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Effect of Seasonal Changes on Predictive Model Assessments of Streams Water Quality with Macroinvertebrates

key words: seasonal variability, macroinvertebrates, predictive models, water quality assessment

Abstract

In this study we investigate how seasonal variability in aquatic macroinvertebrate communities affects the performance of a predictive model developed to assess environmental quality. Macroinvertebrates were sampled from nine not visibly disturbed sites located in different streams of the Mondego catchment across a full year. Organisms were identified to the lowest practicable taxonomic level and their abundances recorded at three taxonomic levels (order, family and lowest level). The seasonal samples were examined with regard to seasonal variation using three predictive models at order, family and lowest taxonomic level. The models showed increasing effect of seasonal changes across taxonomic levels, from order to the lowest level. When using the current models samples should be taken in the same season as the reference sites were sampled. Furthermore, data from more reference sites should be added to the model in order to encompass sufficient natural variation and allow the use of the model in different seasons.

1. Introduction

The Reference Condition Approach (RCA) is an alternative method for assessing aquatic ecosystem condition and for detecting impairment (Barbour *et al.*, 1996). Reference conditions are defined by groups of minimally disturbed sites characterized by physical, chemical and biological attributes (according to Reynoldson *et al.*, 1997). The assessment of water quality is based on the comparison of aquatic invertebrate communities at test sites with the appropriate group of reference sites using a predictive model that matches the test and reference sites.

Selecting the appropriate level of invertebrate identification for biomonitoring depends on the study and the questions being asked (Resh and McElravy, 1993). Zamora-Muñoz and Alba-Tercedor (1996) suggest the family level is sufficient for monitoring water quality in streams, but that species data are required to determine the exact biological response to stress. Monitoring at the family level is used by the Australian River Assessment Scheme (AusRivAS) and has been recommended for marine (Warwick, 1993) and lake (Jackson and Harvey, 1993) ecosystems. Also in assessing the performance of a Fraser River, British Columbia, RCA model Reynoldson *et al.* (2001) report the family level to be most sensitive in detecting community change.

A predictive model using the RCA has been developed for the Mondego River basin in central Portugal (Feio, 2004; Feio *et al.*, 2006). When developing the Mondego River RCA

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model sampling was conducted in the Summer only and models were developed from those samples. Because benthic communities change seasonally and annually (HYNES, 1970; BOULTON and LAKE, 1992; REYNOLDSON and WRIGHT, 2000) this could constrain the applicability of the use of the Mondego predictive model outside the reference season (REECE *et al.*, 2001). FURSE *et al.* (1984) recommend seasonal collections of reference samples when using predictive models. This way, seasonal models or a combined season's model may be developed. However, the additional time and cost of collecting and identifying samples from a large number of reference sites over several seasons are important considerations when opting for this approach (REECE *et al.*, 2001). To determine the need for this additional effort the significance of seasonal variation was investigated by examining a subset of sites sampled seasonally that were representative of the study area (REYNOLDSON *et al.*, 1997; REYNOLDSON and WRIGHT, 2000; REECE *et al.*, 2001).

In this particular study, we were concerned with the sensitivity to temporal variation of the fauna at different levels of identification used in the classification of sites of the Mondego basin. Representatives of families and orders are likely to be present throughout the year while lower taxa (genus or species) may be present in the streams at different times of the year due to their life-cycle. In Canadian rivers, REECE *et al.* (2001) detected a higher sensitivity to seasonal variation at genus level than at family level in exposed sites.

Different precipitation and temperature regimes could lead to different hydrological regimes, species life cycles and consequently to different seasonal variations of aquatic communities. Moreover, central Portuguese streams have high species diversity in invertebrate communities which could result in high differences between predictions to seasonal variations at different taxonomic levels. Therefore, we examined the effect of seasonal changes in benthic invertebrate assemblages of Portuguese streams, through a wide spectrum of identification levels (lowest level, family and order levels), on the accuracy of predictions made with the Mondego predictive model based on summer-reference samples. Reference sites were sampled over one year and also in a second year in summer for comparative purpose.

