TOOL-BOX INFORMATION FROM BENTHIC COMMUNITIES

Introduction

The ECASA study sites extended both over the Mediterranean and the Atlantic and covered an ample gradient of latitudinal locations, water depths, and different target species. Moreover, the complementary sediment analyses made in these locations differ from each other. Only few variables are similar across all sites e.g. Total Organic Matter (TOM) or redox potential. These factors make comparison among sites and variables very difficult to undertake on a global basis.

However, the availability of a data set of species composition and density of a broad set of locations makes it interesting in order to check some of the indicators proposed within this project.

Finally, the data available were sent from the next locations:

Station	Location	Country	Station	Location	Country	Station	Location	Country
So-1	Sounio	Greece	B	Adriatic Italy	Italy	S-0	ICRAM	Italy
So-2	Sounio	Greece	D	Adriatic Italy	Italy	S-1	ICRAM	Italy
So-3	Sounio	Greece	G	Adriatic Italy	Italy	S-2	ICRAM	Italy
So-4	Sounio	Greece	M	Adriatic Italy	Italy	S-3	ICRAM	Italy
So-5	Sounio	Greece	B-1	Garrucha	Spain	S-4	ICRAM	Italy
So-6	Sounio	Greece	B-2	Garrucha	Spain	S-5	ICRAM	Italy
Ce-1	Cephalonia	Greece	B-3	Garrucha	Spain	S-6	ICRAM	Italy
Ce-2	Cephalonia	Greece	B-4	Garrucha	Spain	S-7	ICRAM	Italy
Ce-3	Cephalonia	Greece	B-5	Garrucha	Spain	FWN-2	Slovenia	Slovenia
Ce-4	Cephalonia	Greece	B-6	Garrucha	Spain	FWN-3	Slovenia	Slovenia
Ce-5	Cephalonia	Greece	B-7	Garrucha	Spain	FON-2	Slovenia	Slovenia
Ce-6	Cephalonia	Greece	B-8	Garrucha	Spain	FOS-2	Slovenia	Slovenia
N50	Creran	UK	B-9	Garrucha	Spain	FOS-3	Slovenia	Slovenia
W0	Creran	UK	B-10	Garrucha	Spain	FOCC	Slovenia	Slovenia
N25	Creran	UK	N-0	Norway	Norway	FEN-2	Slovenia	Slovenia
Ref1	Creran	UK	N-50	Norway	Norway	FEN-3	Slovenia	Slovenia
Ref2	Creran	UK	N-100	Norway	Norway			
S25	Creran	UK	N-2000	Norway	Norway			
S50	Creran	UK						

Most of the data are from Mediterranean locations, except those from UK and Norway. All of them are locations for fish cultivation.

The biological data sets, containing the density and species composition, per replicate and station, were homogenised and the structural parameters (density, biomass, etc.) were calculated on the basis of square meter. Then, other univariate variables were derived (Shannon's diversity, AMBI, etc.)[1].

Results

As there is not data for all variables and stations, we have selected only those biological indicators and sediment variables with most coinciding cases. Hence, we have selected density, Shannon's diversity, richness and AMBI, as structural parameters; gravel, mud, sand, redox potential, Total Organic Carbon (TOC) and TOM, as variables characterizing sediments; and depth and distance to the cage.

Correlations between variables

Correlations between the abovementioned variables, using all the stations and locations, can be seen in Table 1. Excepting the distance to the cage the remainder of the variables shows many correlations between them. There are highly (p= 0.001) significant correlations between AMBI and diversity (-0.79) and AMBI and TOM (0.67), but also between richness, diversity and depth; depth and some sediment characteristics, etc. AMBI, as indicator of benthic stress, is positively correlated with density, mud, TOC and TOM, and negatively correlated with diversity, richness, sand and redox potential. It is interesting to note that there is not correlation with distance or depth, when including all data.

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[1] For indicator calculations, see the corresponding tool-box section.

Table 1. Correlation matrix between the variables, showing the correlation (1st row), the number of cases (2nd row) and the p values (3rd row). Colours indicate the level of significance.

