MAYFLY COMMUNITY COMPOSITION AND THE BIOLOGICAL QUALITY OF STREAMS

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An aggregation of the Italian Mayfly species at different levels is proposed: genus level (24 Operational Units), morpho-taxonomic group level (11 O.U.) and species level (*Baetis rhodani* only). On the basis of nymphs sensitivity to water quality and habitat alterations, a score is assigned to each Operational Unit. The scores for all the O.U. are summed to give a cumulative site score that is then divided by the number of O.U. used in the calculation in order to obtain the Mayfly Average Score. By means of calculated average scores, reference classes of mayfly community integrity are defined, so that a biological quality evaluation of streams based on Ephemeroptera can be assessed.

INTRODUCTION

Mayflies can be considered good biological indicators (LANDA & SOLDAN, 1991), they are usually present with many species in lotic environments, and they can contribute up to 25% of the total zoobenthos production (ELLIOTT *et al.*, 1988). Their nymphs are relatively easy to identify, often to the species level, they are present in all types of streams (SOWA, 1975) and show a high morphological and ecological differentiation between families, so that most microhabitats can be colonized (i.e. SOWA, 1975; HEFTI *et al.*, 1985). Furthermore, many mayfly species are sensitive to various forms of habitat alteration and pollution so that they can be used successfully to indicate water quality (i.e. SLADECEK, 1973) and habitat quality (i.e. USSEGLIO-POLATERA & BOURNAUD, 1989; USSEGLIO-POLATERA, 1990). On these bases, it is presumably possible that the order of Ephemeroptera can adequately summarize, in running waters, some aspects of habitat and water quality.

The aim of this work is to define a metric to assess the biological quality of streams using mayflies.

The biological quality of streams is often assessed by means of biotic indices based on the whole macroinvertebrate communities (METCALFE-SMITH, 1994), the study of which is for some aspects difficult. Biotic indices requiring a specific determination of sampled organisms (i.e. Saprobian Index) need a long time for application and are restricted to specialists' use. On the other hand, indices calculated on the basis of family identification (i.e. Biological Monitoring Working Party Score) retain only a part of the biological information present in the community structure, averaging the responses of taxa with different sensitivity (HILSENHOFF, 1988). In this case, for some species tolerance values are lower and for others higher than the fixed family value. This is also true when the genus level is proposed, because within a genus (as within a family) different species may even show opposite characteristics. Some indices (i.e. Extended Biotic Index) use different levels of taxa aggregation (often genus or family) for different invertebrate groups (HELLAWELL, 1986). Even when applying these indices, much biological information is lost. Because individual species are more numerous and give a more detailed environmental reading than families or genera, qualitative «lower than genus» data were found to give better stream site classifications (FURSE *et al.*, 1984) than qualitative or quantitative data at a higher level of identification. Preserving some of the species-level informations without the necessity to identify to species the collected organisms should be then useful. This could be in some cases achieved fixing intermediate levels of identification between genus and species.

METHODS

The mayfly communities of ca. 200 sites located all over Italy in different stream types and of differing altitude and environmental quality were studied. Some of them are reported in Table 7. Sampling was mainly conducted in Northern Italy. In a few cases mayfly communities derived

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from literature data (QUERENA & SOLBIATI, 1979; BELFIORE & GAINO, 1988) were studied. At each collecting site, qualitative samples of mayfly nymphs have been taken from all available habitats with a Surber sampler and a hand net. Mayflies were sorted in the field. Multiple-plate artificial substrata and hand net collecting methods were employed in large rivers. A stereoscope (63x) was used for taxa identification. In 95 sites the whole macroinvertebrate community was collected (Table 1), mayflies were identified to species and the other organisms to the taxonomic level established for the application of EBI (GHETTI, 1986). This index, BMWP Score and ASPT (ARMITAGE *et al.*, 1983) were calculated.

Table 1. Northern Italy streams and sites where the whole macroinvertebrate community was collected. From these samples the standard biotic indices BMWPS, ASPT and EBI were applied.

River	Province	Altitude (m a.s.l.)	Month	Number of sites	Number of samples
Po	Pavia	65	8-9-10	2	7
Staffora	Pavia	150-180	3-7-12	4	12
Ticino	Milano	110	4	2	3
Borretta	Milano	130	2-5	3	4
Grande	Milano	130	2-4-5	2	5
Curone	Lecco	400	4-5-6	6	7
Pioverna	Lecco	650-880	3-7	5	6
Poschiavino	Sondrio	425	7-8-9-12	1	6
Roasco	Sondrio	630	7-8-9-11-12	1	8
Adda	Sondrio	300-1200	2-4-7-9-12	8	22
Frodolfo	Sondrio	1150-1300	2-12	3	6
Viola	Sondrio	1300	7-9	1	2
Spool	Sondrio	1800-1950	7	2	3
Braulio	Sondrio	2300	7-9-10	1	4