2. Methods

2.1. Study Area and Sampling Procedures

The Mondego river catchment (Fig. 1) is located in central of Portugal, between 39°46′ and 40°48′ N and 7°14′ and 8°52′ W (LIMA and LIMA, 2002). The 6670 km^2 catchment includes a wide range of environmental conditions from mountainous areas in the upper (NE) and middle regions to a large alluvial plain (SW) where the river discharges to the Atlantic Ocean (MARQUES *et al.*, 2002).

A total of 36 samples were collected from nine sites from streams reflecting the range of characteristics observed across the Mondego river basin on different seasons (see below) (Fig. 1). The nine "seasonal" sites were chosen from the 75 reference sites sampled in the Summer of 2001 used to construct and test a predictive model for the Mondego river basin. The distance from source of the seasonal sites ranged between 3 km (Tábuas) and 34 km (Trinta) and the altitude between 80 m (Botão) and 1040 m (Sabugueiro). One site is located in sedimentary area (Botão), two sites (Ribamondego and Sabugueiro) in granitic zones and the remaining six sites in metamorphic regions (mainly schist).

Seasonal samples were taken from reference sites in order to assure that any deviation from the reference condition was due to seasonal changes in the community composition and not to changes in water quality. The seasonal samples were obtained in Autumn 2001, Winter, Spring and also Summer 2002. At each site, the sampling procedure followed was the same as that for the collection of the reference data (FEIO, 2004; FEIO *et al.*, 2006). Macroinvertebrates were collected from a 3 or 1.5 minute kick-net sample taken across the stream (for streams of more or less than three meters width, respectively) with a square hand net, 0.30 × 0.30 m opening and 500 μm mesh size. The animals were preserved in formalin and after sorting, conserved in 70% ethanol for later identification and counting.

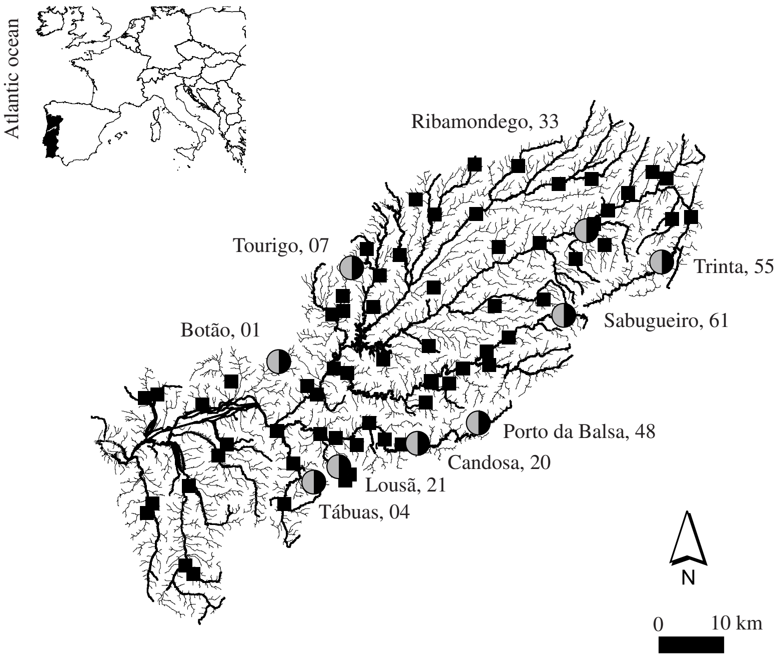

Figure 1. Localization of the Mondego river basin in Portugal and distribution of the reference (black squares) and seasonal test sites with the respective name and code (grey and black circles) in the catchment.

Forty environmental variables were either measured in the field or obtained from cartographic sources for each reference site. These variables represented descriptions of stream hydrology, geology, habitat quality, riparian vegetation, land use in the surroundings of the sampled stream reach, climate, water physics and chemistry, nutrients and organic matter or geographic location (FEIO, 2004; FEIO *et al.*, 2006). Table 1 contains a short description of the variables used. All the measures were repeated in each season for the seasonal test sites.

