

### ***Annex 2.1.5.1 Relationship between pressure data and ICMi in Northern Spain CB GIG rivers***

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Pressure gradients were extracted from a designed dataset of invertebrate samples taken in Galicia (NW Spain) which fulfils the proposed technical aspects of the WFD (Pardo *et al.*, 2005). The dataset comprises a range of sites spanning the pressure gradient ranging from reference sites, which fulfil Northern Spain's reference criteria (in agreement with the REFCOND template agreed with the MS of the CB GIG), to significantly impacted sites. Several pressure gradients were selected and evaluated according to a designed network of samples. In this report only results from the **gradient of general degradation pressure** (including organic, nutrients and hydromorphology) are included, as this is the pressure gradient tested for the biological element of invertebrates in the CB GIG. The dataset comprises samples from IC river types RC2, RC3, RC4 and RC5.

This dataset together with a bigger dataset from Northern Spain allowed the development of classification systems based on multimetrics for the new WFD evaluation of ecological status. A high number of metrics were tested and a few were selected on the basis of their significant relationship with the pressure gradient and for their discriminatory efficiency along the gradient in contrast with the reference population. The metrics chosen were given equal weight but the metrics represented different criteria of the Normative definitions in a balanced way (see Spanish description of Normative definitions in Milestone River CB GIG annex).

The pressure gradients were built using multivariate ordination techniques (PCA, Principal component analysis) of variables comprising organic, nutrient and hydromorphology pressures. Ordination diagrams were checked for consistency with types and known existing pressures. This ordination technique is based on bivariate relationships and regression analyses which allows the extraction of new variables, termed 'PCA axis pressure gradients' in this report.

#### **Gradient of general degradation (organic, nutrients, hydromorphology)**

The first axis of PCA absorbed 40% of variance, and was highly related with Total inorganic nitrogen, electric conductivity, phosphate, DBO<sub>5</sub>, percentage of agriculture in the catchments, chlorophyll a and dissolved oxygen.

#### **Relationship between EQR\_ICM and its metrics components with the gradient of general pressure and related variables**

Relationships between EQR\_ICM and its metric components were established with the gradient of general pressure and related variables by means of regression analyses. For

comparison, the classification of the member state (NSpain) EQR\_MS, was also related in the same way to the pressure gradient and the individual abiotic variables. Relationships were established by regression analyses (linear, quadratic and growth), as a linear relationship is not always found along the whole length of the gradient of pressure.

The relationship ( $r^2$ ) between the gradient of pressure and the ICM and its metrics were significant ( $p<0.05$ ) for most of the regressions along the whole gradient (Table 1). This relationship is curvilinear for most of the metrics and multimetrics. Further analysis with the initial linear part of the pressure gradient (removal of the 4 most distant points in the gradient, located in the positive part of axis I) resulted in slightly lower  $r^2$  values, but the significance ( $p<0.05$ ) of the relationship was maintained (Table 1).

	Whole gradient (curve)			Linear gradient	
	<i>linear</i>	<i>Quadratic</i>	<i>Growth</i>	<i>linear</i>	<i>Quadratic</i>
	PCA I	PCA I	PCA I	PCA I	PCA I
EQR ICMi	<b>0.49</b>	<b>0.50</b>	<b>0.45</b>	<b>0.35</b>	<b>0.35</b>
EQR MS_val	<b>0.46</b>	<b>0.49</b>	<b>0.56</b>	<b>0.38</b>	<b>0.40</b>
EQR Average score per Taxon	<b>0.55</b>	<b>0.55</b>		<b>0.39</b>	<b>0.39</b>
EQR Diversity (Shannon-Wiener-Index)	<b>0.38</b>	<b>0.39</b>	<b>0.34</b>	<b>0.24</b>	<b>0.26</b>
EQR EPT-Taxa	<b>0.38</b>	<b>0.39</b>		<b>0.29</b>	<b>0.29</b>
EQR Number of Families	<b>0.34</b>	<b>0.35</b>	<b>0.42</b>	<b>0.21</b>	<b>0.21</b>
EQR Portuges Gold-Index	<b>0.26</b>	<b>0.30</b>		<b>0.20</b>	<b>0.24</b>
EQR sel EPTD	<b>0.32</b>	<b>0.33</b>		<b>0.19</b>	<b>0.19</b>

Table 1.  $R^2$  values for the different regressions analyses established between ICM and its metrics, the EQR MS\_val and the PCA I gradient of general pressure. All bold figures are significant ( $p<0.05$ ).