RESULTS

Taxa grouping

To approximate the specific composition of the community a definition of mayfly groups with fixed identification level was employed. These groups, either taxonomic or morpho-taxonomic, are named Operational Units (OU). They will be used in the calculation of the metric. A linear regression analysis between species number (s) and OU number on 150 samples collected in Northern Italy was conducted. A correlation coefficient equal to 0.9825 (OU = 0.18 + 0.91s; $p<0.001$) was found, showing OU number can be considered a good approximation of species number. The following aggregation of the Italian mayfly species at different levels is proposed: genus level (24 OU), morpho-taxonomic group level (11 OU) and species level *(Baetis rhodani* only). *B. rhodani* nymphs constitute an Operational Unit themselves. The aggregation of the species belonging to three genera, *Rhithrogena, Caenis, Baetis,* into morpho-taxonomic groups has been made. When possible it was performed on the basis of the most easily visible characters that could be singled out, so as not to complicate nymphal identification. Grouping within the genus *Rhithrogena* has been defined by means of three simple morphological characters (Table 2): presence/absence of a dorsal spot on femurs, of VII gill crenulation and of a dorsal plica on the I gill. For species present in Italy, six groups (defined as OU) can be found: *Rhithrogena* gr. a, b, ... f. Reference species for each group are listed in Table 2. The *Baetis* species, *B. rhodani* excluded, have been aggregated into two morpho-taxonomic groups. One OU (group A) includes the species with reduced paracercus. The remaining species of the genus belong to group B. This OU also includes the species of *Alainites* (*A. muticus)* and *Nigrobaetis* (*N. digitatus, N. niger*) (Table 3). The *Caenis* species are clustered into three OU. The *macrura*-group species constitute the first OU. The second *Caenis* OU (group 5) includes C. *beskidensis* and C. *belfiorei* only. They are distinguishable from the other *Caenis* species for the presence of a light but evident notch on IX sternite, of a well developed gena and of long bristles on the hind margin of fore femurs (see BUFFAGNI, 1997, for details). The third OU (*Caenis* group 3) includes all the remaining *Caenis* species present in Italy (Table 4).

Score attribution

By means of a subjective approach based on literature data and the author's experience, Italian mayflies have been classified into score categories in relation to their potentiality of being environmental indicators. Three score levels have been defined (Table 5). Score 1 is assigned to habitat generalists that can be considered exploiter indicator species, being abundant in polluted waters or altered areas: *Baetis rhodani* (MULLER-LIEBENAU, 1969; JANEVA, 1979; ALBA TERCEDOR *et al.*, 1991) and to the species of the *Caenis macrura*-group

Table 2. Morpho-taxonomic groups, corresponding to six Operational Units, for the Italian species of the genus *Rhithrogena.* Some reference species are reported.

Rhithrogena	Morpho-taxonomic group					
	a	b	c	d	e	f
femur dorsal spot	+	+	+	-	-	+
VII gill margin crenulated	+	+	-	+	+	-
I gill dorsal plica	+	-	+	+	-	-
reference species	*fiorii*, *loyolaea*, *nuragica*	*nivata*, *johannis*	*semicolorata*	*reatina*, *degrangei*, *hybrida*	*alpestris*	*diaphana*, *adrianae*
distribution	alpine piedmont lowland	piedmont lowland	alpine piedmont lowland	alpine piedmont lowland	alpine piedmont lowland	piedmont lowland

Table 3. Morpho-taxonomic groups for the Italian species of the genus *Baetis.*

Baetis	Morpho-taxonomic group				
	B.rhodani	*Baetis group A*			*'Baetis' group B*
bristles on the gill margin	+	-	-	-	-
two bristles on tarsal claws	-	-	+	+	-
middle tail << outer tails	-	+	+(-)	+	-
mandible outer tooth knife shaped	-	-	+	-	-
species	*rhodani*	*pavidus*	*melanonyx*	*cyrneus*, *alpinus*, *lutheri*, *vardarensis*	*buceratus*, *liebenauae*, *vernus*, *fuscatus*, *A.muticus*, *N.niger*, *N.digitatus*
distribution	alpine piedmont lowland	alpine piedmont lowland			(alpine) piedmont lowland

(MALZACHER, 1986; DOLEDEC, 1989). Score 3 is attributed to the majority of the OU. Taxa in this group do not show any peculiar indicator property of evident general interest (i.e. *Ephemerella, Baetis* gr. A, *Baetis* gr. B, etc.: LANDA, 1969; JANEVA, 1979; LEPS *et al.,* 1989; RUSSEV, 1979), the species present in Italy are insufficiently known (i.e. *Ecdyonurus* gr. *venosus:* BELFIORE, 1994; BUFFAGNI & BELFIORE, 1994; *Cloeon, Pseudocentroptilum:* BELFIORE & D'ANTONIO, 1991) or they are represented by rare or uncommon species (i.e. *Brachycercus, Thraulus, Ephoron, Potamanthus, Torleya:* BELFIORE, 1983, 1988). Score 5 is assigned to «pristine» OU**.** These selected detector taxa are supposed to occur in natural conditions and they disappear as a response to man-induced environmental changes (i.e. *Epeorus, Rhithrogena:* RUSSEV, 1979; BELFIORE, 1983; LEPS *et al.,* 1989; ALBA TERCEDOR *et al.,* 1991; *Ephemera:* USSEGLIO-POLATERA & BOURNAUD, 1989; *Caenis* group 5: SOWA, 1973; BUFFAGNI, 1996; *Habrophlebia:* LANDA, 1957; *Electrogena:* BELFIORE, 1995). The absence of each of these taxa could indicate a different habitat or water quality alteration.

