# Anpassung der Phytobenthos-Bewertung an die Fließgewässertypologie Luxemburgs: Charakterisierung von typischen Artgemeinschaften, Referenzwerte und Klassengrenzen

Adaptation of phytobenthos assessment to the Luxembourg river typology : Characterization of typical assemblages, reference values and class boundaries

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# **1. Introduction**

Luxembourg has recently consolidated its river typology ahead of the second River Basin Management Plan (Pottgiesser & Birk 2014). Following this, the Luxembourg water agency asked for a review of the diatom assessment system and, in particular, to validate the metric values used to indicate "expected" conditions as part of EQR calculations. These were originally established as part of the first round of intercalibration (European Union, 2008; Kelly et al., 2009); however, two issues have arisen subsequently that justify a second look at these. The first issue is that tighter rules for screening reference sites were introduced for the second round of intercalibration, and no sites from Luxembourg have been found which fulfil these. The second issue is that the initial scheme adopted a single reference value for the entire territory, despite typological differences being acknowledged (Rimet et al., 2004)

Previous work has established six stream types in Luxembourg based on environmental river features such as catchment size, altitude, slope and bioregion(Ferréol et al. 2005; Table 1, Table 2). Biological quality elements were used to biologically validate this typology (Dohet et al. 2008). The composition of benthic macroinvertebrate, fish and macrophyte communities has already been described. The objective of this report is to characterise the reference diatoms assemblages for these six types and to propose reference values and boundary values of the Luxembourg national metric (the Indice de Polluosensibilité, IPS, Coste in CEMAGREF, 1982).

Туре	Name Original	Deutsche Bezeichnung
I	small high-altitude streams in the Oesling	Bäche der submontanen Stufe des Ösling
П	small mid-altitude streams in the Oesling	Bäche der kollinen Stufe des Ösling
ш	mid-sized mid-altitude streams in the Oesling	Flüsse der kollinen Stufe des Ösling
IV	small mid-altitude streams in the Gutland	Bäche der kollinen Stufe des Gutland
v	mid-sized and mid-altitude streams in the Gutland	Flüsse der kollinen Stufe des Gutland
VI	large lowland streams	Große Flüsse des Tieflands

#### Table 1. Stream types in Luxembourg (after Pottgiesser & Birk, 2014).

Table 2: Comparison between Luxembourg stream types, alternative Luxembourg stream types (Löffler et al. 2003) and stream types adopted in Germany (Pottgiesser & Sommerhäuser 2008) and the EU intercalibration exercise.