Figure 1 compares the performance of the EQR MS\_val and EQR\_ICM vs the pressure gradient. Results show that  $r^2$  values for both comparisons are similar (Table 1). The ICM responds to the increment of the pressure gradient towards the right of the figure in the Spanish datasets. The EQR MS\_val of the MS classification system shows a clearer discrimination of classes along the gradient and lower variability within them, while the EQR\_ICM gives higher values to the higher classes in the negative part (lowest pressure) of the pressure gradient.

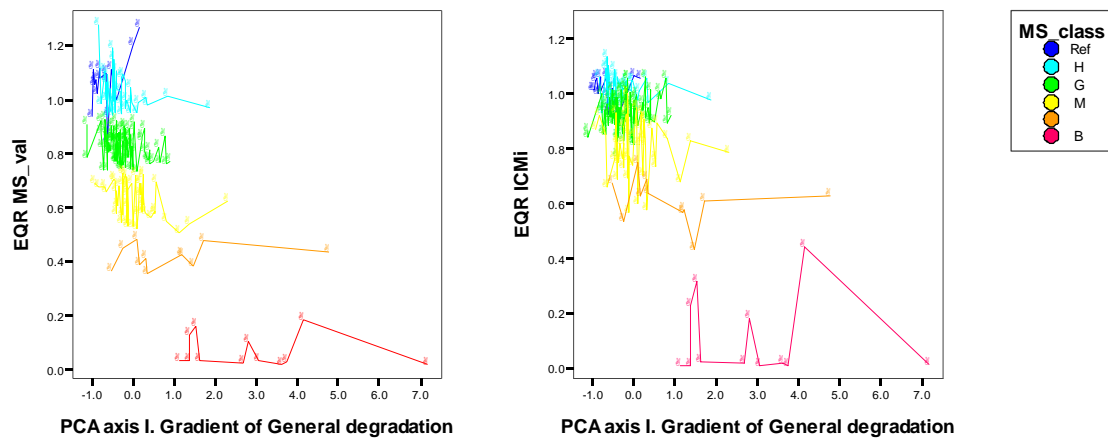


Figure 1. EQR MS\_val and EQR\_ICM vs the pressure gradient, with the corresponding MS\_classes.

The relationship ( $r^2$ ) between the individual pressure variables, the ICM and its metrics, and the EQR MS\_val were significant ( $p < 0.05$ ) for most of the regression analyses (Table 2). This relationship is curvilinear for most of the metrics and multimetrics. Further analysis with the initial linear part of the pressure gradient (removal of the 4 most distant points in the gradient, located in the positive part of axis I) changed the  $r^2$  values in a diverse way, but the significance ( $p < 0.05$ ) of the relationships were mostly maintained (Table 2). The EQR\_ICM shows a slightly better response to ammonia than to nitrate and total inorganic Nitrogen, meanwhile the EQR MS\_val does the opposite.

	Whole gradient (curve)		Linear gradient	
	<i>linear</i>	<i>Cuadratic</i>	<i>linear</i>	<i>Cuadratic</i>
	Nitrate	Nitrate	Nitrate	Nitrate
EQR ICMi	<b>0.02</b>	0.02	<b>0.05</b>	<b>0.06</b>
EQR MS_val	<b>0.08</b>	<b>0.08</b>	<b>0.12</b>	<b>0.14</b>
EQR Average score per Taxon	0.02	0.02	<b>0.05</b>	<b>0.05</b>
EQR Diversity (Shannon-Wiener-Index)	<b>0.03</b>	0.03	<b>0.06</b>	<b>0.07</b>
EQR EPT-Taxa	<b>0.04</b>	<b>0.04</b>	<b>0.07</b>	<b>0.07</b>
EQR Number of Families	0.01	0.01	<b>0.03</b>	<b>0.03</b>
EQR Portuges Gold-Index	<b>0.06</b>	<b>0.07</b>	<b>0.08</b>	<b>0.11</b>
EQR sel EPTD	0.00	0.01	0.02	0.02