Table 4. Morpho-taxonomic groups for the Italian species of the genus *Caenis.*

Caenis	Morpho-taxonomic group					
	C. group macrura	*C. group 3*				*C. group 5*
deep notch on posterior margin of IX sternite	+	-	-	-	-	-
light but evident notch on posterior margin of IX sternite	-	+	-	-(+)	+	+
gena well developed	-(+)	-(+)	-	-	-	+
bristles on posterior margin of fore femur and lateral spines of abdomen short and truncated	-	+	-	-	-	-
species	*luctuosa*, *macrura*, *martae*	*pusilla*	*robusta*, *lactea*	*horaria*	*pseudorivulorum*	*beskidensis*, *belfiorei*
distribution	piedmont lowland	piedmont lowland				(alpine) piedmont lowland

Metric calculation

For any collecting site, the scores for all the sampled OU are summed up to give a cumulative site score (Mayfly Total Score - MTS) that is then divided by the number of OU used in the calculation obtaining the Mayfly Average Score (MAS).

On the basis of species composition of all the studied mayfly communities, threshold values of the Mayfly Average Score and corresponding Integrity Classes of the mayfly community were intuitively identified (Table 6). Integrity is here intended as a measure of the naturalness of the mayfly community.

The obtained values can define classes of mayfly community Integrity (Table 6). Situations without human disturbance belong to Class I (MAS ≥3.5). Class II (3< MAS <3.5) corresponds to a nearly natural community or in some cases to a naturally simplified one (i.e. low discharge small alpine streams or springs). Class III (2.5< MAS ≤3), IV (MAS ≤2.5) and V (1 or 2 OU only) show a mayfly community from altered to very poor. Class VI is obtained when after a repeated sampling no mayfly nymphs are found. When MAS <2.5 there are no detector OU. If MAS <3, number of detector OU < number of exploiter OU. When MAS >3.5 detector OU are usually more numerous than score 3 OU. Examples of MAS application are reported in Table 7.

I assume that a mayfly community should be composed of at least three OU to belong to Integrity Class IV. The presence of two OU only is considered representative of heavily altered environments, high degree of pollution or not yet structured community. Furthermore, the value that could be obtained on the basis of two OU is supposed to be not significant, also for the possibility of collecting isolated score 5 OU not integrated in a stable population, and the sample is attributed to a V Class of integrity of the mayfly community. To avoid over-estimations of environmental quality with increasing MAS values but few OU present (i.e. taxa richness of the benthic community is lower than minimum expected) a minimum number of OU was fixed for each Integrity Class, on the basis of the composition of the studied mayfly communities from different Italian regions. Particular attention was addressed to define the minimum number of OU present in pristine environments. In the author's personal field experience the lowest number of OU in Italian natural environments without human disturbance (high mountain springs excluded) seems to be five OU (i.e. Tordino Stream, see Table 7).

Table 5. Score categories and Operational Units for Italian mayflies.

score	Operational Unit	
1	*Baetis rhodani*	
	Caenis gr. macrura	
3	*Acentrella*	*Torleya*
	Baetis gr. A	*Ecdyonurus*
	Baetis gr. B	*Choroterpes*
	Centroptilum	*Habroleptoides*
	Cloeon	*Paraleptophlebia*
	Procloeon	*Thraulus*
	Pseudocentroptilum	*Oligoneuriella*
	Brachycercus	*Ephoron*
	Caenis gr. 3	*Potamanthus*
	Ephemerella	
5	*Caenis gr. 5*	*Rhithrogena gr. C*
	Ephemera	*Rhithrogena gr. D*
	Epeorus	*Rhithrogena gr. E*
	Heptagenia	*Rhithrogena gr. F*
	Electrogena	*Habrophlebia*
	Rhithrogena gr. A	*Siphlonurus*
	Rhithrogena gr. B	

To evaluate the effectiveness of the threshold values proposed to separate the mayfly community Integrity Classes, reference biological quality classes were defined by integrating values of BMWPS, EBI and ASPT. Class 1 (pristine environment) is identified by the simultaneous presence of BMWPS≥130, EBI≥10 and ASPT≥6.5. Class 2 (nearly natural env.): 100≤BMWPS<130, EBI≥9 and ASPT≥6. Class 3 (fairly altered env.): 70<BMWPS<100, EBI≥8 and ASPT≥5.5. Class 4 (altered env.): 40≤BMWPS<70, EBI≥7 and ASPT≥4.5. Class 5 (heavily altered env.): BMWPS<40 or EB<7 or ASPT<4.5. If even only one of the indices values was under the fixed threshold the sample was assigned to the lower class.