Table 1. Environmental parameters obtained for each sampling site and sources.

Environmental Variables	Description and Source
Stream Order	Military maps 1 : 250000 (Strahler system) (*Inst. Geográfico do Exército*)
Distance to Source (km)	Digital military maps (1 : 25000; DRAOT-Centro)
Decimal Latitude and Decimal Longitude	GPS (GARMIN) and digital military maps (1 : 25000; DRAOT-Centro)
Altitude(m)	idem
Valley Form	Field observations; Categories: 1 for V shapes; 2 for U shape, meander and plain floodplain)
Mean Annual Temperature (°C)	*Atlas Digital do Ambiente* – Instituto do Ambiente (data from 1931–1960).

Table 1. Condinued

Environmental Variables	Description and Source
Mean Annual Total Precipitation (mm)	*Atlas Digital do Ambiente* – Instituto do Ambiente (data from 1931–1960).
Mean Annual Precipitation (days/year)	*Atlas Digital do Ambiente* – Instituto do Ambiente (data from 1931–1960).
Mean Stream Width(m)	Field measurements (six measurements each transept)
Mean Stream Depth (m)	Idem
Current Velocity ($m\ s^{-1}$)	six field measurements (VALEPORT 15277)
Mean Discharge ($m^3\ s^{-1}$)	Stream width × Stream Depth x Current Velocity ($n = 6$)
Water Temperature (°C)	Field measurement (WTW OXI 92)
pH	Field measurement (JENWAY 3310)
Conductivity ($\mu S\ cm^{-1}$)	Field measurement (WTW LF 330)
O_2 ($mg\ l^{-1}$) and O_2 (%)	Field measurement (WTW OXI 92)
Total Dissolved Solids ($mg\ l^{-}$) (TDS)	Field measurement (WTW LF 330)
Chloride ($mg\ l^{-1}$)	Ion Chromatograph Dionex DX–120
Nitrate ($mg\ l^{-1}$)	Ion Chromatograph Dionex DX–120
Nitrite ($mg\ l^{-1}$)	Ion Chromatograph Dionex DX–120
Sulphate ($mg\ l^{-1}$)	Ion Chromatograph Dionex DX–120
P-Phosphate ($mg\ l^{-1}$)	Ion Chromatograph Dionex DX–120
N-Ammonia ($mg\ l^{-1}$)	Ion Chromatograph Dionex DX–120
Alkalinity ($mg\ l^{-1}$)	Titration to an end pH of 4.5 (A.P.H.A., 1995)
CPOM >1mm (AFDM, g)	Collected in benthos samples, dried, and burned to ashes, 500 °C, 2 h.
Chlorophyll in Periphyton ($mg\ m^{-2}$)	Collection by stone scraping; washed with 300 ml of water and kept in WHATMAN GFC fibre-glass filters. Analysis according to A.P.H.A., 1995.
Substrate Quality	Field observation. Categories: 1: poor; 2: marginal; 3: sub-optimal; 4: optimal. Based in Barbour *et al.*, 1999.
Mean Substrate Size (mm)	Field measurements of 18 average stones.
Habitat Complexity	Field observation. Categories: 1: poor; 2: marginal; 3: sub-optimal; 4: optimal. Based in Barbour *et al.*, 1999
Pool Quality	Field observation. Categories: 1: poor; 2: marginal; 3: sub-optimal; 4: optimal. Based in Barbour *et al.*, 1999
Lithology	Atlas Digital do Ambiente – DGA (1982). Categories: 1 = sedimentary; 2 = sedimentary + metamorphic; 3 = plutonic rocks
Riparian Vegetation (total width; m)	Field measurement.
Woody vegetation (%)	Field observation. Woody vegetation in the riparian corridor.
Shading at zenith (%)	Field observation. Shading done by the riparian vegetation in the stream.
Forest (%)	
Eucalyptus (%)	Measured in the area of a circle of 1km radius marked around each sampling site.
Industrial, urban and degraded areas (%)	Data from Plano de Bacia Hidrográfica do Mondego
Agriculture (%)	(Maot, 2002)

