecological theory.

Moreover, Orfanidis (2007) indicates that descriptive approaches and expert judgement should be as much as possible avoided to develop. This was rebutted by Ballesteros et al. (2007a). Hence, these authors argue that “no experimental science, nor predictive models, is absolutely necessary to develop biotic indices, but descriptive studies showing the relationships between species abundances and environmental variables are essential”. This debate and others proposals reflect that there is not an agreement about the requirements for developing a new methodology or index.

However, for the development of a new assessment method, the steps described by Borja and Dauer (2008) can be followed, which included: (i) the spatio-temporal scale of the intended application; (ii) the selection of the candidate metrics; (iii) the metric combination; and (iv) the index validation, by testing it using an independent data set, different than the index development data set (calibration data set).

Most of the above mentioned methodologies/indices are based upon knowledge of the relationships between biota and ambient/pressures. However, the interactions between biota and different environmental factors are complex and often non-linear, making it difficult to produce robust predictions (e.g., Fernandes et al., 2010). 'Machine-learning' techniques (in particular, supervised classification methods) have been proposed as useful tools, to overcome such difficulties (e.g, Fernandes et al., 2010).

The novelty of the present research is based upon the well-known fact that structural parameters of biota are related with the degree of pressures and therefore can predict the quality, but taking into account that the relationships between biota and ambient/pressures are complex. Here, the 'machine-learning' techniques (in particular, supervised classification methods) have been applied for identifying a set of biological elements that can predict the variability in the status of the biota, trying to develop a new approach in assessing the ecological status, within the WFD, in hard-bottom substrata.

# 2 Materials and methods

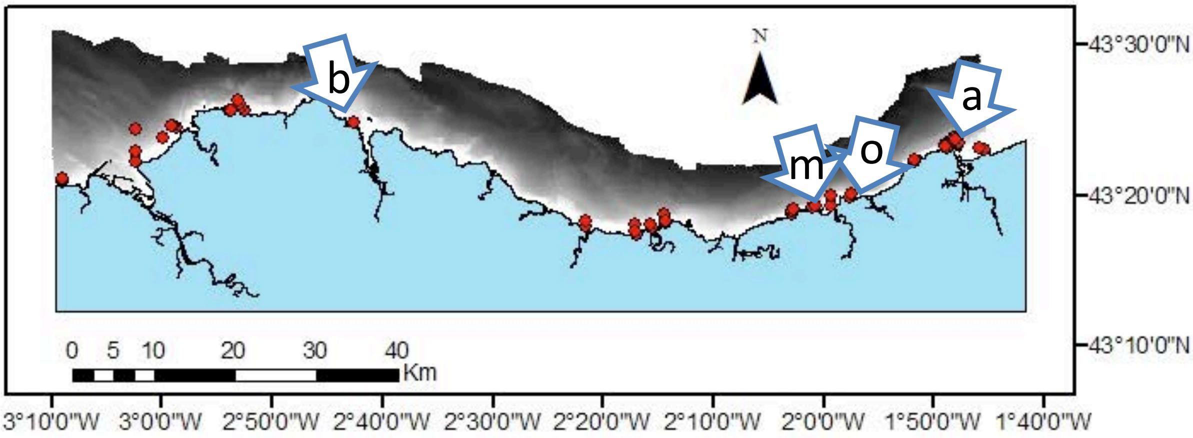
## 2.1. Sites description used to develop the method

The Basque coast is located in the south-eastern Bay of Biscay and extends along 150 km (approximately), between 43°22′ N, 1°46′ W and 43°21′ N, 3°9′ W. This coast is of high energy and mostly erosional, with extensive cliffs. It has been affected by anthropogenic impacts, especially urban and industrial wastewater discharges. In recent times, water treatment programmes has been carried out, with an improvement in the status of the ecosystems.

One of the main pressures in the hard-bottom habitats is the presence of sewage outfalls (Borja et al., 2006; Franco et al., 2004). These include submarine outfalls and intertidal outfalls.

Previous data from several monitoring programs include sampling sites have been sampled yearly during 1992-2009. This data was evaluated and it was concluded that more sampling sites was required due to the fact that the statistical approach used (see Section 2.3) requires a minimum data of samples in each of the classes of the variable to be predicted (i.e., ‘high’, ‘good’, ‘moderate’, and ‘poor’ status[1]). Hence, some of the classes had a low number of data, and therefore new sampling sites were sampled in order to achieve the requirement that all the classes have an enough number of data. These new samplings were carried mainly in the proximities of the outfalls of Oiartzun, Atalerreka, Mompas-Pasaia and Bermeo (all of them in the Basque coast). In Figure 1 it is shown the localities included in this study (i.e, those sampled previously together those sampled for this study).

*Figure 1. Study sites, showing all locations sampled.Key: o - Oiartzun, a - Atalerreka, m -Mompas-Pasaia, b -Bermeo*



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[1] Those azoic sampling sites were classified as ‘bad’ status and were not included in this research.

The sampling sites included intertidal (e.g., Figures 2, 3 and 4) and subtidal localities. Hard-bottom macrophytobenthos and macrozoobenthos were sampled in replicated squares of 0.25 m x 0.25 m, in intertidal areas, and 0.5 m x 0.5 m, in subtidal areas. Biota was counted and identified to the lowest taxonomic level possible. Biomass (dry weight, 60ºC 48 h) was measured for each taxon.

*Figure 2. Intertidal sampling site in Bermeo.*

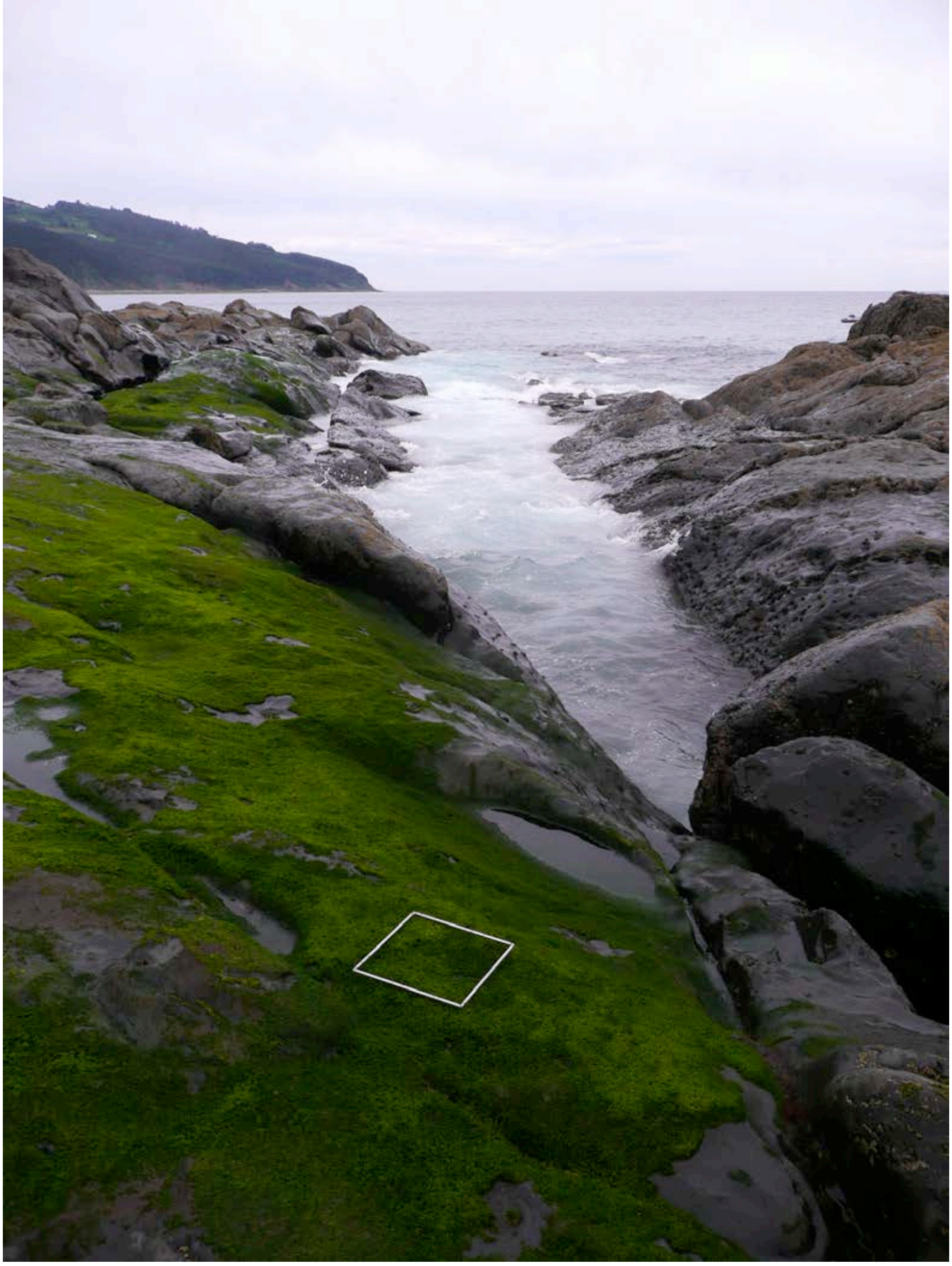


Figure 3. Intertidal sampling site in Bermeo.

Figure 4. Intertidal outfall in Bermeo.

## 2.2. Preparation of data to be used in the method

All the analyses carried out were based upon the biomass data (dry weight per square meter of each taxon). The taxonomy of data was verified by means of the WoRMS (World Register of Marine Species, www.marinespecies.org) and ERMS (European Register of Marine Species; http://www.marbef.org/data/erms.php). Those azoic sampling sites were classified as 'bad' status and were not included in this research.