The minimum MAS value observed in quality class 1 is 3.5 (see Table 8). In class 2 the minimum and the lower quartile values are 3.28, while the upper quartile is 3.33 and the maximum 3.57. The separation between Integrity Classes I and II can then be confirmed at 3.5 (included in Class I). Also the separation between Classes II and III can be maintained at 3, that is the upper quartile of the 3th class of integrated indices of biological quality. Its lower quartile is 2.42 and the upper quartile of class 4 is 3 so that the further separation can be supposed at 2.5 (included in Class IV). Mini-

Table 6. Classes of mayfly community integrity with score threshold values and attributes. In the first column the minimum Operational Unit number for each class is reported.

	Mayfly Average Score (MAS)	Mayfly community	Integrity Class	Attributes
at least 5 O.U.	≥ 3.5	natural	I	comparable to the best situations without human disturbance; many indicator taxa are present
at least 4 O.U.	3 < σ < 3.5	nearly natural (or low diversity env.)	II	somewhat below expectation, especially due to the loss of the indicator taxa
at least 3 O.U.	2.5 < σ ≤3	altered	III	notable loss of indicator taxa; fewer taxa
at least 3 0,U.	≤ 2.5	heavily altered	IV	dominated by habitat generalists no indicator taxa
1 or 2 O.U. only	–	very poor	V	a structured community does not exist; usually just tolerant taxa are found
no mayflies	–	absent	VI	repeated sampling finds no mayflies

Table 7. Examples of application of the Mayfly Average Score (MAS) class distinction for mayfly communities of different sites all over Italy. Sites are class aggregated. MAS values in brackets indicate no usable average score: class inclusion is forced by Operational Unit number.

Integrity Class I

Collecting site	Operational Units	
T.Curone	*Rhithrogena gr. C*	
28.05.1992	*Ecdyonurus*	
Olgiate M. (CO)	*Electrogena*	
250 m	*Ephemerella*	total score
	Torleya	25
	Baetis rhodani	MAS
	Habrophlebia	3.57
Collecting site	**Operational Units**	
T.Val Vigezzo	*Rhithrogena gr. A*	
1.01.1993	*Rhithrogena gr. C*	
S.Maria M. (NO)	*Rhithrogena gr. D*	
920 m	*Ecdyonurus*	total score
	Epeorus	27
	Baetis gr. A	MAS
	Baetis rhodani	3.85
Collecting site	**Operational Units**	
T.Trebbia	*Rhithrogena gr. A*	
19.06.1994	*Rhithrogena gr. C*	
Bobbio (PC)	*Ecdyonurus*	
350 m	*Ephemerella*	
	Siphlonurus	
	Baetis gr. B	
	Baetis rhodani	
	Acentrella	total score
	Habrophlebia	39
	Paraleptophlebia	MAS
	Potamanthus	3.54
Collecting site	**Operational Units**	
T.Pioverna	*Rhithrogena gr. C*	
26.03.1995	*Rhithrogena gr. D*	
Introbio (LC)	*Rhithrogena gr. E*	
980 m	*Ecdyonurus*	
	Baetis gr. A	total score
	Betis gr. B	28
	Baetis rhodani	MAS
	Habroleptoides	3.5
Collecting site	**Operational Units**	
F.Grande	*Rhithrogena gr. C*	
22.02.1992	*Ecdyonurus*	
Vittuone (MI)	*Ephemerella*	
50 m	*Baetis gr. B*	
	Baetis rhodani	total score
	Caenis gr. 5	28
	Habroleptoides	MAS
	Ephemera	3.5
Collecting site	**Operational Units**	
T.Tordino	*Ecdyonurus*	
14.10.1993	*Epeorus*	total score
Padula (TE)	*Baetis gr. A*	19
950 m	*Caenis gr. 5*	MAS
	Habroleptoides	3.8

Integrity Class II

Collecting site	Operational Units	
T:Brembo	*Rhithrogena gr. A*	
2.02.1992	*Ecdyonurus*	total score
Mezzoldo (BG)	*Epeorus*	17
1200 m	*Baetis gr. A*	MAS
	Baetis rhodani	3.4
Collecting site	**Operational Units**	
T.Frodolfo	*Rhithrogena gr. A*	total score
13.10.1992	*Rhithrogena gr. C*	16
S.Antonio (SO)	*Ecdyonurus*	MAS
1500 m	*Baetis gr. A*	[4]
	4 OU only	Class II
Collecting site	**Operational Units**	
T.Agogna	*Rhithrogena gr. C*	
10.11.1992	*Ecdyonurus*	
Gozzano (NO)	*Epeorus*	total score
300 m	*Ephemerella*	17
	Baetis rhodani	MAS
	Habroleptoides	3.4
Collecting site	**Operational Units**	
T.Flumendosa	*Electrogena*	
30.05.1985	*Ephemerella*	
Gadoni (NU)	*Procloeon*	
550 m	*Baetis rhodani*	total score
	Caenis gr. 1	23
	Habrophlebia	MAS
	Siphlonurus	3.28