	AMBI	Density	Diversity	Richness	Depth	Distance	Gravel	Mud	Sand	redox	TOC	TOM
Density	0.340											
	45											
	0.022											
Diversity	-0.791	-0.120										
	53	45										
	0.000	0.434										
Richness	-0.420	0.090	0.675									
	53	45	53									
	0.002	0.556	0.000									
Depth	-0.259	-0.431	0.418	0.513								
	53	45	53	53								
	0.061	0.003	0.002	0.000								
Distance	-0.239	-0.120	0.156	0.186	0.151							
	53	45	53	53	53							
	0.085	0.433	0.263	0.183	0.282							
Gravel	0.080	0.491	-0.039	0.058	-0.254	0.059						
	39	31	39	39	39	39						
	0.628	0.005	0.815	0.725	0.118	0.720						
Mud	0.332	-0.132	-0.460	-0.461	-0.513	0.042	-0.439					
	39	31	39	39	39	39	39					
	0.039	0.478	0.003	0.003	0.001	0.798	0.005					
Sand	-0.365	0.032	0.494	0.479	0.585	-0.055	0.303	-0.989				
	39	31	39	39	39	39	39	39				
	0.022	0.865	0.001	0.002	0.000	0.742	0.060	0.000				
redox	-0.325	0.184	0.367	0.313	0.330	0.047	0.489	-0.744	0.708			
	48	41	48	48	48	48	38	38	38			
	0.024	0.249	0.010	0.030	0.022	0.751	0.002	0.000	0.000			
TOC	0.433	0.877	-0.056	0.435	-0.648	-0.276	0.400	-0.351	0.297	0.501		
	24	24	24	24	24	24	21	21	21	24		
	0.035	0.000	0.796	0.034	0.001	0.192	0.073	0.119	0.191	0.013		
TOM	0.671	0.399	-0.679	-0.319	-0.615	-0.014	0.117	0.697	-0.767	-0.388	0.423	
	41	41	41	41	41	41	31	31	31	41	24	
	0.000	0.010	0.000	0.042	0.000	0.933	0.533	0.000	0.000	0.012	0.040	

🟥 p<0.001
🟩 0.001<p<0.005
🟨 0.005<p<0.05

One of the problems when studying all data together comes from the different characteristics of the locations. Some of them do not show clear gradients of impact, from the cage as source of pressure over the benthic communities. As it is shown below, in other sections of the results, the locations without clear gradients are ICRAM, Spain and Norway. When calculating the correlations only with clear gradient locations, the results obtained are shown in Table 2. Now AMBI has high significant correlation with diversity (-0.79) and TOM (0.70), and also with distance (-0.41), redox (-0.45) and richness (-0.4).

Table 2. Correlation matrix between the variables, removing locations without clear impact gradient, showing the correlation (1st row), the number of cases (2nd row) and the p values (3rd row). Colours indicate the level of significance.

	AMBI	Density	Diversity	Richnes	Depth	Distance	Gravel	Mud	Sand	redox	TOC	TOM
Density	0.184											
	23											
	0.400											
Diversity	-0.791	0.220										
	31	23										
	0.000	0.314										
Richnes	-0.402	0.579	0.673									
	31	23	31									
	0.025	0.004	0.000									
Depth	0.159	-0.639	-0.375	-0.203								
	31	23	31	31								
	0.395	0.001	0.038	0.274								
Distance	-0.407	-0.411	0.199	-0.047	0.229							
	31	23	31	31	31							
	0.023	0.051	0.283	0.803	0.216							
Gravel	-0.046	0.281	0.143	0.279	-0.175	0.008						
	24	16	24	24	24	24						
	0.832	0.292	0.504	0.187	0.414	0.970						
Mud	0.245	-0.349	-0.338	-0.450	-0.246	-0.002	-0.673					
	24	16	24	24	24	24	24					
	0.249	0.185	0.106	0.028	0.247	0.993	0.000					
Sand	-0.265	0.323	0.351	0.450	0.307	0.001	0.564	-0.990				
	24	16	24	24	24	24	24	24				
	0.210	0.222	0.093	0.027	0.144	0.998	0.004	0.000				
redox	-0.446	0.080	0.450	0.430	0.262	0.236	0.632	-0.785	0.756			
	30	23	30	30	30	30	23	23	23			
	0.014	0.715	0.013	0.018	0.162	0.210	0.001	0.000	0.000			
TOC	0.303	0.811	0.233	0.813	-0.625	-0.482	0.230	-0.277	0.255	0.047		
	16	16	16	16	16	16	16	16	16	16		
	0.255	0.000	0.386	0.000	0.010	0.059	0.391	0.300	0.341	0.864		
TOM	0.698	0.182	-0.585	-0.122	0.118	-0.209	-0.219	0.704	-0.734	-0.655	0.359	
	23	23	23	23	23	23	16	16	16	23	16	
	0.000	0.406	0.003	0.581	0.593	0.339	0.415	0.002	0.001	0.001	0.173	

🟥 p<0.001

🟩 0.001<p<0.005

🟨 0.005<p<0.05

AMBI as indicator

Although the abovementioned gaps in the data (mainly in sediment variables), several patterns in the cage impacts over the benthic communities can be found, when studying all data together.

As seen in Table 1, there is not correlation between AMBI and **water depth**. However, deep areas (>40 m water depth) have always low AMBI values (<2.5), showing no or slight disturbance. Probably this is related with a better dispersion of faeces, organic matter, etc., in deep locations. Conversely, shallow stations present a wide range of AMBI values.