A total of 288 samples were taken into account. Data was classified in the following shore levels: (i) Supralittoral zone (intertidal area, only covered by water in high tides); (ii) Midlittoral zone (intertidal area, subjected to daily tides); (iii) Infralittoral zone (intertidal area, only discovered in very low tides); (iv) Subtidal zone depth 5-15 m; and (v) Subtidal zone depth >15m. However, from the supralittoral zone a low number (9) of samples was available and therefore this data was excluded.

## 2.3. Bayesian method used to assess the status

### 2.3.1. Classification of data based upon expert judgment.

The Bayesian method of classification requires a set of data with values of predictor variables (i.e, biological characteristics) and response variables (i.e., ecological status). In order to build the required data set classification of data ecological status was assigned taking into account only macrofauna, only macroalgae and both together (macroalgae+macrofauna). This was done based by means of expert judgment.

A total of 279 samples were sent to four researchers with previous experience in benthic studies relating biota status to pressures, including: (i) shore level; (ii) composition of species (macrofauna and macroalgae); and (iii) biomass of each taxon.

Each of the researchers classified each of the samples in one status (i.e., 'high', 'good', 'moderate', and 'poor') taking into account: (i) only macrofauna data; (ii) only macroalgae data; and (iii) macroalgae and macrofauna data.

After the classification of the researchers it was found samples: (i) with total coincidence in the four researchers' classification; (ii) samples with (partial) coincidence in three researchers' classification; (iii) samples with (partial) coincidence in two researchers' classification; and (iv) samples without agreement.

These last ones were removed, with a final set of 268 data. In those samples without 100% of agreement the classification assigned was done following: (i) sample with partial coincidence in three researchers' classification. The final classification is those in agreement by the three researchers; and (ii) samples with partial coincidence in two researchers. Final classification is those averaged, using values of 'high'=4, 'good'=3, 'moderate'=2, and 'poor'=1. Averaged values were used for final classification: criteria: high' >3.5 >'good' >2.5 > 'moderate' >1.5 'poor'.

### 2.3.1. Parameters used as predictor variables.

The Bayesian method allows selecting those variables (i.e., biological parameters) that better predict the response variable (i.e., ‘high’, ‘good’, ‘moderate’, and ‘poor’). This does not imply that the selected variables are those that have a more ecological significance or that better response to pressures (i.e., individual response).

A total of 186 parameters were calculated from biomass (Table 1), using PRIMER 6 (Plymouth Routines In Multivariate Ecological Research, www.primer-e.com) software.

*Table 1. Parameters derived from biotic data (based upon biomass). Key: S-number of taxa, d - richness(Margalef), Jbis - Pielou’s eveness, Brillouin - H index, H(log2) - Shannon-Wiener diversity index (H‘), Lambda - Simpson’s Lambda, 1-Lambda - Simpson’s 1-Lambda, Lambda Bis - Simpson’s Lambda‘, 1-Lambda Bis - Simpson’s 1-Lambda‘, N10 - Hill’s N10, N10Bis - Hill’s N10‘, N21 - Hill’s N21, N21Bis - Hill’s N21‘, Delta -Taxonomic diversity, Delta* - Taxonomic distinctness, Delta+ - Average Taxonomic distinctness (Presence/Absence), sDelta+ - Total taxonomic distinctness (Presence/Absence), Lambda+ - Variation in taxonomic distinctness (Presence/Absence), Phi+ - Average Phylogenetic diversity averaged over number of species in sample, sPhi+ - Total Phylogenetic diversity.*

| | S | d | JBis | Brillouin | H(log2) | Lambda | 1-Lambda | Lambda Bis | 1-Lambda Bis | N10 | N10Bis | N21 | N21Bis | Delta | Delta* | Delta+ | sDelta+ | Lambda+ | Phi+ | sPhi+ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **All taxa** | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **All taxa without Nematoda, Platyhelminthes, Foraminifera and Copepoda** | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Animalia** | Yes | No | Yes | No | Yes | Yes | Yes | No | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Animalia without Nematoda, Platyhelminthes, Foraminifera and Copepoda** | Yes | No | Yes | No | Yes | Yes | Yes | No | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Arthropoda without Copepoda** | No | No | No | No | Yes | No | No | No | No | No | No | No | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Porifera** | Yes | No | No | No | Yes | No | No | No | No | No | No | No | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Mollusca** | Yes | No | No | No | Yes | Yes | Yes | No | No | Yes | No | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Cnidaria** | Yes | No | No | No | Yes | No | No | No | No | No | No | No | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Bryozoa** | Yes | No | No | No | Yes | No | No | No | No | No | No | No | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Polychaeta** | Yes | No | No | No | Yes | No | No | No | No | No | No | No | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Plantae, Bacteria and Chromista** | Yes | No | No | No | Yes | No | No | No | No | No | No | No | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Plantae** | Yes | No | No | No | Yes | No | No | No | No | No | No | No | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Chromista** | Yes | No | No | No | Yes | No | No | No | No | No | No | No | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Bacteria** | Yes | No | No | No | No | No | No | No | No | No | No | No | No | No | No | No | No | No | Yes | Yes |
| **Chlorophyta** | Yes | No | No | No | Yes | No | No | No | No | No | No | No | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Rhodophyta** | Yes | No | No | No | Yes | No | No | No | No | No | No | No | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Florideophyceae** | Yes | No | No | No | Yes | No | No | No | No | No | No | No | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

Some of these parameters require an aggregation file that contains the appropriate taxonomic structure of the taxa included in the analyses. The taxonomic structure was created at seven levels, and the weights used for the analyses were of branch length of value 1. The seven taxonomic levels were: Kingdom; Phylum or Division (depending upon the taxa); Class; Subclass, Order or Suborder (depending upon the taxa); Family; Genus and Species.

Some of the biota was not identified at species level. Hence, for the taxonomic levels below, virtual taxa were use. As an example, if an individual was identified as Ascidiacea the following taxa levels were used:

1) Aminalia
2) Chordata
3) Ascidiacea
4) AscidiaceaVirtualSubclass
5) AscidiaceaVirtualFamily
6) AscidiaceaVirtualGenus
7) AscidiaceaVirtualSpecies

Moreover for some of the biota it is not defined all the taxonomic levels, and virtual taxa was also used. A total of 1579 taxa were used.

Based upon the datasets built for learning, 12 Bayesian models (Table 2) were created.

Table 2. Preliminary Bayesian models.

| | Macrofauna | Macroalgae | Macrofauna+ macroalgae |
|---|---|---|---|
| **Supralittoral zone** | Yes | Yes | Yes |
| **Midlittoral zone** | Yes | Yes | Yes |
| **Infralittoral zone** | Yes | Yes | Yes |
| **Subtidal zone** | Yes | Yes | Yes |

It was found that some of these models had not good confidence in predictions. One of the reasons was that some of the classes to be predicted (e.g., ‘poor’ status) had low number of data in the learning data set. Hence, if the number of data was low, for some models the response variable was grouped into two classes (i.e., ‘high-good’ and ‘moderate-poor’) or three classes (i.e., ‘high’, ‘good’, and ‘moderate-poor’), instead of four response classes (i.e., ‘high’, ‘good’, ‘moderate’, and ‘poor’).

The Bayesian Network models where fitted in three steps:

1) Hall’s multivariate Correlation-based Feature subset Selection (CFS) method (Hall and Smith, 1997) was used to select the predictive factors. CFS is based upon an intuitive formulation, the assumption that a good subset of factors is one that is highly correlated with the response variable and, at the same time, the predictive factors have low correlation between them. The method is implemented in the machine learning free software Weka.

2) The predictor variables were discretized into levels (less than x, more than x) using the Fayyad and Irani's Multi-Interval Discretization (MID) method (Fayyad and Irani, 1993). It searches, in each predictor, for a set of cut-off points that reduces the response variable uncertainty measured in terms of entropy and conditional entropy (Information Theory based).

3) A naive Bayes model was fitted (Minsky, 1961; Duda et al., 2001). Naive Bayes is one of the simplest Bayesian Networks Classifiers (Larrañaga et al., 2005). The Naive Bayes is a probabilistic model, which assumes that, given the class variable, all of the factors are independent. This assumption implies that a naive Bayes classifier requires the specification of a small number of parameters. This leads to robust models and parameter estimation when sparse training data available. The commercial software Bayesia was used to fit the models and perform the inference showed in the figures.

## 2.4. Data used for the validation of the method

The Bayesian models created with data set from Basque Country was validated with data from Denmark and Portugal.

Data from Denmark was provided by Karsten Dahl (Department of Bioscience - Section for Marine Ecology, National Environmental Research Institute). These data include macrofauna and macroalgae biomass aggregated in 3 depth intervals each representing 12 (0.1 $m^2$) subsamples. Data were from a subtidal zone.

Data from Portugal include macrofauna biomass from a re-colonisation field experiment in intertidal rocky shore. Details of sampling are given in Patrício et al. (2006).

# 3 Results

## 3.1.Bayesian models based upon macrofauna

### 3.1.1. Midlittoral zone

The response variable (status) included two classes: ‘high-good’ and ‘moderate-poor’ status. The selected variables (i.e, those variables that better predict the status) were (Figure 5):

H’ [Polychaeta]

H’ [Arthropoda without Copepoda]

H’ [Animalia without Nematoda, Platyhelminthes, Foraminifera and Copepoda]

Total taxonomic distinctness (Presence/Absence) [Mollusca]

Hill’s N21 [Animalia without Nematoda, Platyhelminthes, Foraminifera and Copepoda]

Regarding to the performance of the model, the accuracy was of 75.3±3.8 and the Brier score was of 0.11 (‘superior’ performance). A ‘superior’ performance in accuracy is the highest (between 0 and 100); whereas in Brier score is the lowest (between 0 and 1, or 2 if not normalized). An interpretation of Brier score in terms of ‘excellent’, ‘superior’, ‘adequate’, ‘acceptable’ or ‘insufficient’ can be consulted in Fernandes (2011).