Integrity Class III

Collecting site	Operational Units	
F.Po	*Heptagenia*	
6.10.1994	*Ephemerella*	
Mezzana C. (PV)	*Baetis gr. A*	
40 m	*Baetis gr. B*	total score
	Baetis rhodani	19
	Caenis gr. 1	MAS
	Caenis gr. 3	2.71
Collecting site	**Operational Units**	
T. Roasco	*Rhithrogena gr. C*	total score
13.10.1992	*Ecdyonurus*	12
Grosotto (SO)	*Baetis gr. A*	MAS
650 m	*Baetis rhodani*	3
Collecting site	**Operational Units**	
F.Isarco	*Rhithrogena gr. C*	total score
05.1976	*Ecdyonurus*	12
Vipiteno (TN)	*Baetis gr. A*	MAS
975 m	*Baetis rhodani*	3
Collecting site	**Operational Units**	
F.Ticino	*Ecdyonurus*	
11.02.1993	*Ephemerella*	total score
Boffalora T. (MI)	*Baetis gr. A*	13
100 m	*Betis gr. B*	MAS
	Baetis rhodani	2.6
Collecting site	**Operational Units**	total score
F.Crati	*Ecdyonurus*	11
17.01.1991	*Epeorus*	MAS
Cosenza (CN)	*Ephemerella*	[3,6]
250 m	3 OU only	Class III
Collecting site	**Operational Units**	
T.Mera	*Ecdyonurus*	total score
2.02.1992	*Epeorus*	12
Averara (BG)	*Betis gr. A*	MAS
680 m	*Baetis rhodani*	3

Integrity Class IV

Collecting site	Operational Units	
T.Poschiavino	*Ecdyonurus*	total score
13.10.1992	*Baetis gr. A*	10
Tirano (SO)	*Baetis gr. B*	MAS
425 m	*Baetis rhodani*	2.5
Collecting site	**Operational Units**	
T.Nervia	*Cloeon*	total score
8.04.1993	*Baetis rhodani*	5
Ventimiglia (IM)	*Caenis gr. 1*	MAS
25 m		1.66
Collecting site	**Operational Units**	
F:Adige	*Ecdyonurus*	total score
05.1976	*Ephemerella*	10
Peri (VR)	*Baetis gr. A*	MAS
155 m	*Baetis rhodani*	2.5
Collecting site	**Operational Units**	total score
F.Crati	*Ecdyonurus*	8
16.01.1991	*Baetis gr. A*	MAS
Cosenza (CS)	*Baetis rhodani*	2
250m	*Caenis gr. 1*	
Collecting site	**Operational Units**	
Naviglio Grande	*Ephemerella*	total score
14.06.1993	*Baetis gr. B*	7
Milano	*Baetis rhodani*	MAS
100 m		2.33

Integrity Class V

Collecting site	Operational Units	
T.Frodolfo	*Baetis rhodani*	total score
13.10.1992		1
Bormio (SO)		MAS
1170 m	1 OU only	[1]
Collecting site	**Operational Units**	
T.Valaperta	*Baetis gr. B*	total score
5.08.1993	*Baetis rhodani*	4
Valaperta (CO)	2 OU only	MAS
200 m		[2]
Collecting site	**Operational Units**	
F.Adige	*Cloeon*	total score
08.1976		3
Verona (VN)		MAS
59 m	1 OU only	[3]
Collecting site	**Operational Units**	
F:Adda	*Baetis rhodani*	total score
16.01.1991		1
Grosio (SO)		MAS
600 m	1 OU only	[1]

Table 8. Values of OU numbers, Mayfly Average Score (MAS) (corrected and uncorrected), Mayfly Total Score (MTS) and mayfly community Integrity Classes (corrected and uncorrected) in the reference biological quality classes defined by integrating values ofBMWPS, EBI and ASPT (class 1: pristine environment; class 5: heavily altered environment).