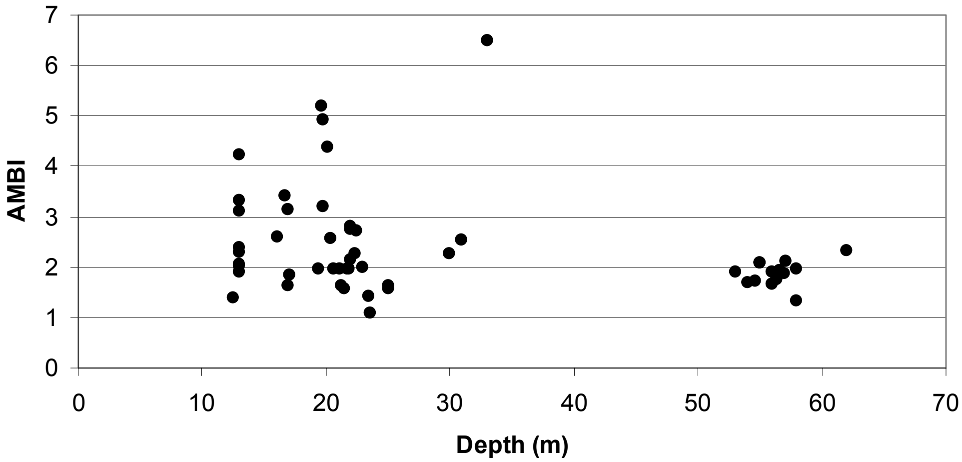

Figure 1. Relationships between AMBI and water depth, including all stations.

There is a decrease of AMBI values with the **distance to the cage** (Figure 2), although the correlation was not significant (Table 1). However, the higher AMBI values, showing disturbance, extend until 20-50 m. Stations >50 m from the cages present always AMBI values <3, showing no or slight disturbance. In some cases, under the cage, there are low AMBI values (<3); being most of them deep locations. This pattern is very similar to the previous one (depth).

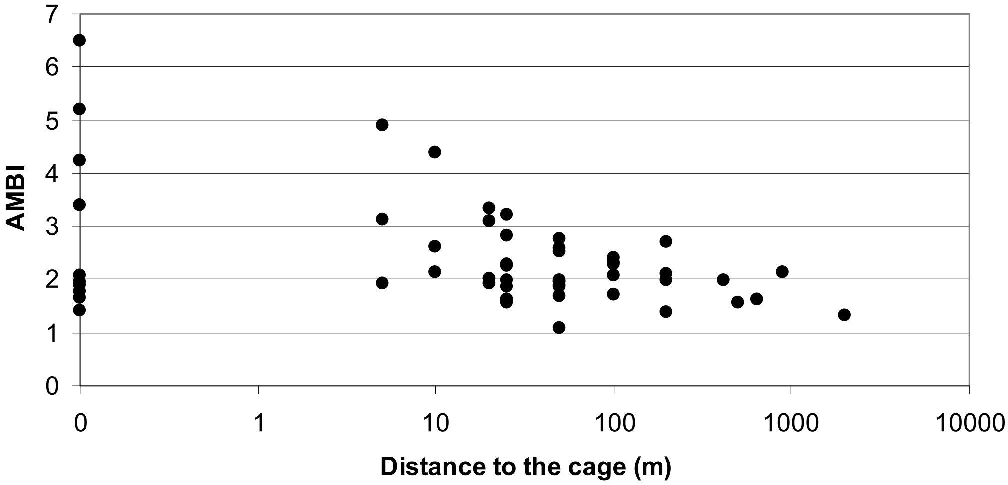

Figure 2. Relationships between AMBI and distance to the cage, including all stations (note that distance data are in logarithmic scale).

This pattern of distribution AMBI with **distance to the cage and depth** can be seen in Figure 3. The 3.3 AMBI value represents the boundary between slightly disturbed and moderately disturbed. Hence, the influence of the cages extends until 25-30 m, and stations with depths under 45-50 m are less disturbed.

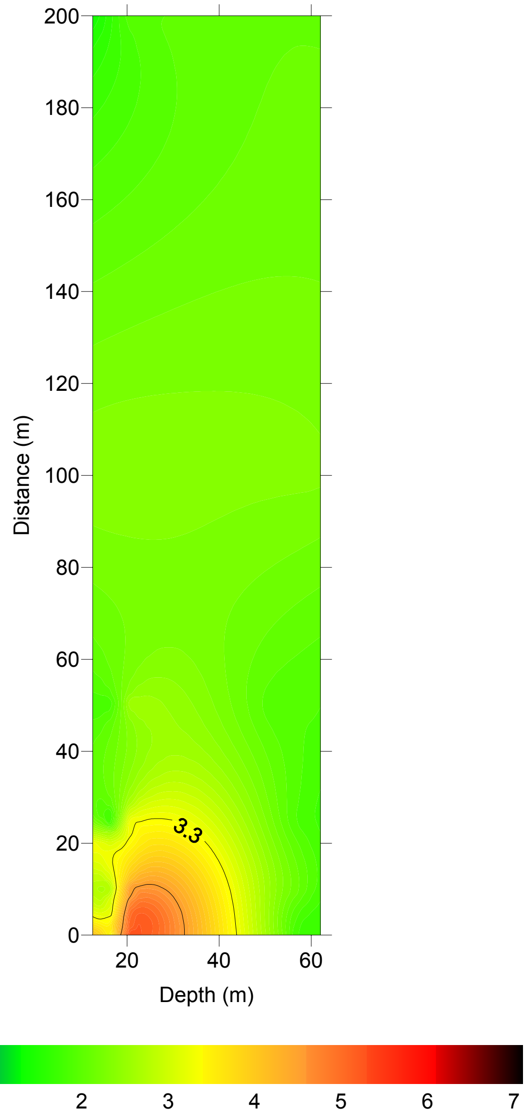

Figure 3. AMBI geographical distribution, related with water depth and distance to the cage (only until 200 m), including all stations.