### 3.1.2. Infralittoral zone

The response variable (status) included three classes: ‘high-good’, ‘moderate’ and ‘poor’ status. The selected variables (i.e, those variables that better predict the status) were (Figure 6):

Hill’s N21 [Animalia]

Average Taxonomic distinctness (Presence/Absence) [Arthropoda without Copepoda]

Taxonomic diversity [Mollusca]

Hill’s N21 [Mollusca]

Total taxonomic distinctness (Presence/Absence) [Animalia without Nematoda, Platyhelminthes, Foraminifera and Copepoda]

Regarding to the performance of the model, the accuracy was of 57.6±1.3 and the Brier score was of 0.31 (‘adequate’ performance).

*Figure 5. Midlittoral zone. Bayesian variable selection. Macrofauna. Key: A – 'moderate-poor' status; B – 'high-good' status.*

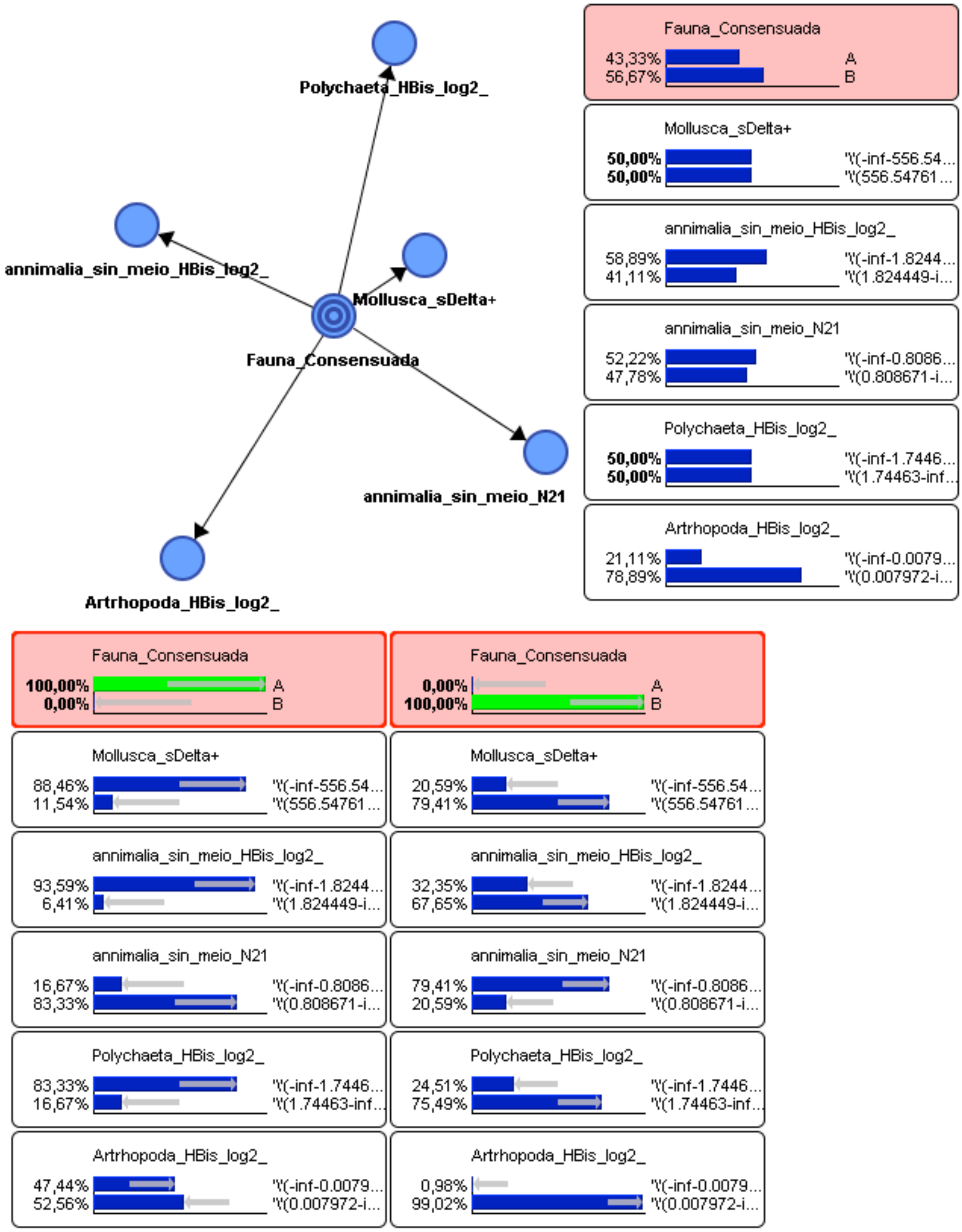
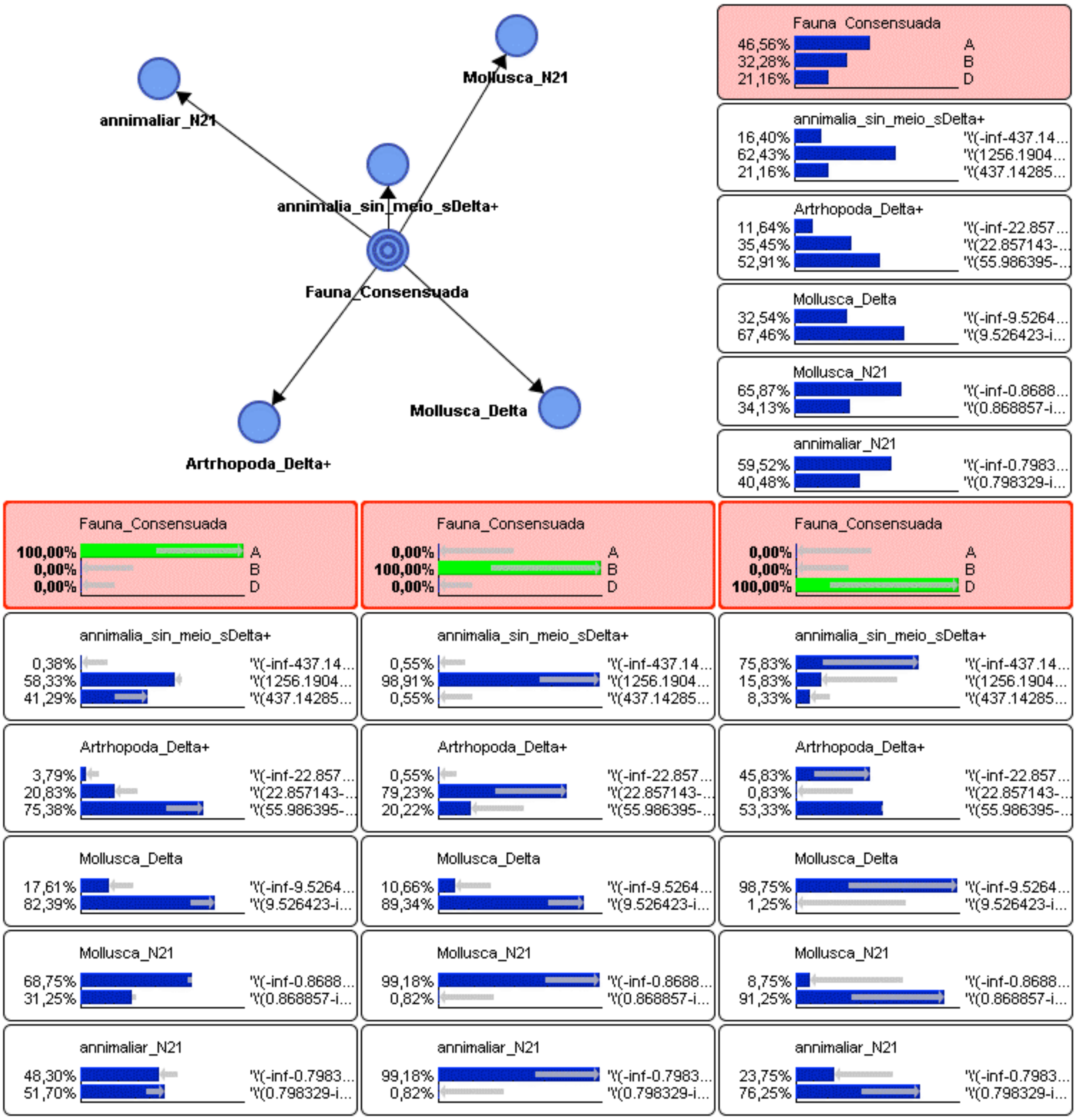


*Figure 6. Infralittoral zone. Bayesian variable selection. Macrofauna. Key: A – 'moderate' status; B – 'high-good' status, D - 'poor' status.*



### 3.1.3. Subtidal zone depth 5-15 m

The response variable (status) included three classes: 'high', 'good', and 'moderate-poor' status. The selected variables (i.e, those variables that better predict the status) were (Figure 7):