		Quality classes of integrated indices				
		class 1	class 2	class 3	class 4	class 5
MAS Operational Units	*average*	7.6	6.72	5.21	3.55	2.47
	st.deviation	0.89	0.46	1.76	1.75	1.13
	minimum	6	6	2	1	1
	maximum	8	7	9	7	5
	median	8	7	5	3	2
	lower quart.	8	6	4	2	2
	upper quart.	8	7	6	5	3
Mayfly Total Score (MTS)	*average*	27.2	22.54	14.64	9.11	5.33
	st.deviation	1.78	1.8	5.86	5.6	3.34
	minimum	24	20	4	1	1
	maximum	28	25	29	19	15
	median	28	23	14	9	4
	lower quart.	28	20	12	4	4
	upper quart.	28	23	17	12	7
Uncorrected MTS	*average*	27.2	22.54	14.92	10.05	5.66
	st.deviation	1.78	1.8	5.38	4.77	3.23
	minimum	24	20	8	1	1
	maximum	28	25	29	19	15
	median	28	23	14	9	5
	lower quart.	28	20	12	7	4
	upper quart.	28	23	17	12	7
Mayfly Average Score (MAS)	*average*	3.6	3.34	2.76	2.34	1.96
	st.deviation	0.22	0.11	0.42	0.63	0.53
	minimum	3.5	3.28	2	1	1
	maximum	4	3.57	3.3	3	3
	median	3.5	3.28	3	2.33	2
	lower quart.	3.5	3.28	2.42	2	2
	upper quart.	3.5	3.33	3	3	2.33
Uncorrected MAS	*average*	3.6	3.34	2.9	2.84	2.17
	st.deviation	0.22	0.11	0.47	0.73	0.65
	minimum	3.5	3.28	2	1	1
	maximum	4	3.57	4	4	4
	median	3.5	3.28	3	3	2
	lower quart.	3.5	3.28	2.66	2.33	2
	upper quart.	3.5	3.33	3	3	2.5
Mayfly community Integrity Class	*average*	1	1.81	3.21	3.94	4.54
	st.deviation	0	0.4	0.8	0.87	0.59
	minimum	1	1	2	3	3
	maximum	1	2	5	5	5
	median	1	2	3	4	5
	lower quart.	1	2	3	3	4
	upper quart.	1	2	4	5	5
Uncorrected Integrity Class	*average*	1	1.81	2.92	3	3.76
	st.deviation	0	0.4	0.82	1.02	0.57
	minimum	1	1	1	1	1
	maximum	1	2	4	4	4
	median	1	2	3	3	4
	lower quart.	1	2	3	3	4
	upper quart.	1	2	3	4	4
	sample size	5	11	14	18	47

mum and maximum values in classes 4 and 5 are the same and hereby it could be hardly suggested a separation between the correlative Integrity Classes on the basis of MAS values.

Values of standard biotic indices and of mayfly parameters in the different Integrity Classes are reported in Table 9. Relatively few quantitative differences are present between Class I and Class II, whilst more relevant differences of indices are evident between Class II and Class III. A gradual decrease of indices values, number of mayfly species, OU, MTS and MAS is evident since Class III to Class VI.

To verify if the imposed taxa minimum numbers are justified and lead to a better fitting of environmental quality, correlations between the applied standard biotic indices and the corrected (with minimum OU number fixed) or uncorrected (without minimum OU number) formulations of MAS and Integrity Classes were calculated (Table 10). In all the cases the adopted corrections determined a higher correlation with both standard indices and mayfly parameters (species number, OU number, Mayfly Total Score). High correlation coefficients were found between the different biotic indices and between these and MAS, Integrity Classes and MTS. Coefficients of 0.8986 and 0.8852 were found between the integrated indices quality classes and MTS and Integrity Classes respectively.

DISCUSSION AND CONCLUSIONS

Without significantly increasing the taxonomic identification effort, it is useful, in some cases, to aggregate similar sensitivity species at a «lower than genus» level. This is the case with the *Caenis* species. While the *macrura-group* species (score 1 in the present work) can tolerate organic pollution and can be favoured in altered environmental conditions, the group 5 taxa (score 5) indicate relatively good water and habitat quality. The score 3 has been assigned to the other species of the genus, those which show less evident indicator properties. The *macrura*-group OU is very easily recognizable through the presence of a deep incision on the IX sternite of the nymphs. With respect to some currently employed indices (i.e. E.B.I.), the discrimination between the score 5 and the score 3 *Caenis* OU seems to be the only additional effort of some importance needed by MAS application.

Nymphs of *Baetis rhodani* have been separated from those of the other *Baetis* species, as suggested by many authors (i.e. WOODIWISS, 1964; TONER, 1984) and they can be identified easily by the pointed short bristles on the gill margins.

In some stream types a single mayfly genus may be present locally with various species that could represent the dominant portion of the Ephemeropteran community (SOWA, 1975). Therefore, underestimations of community diversity, and thereby of habitat diversity and quality, could arise from a genus level identification of nymphs. In order to partially reduce this over-simplification, the *Baetis* species *(B. rhodani* excluded) are here assigned to two distinct Operational Units on the basis of differing ecological characteristics of the nymphs (MULLER-LIEBENAU, 1969). Group A includes species of fast-flowing areas, while group B those of slow-flowing areas. The species of the two recently revised Baetidae genera *Alainites* and *Nigrobaetis* (WALTZ *et al*., 1994) have been provisionally put in the *Baetis* group B. Their belonging to this group or to a separate one will be investigated further. A similar, but more detailed, ordination than of the *Baetis* species, has been carried out for the genus *Rhithrogena*. In this case, an additional purpose of the partitioning was to extend MAS applicability to alpine streams, often dominated by *Rhithrogena* species. One of the main limitations of many biotic indices is indeed the fact that some indicator taxa are present only in some stream typologies (ARMITAGE *et al*., 1990). For instance, Plecoptera nymphs, that play a significant role in detecting pollution in most bioassessment techniques applied in Europe, are usually confined to cool and well oxygenated waters (BRITTAIN, 1990), and are therefore naturally excluded in lowland rivers. Being detector OU, the *Rhithrogena* morpho-taxonomical groups compensate the natural absence of other detector OU of mayflies which are confined to lower altitudes and to warmer waters. On the other hand, detector OU like *Ephemera* and *Heptagenia* allow MAS application in lowland large rivers, while *Habrophlebia* and *Electrogena* can indicate

Table 9. Values of standard biotic indices and mayfly parameters in the Integrity Classes of the mayfly community (*fixed value).