This pattern is related with the discharges from cages. Hence, AMBI values increase with increasing **TOM percentages** (with some exceptions), as shown in Figure 4a; however, the pattern is not so clear when studying **TOC** (Figure 4b). Percentages of organic matter <4% correspond to AMBI values <2.5 (most stations are undisturbed or slightly disturbed); organic matter between 4 and 10% correspond to AMBI values between 1.5 and 3.3 (slightly disturbed communities); and organic matter values >10% correspond to AMBI values >3.3 (moderate to heavily disturbed communities) (Figure 4a).

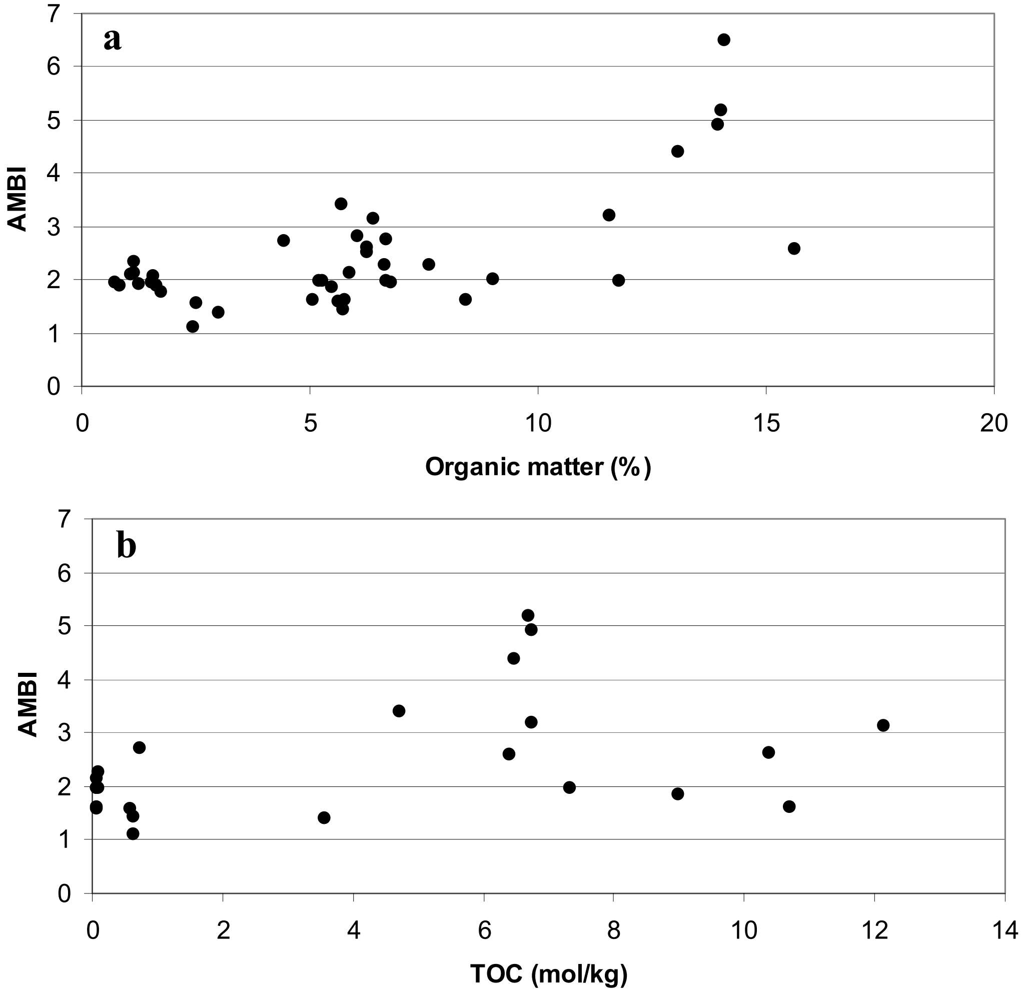

Figure 4. Relationships between AMBI and organic matter (TOM) (fig. 4a), and AMBI and TOC (fig. 4b), including all stations.

As shown in Table 1, organic matter and TOC are correlated with **redox potential**. The higher TOM values produce lower redox potential, due to oxygen consumption. When studying all data together and compare AMBI and redox potential, it seems that there is no pattern of distribution (Figure 5). However, high redox potential values (in general positive values) show always AMBI values <3.3, corresponding to undisturbed or slightly disturbed benthic communities. Negative redox potential values show a higher dispersion in AMBI values, but all values >3.3 (moderate to heavily disturbed communities) correspond to negative redox potentials.