Ferreol et a	Ferreol et al (2005)		et al. (2003)	Pottgie	sser & Sommerhäuser (2008)		Interkalibrierungstypen		
Code	Name	Code	Name	Code	Name		Code	Name	
Туре І	Oesling	Typ 1	kleine und große Bäche des	Typ 5	Grobmaterialreiche, silikatische		Interkalii Code RC-3 RC-4 RC-6 RC-6 RC-6 RC-6	Silikatische Mittelgehirgshäche	
Type II	small mid-altitude streams in the Oesling	турт	Schiefergebirges (Salmonidenregion)	Typ 5	Mittelgebirgsbäche		KC-3		
	mid-sized mid-altitude streams in the	Тур 2	kleine Flüsse des Schiefergebirges (Zwischenregion)	Тур 9	Typ 9         Silikatische, fein- bis grobmaterialreiche Mittelgebirgsflüsse		RC-4	Flüsse des Tieflands	
i ype in	Oesling	Тур 1	große Bäche des Schiefergebirges (Salmonidenregion)	Тур 5	Grobmaterialreiche, silikatische Mittelgebirgsbäche		RC-3	Silikatische Mittelgebirgsbäche	
		Löffler et al. (2003) CodeCodeNameTyp 1kleine und große Bäche des Schiefergebirges (Salmonidenregion)Typ 2kleine Flüsse des Schiefergebirges (Zwischenregion)Typ 1große Bäche des Schiefergebirges (Salmonidenregion)Typ 4kleine und große Bäche des Muschelkalks (Salmonidenregion)Typ 7kleine und große Bäche des Luxemburger Sandstein (Salmonidenregion)Typ 9kleine und große Bäche des Luxemburger Sandstein (Salmonidenregion)Typ 5kleine und große Bäche der Keuper- /Liaslandschaften (Salmonidenregion)Typ 8kleine Flüsse des Muschelkalks (Zwischenregion)Typ 7große Bäche des Muschelkalks (Zwischenregion)Typ 8kleine Flüsse der Keuper-/Liaslandschaften (Zwischenregion)Typ 7große Bäche des Luxemburger Sandstein (Salmonidenregion)Typ 7große Bäche der Keuper-/Liaslandschaften (Salmonidenregion)Typ 9große Bäche der Keuper-/Liaslandschaften (Salmonidenregion)Typ 9große Bäche der Keuper-/Liaslandschaften (Salmonidenregion)Typ 9große Bäche der Keuper-/Liaslandschaften (Salmonidenregion)Typ 6große Flüsse des Muschelkalks (Cyprinidenregion)	Тур 7	Grobmaterialreiche, karbonatische Mittelgebirgsbäche					
Type IV	small mid-altitude streams in the Gutland	Тур 7	kleine und große Bäche des Luxemburger Sandstein (Salmonidenregion)	Typ 6	Feinmaterialreiche, karbonatische		RC-6	Karbonatische Bäche des Tieflands	
		Тур 9	kleine und große Bäche der Keuper- /Liaslandschaften (Salmonidenregion)	lyp 0	Mittelgebirgsbäche				
		Тур 5	kleine Flüsse des Muschelkalks (Zwischenregion)		Karhonatische fein-his	1			
		Тур 8	kleine Flüsse der Keuper-/Liaslandschaften (Zwischenregion)	Тур 9.1	grobmaterialreiche Mittelgebirgsflüsse	•	RC-4	Flüsse des Tieflands	
Type V	mid-sized and mid-altitude streams in the Gutland	Тур 4	große Bäche des Muschelkalks (Salmonidenregion)	Тур 7	Grobmaterialreiche, karbonatische Mittelgebirgsbäche				
		Тур 7	große Bäche des Luxemburger Sandstein (Salmonidenregion)	Typ 6	Feinmaterialreiche, karbonatische		RC-6	Karbonatische Bäche des Tieflands	
		Тур 9	große Bäche der Keuper-/Liaslandschaften (Salmonidenregion)	Typ 0	Mittelgebirgsbäche				
Type VI	large lowland streams	Тур 6	große Flüsse des Muschelkalks (Cyprinidenregion)	Тур 9.2	Typ 9.2 Große Flüsse des Mittelgebirges		RC-5	Große Flüsse des Tieflands	
		Тур 6	Ströme des Muschelkalks (Cyprinidenregion)						

# 2. Methods

Diatom assemblages and associated environmental variables for 134 sites with low anthropogenic impact in Luxembourg ("near-reference dataset") was provided for this project. Land use data in the vicinity of the streams was used to screen these sites according to strict ECOSTAT criteria (Pardo et al., 2012) and also with these criteria each relaxed by 10% and 25%. These were then further screened against chemical state, again using ECOSTAT criteria. In view of the limited amount of chemical data available per type, thresholds based on mean values, rather than 90<sup>th</sup> percentiles were used. Where a Luxembourg type straddles two intercalibration types, the more stringent of the two thresholds was used.

In addition, a dataset of diatom assemblage composition from 411 sites from 15 Member States that fulfilled the ECOSTAT land use and chemical criteria from throughout Europe was used (the "X-GIG database": Kelly et al., 2012). 132 of these samples, representing 9 Member States, were from the Central-Baltic Geographical Intercalibration Group (GIG), which includes Luxembourg.

Statistical analyses were performed using the R software package (R Core Team, 2012) using the vegan package (Okensanen et al., 2011) for multivariate analyses. Non-metric MultiDimensional Scaling (NMDS) was used on square-root transformed data after rare and infrequent species (recorded in < 10% of samples and/or maximum relative abundance < 1%) were omitted.

# 3. Results

# 3.1 Preliminary screening of the near-reference dataset

The dataset was first screened using ECOSTAT land use and chemical critiera (see Pardo et al., 2012) to establish whether there were sites within Luxembourg from which estimates of index values at reference conditions could be determined.

No sites survived the ECOSTAT screening process, although 11 sites survived when all thresholds (land use and chemical) were relaxed by 25% (Table 3). However, two types (5,6) were not represented at all and two others (2,4) were only represented by a single sample. The only site that survived the screening based on chemical criteria was "amont Horas, Pratz", in the Breschterbaach (location code: L106044A01) although it was only designated as a "potential reference site" in the original screening process as it exceeds the threshold for artificial land cover (3.5% recorded, cf 0.8% rejection threshold).