		Mayfly community Integrity Classes					
		Class 1	Class II	Class III	Class IV	Class V	Class VI
Systematic Units for EBI	*average*	24.14	22.81	11.7	8.13	5.84	3.2
	st deviation	7.77	4.64	4.71	4.46	2.91	1.48
	minimum	18	16	4	3	2	1
	maximum	38	34	22	23	14	5
	median	21	22	11	7	5	3
	lower quart.	18	21	8	6	4	3
	upper quart.	31	24	15	9	7	4
BMWP Score	*average*	132.14	120.63	67.58	40.86	28.21	15.6
	st.deviation	40.13	23.62	21.28	25.91	17.48	16.24
	minimum	89	82	31	7	6	2
	maximum	210	178	98	119	73	37
	median	132	121	67	34	25	7
	lower quart.	104	107	53	25	18	3
	upper quart.	148	126	83	53	38	29
ASPT	*average*	6.88	6.29	6.6	5.03	4.42	4.33
	st.deviation	0.29	0.21	0.67	1.13	1.31	2.83
	minimum	6.5	5.63	5.25	2.33	2.3	1.5
	maximum	7.41	6.73	7.8	7	7.3	7.4
	median	6.8	6.29	6.57	5.16	4.19	3.5
	lower quart.	6.72	6.25	6.22	4.16	3.8	2
	upper quart.	7.06	6.36	6.99	5.87	5.12	7.25
EBI	*average*	10.71	10.18	8	6.65	5.87	3
	st.deviation	1.97	0.87	1.45	1.55	1.53	1.87
	minimum	9	9	5	4	4	1
	maximum	14	12	11	10	9	5
	median	10	10	8	7	5	2
	lower quart.	9	10	7	6	5	2
	upper quart.	13	11	9	8	7	5
Quality classes of integrated indices	*average*	1.28	2.18	3.64	4.52	4.75	5
	st.deviation	0.48	0.4	0.7	0.73	0.5	0
	minimum	1	2	3	3	3	5
	maximum	2	3	5	5	5	5
	median	1	2	4	5	5	5
	lower quart.	1	2	3	4	5	5
	upper quart.	2	2	4	5	5	5
Number of Mayfly species	*average*	7.85	7	5.23	3.95	1.81	no mayflies
	st.deviation	1.34	1.18	1.56	1.14	0.59	
	minimum	6	6	3	3	1	
	maximum	10	10	9	7	3	
	median	8	7	5	4	2	
	lower quart.	7	6	4	3	1	
	upper quart.	9	7	6	4	2	
MAS Operational Units	*average*	7.42	6.81	4.82	3.91	1.68	no mayflies
	st.deviation	0.78	0.87	1.18	1.08	0.47	
	minimum	6	6	3*	3*	1*	
	maximum	8	9	7	7	2*	
	median	8	7	4	4	2	
	lower quart.	7	6	4	3	1	
	upper quart.	8	7	6	4	2	
Mayfly Total Score (MTS)	*average*	26.57	22.45	14.11	8.95	3.06	no mayflies
	st.deviation	1.81	2.62	3.01	2.6	1.41	
	minimum	24	20	9	7	1	
	maximum	28	29	19	17	4	
	median	28	23	12	8	4	
	lower quart.	25	20	12	7	1	
	upper quart.	28	23	16	10	4	
Mayfly Average Score (MAS)	*average*	3.59	3.28	2.94	2.28	1 or 2 O.U. only	no mayflies
	st.deviation	0.18	0.02	0.12	0.16		
	minimum	3.5	3.22	2.66	2		
	maximum	4	3.33	3	2.5		
	median	3.5	3.28	3	2.33		
	lower quart.	3.5.	3.28	3	2.2		
	upper quart.	3.57	3.3	3	2.33		
	sample size	7	11	17	23	32	5

good habitat conditions in smaller streams. The role of detector OU has also been given to *Siphlonurus* nymphs because they colonise marginal clear pools whose presence along a stream often indicates natural channel morphology and high habitat diversity.

Establishing minimum OU numbers for each Integrity Class of the mayfly community allowed a better correlation between these classes, MAS values and the other calculated biological indices.

A significant high correlation of 0.8852 was found between Integrity Classes and the integrated BMWPS, EBI and ASPT quality classes. High correlation values were also found with BMWPS and EBI directly. The mayfly parameter that shows the best correlation coefficients with the other biotic indices is Mayfly Total Score (nearly 90% with integrated indices quality classes and about 83% with EBI Systematic Units). This seems to suggest that the information present in the structure of the mayfly community, even if very simply codified through OU definition and score attribution, can offer a representative synthesis of biological quality assessed by means of the whole benthic community. The values of the standard biotic indices observed in the Integrity Classes I and II of the mayfly community, however different, are close and correspond in most cases to high water quality standard classes (ARMITAGE *et al.*, 1983; GHETTI, 1986). This seems to denote the possibility that the studied mayfly parameters could reveal themselves especially useful in pointing out situations of particularly significant environmental quality.