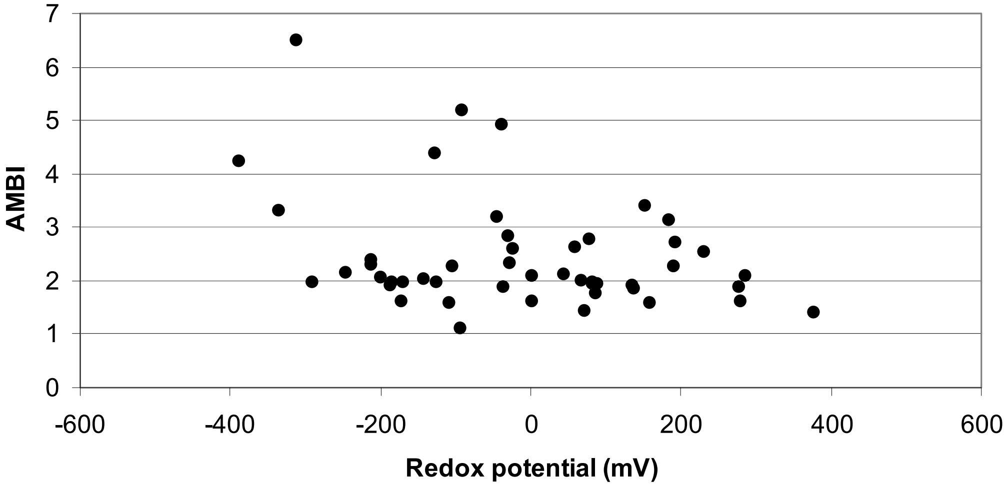

Figure 5. Relationships between AMBI and redox potential, including all stations.

Most of the problems associated to the absence of clear patterns probably are due to the analysis of all data together. When representing data for each of the locations other patterns can be detected (Figure 6).

Although ICRAM presents clear redox potential gradients (all of them negative) and TOM concentrations between 5 and 8%, there is not a clear gradient with AMBI, showing low AMBI values, being water depths around 21 m. This could indicate absence of impact or inability of AMBI to detect changes, probably due to a good dispersion due to strong currents.

Adriatic Italy shows most of locations with positive redox, lower TOM values (2-6%) and water depths around 23 m. Probably these values can explain the absence of AMBI gradients with the distance to the cage.