This preliminary screening exercise demonstrate that there are not enough sites within Luxembourg to permit the derivation of meaningful reference values. Those sites that do survive the relaxed screening are mostly found in the low alkalinity types (1,2,3), with just one high alkalinity site represented.

Туре	ECOSTAT criteria		ECOSTAT cri	teria + 10%	ECOSTAT criteria + 25%		
	Land use	+ chem	Land use	+ chem	Land use	+ chem	
1	6	1	9	1	30	7	
2	3	0	4	0	13	1	
3	0	0	0	0	3	2	
4	12	0	12	0	25	1	
5	0	0	0	0	0	0	
6	0	0	0	0	0	0	
Total	21	1	25	1	68	11	

Table 3. Number of sites per type that fulfil criteria for reference conditions, as determined by ECOSTAT.

# 3.2 Diatom assemblage composition within the near-reference dataset

The structure of the data was then investigated using Non-metric MultiDimensional Scaling (NMDS). The result of this analysis was a robust ordination (stress: 0.18) which separates the national types along axis 1. The three types associated with low alkalinity water have low scores on axis 1 whilst the three associated with high alkalinity water have high scores on axis 1. It was not clear, based on information available, what factors were most strongly associated with axis 2.

No significant difference was found in the composition of the three low alkalinity types (Anosim: R = 0.139; N.S.) nor of the three high alkalinity types (Anosim: R = 0.033; N.S.) suggesting that other typological factors (e.g. catchment size) have little influence on diatom assemblages. For all future analyses, types 1,2 and 3 are treated as a single "low alkalinity" supertype, whilst types 4,5 and 6 are combined into a single "high alkalinity" supertype.



# Fig. 1. Axes 1 and 2 of a Non-metric MultiDimensional Scaling analysis of the near-reference dataset; sites are grouped according to the Luxembourg national typology.

The three low alkalinity types (1,2,3) are associated with lower IPS scores than the high alkalinity types (4,5,6). Types 1 and 2, in particular, seem to have IPS values that are much lower than one

would expect in a "reference site". Eight samples in type 1, for example, have IPS < 10 and assemblage composition suggests some enrichment (abundant taxa include *Reimeria sinuata, Navicula gregaria, Mayamaea permitis, Navicula lanceolata, Eolimina minima, Planothidium lanceolatum*). More significantly numbers of indicators of high and good status are only present in low numbers and typical indicators of soft water are also rare. Overall, the composition of these samples suggests higher alkalinity than would be predicted from the thresholds for the types.



Figure 2. IPS values for samples in the near-reference dataset, plotted by stream type. The horizontal red line is the present reference value for Luxembourg (17.4).

### 3.3. Comparison with reference sites elsewhere in Europe

As the number of minimally-impacted sites in Luxembourg is very low, the next exercise uses evidence from elsewhere in Europe to select samples from the near-reference dataset that have assemblages consistent with "high status". If associated with suitably low levels of pressure, these sites could provide a baseline that, whilst not strictly "reference", would at least give a plausible estimate of the expected IPS for Luxembourg streams. Each Luxembourg type was matched to the appropriate intercalibration type (see Tab. 2) and the 90<sup>th</sup> percentile of the IPS values of screened sites was used as a working limit for "high ecological status". In total, 17 sites from Luxembourg fulfilled this criterion (Table 4).

Ту	/pe	Ν		I	PS		LU sites	
LU	IC	Samples	MS	Mean	90 <sup>th</sup> %ile	Total	Pass	supertype
1	RC3	32	4	18.4	16.05	37	7	
2	RC3	32	4	18.4	16.05	14	0	7
3	RC3	32	5	18.4	16.05	4	0	/
	RC4	24	4	16.5	14.69	1	0	
4	RC6	14	4	17.9	16.57	60	6	
5	RC4	24	4	16.5	14.69	5	4	- 10
	RC6	14	4	17.9	16.57	8	0	10
6	RC5	12	3	16.6	14.35	1	0	

Table 4.Sites in Luxembourg that approximate to High Ecological Status, following criteriaestablished from the X-GIG database (= 90<sup>th</sup> percentile of IPS values from screened referencesites).Table also indicates the number of samples per intercalibration type, and the number ofMember States (MS) from which these were derived.