	Whole gradient (curve)			Linear gradient		
	<i>linear</i>	<i>Cuadratic</i>	<i>Growth</i>	<i>linear</i>	<i>Cuadratic</i>	<i>Growth</i>
	NH4+	NH4+	NH4+	NH4+	NH4+	NH4+
EQR ICMi	<b>0.42</b>	<b>0.56</b>	<b>0.59</b>	<b>0.35</b>	<b>0.45</b>	<b>0.40</b>
EQR MS_val	<b>0.28</b>	<b>0.28</b>	<b>0.54</b>	<b>0.24</b>	<b>0.32</b>	<b>0.43</b>
EQR Average score per Taxon	<b>0.48</b>	<b>0.62</b>		<b>0.39</b>	<b>0.50</b>	
EQR Diversity (Shannon-Wiener-Index)	<b>0.33</b>	<b>0.45</b>	<b>0.55</b>	<b>0.26</b>	<b>0.37</b>	<b>0.38</b>
EQR EPT-Taxa	<b>0.24</b>	<b>0.33</b>		<b>0.19</b>	<b>0.24</b>	
EQR Number of Families	<b>0.31</b>	<b>0.40</b>	<b>0.52</b>	<b>0.21</b>	<b>0.27</b>	<b>0.33</b>
EQR Portuges Gold-Index	<b>0.15</b>	<b>0.22</b>		<b>0.13</b>	<b>0.18</b>	
EQR sel EPTD	<b>0.32</b>	<b>0.43</b>		<b>0.26</b>	<b>0.33</b>	

	Whole gradient (curve)		Linear gradient	
	<i>linear</i>	<i>Cuadratic</i>	<i>linear</i>	<i>Cuadratic</i>
	Ntot	Ntot	Ntot	Ntot
EQR ICMi	<b>0.19</b>	<b>0.21</b>	<b>0.13</b>	<b>0.14</b>
EQR MS_val	<b>0.25</b>	<b>0.29</b>	<b>0.20</b>	<b>0.23</b>
EQR Average score per Taxon	<b>0.43</b>	<b>0.21</b>	<b>0.15</b>	<b>0.14</b>
EQR Diversity (Shannon-Wiener-Index)	<b>0.17</b>	<b>0.21</b>	<b>0.12</b>	<b>0.14</b>
EQR EPT-Taxa	<b>0.17</b>	<b>0.17</b>	<b>0.12</b>	<b>0.12</b>
EQR Number of Families	<b>0.13</b>	<b>0.14</b>	<b>0.08</b>	<b>0.08</b>
EQR Portuges Gold-Index	<b>0.16</b>	<b>0.21</b>	<b>0.12</b>	<b>0.17</b>
EQR sel EPTD	<b>0.10</b>	<b>0.11</b>	<b>0.05</b>	<b>0.06</b>

Table 2. R<sup>2</sup> values for the different regressions analyses established between ICM and its metrics and the EQR MS\_val, and Nitrate, Ammonia and total inorganic nitrogen. All bold figures are significant (p<0.05).

Relationships with Phosphate, DBO5 and % of agriculture (in the latter case with very low r<sup>2</sup> values) were also significant for all invertebrate metrics and multimetrics (Table 3).

	Whole gradient (curve)			Linear gradient		
	<i>linear</i>	<i>Cuadratic</i>	<i>Growth</i>	<i>linear</i>	<i>Cuadratic</i>	<i>Growth</i>
	<b>PO4</b>	<b>PO4</b>	<b>PO4</b>	<b>PO4</b>	<b>PO4</b>	<b>PO4</b>
EQR ICMi	<b>0.37</b>	<b>0.44</b>	<b>0.37</b>	<b>0.26</b>	<b>0.36</b>	<b>0.17</b>
EQR MS_val	<b>0.31</b>	<b>0.39</b>	<b>0.44</b>	<b>0.25</b>	<b>0.34</b>	<b>0.29</b>
EQR Average score per Taxon	<b>0.41</b>	<b>0.47</b>		<b>0.30</b>	<b>0.38</b>	
EQR Diversity (Shannon-Wiener-Index)	<b>0.29</b>	<b>0.35</b>	<b>0.31</b>	<b>0.19</b>	<b>0.29</b>	<b>0.13</b>
EQR EPT-Taxa	<b>0.27</b>	<b>0.33</b>		<b>0.22</b>	<b>0.27</b>	
EQR Number of Families	<b>0.26</b>	<b>0.31</b>	<b>0.33</b>	<b>0.16</b>	<b>0.21</b>	<b>0.15</b>
EQR Portuges Gold-Index	<b>0.17</b>	<b>0.22</b>		<b>0.14</b>	<b>0.19</b>	
EQR sel EPTD	<b>0.25</b>	<b>0.30</b>		<b>0.15</b>	<b>0.24</b>	