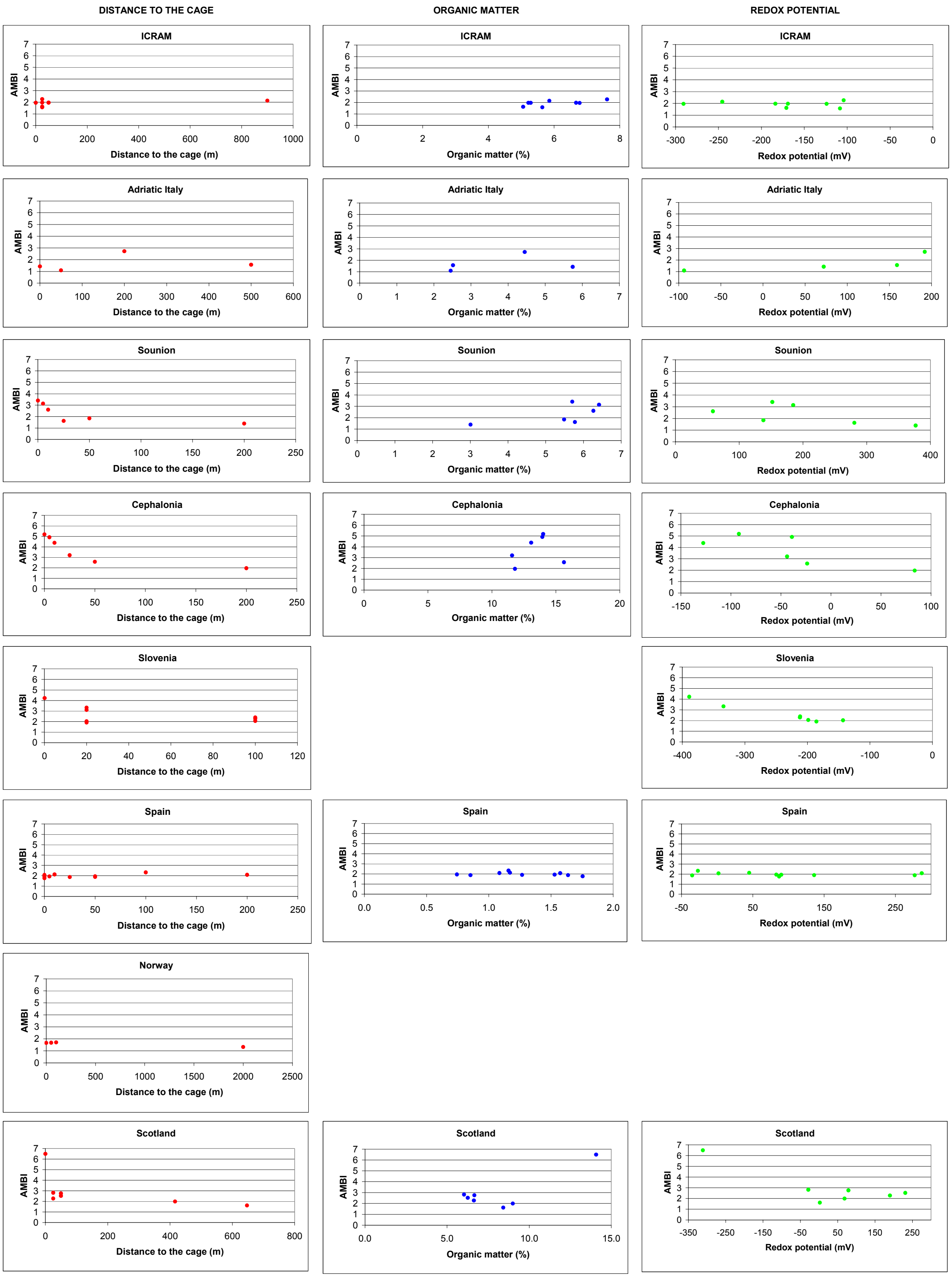

Figure 6. Relationships between AMBI and several variables (distance to the cage, organic matter and redox potential), at each of the locations.

Sounio and Cephalonia (Greece) show a clear AMBI gradient with the distance to the cage, probably influenced by the gradients in organic matter and redox potential. Cephalonia shows higher AMBI values than Sounio, corresponding to the higher organic matter concentrations (11-16% and 3-7%, respectively) and differences in redox potential (negative in Cephalonia and positive in Sounio). Sounio has water depths between 12 and 17 m, and Cephalonia around 20 m.

Slovenia shows a clear gradient, related with redox potential (all data negative) and distance to the cage. The water depth in the area is 13 m.

Spain shows very low AMBI values, without gradient with the distance to the cage, related with the low TOM values (<2%), and positive or slightly negative redox potential values. In this case the area is very deep (50-65 m), and the dispersion very high, due to strong currents.

Norway do not show AMBI gradient with distance (no other data available), but as in Spain the area is very deep (54-58 m). Probably this is related with a good dispersion of discharges.

The area under the cage in Creran (UK) is very disturbed (in terms of AMBI), due to high TOM concentrations (near 15%) and low redox potential (near -350 mV). The rest of the area shows AMBI values <3, with positive redox potential and TOM concentrations between 6 and 9%.

Multiple regression between variables

Taking into account the previous analyses it is possible to develop several multiple regression models, trying to explain the variability of AMBI as indicator. As abovementioned, the most important problem is the availability of data for all variables and locations.

Hence, using depth, distance to the cage, mud, redox potential, TOM, and TOC as independent variables, there are only 21 complete cases. In this particular case, most of the AMBI variability (51.9%) is explained by mud and TOM, or TOM alone (49.5%) (Table 3).

Table 3. Multiple regression models with largest adjusted r^2, obtained using depth (A), distance (B), mud (C), redox potential (D), TOM (E), and TOC (F) as independent variables and AMBI as dependent variable.

```
----------------------------------------------------------------
                        Adjusted                Included
MSE         R-Squared   R-Squared   Cp          Variables
----------------------------------------------------------------
0.657432    56.6896     51.8774     -0.720252   CE
0.689374    52.0623     49.5392     -1.19457    E
0.690015    57.0685     49.4924     1.15483     CEF
0.691248    54.4619     49.4021     0.0142508   AE
0.692879    56.8903     49.2827     1.21359     ACE
0.695369    56.7354     49.1004     1.26467     CDE
0.695765    56.7108     49.0715     1.27279     BCE
0.696098    54.1424     49.0471     0.119596    EF
0.717111    52.7581     47.509      0.576017    DE
0.718936    52.6378     47.3754     0.615661    BE
0.728213    54.6919     46.6963     1.93843     AEF
0.729314    57.2926     46.6158     3.08094     BCEF
0.730329    57.2332     46.5415     3.10054     ACEF
0.732931    57.0808     46.351      3.15078     CDEF
0.735263    56.9442     46.1803     3.19581     ABCE
0.736026    56.8996     46.1245     3.21053     BCDE
0.773475    57.5375     43.3833     5.00022     ABCEF
0.77707     57.3401     43.1201     5.06529     BCDEF
0.777319    57.3264     43.1019     5.06981     ACDEF
0.782901    57.02       42.6933     5.17084     ABCDE
0.821962    54.8756     39.8341     5.87787     ABDEF
1.19388     16.9798     12.6103     10.3724     F
1.29539     9.92086     5.17985     12.6998     B
1.31942     8.24988     3.42092     13.2507     C
1.36616     5.0         0.0         15.0169     D
1.36616     0.0         0.0         13.9707
----------------------------------------------------------------
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Using only depth, distance, TOM, and redox potential as independent variables, 41 complete samples are obtained. In this particular case, the AMBI explained variability increases (53.6%), being depth, distance, and TOM the variables explaining such variability (Table 4).

Table 4. Multiple regression models with largest adjusted r^2, obtained using depth (A), distance (B), TOM (C), and redox potential (D), as independent variables and AMBI as dependent variable.