The chemical composition of these "HES" sites was then examined using the screening limits established by CB-GIG as indicators of the level of impairment present. A series of boxplots shows how sites that fulfil the HES criterion compare to those that do not for the low and high alkalinity supertypes.

BOD values are mostly within the limits for both low and high alkalinity supertypes, although several dissolved oxygen concentrations are below the lower limit (Fig. 3). The significance of this, however, should not be over-estimated due to the relatively sparse nature of the chemical dataset. Several records for ammonia-N exceed the limit at high alkalinity sites whilst nitrate-N concentrations frequently exceed the limits at both low and high alkalinity sites (Fig. 4). Orthophosphorus is largely below the screening limit for low alkalinity sites but several records exceed this at high alkalinity sites (Fig. 5). Overall, there is evidence that several of the sites that have diatom assemblages consistent with high status still have evidence of elevated pressures.



Figure 3. Box-and-whisker plots showing the range of BOD (top) and dissolved oxygen (bottom) values recorded in sites that fulfilled the HES criterion compared to those that do not for the low and high alkalinity supertypes. Horizontal lines are the thresholds for reference conditions for each determinant (after Pardo et al., 2012)



Figure 4. Box-and-whisker plots showing the range of ammonium-N (top) and nitrate-N (bottom) values recorded in sites that fulfilled the HES criterion compared to those that do not for the low and high alkalinity supertypes. Horizontal lines are the thresholds for reference conditions for each determinant (after Pardo et al., 2012)



Figure 5. Box-and-whisker plots showing the range of ortho-phosphorus values recorded in sites that fulfilled the HES criterion compared to those that do not for the low and high alkalinity supertypes. Horizontal lines are the thresholds for reference conditions for ortho-phosphorus (after Pardo et al., 2012)

# 3.4 Use of X-GIG database to establish reference conditions for Luxembourg

As there are not enough sites with low levels of pressure within Luxembourg from which an "expected" value for EQR calculations can be derived, the focus moved to looking at reference sites elsewhere in CB-GIG and considering whether these might provide robust estimates for Luxembourg. In this section, the composition of three of the four intercalibration types that are represented in Luxembourg will be analysed in order to evaluate their suitability. RC5 cannot be analysed in this way as none of the RC5 sites have assemblages that resemble HES.

### 3.4.1. RC3

There was considerable variation in IPS values for reference sites between MS (Kruskal-Wallis  $\chi^2$ : 16.05; P = 0.003), with Spain and Sweden generally having higher IPS values than Belgium (Wallonia). Austria and UK were only represented by a single sample each. The NMDS, including Luxembourg HES sites, separated the Spanish and Swedish sites (high axis 1 values) from the Belgium and Luxembourgish sites (low axis 1 values), with further separation of Belgium and Luxembourg, and of Spain and Sweden, along axis 2.



Fig. 6. Box-and-whisker plot showing variation between IPS values for RC3 reference sites in the X-GIG database. Red line: IPS = 17.4 (current Luxembourg reference value).



Fig. 7. Axes 1 and 2 of a Non-metric MultiDimensional Scaling analysis of RC3 reference sites along with HES sites from Luxembourg (stress = 0.09).

Table 5: Association table for reference sites and Luxembourg HES sites in RC3. Black: maximum relative abundance  $\geq$  10%; red: maximum relative abundance  $\geq$  5% and < 10%; +: present.

		LU	BE_	RW		ES		SE	ŀ	٩T	l	UK
DENOM	Ν	max	Ν	max	Ν	max	Ν	max	Ν	max	Ν	max
Cocconeis placentula	6	56.99	7	50.27	1	16.20		-	1	7.11		+
Achnanthidium subatomus	2	35.75	4	39.40	2	69.91		+		-		+
Achnanthidium minutissimum	2	22.69	8	50.33	16	91.87	1	30.63		-		-
Gomphonema elegantissiumum	1	40.40		-		-		-		-		-
Gomphonema pumilum	1	13.97		-		+		-		-		-
Planothidium lanceolatum	1	22.94		+		+		-		+		-
Rhoicosphenia abbreviata	1	12.75		+		+		-		-		
Planothidium frequentissimum	7	7.48	1	13.51		+		-		-		-
Reimeria sinuata	7	9.56	1	14.45		+		-		-		+
Eunotia subarcuatoides	_	-		-	7	60.68		-		-		-
Eunotia intermedia	_	-		-	5	37.45		-		-		-
Surirella roba	_	-		-	4	22.39		-		-		-
Diatoma mesodon	_	-		+	2	14.73		-	1	14.67		-
Fragilaria rumpens	_	+		+	2	49.64		-		-		-
Achnanthes oblongella		-		-	1	18.21		-		-	1	36.22
Gomphonema exilissimum		-		+	1	26.62		+		-		-
Navicula angusta		-		-	1	19.08		+		-		_
Nitzschia palea var.debilis		+		+	1	13.43		-		-		-
Gomphonema angustatum		-		-		-		-	1	23.56		-
Navicula lanceolata		+	9	7.1		-		-	1	13.33		+
Brachysira neoexilis		-		-		-	2	22.26		-		+
Frustulia crassinervia		-		-		-	1	20.12		-		-
Peronia fibula		-		-		+	1	13.11		-		-