Together the ordination of organisms according to their pollution tolerance, the total number of systematic units present in the sample, as a measure of species richness, is often the second parameter to calculate a biotic index (i.e. WOODIWISS, 1964; VERNEAUX & TUFFERY, 1967; VERNEAUX *et al.,* 1982; DE PAUW & VANHOOREN, 1983; GHETTI, 1986). The total number of taxa and the number of mayfly taxa are also included in the Invertebrate Community Index (OHIO EPA, 1987), in the Rapid Bioassessment Protocols II and III (PLAFKIN *et al.*, 1989) and in the Benthic Index of Biotic Integrity (KERANS & KARR, 1994) relative to benthic communities. The number of mayfly species and of OU in pristine Italian running water environments can vary greatly (see Table 5). This parameter is a function, other than of human-induced habitat alterations, of zoogeographic and ecological factors (FAUSCH *et al.*, 1984). Data on Italian mayfly ecology, zoogeography and distribution, even if rapidly increasing (BUFFAGNI & BELFIORE, 1994), are scarce (BELFIORE, 1994). The use of Mayfly Total Score, the better correlating mayfly parameter, to assess the biological quality of Italian streams is therefore not yet possible, since the observed high correlations are good for the geographical region in which the presented methodology has been till now tested. Further studies will try to evaluate their validity in different regions.

Table 10. Correlation coefficients (p<0.001) between the studied mayfly parameters and the standard biotic indices.

	SU EBI	BMWP	ASPT	EBI	QC int. indices	Mayfly species	MAS OU	MTS	MAS	Uncor. MAS	Integrity Class
Systematic Units for EBI	1.0000										
BMWP Score	0.9765	1.0000									
ASPT	0.5411	0.65551	1.0000								
EBI	0.9179	0.9321	0.7182	1.0000							
Quality class of integrated indices	-0.8908	-0.9144	-0.6162	-0.8890	1.0000						
Number of Mayfly species	0.8367	0.8339	0.5658	0.8241	-0.8274	1.0000					
Number of MAS Operational Units	0.8306	0.8403	0.5863	0.8268	-0.8417	0.9866	1.0000				
Mayfly Total Score (MTS)	0.8764	0.8896	0.6201	0.8459	-0.8986	0.9560	0.9680	1.0000			
Mayfly Average Score (MAS)	0.6744	0.7001	0.5890	0.7656	-0.7851	0.8340	0.8552	0.8557	1.0000		
Uncorrected MAS	0.5883	0.6185	0.5702	0.6982	-0.7005	0.6610	0.6715	0.7259	0.8518	1.0000	
Mayfly community Integrity Class	-0.8059	-0.8246	-0.5983	-0.8071	0.8852	-0.8947	-0.9146	-0.9499	-0.8967	-0.7133	1.0000
Uncorrected Integrity Class	-0.7124	-0.7263	-0.5354	-0.7466	0.8070	-0.6700	-0.6738	-0.7798	-0.7635	-0.9019	0.7646

A possibility of weighting all the OU present and MTS without the necessity of relating to an expected total number of OU is presented by the BMWPS approach (ARMITAGE *et al.*, 1983). A site score is obtained by summing up the individual scores, that reflect pollution tolerances, of all families present. This value is obviously influenced by the total number of families collected. A second step is then proposed: the computation of the Average Score Per Taxon (ASPT) by dividing the site score by the number of scoring taxa. The same procedure, frequently applied to the Chandler's score (METCALFE-SMITH, 1994), has been adopted to calculate the Mayfly Average Score (MAS). ASPT has been considered more informative than BMWPS (ARMITAGE *et al.*, 1983; FRAKE, 1988) and less affected by sample size, stream type and season (i.e. PINDER *et al.*, 1987) though related to an upstream-downstream gradient (ROSSARO & PIETRANGELO, 1993). This averaging calculation avoids having to define expected numbers of OU.

Extensive correlations between species presence and water or habitat quality are necessary for quantitative tolerance determinations (HILSENHOFF, 1987). In this paper a subjective approach to classifying organism tolerance has been adopted, but the acquisition of a national database is being carried out, with the aim of defining characteristic communities and OU tolerances on the basis of more precise and quantitative methods. This will allow the verification of the applicability of the Mayfly Average Score values to all the different Italian regions and stream types and to better define which environmental modifications MAS is able to detect. Possibly, after the validation of their relevancy, further definitions of new Operational Units and modifications of score attribution will take place.

Often used for other types of perturbation or pollution, most biotic indices should properly apply only to organic pollution assessment (METCALFE-SMITH, 1994). New metrics from all the aquatic organisms should be developed to allow a comprehensive biological integrity evaluation (KARR, 1991). These indices and metrics relative to single aquatic components could be useful, if integrated, to support a broader approach to biological monitoring and assessment (KARR, 1991).

The Mayfly Average Score could be a simple preliminary proposal for an extensive application of mayfly nymphs as indicators of the biological quality of streams.

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