```
------------------------------------------------------------------
                         Adjusted                Included
MSE          R-Squared   R-Squared   Cp          Variables
------------------------------------------------------------------
0.553846     57.1134     53.6361     3.04058     ABC
0.56859      57.1617     52.4019     5.0         ABCD
0.571632     54.5398     52.1472     3.20335     AC
0.586766     54.5642     50.8803     5.1828      ACD
0.634763     49.5192     46.8623     7.42248     BC
0.648491     49.7846     45.7131     9.19943     BCD
0.67445      44.9515     43.54       9.26104     C
0.689396     45.1744     42.2889     11.0737     CD
1.07615      16.6692     9.91261     37.0287     ABD
1.07661      14.3802     9.87388     36.9522     BD
1.11203      9.23614     6.90886     39.2751     D
1.12332      10.6661     5.96433     40.0734     AD
1.1644       4.96194     2.52506     42.8671     B
1.19064      2.82009     0.328294    44.667      A
1.19456      0.0         0.0         45.0369
------------------------------------------------------------------
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Fitting this multiple linear regression model to describe the relationship between AMBI and those independent variables, the equation of the fitted model is

AMBI = 0.355 - 0.0009*Distance + 0.0247*Depth + 0.2347*TOM

The P-value in the ANOVA is less than 0.0001, so, there is a statistically significant relationship between the variables at the 99.9% confidence level.

Multivariate analysis

In this section, a Principal Component Analysis (PCA) was undertaken. In the first exercise AMBI, distance, depth, redox, TOM, and mud were used. The first component explains 47.1% of the variability, the second one 21.4%, and the third one 15.6% (in total 84%).

The first component seems to be related with the disturbance (including TOM and AMBI as the variables explaining most of the variability) (Figure 7a). The second one is clearly related with distance to the cage (Figure 7a), and the third one with characteristics of the location (mud and redox) (Figure 7b).

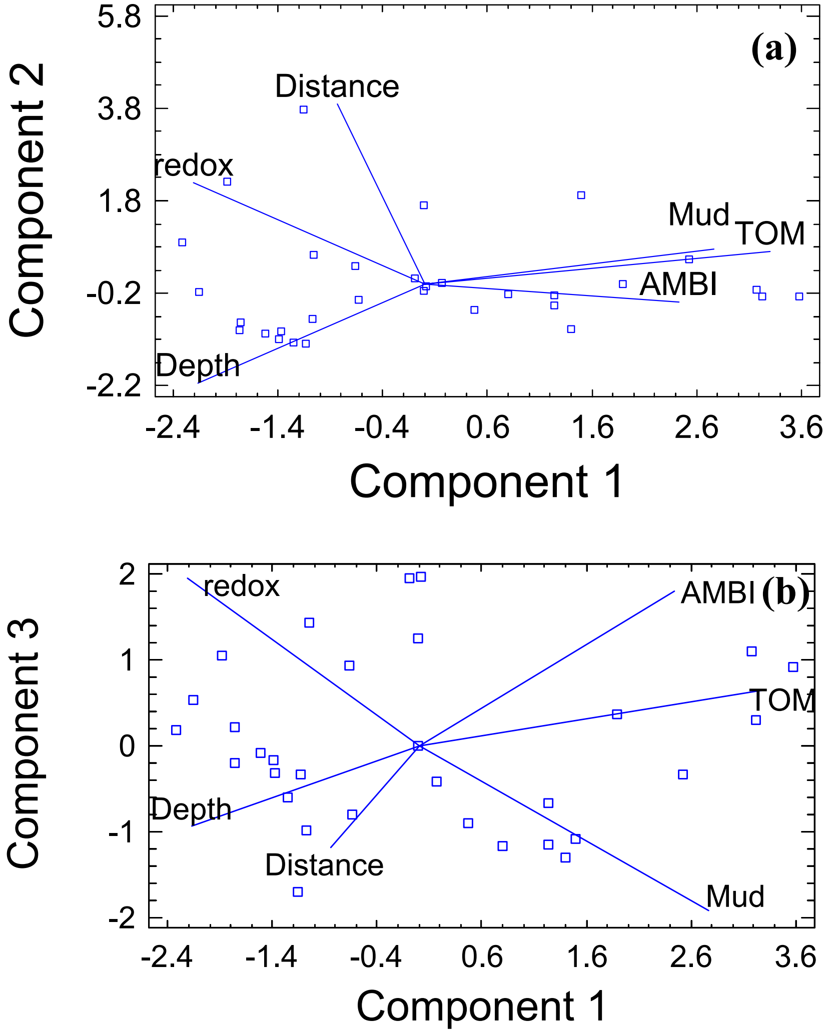

Figure 7. Principal Component Analysis using AMBI, redox, TOM, depth, distance and mud, from all locations. (a) Components 1 and 2; (b) components 1 and 3.