Differences in composition between assemblages were sufficiently marked that they are presented as an "association table" (Table 5) based on those taxa present in numbers (>10% of total) rather than via a statistical technique such as Indicator Value analysis. The Luxembourg samples are on the left of Table 4, with data from other Member States presented in order of decreasing similarity. There are several similarities between Luxembourg and Belgium samples, particularly when members of the *Cocconeis placentula* complex are combined (differences are as likely to represent taxonomic conventions as ecological/biogeographical variation). On the other hand, Spanish samples in particular, seem to suggest softer waters, with several *Eunotia* species and *Achnanthes oblongella* occurring in numbers. Sweden and UK, though represented by fewer samples, also suggest softer waters. It is possible that the 0.4 meq L-1 threshold for RC3 encompasses natural variation along an alkalinity gradient. Nonetheless, the similarity between axis 1 scores for Luxembourg and Belgium samples as surrogate reference samples for Luxembourg.

#### 3.4.2 RC4

As was the case for RC3, there is a clear difference between Member States (Fig. 8; Kruskal Wallis  $\chi^2$ : 13.27; P = 0.004) with Spain having higher values than Poland, whilst Estonia and Sweden are each represented by a single sample. These differences continue in the NMDS (Fig. 9) though, unlike RC3, there are no close geographical neighbours represented, and Poland, the closest in the ordination space, is represented by just two samples.

The most abundant species in samples from Luxembourg suggest enrichment (e.g. *Navicula cryptotenella, Nitzschia soratensis*) and are not abundant in any of the reference samples for this type (Table 6).



Fig. 8. Box-and-whisker plot showing variation between IPS values for RC4 reference sites in the X-GIG database. Red line: IPS = 17.4 (current Luxembourg reference value).



Fig. 9. Axes 1 and 2 of a Non-metric MultiDimensional Scaling analysis of RC4 reference sites along with HES sites from Luxembourg (stress = 0.11).

Table 6: Association table for reference sites and Luxembourg HES sites in RC4. Black: maximum relative abundance  $\geq$  10%; red: maximum relative abundance  $\geq$  5% and 10%; +: present.

Taxon	I	LU		PL		ES		SE		EE
	N	max	Ν	max	Ν	max	Ν	max	Ν	max
Amphora pediculus	4	55.45	1	19.76		+		-		+
Navicula cryptotenella	2	28.78		+	7	9.15		+		+
Achnanthidium minutissimum	1	13.40	1	22.12	6	64.37	1	39.23	1	70.91
Navicula tripunctata	1	13.46	1	17.99		+		+		+
Achnanthidium subatomus	1	44.30		+	5	41.45	-	+		-
Nitzschia soratensis	1	10.74		+		+		+		-
Fragilaria construens and varieties		-	2.	27.05		+		+		-
Cocconeis placentula and varieties	4	5.37	1	12.16	6	54.84		+	1	5.90
Pseudostaurosira brevistriata		-	1	20.06		+	1	9.73		-
Fragilaria pinnata		-	1	11.55		+	1	6.19		-
Achnanthidium pyrenaicum	2	6.73		+	2	92.51		-		-
Fragilaria capucina		-		+	1	10.87		1 -		-
Gomphonema rhombicum		-		+	1	17.37		-		-

#### 3.4.3. RC6

The potential for analysing differences between MS for RC6 is limited by the low numbers of samples from all Member States except Spain (Fig. 10). Two clusters of sites are revealed by NMDS ordination (Fig. 11): Spain, UK and Ireland (high scores on axis 1) and Belgium and Luxembourg. There are, however, just two reference sites for Belgium and one each for Ireland and UK, which complicates the comparison of species (Table 7)..