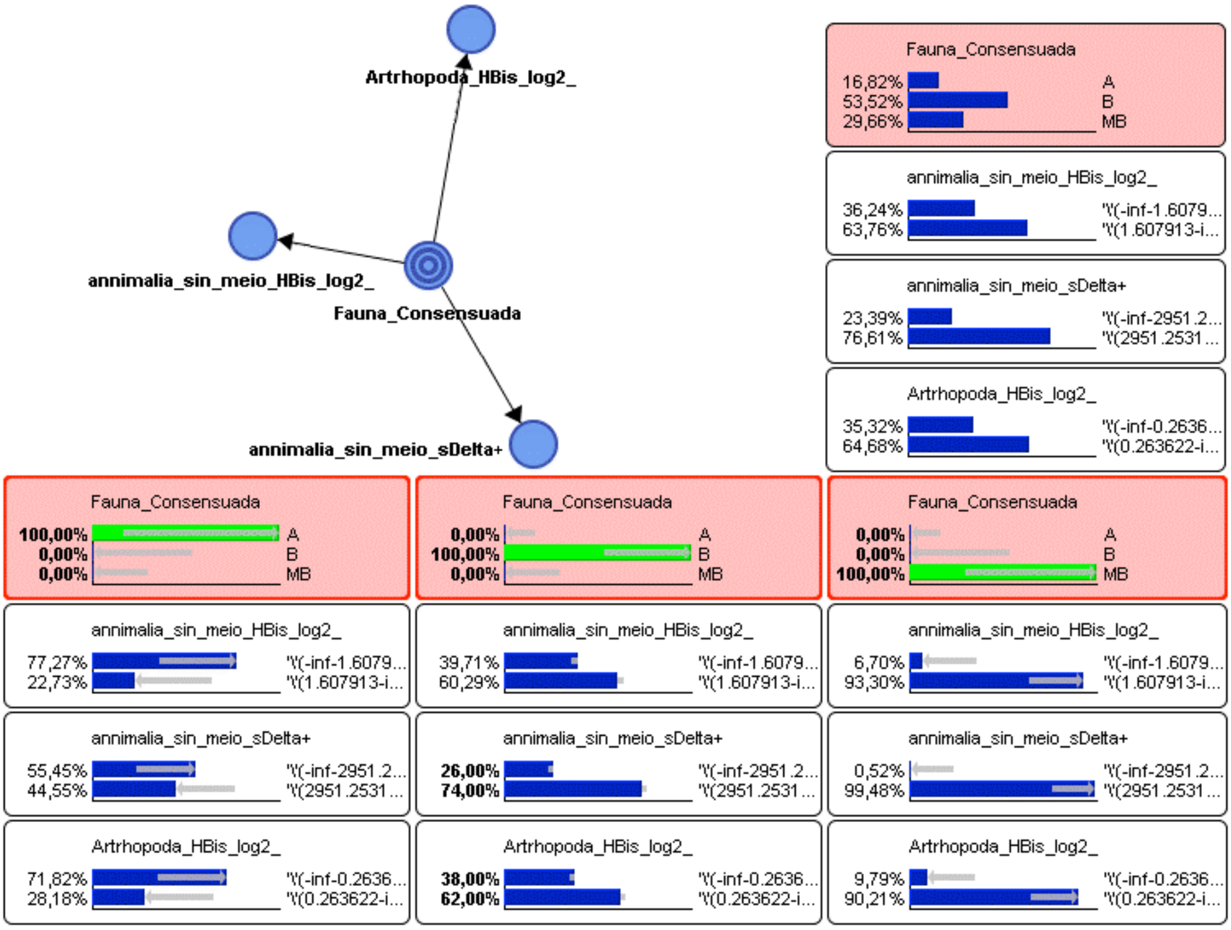
H' [Arthropoda without Copepoda]

H' [Animalia without Nematoda, Platyhelminthes, Foraminifera and Copepoda]

Total taxonomic distinctness (Presence/Absence) [Animalia without Nematoda, Platyhelminthes, Foraminifera and Copepoda]

Regarding to the performance of the model, the accuracy was of 49.6±3.8 and the Brier score was of 0.32 ('adequate' performance).

*Figure 7. Subtidal zone depth 5-15 m. Bayesian variable selection. Macrofauna. Key: A – 'moderate-poor' status; B – 'good' status, MB - 'high' status.*



### 3.1.4. Subtidal zone depth >15m

The response variable (status) included three classes: 'high', 'good', and 'moderate-poor' status. The selected variables (i.e, those variables that better predict the status) were (Figure 8):

Taxonomic diversity [Arthropoda without Copepoda]

Total taxonomic distinctness (Presence/Absence) [Animalia]

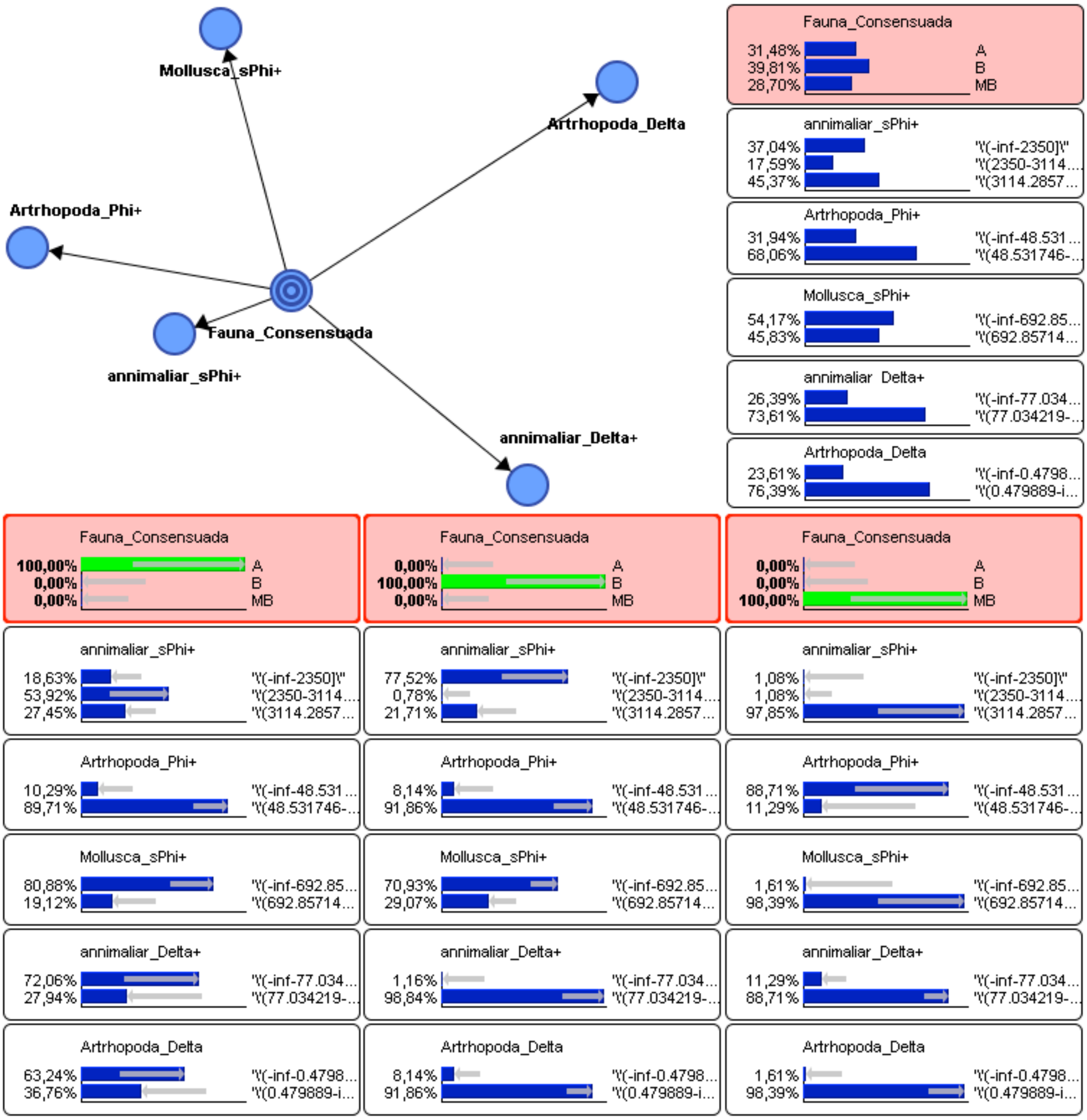
Total Phylogenetic diversity [Animalia]

Total Phylogenetic diversity [Mollusca]

Average Phylogenetic diversity [Arthropoda without Copepoda]

Regarding to the performance of the model, the accuracy was of 70.2±4.8and the Brier score was of 0.22 (‘superior’ performance).