2.2. *Data Analysis*

Invertebrate abundance was converted to animals/minute (sample unit) and recorded at three taxonomic levels: order, family and the lowest practical level (mostly genus and species). These levels were used for the majority of the taxa, especially for insects, but in some cases animals were left at a higher taxonomic level (Oligochaeta, Hydracarina, Colembolla, Copepoda, Ostracoda, Hydridae). At all taxonomic levels, rare taxa (representing ≤0.01% of the total abundance in all samples and not more than five animals per sample unit) were eliminated and the biological data were transformed using double square root transformation to downweight taxa with very high abundance. For data analysis, all environmental variables were transformed to approximate normality.

2.2.1. Summary of the Model Building Procedure

The model building methodology is summarised in Figure 2. The approach is based on the Benthic Assessment of SedimenT (BEAST) predictive model methodology developed by REYNOLDSON *et al.* (1995, 2001) for the Great Lakes and Fraser River, Canada. The BEAST is a three stages process: 1) analysis and classification of reference sites based on community structure; 2) analysis of the relationship between community structure and environmental features; 3) evaluation of test sites by comparison with the appropriate subset of reference sites (ROSENBERG *et al.*, 2000).

For the present study three models were used, built with 51 (lowest level model), 52 (family level) and 53 (order level models) reference sites distributed across the catchment and sampled in the Summer 2001 (FEIO, 2004; FEIO *et al.*, 2006). Table 2 shows the models performance, the number of reference biotic groups and the best discriminant variables used in each model. The performance of the models was determined from the correct assignment of reference sites based on habitat attributes to groups defined by stepwise forward Discriminant Analysis with Jaccknifed cross-validation (Systat 8.0, Systat Software Inc., Point Richmond California).

Table 2. Resume of the three models characteristics with the groups resultant from the classification of reference sites (and number of sites), the variables selected by the Stepwise Forward Discriminant analysis (with respective F-to-remove statistic and Tolerance) and the models discriminant performance (Jackknifed classification).

Model Taxonomic Level	Groups	Variables (F-to-remove, Tolerance)	Performance (%)
Order level	Group 1: 42 reference sites Group 2: 11 reference sites	substrate quality (15.99, 1.000)	81%
Family level	Group 1: 35 reference sites Group 2: 17 reference sites	stream order (12.06, 0.686) current velocity (8.37, 0.809) substrate quality (4.37, 0.806) pool quality (3.68, 0.778) mean annual precipitation (days/year; 2.50, 0.842)	81%
Lowest level	Group 1: 34 reference sites Group 2: 17 reference sites	stream order (9.76, 0.739) current velocity (7.35, 0.803) pool quality (3.85, 0.771) substrate quality (2.59, 0.834)	78%