When adding other structural parameters to the PCA (such as diversity, richness and density), the explained variability decreases (first component 41.6%, second 18.1%, and third 16.3%).

The explanation of the components is very similar. The first component is related with TOM, diversity and AMBI (Figure 8a); the second one with density, mud and richness (Figure 8a); and the third one with redox and distance (Figure 8b).

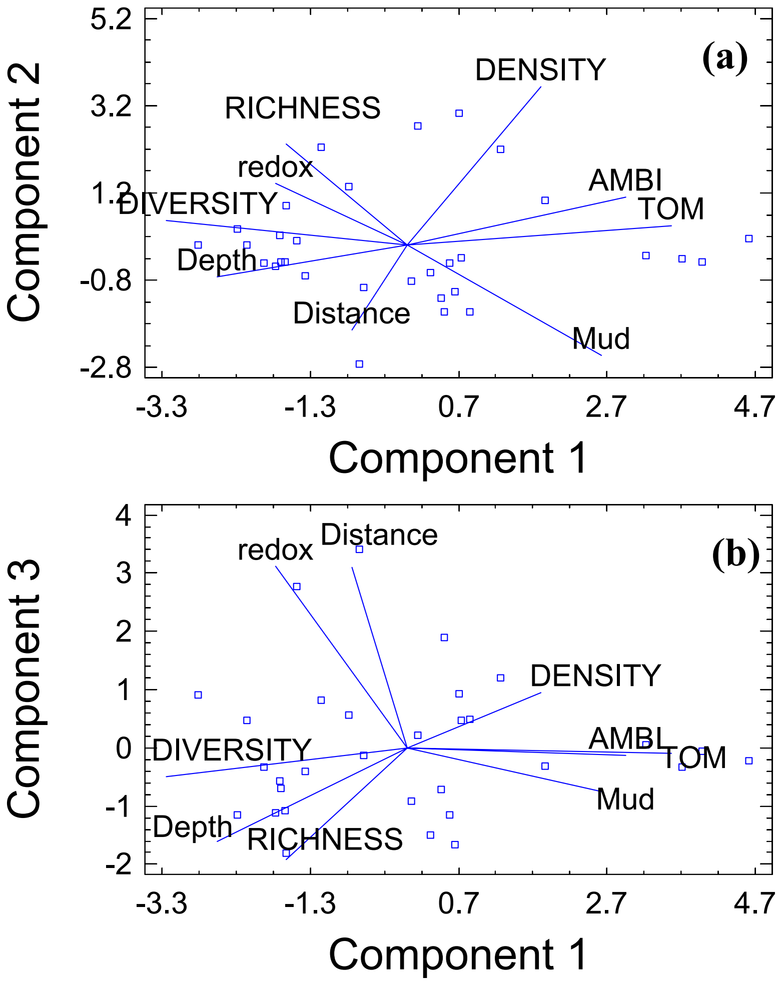

Figure 8. Principal Component Analysis using AMBI, richness, density, diversity, redox, TOM, depth, distance and mud, from all locations. (a) Components 1 and 2; (b) components 1 and 3.

Finally, assuming that stations without a clear gradient of impact have good dispersion and the impacts are minimised, the same analyses as above were undertaken using only those locations with a clear gradient. Now, with AMBI alone as structural parameter, the components and explained variability of the PCA were as follows:

Component	Explained variability	Variables
First	50.4%	TOM, Redox, AMBI
Second	29.4%	Depth, distance
Third	11.4%	Distance, redox
Total	**91.2%**	

When adding other structural parameters, such as diversity, richness, and density, the explained variability decreases:

Component	**Explained variability**	**Variables**
First	42.3%	Diversity, Mud, Redox
Second	30.9%	Density, depth, distance
Third	10.8%	Richness
Total	**83.9%**	

Conclusions

The most important problem when analysing the data is the absence of the same variables at all locations. However, when studying those variables which are common to all datasets, several conclusions can be obtained:

- Some locations do not show clear gradients of impact, in terms of the benthic indicators selected in ECASA. In these cases it seems that the good dispersion of the discharges from the cages avoids any damage to the benthic communities. This good dispersion seems to be related with: (i) the situation of the cages on high water depths (>40-50 m), such as in Spain or Norway; (ii) the strong dynamics of the area e.g. currents, such as in Spain and ICRAM.

- The gradients of impact are related with the distance to the cage and depth, being the most impacted area that extending until 25-50 m from the cages.

- Most structural parameters are correlated between them.

- AMBI seems to be a good indicator of the benthic stress, related with organic matter percentages. This is also related with redox potential (showing the oxygen consumption under the cages).

- Multiple regression using AMBI as dependent variable and distance to the cage, depth, and TOM as independent, explains 53.6% of the total variability.