*Figure 8. Subtidal zone depth >15 m. Bayesian variable selection. Macrofauna. Key: A – ‘moderate-poor’ status; B – ‘good’ status, MB - ‘high’ status.*



## 3.2.Bayesian models based upon macroalgae

### 3.2.1. Midlittoral zone

The response variable (status) included four classes: ‘high’, ‘good’, ‘moderate’ and ‘poor’ status. The selected variables (i.e, those variables that better predict the status) were (Figure 9):

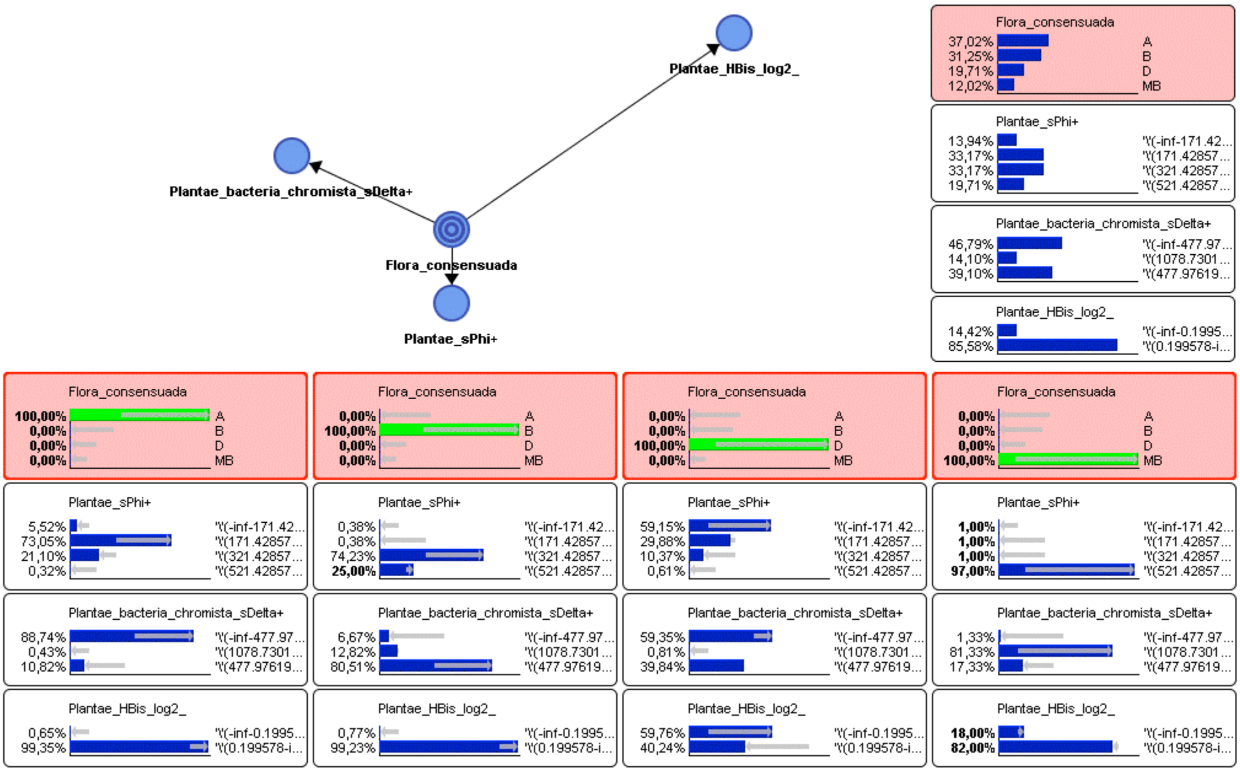
H’ [Plantae]

Average Phylogenetic diversity [Plantae]

Total taxonomic distinctness (Presence/Absence) [Plantae, Bacteria and Chromista]

Regarding to the performance of the model, the accuracy was of 64.4±3.2 and the Brier score was of 0.21 (‘superior’ performance).

*Figure 9. Midlittoral zone. Bayesian variable selection. Macroalgae. Key: D – ‘bad’ status, A – ‘moderate’ status; B – ‘good’ status, MB - ‘high’ status.*



### 3.2.2. Infralittoral zone

The response variable (status) included three classes: ‘high-good’, ‘moderate’ and ‘poor’ status. The selected variables (i.e, those variables that better predict the status) were (Figure 10):

Average Phylogenetic diversity’ [Rhodophyta]

Total taxonomic distinctness (Presence/Absence) [Plantae, Bacteria and Chromista]

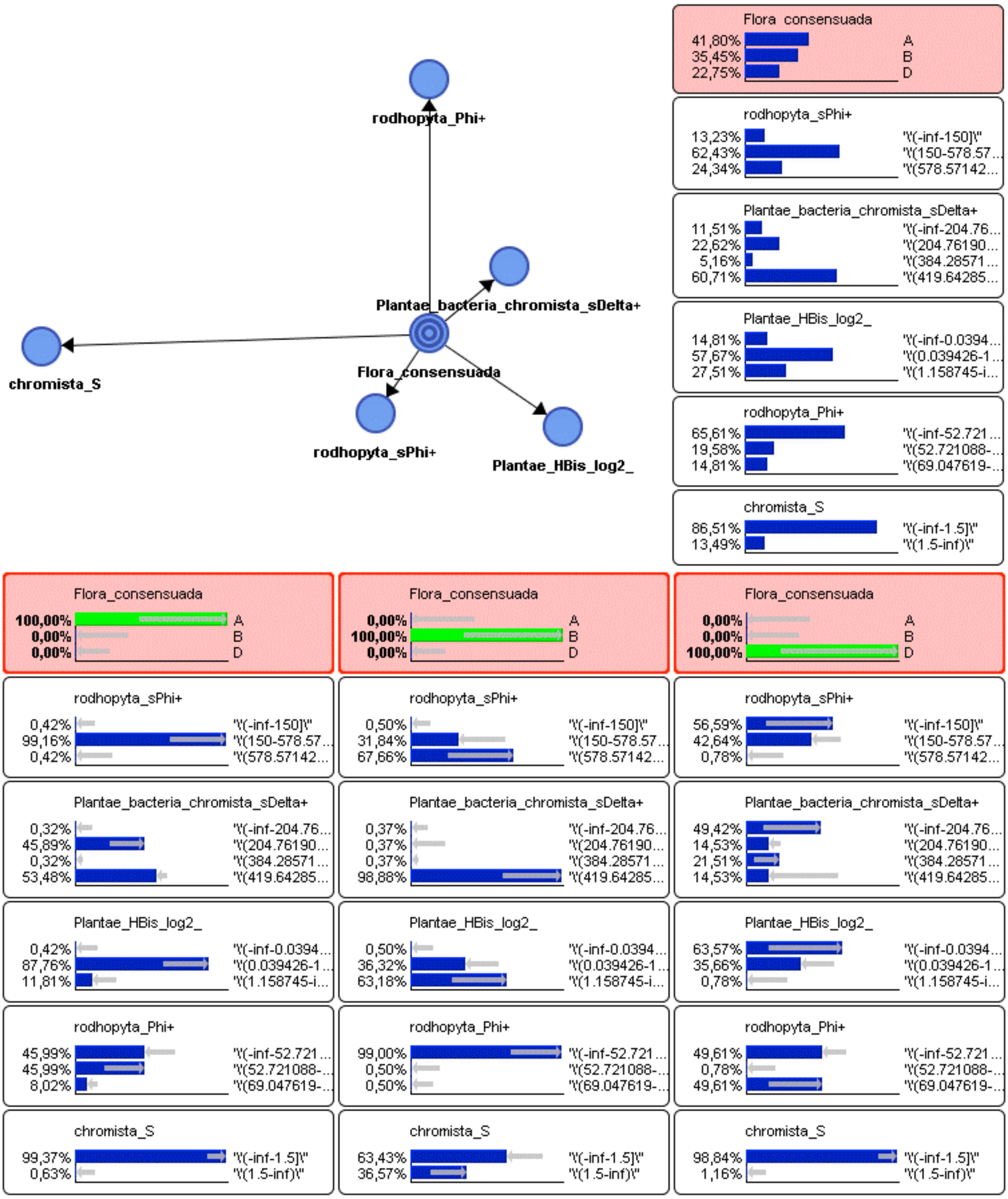
H’ [Plantae]

Total Phylogenetic diversity [Rhodophyta]

Number of taxa [Chromista]

Regarding to the performance of the model, the accuracy was of 67.8±3.9 and the Brier score was of 0.16 (‘excellent’ performance).

*Figure 10. Infralittoral zone. Bayesian variable selection. Macroalgae. Key: D – 'bad' status, A – 'moderate' status; B – 'high-good' status.*



### 3.2.3. Subtidal zone depth 5-15 m

The response variable (status) included two classes: 'high-good' and 'moderate-poor' status. The selected variables (i.e, those variables that better predict the status) were (Figure 11):

H' [Chromista]