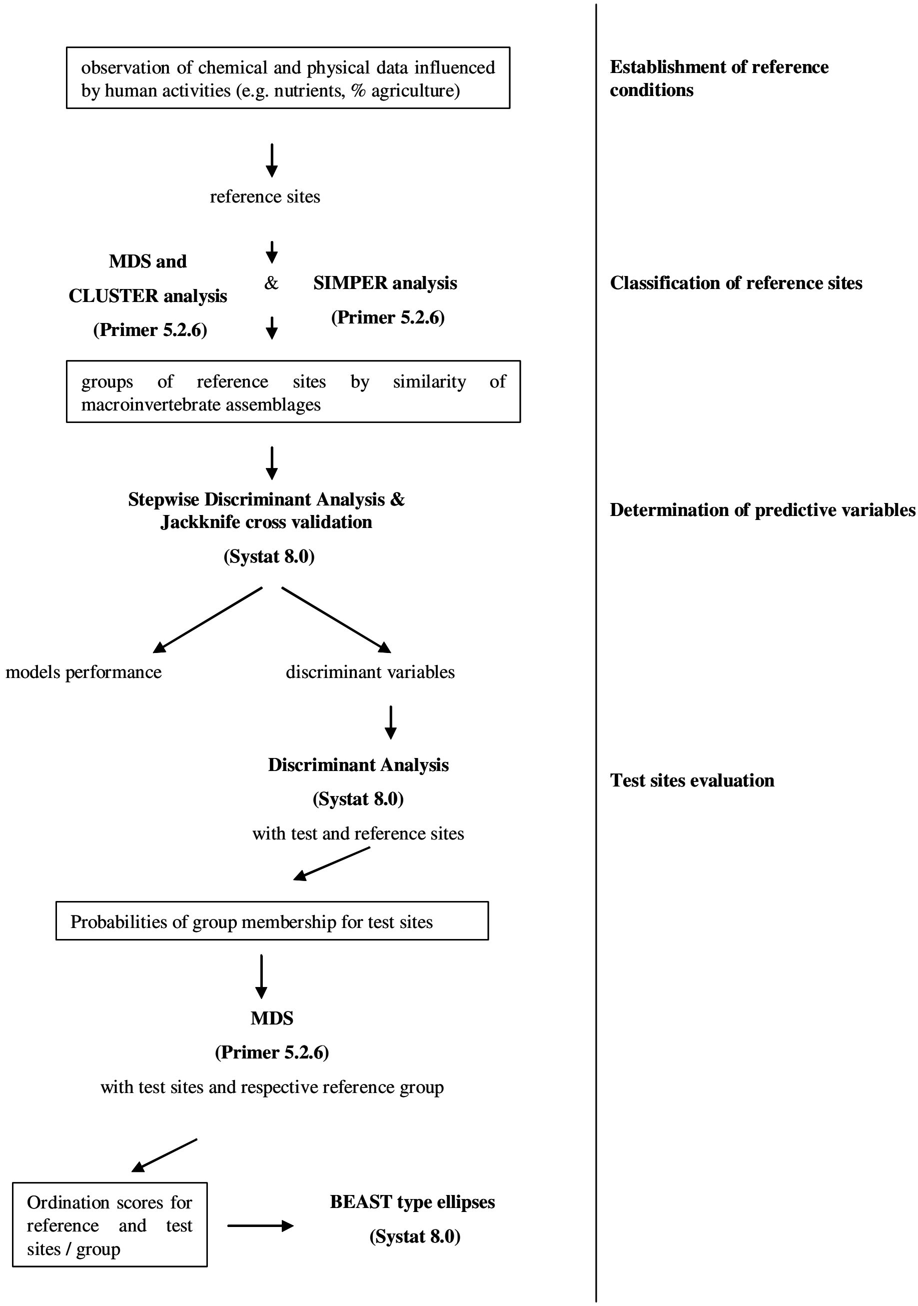

Figure 2. Methodology followed to the Mondego model building and evaluation of the test sites.

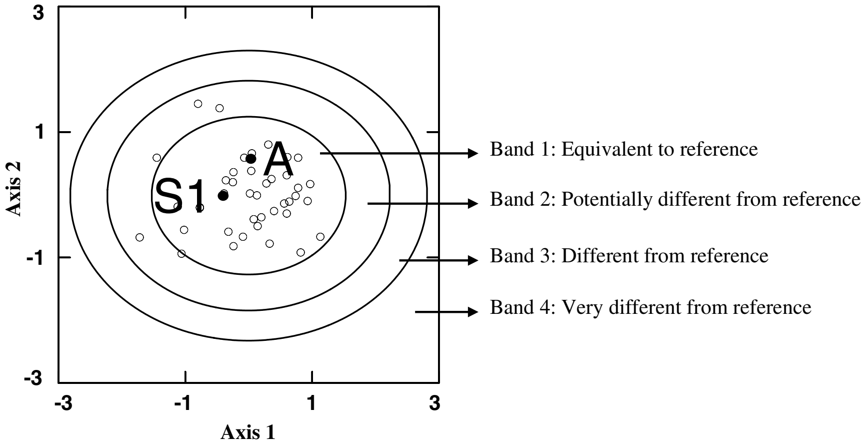

Figure 3. Comparison of an Autumn (A) seasonal sample (site Botão) with the Summer 2001 reference group 1 (order level model). Both samples are in the same band (Band 1, equivalent to reference) and therefore no seasonal effect was detected.