	Whole gradient (curve)			Linear gradient		
	<i>linear</i>	<i>Cuadratic</i>	<i>Growth</i>	<i>linear</i>	<i>Cuadratic</i>	<i>Growth</i>
	<b>DBO5</b>	<b>DBO5</b>	<b>DBO5</b>	<b>DBO5</b>	<b>DBO5</b>	<b>DBO5</b>
EQR ICMi	<b>0.35</b>	<b>0.51</b>	<b>0.48</b>	<b>0.33</b>	<b>0.46</b>	<b>0.47</b>
EQR MS_val	<b>0.23</b>	<b>0.34</b>	<b>0.50</b>	<b>0.19</b>	<b>0.29</b>	<b>0.46</b>
EQR Average score per Taxon	<b>0.41</b>	<b>0.59</b>		<b>0.40</b>	<b>0.51</b>	
EQR Diversity (Shannon-Wiener-Index)	<b>0.25</b>	<b>0.36</b>	<b>0.33</b>	<b>0.20</b>	<b>0.26</b>	<b>0.36</b>
EQR EPT-Taxa	<b>0.20</b>	<b>0.32</b>		<b>0.19</b>	<b>0.32</b>	
EQR Number of Families	<b>0.26</b>	<b>0.37</b>	<b>0.40</b>	<b>0.21</b>	<b>0.27</b>	<b>0.36</b>
EQR Portuges Gold-Index	<b>0.11</b>	<b>0.17</b>		<b>0.09</b>	<b>0.13</b>	
EQR sel EPTD	<b>0.27</b>	<b>0.41</b>		<b>0.25</b>	<b>0.38</b>	

	Whole gradient (curve)			Linear gradient		
	<i>linear</i>	<i>Cuadratic</i>	<i>Growth</i>	<i>linear</i>	<i>Cuadratic</i>	<i>Growth</i>
	<b>%agricult.</b>	<b>%agricult.</b>	<b>%agricult.</b>	<b>%agricult.</b>	<b>%agricult.</b>	<b>%agricult.</b>
EQR ICMi	<b>0.05</b>	<b>0.06</b>		<b>0.05</b>	<b>0.06</b>	<b>0.04</b>
EQR MS_val	<b>0.09</b>	<b>0.09</b>	<b>0.07</b>	<b>0.09</b>	<b>0.09</b>	<b>0.07</b>
EQR Average score per Taxon	<b>0.05</b>	<b>0.06</b>		<b>0.05</b>	<b>0.06</b>	
EQR Diversity (Shannon-Wiener-Index)	<b>0.05</b>	<b>0.05</b>	<b>0.03</b>	<b>0.05</b>	<b>0.05</b>	<b>0.03</b>
EQR EPT-Taxa	<b>0.05</b>	<b>0.05</b>		<b>0.05</b>	<b>0.05</b>	
EQR Number of Families	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>
EQR Portuges Gold-Index	<b>0.04</b>	<b>0.04</b>		<b>0.04</b>	<b>0.04</b>	
EQR sel EPTD	<b>0.03</b>	<b>0.03</b>		<b>0.03</b>	<b>0.03</b>	

Table 3.  $R^2$  values for the different regressions analyses established between ICM and its metrics and the EQR MS\_val, and Phosphate, DBO5 and % agriculture. All bold figures are significant ( $p < 0.05$ ).

The ICM metrics showing a better fit with either the PCA I pressure gradient, or the individual pressure variables, were the ASPT, the Shannon-Wiener diversity index and the EPT taxa (Table 1, 2 and 3). The EPT taxa had the better adjustment along the whole gradient in correspondence with the MS\_classes. The ASPT showed instead a broader class overlap at the initial part (lower pressure) of the pressure gradient (Figure 2). In general abundance metrics did not perform as well; the Portuguese Gold-Index and the EQR sel EPTD had lower  $r^2$  values and greater class overlap along the whole pressure gradient (Figure 2). The relationship between the number of families and % of agriculture in the catchments was not a significant.