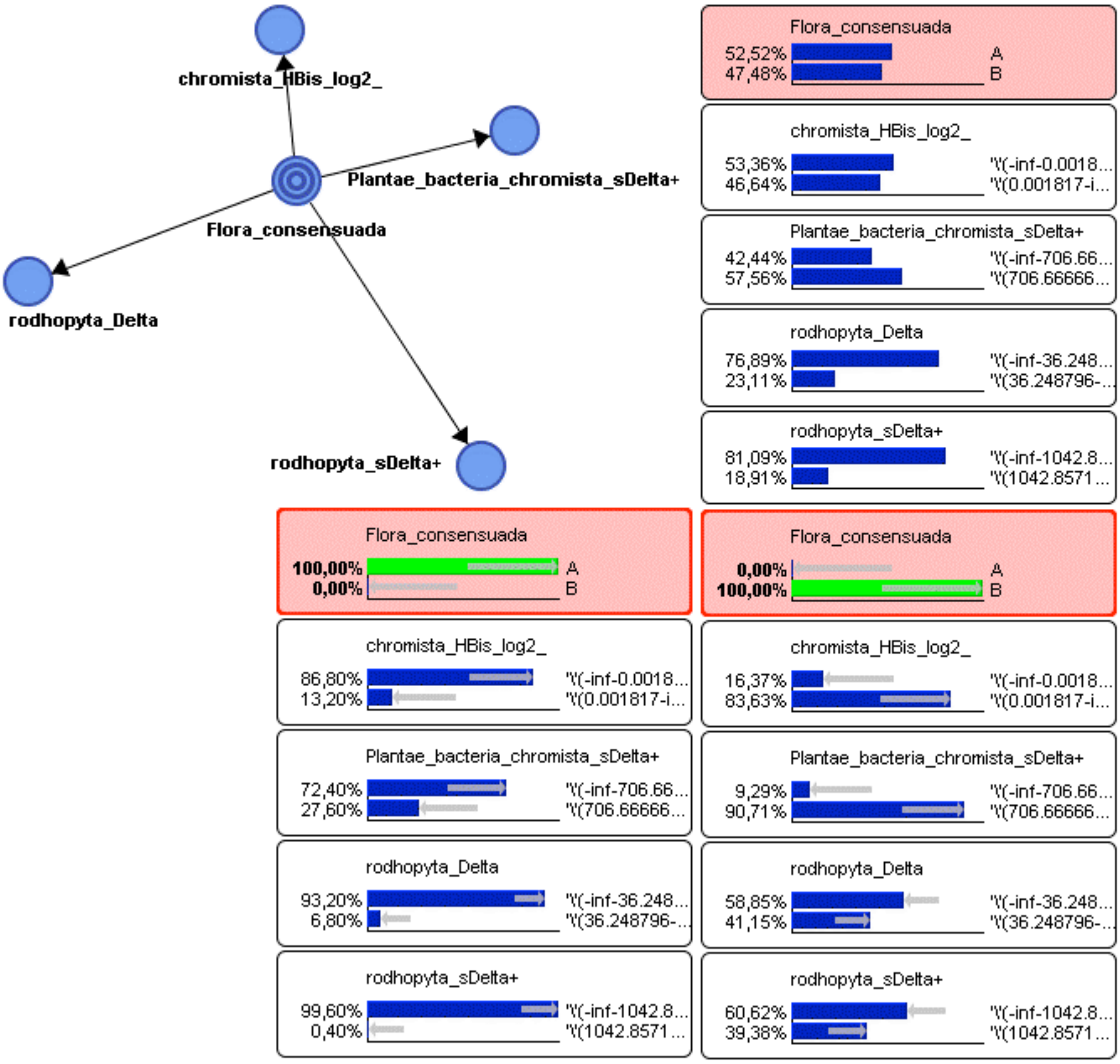
Total taxonomic distinctness (Presence/Absence) [Plantae, Bacteria and Chromista]

Total Phylogenetic diversity [Rhodophyta]

Taxonomic diversity [Rhodophyta]

Regarding to the performance of the model, the accuracy was of 86.7±0.8 and the Brier score was of 0.08 (‘excellent’ performance).

*Figure 11. Subtidal zone depth 5-15 m. Bayesian variable selection. Macroalgae. Key: A – ‘moderate-bad’ status; B – ‘high-good’ status.*



### 3.2.4. Subtidal zone depth >15m

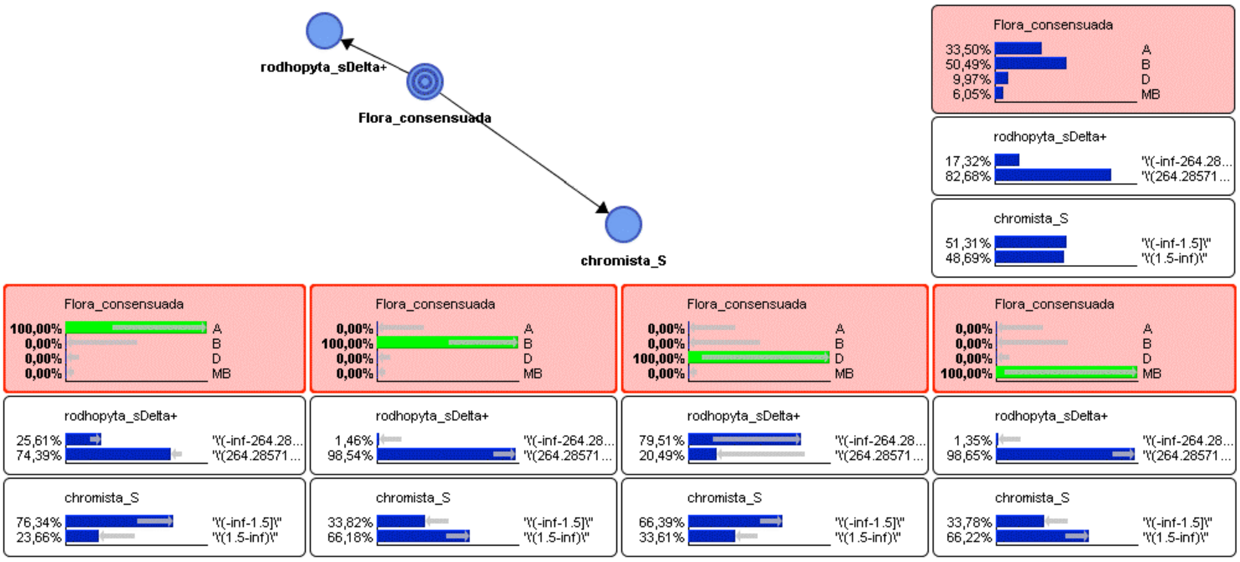
The response variable (status) included four classes: ‘high’, ‘good’, ‘moderate’ and ‘poor’ status. The selected variables (i.e, those variables that better predict the status) were (Figure 12):

Number of taxa [Chromista]

Total taxonomic distinctness (Presence/Absence) [Rhodophyta]

Regarding to the performance of the model, the accuracy was of 84.8±3 and the Brier score was of 0.07 (‘excellent’ performance).

*Figure 12. Subtidal zone depth >15 m. Bayesian variable selection. Macroalgae. Key: A – ‘moderate’, D – ‘bad’ status; B – ‘good’ status and MB - ‘high’ status.*



## 3.3. Bayesian models based upon macroalgae and macrofauna

### 3.3.1. Midlittoral zone

The response variable (status) included two classes: ‘high-good’, ‘moderate-poor’ status. The selected variables (i.e, those variables that better predict the status) were (Figure 13):

H’ [Plantae]

Average taxonomic distinctness (Presence/Absence) [Plantae, Bacteria and Chromista]

Total Phylogenetic diversity [Arthropoda]

Total taxonomic distinctness (Presence/Absence) [Plantae]

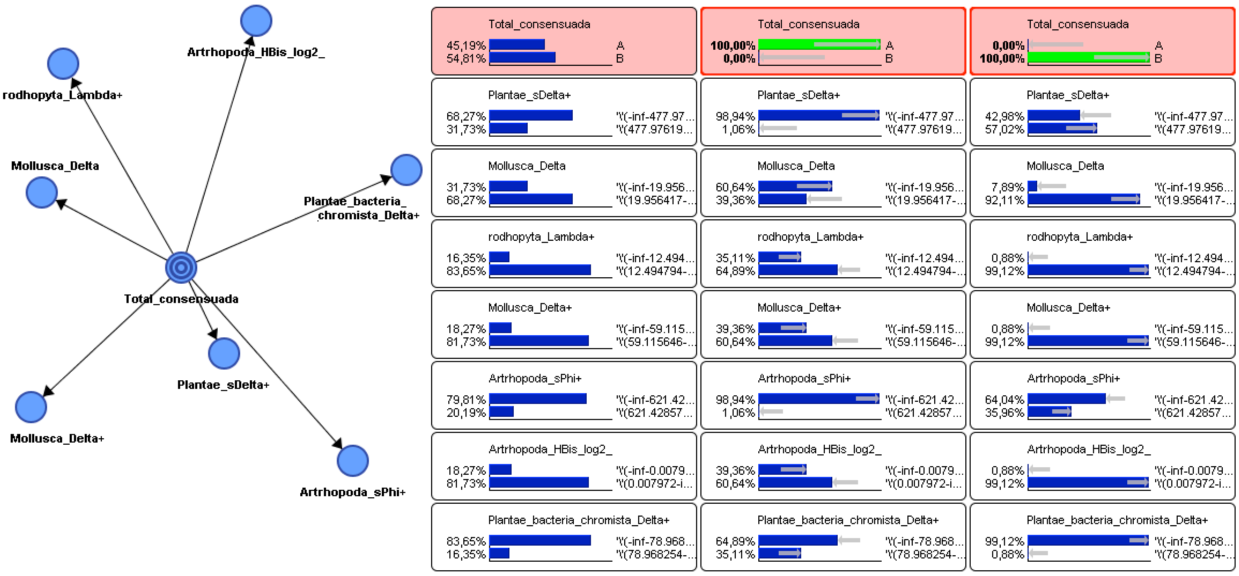
Average taxonomic distinctness (Presence/Absence) [Mollusca]

Total taxonomic distinctness (Presence/Absence) [Mollusca]

Variation in taxonomic distinctness (Presence/Absence) [Rhodophyta]

Regarding to the performance of the model, the accuracy was of 73.9±2.1 and the Brier score was of 0.10 (‘excellent’ performance).