2.2.2. Assessment of Seasonality

Seasonal samples were compared to the reference group where the site was allocated in the reference season (Summer 2001). The only exception was the seasonal site Lousã, which was not used to build the model. Therefore its reference group needed to be determined by a different procedure, as it would be necessary for any new test site. This was done by calculation of the probability of group membership (Discriminant Analysis, complete) based on the optimum set of discriminating environmental variables (see Fig. 2). These procedures were followed for each model.

In the BEAST models, the diference between test sites and the reference groups is determined using probability ellipses that allow the response of invertebrate communities to be viewed along a gradient of response levels (REECE and RICHARDSON, 2000). Seasonal sites similarity between seasons was first obtained by ordination (non-metric MDS, Bray-Curtis coefficient) of the reference sites with the respective seasonal test sites, one by one. The ordination scores of the reference sites of each group were used to calculate three Gaussian bivariate probability ellipses (90, 99 and 99.9%) that were plotted against the ordination space (REECE *et al.*, 2001) in Systat 8.0. The area between each ellipse is a Band and the sites located inside the first ellipse (Band 1) are considered equivalent to reference (see Fig. 3). In Band 2 sites are considered potentially different, in Band 3 different and in Band 4 very different (REYNOLDSON *et al.*, 2000). The greater the distance between seasonal sites and the centre of the "cloud" of reference sites, the less potential has the model for water quality evaluation of samples collected out of the reference season. In such a situation, the model may be assessing not only water quality but also the temporal variability in aquatic macroinvertebrate communities.

Only two axis scores (from the MDS) were used to build the ellipses except when the stress value was equal or greater than 0.200 when three axes were used. In those cases three graphs were plotted, equivalent to the three possible combinations of two axes (1–2, 2–3 and 1–3). The worse situation for the test site (corresponding to the higher band) was always chosen as a safety measure.

3. Results

3.1. Order Model

All seasonal samples were compared to reference group 1, except for samples collected at Lousã. From all the seasonal samples from the nine sites, 21 of 27 (78%) were equivalent

Table 3. Distance (number of bands) from the Summer 2001 reference situation band. The seasons are indicated by: A for Autumn 2001, W for Winter 2002, Sp for Spring 2002 and Su for Summer 2002.

Non seasonal samples: with a difference of	Order model				Family model				Lowest level model			
	A	W	Sp	Su 02	A	W	Sp	Su 02	A	W	Sp	Su 02
0 Bands	6	8	7	5	1	3	4	1	0	0	1	2
1 Bands	1	1	2	1	4	3	5	4	1	2	3	4
2 Bands	0	0	0	2	3	3	0	1	4	4	4	1
3 Bands	2	0	0	1	1	0	0	3	4	3	1	2

to reference season (Summer 2001). One would expect 90% to be within Band 1 and therefore there is 12% error due to seasonal variation. Four samples (15%) were potentially different (one Band of difference) and two (7%) are very different (three Bands of difference). The Autumn samples showed the greatest variation from the reference season, with an average difference of 0.8 bands, with Spring at 0.2 bands and Winter at 0.1 bands (Table 3).

The variation between the two successive summers did not occur in five (56%) of the sites. The differences fall in one Band (one site), two Bands (two sites) and three Bands (one site), meaning that samples are potentially different, different and very different from the previous year samples, respectively. It is noteworthy that this variation was greater than seasonal variation, because more invertebrates were sampled in 2004 (Fig. 4).

3.2. Family Model

In the family model, the seasonal samples were all compared to reference group 1. For all the seasonal samples (Autumn, Winter and Spring) from eight of 27 (30%) were equivalent to reference season (Summer 2001), 12 (44%) were potentially different (one Band of difference), six (22%) were in different (two Bands of difference) and one (4%) was very different (three Bands of difference). As in the case of the order level, the Autumn samples showed the greatest seasonal difference (average 1.4 Bands) while Winter and Spring were less different (Table 3).