Fig. 10. Box-and-whisker plot showing variation between IPS values for RC6 reference sites in the X-GIG database. Red line: IPS = 17.4 (current Luxembourg reference value).



Fig. 11. Axes 1 and 2 of a Non-metric MultiDimensional Scaling analysis of RC6 reference sites along with HES sites from Luxembourg (stress = 0.07).

Table 7: Association table for reference sites and Luxembourg HES sites in RC6. Black: maximum relative abundance  $\geq$  10%; red: maximum relative abundance  $\geq$  5% and 10%; +: present.

Name		LU		ES		BE	l	UK		IE
	n	max								
Amphora pediculus	5	44.10	1	14.17	2	9.23		+		+
Achnanthidium minutissimum	3	37.41	8	86.35	2	11.38	1	49.20	1	69.08
Achnanthidium pyrenaicum	2	19.00	5	57.32		-		-	1	9.9
Cocconeis placentula and varieties	1	10.86	6	59.07		+		-		+
Achnanthidium atomoides	1	29.88	2	12.26		+		-		-
Navicula tripunctata	1	14.25		+	2	14.84		-		-
Amphora inariensis	1	25.78		+		+		-		-
Denticula tenuis	1	16.24		+		+		-		-
Rhoicosphenia abbreviata	1	14.96		+	2	6.85		+		-
Achnanthidium subatomus		-	1	43.08		-		-		-
Gomphonema pumilum var. elegans		-	1	10.63		-		-		-
Cocconeis pediculus		+		+	2	17.81		-		-
Navicula cryptotenella	4	7.25	5	5.44	2	17.08		-		-
Encyonema reichardtii				-		-	1	18.18		-

#### 3.4.4. Recommendation for a revised reference value for Luxembourg

Two possibilities emerge from the analysis: either the average IPS value for the corresponding IC type(s) could be used as an alternative reference value for EQR calculations, or a value based on the Belgium (Wallonia) reference value could be used. There is some evidence in all three IC types of between-Member State differences; this probably reflects a combination of methodological and biogeographical factors that are difficult to unravel but which could be circumvented, to some extent, by using only values from a near-neighbour.

Table 8 summarises the alternatives. For the low alkalinity supertype, the GIG mean is higher than the present reference value. However, there is evidence of a gradient within RC3 references sites, perhaps reflecting alkalinity, and the high IPS values associated with ES and SE may reflect very soft waters. Therefore, it is not appropriate to use the GIG mean as a reference value for LU. The average value for RC3 reference sites in Belgium (Wallonia) may be a more appropriate value, but this involves a slight drop in IPS compared to the present value.

The situation for the high alkalinity supertype is more complicated. High alkalinity sites straddle three intercalibration types and there are few good reference sites from neighbouring countries (just two reference sites in Wallonia). There are no strong reasons to treat the diatom assemblages of national types 4-6 separately but the mean IPS values of these types range from 16.5 (RC4) to 17.9 (RC6). The average for all three types combined is 16.9, which is still slightly higher than the average of the two Walloon reference sites but lower than the current reference value of 17.4 (Table 9).

This means that there are four options for selecting a new reference value for Luxembourg. Of these options, the "hybrid" is recommended. This means a slight relaxation of the reference value for both low and high alkalinity stream types, but has the advantage of a stronger justification, based on comparisons between Luxembourg samples with fully-screened reference samples from the X-GIG database. The final issue, therefore, is to examine the consequences for such a change on high/good (H/G) and good/moderate (G/M) boundaries, to ensure that there is no drop in stream quality as a result of this step.

	Current LU reference value	GIG mean	Belgium (Wallonia) mean
Low alkalinity	17.4	18.4	17.1
		16.5 (RC4)	
Llich alkalinity	47.4	16.6 (RC5)	16.2
High alkalinity	17.4	17.9 (RC6)	10.3
		16.9 (RC4-6 combined)	

# Table 8: Current IPS reference value for Luxembourg, compared with corresponding mean values based on screened reference sites in CB-GIG and Belgium (Wallonia)

Option	Low alk	High alk	
Do nothing	17.4	17.4	Intercalibrated and accepted
Use GIG means	18.4	16.9	Low alkalinity value is skewed by softwater sites in ES and SE
Use Walloon means	17.1	16.3	Slightly more relaxed than present values; high alkalinity value is based on just two samples
"Hybrid"	17.1	16.9	Belgium (Wallonia) value is appropriate for low alkalinity types; IC mean for high alkalinity types; both still slightly more relaxed than present values

#### Table 9. Options for selecting revised reference values for Luxembourg

## 3.5 Adjustment of H/G and G/M boundaries

The current intercalibrated boundaries for phytobenthos in Luxembourg are 0.9 and 0.7, which equate to absolute IPS values of 15.7 and 12.2 respectively. These are similar to boundary values used in Wallonia but G/M boundaries in particular are lower than used in comparable stream types in France (Table 10). A direct comparison with German boundaries is not possible, due to the complexities of the German assessment system.