*Figure 13. Midlittoral zone. Bayesian variable selection. Macroalgae and macrofauna. Key: A – 'moderate-bad' status; B – 'high-good' status.*



## 3.3.2. Infralittoral zone

The response variable (status) included three classes: 'high-good', 'moderate' and 'poor' status. The selected variables (i.e, those variables that better predict the status) were (Figure 14):

Pielou's eveness [Animalia]

Hill's N21 [all taxa]

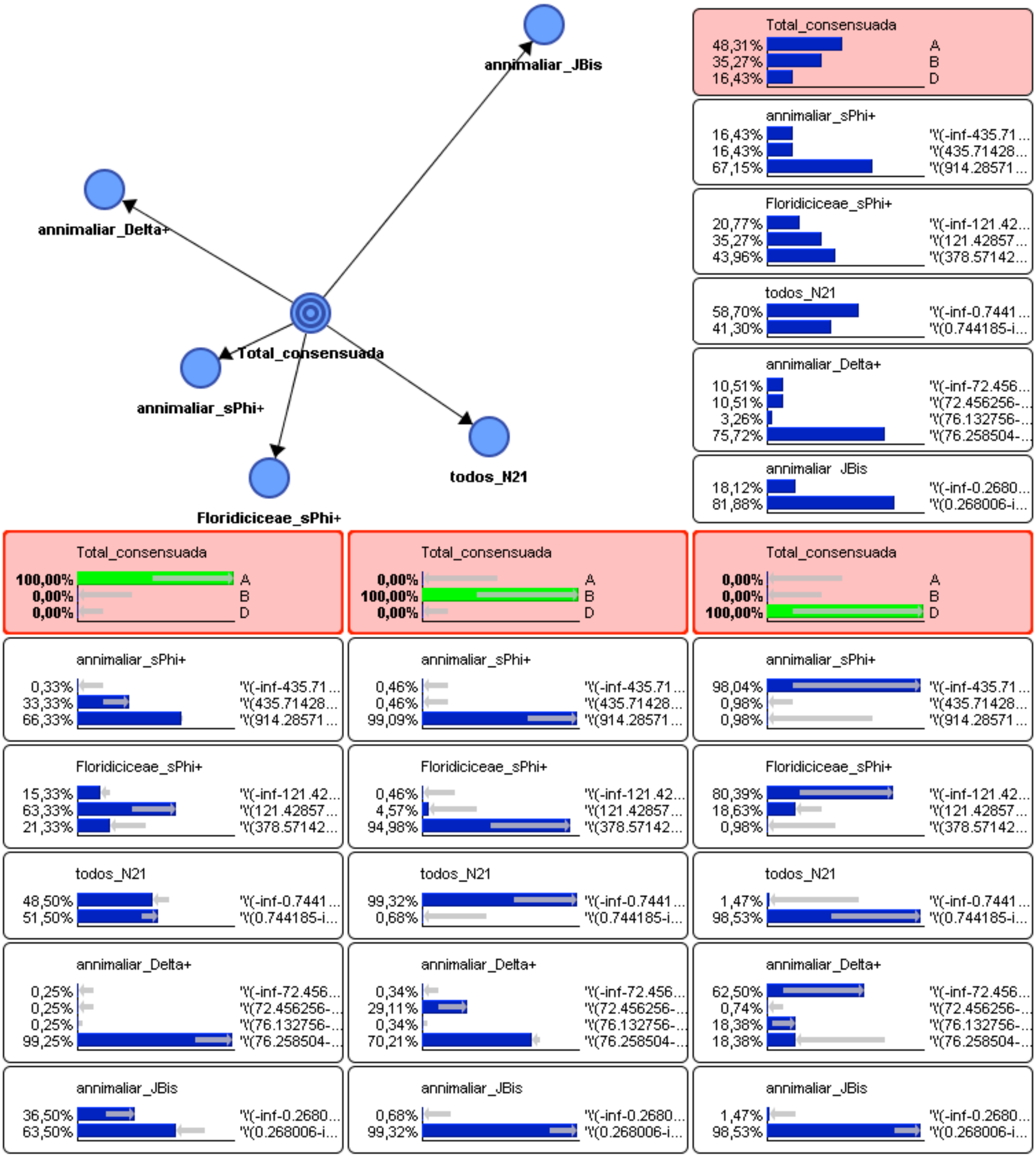
Total Phylogenetic diversity [Florideophyceae]

Total Phylogenetic diversity [Animalia]

Average Taxonomic distinctness (Presence/Absence) [Animalia]

Regarding to the performance of the model, the accuracy was of 68.4±1 and the Brier score was of 0.25 ('superior' performance).

*Figure 14. Infralittoral zone. Bayesian variable selection. Macroalgae and macrofauna. Key: A – 'moderate' statuts; D – 'bad' status; B – 'high-good' status.*



### 3.3.3. Subtidal zone depth 5-15 m

The response variable (status) included three classes: 'high', 'good' and 'moderate-poor' status. The selected variables (i.e, those variables that better predict the status) were (Figure 15):

Richness (Margalef) [all taxa]

Brillouin-H index [all taxa]

H' [Cnidaria]

Average Phylogenetic diversity [Bryozoa]

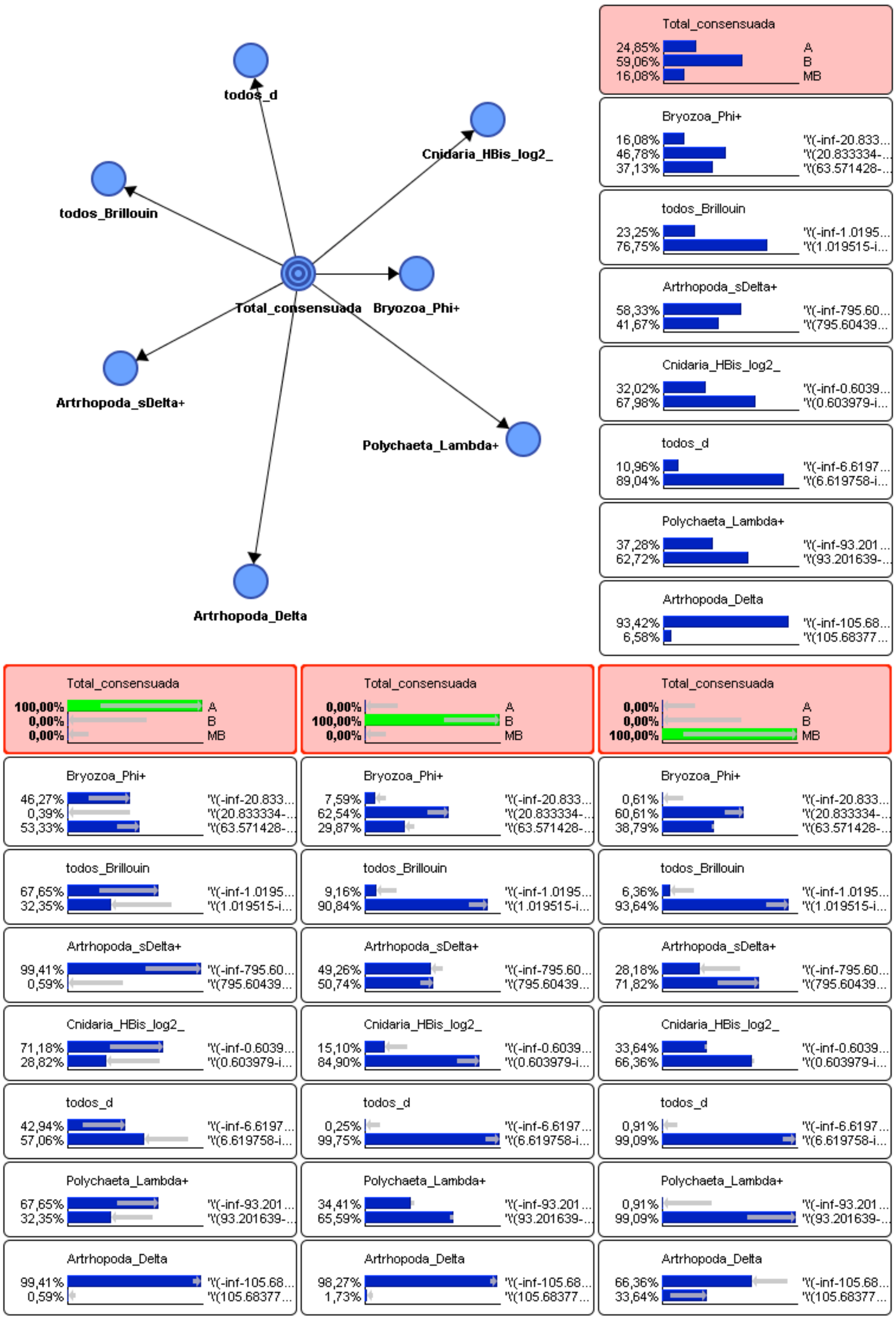
Simpson’s Lambda‘ [Polychaeta]

Taxonomic diversity [Arthropoda]

Total taxonomic distinctness (Presence/Absence) [Arthropoda]

Regarding to the performance of the model, the accuracy was of 61.9±1.8 and the Brier score was of 0.26 (‘superior’ performance).