Again at the family level variation across two consecutive years was greater than seasonal variation (average 1.7 Bands). Only one site (Porto da Balsa) showed no differences between the two years. In the sites with different invertebrate assemblages, four are classified as potentially different, one different and three very different. Figure 4C shows the total number of families found at each site in the two Summers, with a mean decrease of 27% between one year and the next.

3.3. Lowest Level Model

Reference group 1 was attributed to the seasonal samples for the lowest level model. At the lowest possible taxonomic level, none of the seasonal samples had a whole set of seasonal samples (Autumn, Winter and Spring) equivalent to the reference season (Summer 2001). Of the 27 seasonal samples (Autumn, Winter and Spring) only one (4%) was equivalent to reference season (Summer 2001), six (22%) were potentially different (one Band of difference), 12 (44%) were different (two Bands of difference) and eight (30%) were very different (three Bands of difference). Again, Autumn was the season more frequently different from the reference (in average 2.3 Bands), followed by Winter.

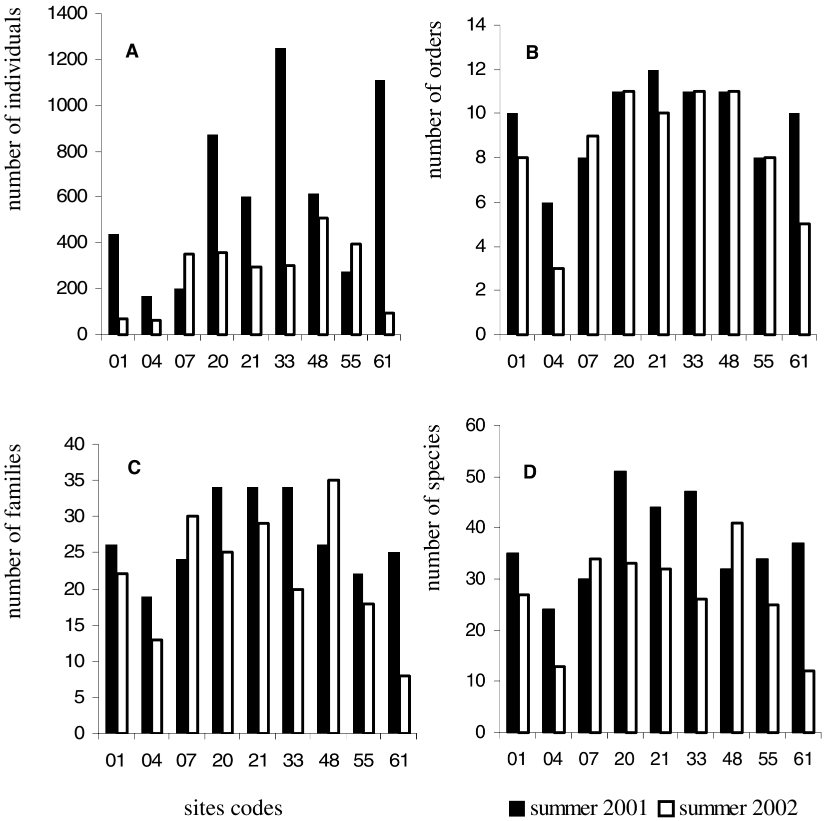

Figure 4. Total number of individuals (A) found in the Summer 2001 and Summer 2002 samples at the nine seasonal test sites, represented by their code; number of orders (B), families (C) and lowest level taxa ("species", D) found in the same samples.

The lowest level model detected no changes across the two summers in only two sites (Candosa and Trinta). From the sites classified as not equivalent to reference condition, four were potentially different, one was different and two were very different. Figure 4D shows a decrease in species number from the year 2001 to the year 2002 of 18%.

4. Discussion

One of the potential sources of uncertainty in estimating the biological quality with predictive models is the temporal variation of the observed fauna rather than stress or pollution (CLARKE, 2000). Invertebrate communities change seasonally largely as result of variation in their species life cycles (SOULSBY *et al.*, 2001). In this study the models built at three taxonomic levels showed seasonal changes in the invertebrate communities with different directions and amount of variation depending on the site and the taxonomic level used.