If the reference values are adjusted, then the EQR values for H/G and G/M boundaries will also need to be adjusted in order to ensure that there is not a drop in absolute quality for Luxembourg streams.

Table 10. Comparison between reference and boundary values for Luxembourg, Belgium(Wallonia) and French stream types. Note that "Ardennes" corresponds to the low alkalinitystreams in Luxembourg whilst Côtes Calcaires Est corresponds to high alkalinity streams.

MS	Reference value	H/G	G/M	Notes
LU	17.4	15.7	12.2	
BE	16.4	16	12	
FR – Ardennes	(16.7)	15.88	13.66	Inferred from IBD v IPS
FR – Côtes Calcaires Est	(17.2)	16.32	14.11	regression



Figure 12. Scatterplot showing Luxembourg near-reference dataset expressed as IPS and phytobenthos intercalibration common metric (ICM). Positions of current reference value (black), H/G boundary (blue) and G/M boundary (green) are superimposed on this.

Using the relationship phytobenthos ICM = 0.044\*IPS + 0.1014 it is possible to recalculate the EQR values that preserve the current boundaries in absolute terms and to explore alternative options (Table 11). Whilst some caution is needed here (as there are no ECOSTAT-compliant reference sites for these two types), the possibility of applying more stringent boundaries within Luxembourg should be considered.

Options are set out in Table 10:

Option 1, to retain the current reference value and boundaries, is included here to aid comparison with other possibilities.

Option 2 shows the consequences of adjusting reference values, as discussed above, but retaining the current EQR boundary values. This would result in a drop in boundary values, as IPS, of almost 0.4 units, in the case of high alkalinity streams.

Option 3 is the same as Option 2, except that the EQR values have been adjusted in order to preserve the current boundaries, as IPS. This results in slight increases (0.01 – 0.03 EQR units). Both H/G and G/M boundary values are very similar in absolute terms to those in Belgium (Wallonia) and the H/G boundary is less than one IPS unit from both French boundaries. However, the G/M boundary is 1.5 and 1.9 units lower than the two comparable French types (Table 9).

Option 4 adjusts the position of both H/G and G/M boundaries so that both are now at the top of the "acceptable band", relative to other Member States in CB-GIG. The "acceptable band" is based on the criterion adopted in intercalibration that all boundaries should be within a quarter of a class of the average value for the GIG. Luxembourg's high/good boundary was close to the GIG mean, whilst the good/moderate boundary was 0.14 class widths below the mean. Both of these were raised so that they were 0.25 class widths above the GIG mean. This raises the threshold IPS by approximately one unit for both stream types. The H/G boundary is now slightly higher than that for the two French types, but the G/M boundaries are still slightly lower than those set for France. There is no reason, indeed, why the boundary values for Luxembourg should not be raised to higher levels, but this option does, at least, keep Luxembourg broadly aligned with the rest of CB-GIG.

	Reference		H	/G	G/	М
	EQR	IPS	EQR	IPS	EQR	IPS
Current value (option 1)						
All streams	1.00	17.4	0.90	15.66	0.70	12.2
Revised value (option 2)						
Low alkalinity streams	1.00	17.1	0.90	15.39	0.70	11.97
High alkalinity streams	1.00	16.9	0.90	15.21	0.70	11.83
Revised value (option 3)						
Low alkalinity streams	1.00	17.1	0.92	15.66	0.71	12.2
High alkalinity streams	1.00	16.9	0.93	15.66	0.72	12.2
Revised values (option 4)						
Step 1: move LU to top of "acceptable bar	nd" using c	urrent ref	erence val	ue		
All streams	1.00	17.4	0.965	16.8	0.762	13.26
Step 2: adjust EQRs using revised reference	ce values					
Low alkalinity streams	1.00	17.1	0.98	16.8	0.78	13.26
High alkalinity streams	1.00	16.9	0.99	16.8	0.78	13.26