*Figure 15. Subtidal zone depth 5-15 m. Bayesian variable selection. Macroalgae and macrofauna. Key: A – ‘moderate-bad’ status; MB – ‘high’ status; B – ‘high-good’ status.*



### 3.3.4. Subtidal zone depth >15m

The response variable included two classes: ‘high-good’ and ‘moderate-poor’ status. The selected variables (i.e, those variables that better predict the status) were (Figure 16):

Average Taxonomic distinctness (Presence/Absence) [all taxa]

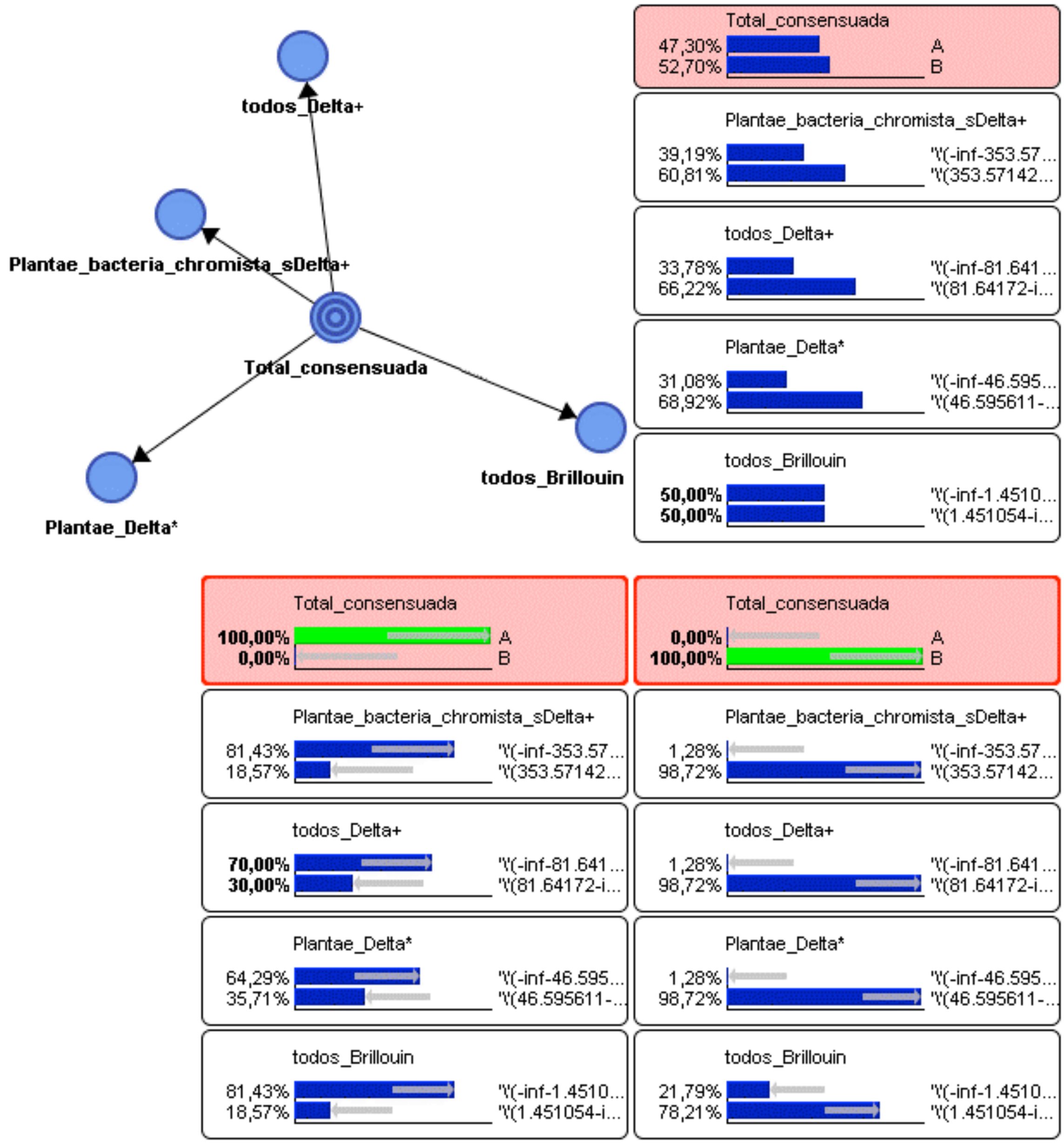
Brillouin-H index [all taxa]

Taxonomic distinctness [Plantae]

Total taxonomic distinctness (Presence/Absence) [Plantae, Bacteria and Chromista]

Regarding to the performance of the model, the accuracy was of 89.9±2.1and the Brier score was of 0.09 (‘excellent’ performance).

*Figure 15. Subtidal zone depth >15 m. Bayesian variable selection. Macroalgae and macrofauna. Key: A – ‘moderate-bad’ status; B – ‘high-good’ status.*



## 3.2. Validation of the created Bayesian models

### 3.2.1. Denmark

The results of the Bayesian models for: (i) only macrofauna, (ii) only macroalga, and (iii) macrofauna and macroalgae applied upon data found that the status was ‘good’ or ‘high’ (Table 3).

These results do not agree with the status observed in biota, since data are from an area affected by eutrophication (Karsten Dahl, pers. com.).

*Table 3. Results of the Bayesian models for macrofauna, macroalgae and macrofauna+macroalgae. Probability of each quality class is shown. Key: A – ‘moderate-bad’ status; B – ‘high-good’ status.*

| Sampling site | Model | 'Moderate-Bad' | 'Good' | 'High' | 'High-good' |
|---|---|---|---|---|---|
| **Sample3,4-5,4** | **Macroalgae and macrofauna** | 0 | 0.77 | 0.23 | - |
| | **Macrofauna** | 0.01 | 0.37 | 0.62 | - |
| | **Macroalgae** | 0.01 | - | - | 0.99 |
| **Sample5,4-7,4** | **Macroalgae and macrofauna** | 0 | 0.77 | 0.23 | - |
| | **Macrofauna** | 0.01 | 0.37 | 0.62 | - |
| | **Macroalgae** | 0.01 | - | - | 0.99 |
| **Sample7,4-9,4** | **Macroalgae and macrofauna** | 0 | 0.77 | 0.23 | - |
| | **Macrofauna** | 0.01 | 0.37 | 0.62 | - |
| | **Macroalgae** | 0.01 | - | - | 0.99 |

### 3.2.2. Portugal

The results of the Midlittoral zone Bayesian model for macrofauna applied upon data from succession experiment carried out at Portugal did not find differences in the status between the control and the treatment zones (Table 4). The Bayesian model predicts ‘high-good’ status in all dates. These results do not agree with the successional pattern found (Patrício et al., 2006). Hence, in the two first samples after removing biota, number of taxa and eco-exergy was lower in the succession samples. Moreover, multidimensional analysis carried out on the macrofauna data did found different species assemblages in control and treatment zones. In other words, a lower status was expected in the first samplings after removing biota.

*Table 4. Results of the Midlittoral zone Bayesian model for macrofauna. Probability of each quality class is shown. Key: A – 'moderate-bad' status; B – 'high-good' status.*

| Sampling site | Date | A | B |
|---|---|---|---|
| **Cleared** | **21/03/1999** | 0.01 | 0.99 |
| **Cleared** | **17/05/1999** | 0.00 | 1.00 |
| **Cleared** | **16/06/1999** | 0.01 | 0.99 |
| **Cleared** | **14/07/1999** | 0.01 | 0.99 |
| **Cleared** | **29/09/1999** | 0.00 | 1.00 |
| **Cleared** | **25/11/1999** | 0.00 | 1.00 |
| **Cleared** | **23/01/2000** | 0.00 | 1.00 |
| **Cleared** | **21/03/2000** | 0.01 | 0.99 |
| **Cleared** | **17/05/2000** | 0.00 | 1.00 |
| **Control** | **18/02/1999** | 0.00 | 1.00 |
| **Control** | **17/05/1999** | 0.00 | 1.00 |
| **Control** | **16/06/1999** | 0.00 | 1.00 |
| **Control** | **29/09/1999** | 0.01 | 0.99 |
| **Control** | **25/11/1999** | 0.01 | 0.99 |
| **Control** | **23/01/2000** | 0.01 | 0.99 |
| **Control** | **21/03/2000** | 0.02 | 0.98 |
| **Control** | **17/05/2000** | 0.00 | 1.00 |

# 4 Discussion

The statistical approach tested in this investigation has *a priori* several advantages:

i) Can create a model even if relationships between biota and different environmental factors are complex or non-linear (e.g., Fernandes et al., 2010).

ii) The used 'machine-learning' techniques allow to identify those biotic parameters that can predict better the ecological status (e.g, Fernandes et al., 2010).

iii) The number of biotic parameters that can predict with high confidence can be low.

iv) The biotic parameters can be highly correlated.

v) The predictions allow knowing probability of occurrence in different statuses.

The point (i) is important, within a statistical point of view, since relationships between the ecological status and the environment are often complex. Additionally, points (ii) and (iv) are important when the number of biotic variables is high.

The point (iii) is of interest within monitoring networks. As example, if biotic parameters related with only three taxonomic groups (and not the whole biota) are enough to predict the status, the monitoring can be focussed only in those taxa.

In turn, we have found the following disadvantages using this method: (i) this statistical approach requires a high number of data for build a ‘learning’ data set; and (ii) in some cases, different statuses have to be joined to increase confidence level of the model.

On the other hand, the validation of the models in areas from Portugal and Denmark did not find coherent results. This suggests that the Bayesian models developed in this study can be useful only for the Basque Country area. This can be due to the fact that the pressure taken into account in the Basque coast is related mainly to outfalls. Additionally, biogeographical differences in taxonomic composition (or differential responses to pollution) can be related also to the lack of response in Portugal and Denmark. On the other hand, differences in sampling strategy can also have influence on the approach.

Nevertheless, we have found that it is possible to build Bayesian models that can predict the ecological status in hard-bottom substrata. Therefore, in those countries with a high number of data, obtained with the same sampling methodology, Bayesian models could be applied. However, this approach can imply difficulties in the intercalibration procedures required within the WFD, since it is possible that the Bayesian models can be only useful for a specific area and not applicable to another.

# 5 Conclusions

Bayesian models were built for predicting the ecological status of macroalgae, macrofauna, and macroalgae+macrofauna, within hard-bottom areas of the Basque Country, at different shore levels, within intertidal and subtidal areas.

- The number of statuses that can be predicted varied from two to four.
- The accuracy of the models differs between shore levels.
- The results of the Bayesian models applied to data from Portugal and Denmark, as validation exercises, were not coherent.
- Bayesian models can be useful if a long data set is available. However, the results obtained from a particular area could be not extended to another. Hence, local models should be developed, making difficult to apply the same approach to large areas.
- More research is required to apply this approach to other areas, looking for similar sampling methods and data availability.

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