The order level model was the least sensitive to seasonal changes in the aquatic invertebrate community while lowest level model was most sensitive. Yet, there is still a substantial chance (in the order of 12%) of one to three Bands of error in some evaluations of the order model. In that case the error would be the classification of unstressed of a site that was stressed.

The present RCA model at family and lowest level models should not be used to evaluate non-Summer samples. Similar results were obtained by Reece *et al.* (2001), with the BEAST model, for Fraser river samples. They evaluated family and genus models and in both cases seasonal changes caused some seasonal test site samples to fall outside of Band 1, with higher incidence in the genus level.

Alternatives to the one-time evaluation could be the construction of seasonal models or/and the additional collection of samples from other seasons of the year (Humphrey *et al.*, 2000; Wright, 2000; Reece *et al.*, 2001). In the Mondego catchment precipitation is highly variable over a wide range of time and space and the strong irregular fluctuations of precipitation lead, in certain cases to the occurrence of floods and droughts (Lima and Lima, 2002) and this study point to an annual variation even greater than seasonal variation. Therefore, building seasonal models might not be the best option for the Mondego catchment, as seasonal environmental conditions are highly changeable from year to year (e.g. water velocity, wetted width and stream depth, as consequence of an irregular precipitation regime). On the contrary, the addition of data from other periods of the year to the original Summer data set might contribute to the inclusion of the natural variability over the year in the model, enabling its use in other periods of the year, to test for biological water quality. A disadvantage of this approach might be the construction of a model so robust to natural environmental change that only detects severe impacts in the communities (Humphrey *et al.*, 2000). Therefore, this would have to be carefully tested in future work.

Another critical issue is the variables used in the models to predict the group membership of a test site and therefore the community type to which the observed fauna should be compared (Reynoldson and Wright, 2000). Some variables are constant in time, such as stream order, latitude but others such as, water depth of the channel and current velocity are variable and again depend on climate changes. Therefore, it was predictable that invertebrate communities associated to environmental variables such as discharge and pool quality would be different over the year. So, running the model for the seasonal samples in order to obtain the reference group to which they should be compared rather than assume the same group as the Summer 2001 sample might be a better option to evaluate the water quality of samples collected in other seasons.

Regarding the variability across consecutive years, the three models detected changes comparable to seasonal variation at order and family levels, which is consistent with the reported inter-annual differences in invertebrate communities reported to Scotland, by Soulsby *et al.* (2001). December, January and March of 2000–2001 was a period of very high precipitation in Portugal. Therefore, in the Summer of 2001, when the reference samples were collected, the rivers had high water levels. In contrast, the following year was particularly dry, with a decrease in 45.6% of the mean discharge for the same sites, which could explain the biological differences. Previously, other authors (e.g. Resh *et al.*, 1988; Bunn and Arthington, 2002; Hieber *et al.*, 2003) suggested that physical disturbances are a major determinant of spatial and temporal variability of benthic communities in streams where flow regime in streams has been related to invertebrate density and taxa richness. Therefore, relying on one single year data is not recommended and the role of inter-annual variation should be evaluated in order to include more natural variability (for example, a group of sites that represent high flow under reference and a group of sites that represent low flow under reference) and not only one type of reference.

In conclusion, examination of the sensitivity of the different taxonomic models to seasonal and annual-changes of the communities revealed that: 1) all models were sensitive to temporal variability of the fauna with the lowest taxonomic level being most sensitive; 2) errors (classifying a site of impaired when it is not) could occur in the evaluation of the sites with samples taken from seasons other than Summer and with samples from "atypical" years. To prevent these errors, with the present model, test samples should be taken in the same season (Summer) as the reference samples. To confer more flexibility in the evaluation period, seasonal data could be added to the predictive model. To increase the predictive power of the model, the addition of data from more reference sites may be a solution to encompass sufficient natural variation.

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