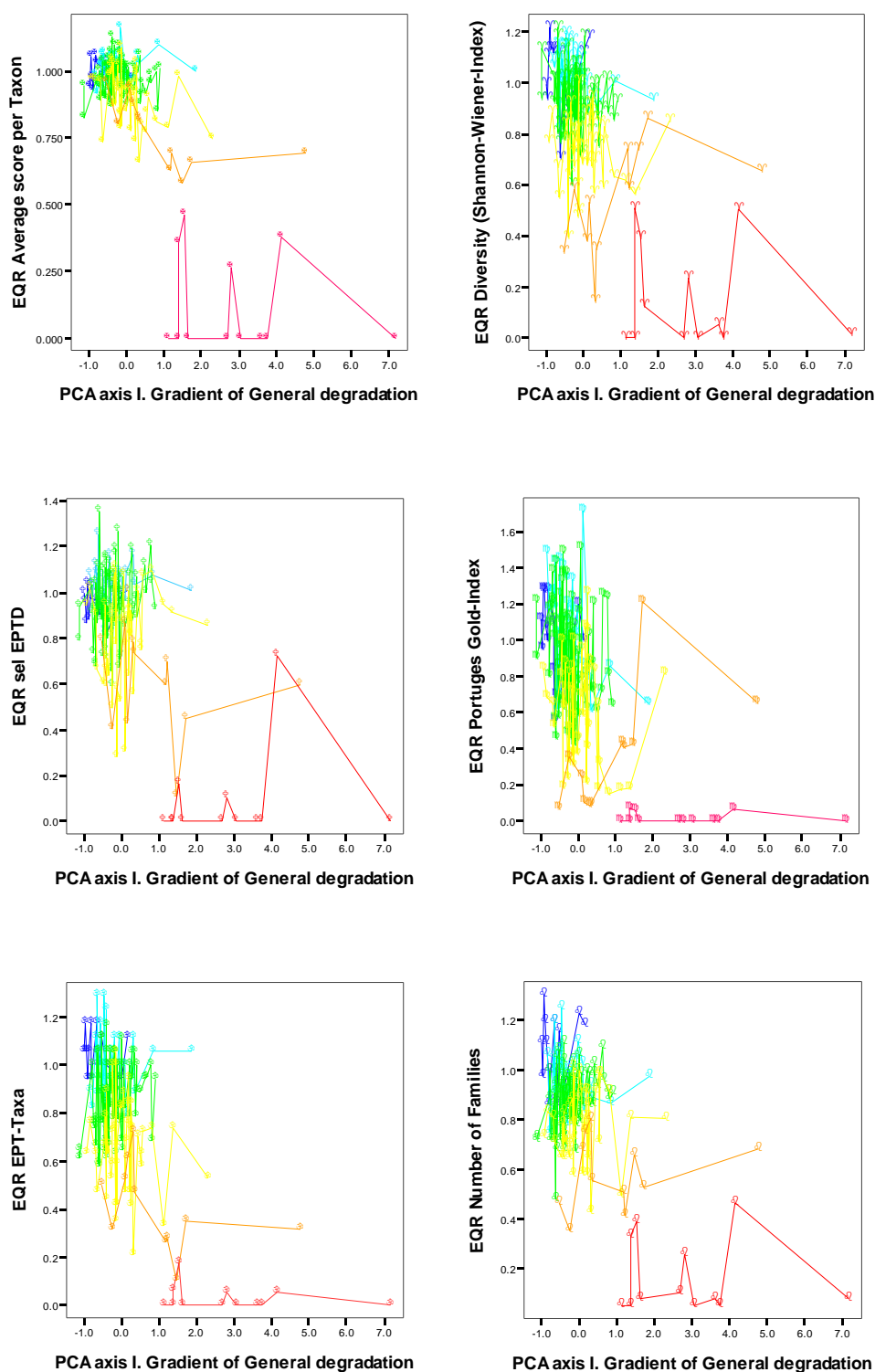


Figure 2. EQR values of the metrics comprising the EQR\_ICM and their relationship with the pressure gradient; corresponding MS\_classes are quoted in colours as for Figure 1.

#### References

Pardo, I. A. M. Olsen, C. Delgado, L. García, A. Nebra & M. Domínguez. 2005. Implantación da Directiva Marco da Auga 2000/60/CE no Ámbito territorial Galicia-Costa. Technical report.