# Table 11. Revised EQR values for Luxembourg streams, with boundaries expressed as both EQR and IPS.

# 3.6 Characterisation of diatom assemblages

Based on these analyses, the descriptions of characteristic diatoms for the running water types in Luxembourg, as presented in the Steckbriefe can be updated. The lack of differentiation between types 1,2 and 3 ("low alkalinity supertype"), and between types 4,5 and 6 ("high alkalinity supertype", see section 3.2) means that just two descriptions are presented here. These are based on the taxa associated with sites defined as high ecological status, assuming option 4 is adopted (see section 3.5).

Five sites fulfilled this criterion for each of the supertypes, and descriptions are based on the composition of these. The taxa are selected on the basis of consistency and relative abundance,

with those forming  $\geq$ 10% of the total being considered to be dominant, and those between 5 and 10% as sub-dominants. Taxa found in less than three samples have been omitted. It is hard to make detailed descriptions based on just five samples and additional sampling of these sites is recommended in order to characterise these sites more fully.

### 3.6.1. Low alkalinity supertype (Luxembourg types 1,2 and 3)

These streams are dominated by attached diatoms, including *Cocconeis lineata, C. pseudolineata, Remieria sinuata* and *Planothidium frequentissimum. Achnanthidium minutissimum, A. subatomoides* and *Planothidium lanceolatum* are also often found in large numbers. Sub-dominants include *Amphora pediculus* and *Nitzschia soratensis*. Acid-tolerant and softwater forms are not common.

## 3.6.2. High alkalinity supertype (Luxembourg types 4,5 and 6)

These streams are dominated by attached diatoms, with *Amphora pediculus* and *Achnanthidium minutissimum* both occurring in numbers, along with the motile diatom *Navicula tripunctata*. *Reimeria sinuata* is consistently present, though in lower numbers. *Achnanthidium pyrenaicum, Cocconeis euglypta, Denticula tenuis, Navicula cryptotenella* and *Rhoicosphenia abbreviata* are also often found in large numbers.

# 4. Summary and Conclusions

- 1. The objective of this study was to characterise the reference diatoms assemblages for the six stream types recognised in Luxembourg and to propose reference values and boundary values of the Luxembourg national metric for these.
- 2. Characterising diatom assemblages in Luxembourg streams is, however, complicated by the absence of streams in Luxembourg that conform to the ECOSTAT screening criteria. Even when criteria were relaxed by up to 25%, few sites conformed to the criteria. These were most associated with stream type 1; stream types 5 and 6 had no reference sites, even with these relaxed criteria. For this reason, the search for appropriate reference sites was extended beyond the Luxembourg borders using a dataset compiled for the phytobenthos intercalibration exercise.
- 3. Preliminary ordinations of a dataset of "best available" sites from Luxembourg suggested that the six stream types could be consolidated into two "supertypes" based on their diatom assemblages. Types 1, 2 and 3 formed a low alkalinity supertype, whilst types 4,5 and 6 formed a high alkalinity supertype.
- 4. There were significant differences in IPS values for ECOSTAT-compliant reference sites between Member States for the intercalibration stream types represented in Luxembourg. This precludes the use of the GIG mean IPS value as a surrogate reference value for Luxembourg. However, the dataset could be used to screen the Luxembourg data in order to identify sites that approximately correspond to a GIG-wide view of "high ecological status" (HES).
- 5. The composition of these HES sites was then compared with the composition of reference sites from this type from elsewhere in the GIG. In the case of low alkalinity RC-3 sites, Luxembourg HES sites had a similar composition to reference sites in Belgium (Wallonia). There were fewer

reference sites for high alkalinity types, and similarities with neighbouring countries were not so clear-cut.

- 6. On the basis of this, we propose the adoption of the average value of ECOSTAT-compliant sites in Belgium (Wallonia) for low alkalinity sites in Luxembourg, and of the average of all reference sites in RC4, RC5 and RC6 for the high alkalinity sites in Luxembourg.
- 7. Based on these revised reference values, new boundary values are proposed to ensure no change in ambition for Luxembourg streams based on these new reference values. The possibility of raising the ambition for Luxembourg streams is also discussed. The values proposed (option 4) are still broadly aligned with the rest of CB-GIG but now veer to "stringent" rather than "relaxed".

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