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The Combination of a Biotic and a Chemical-Microbiological Index Shows the Environmental Risks of a high Quality Mediterranean Stream (Tellesimo; S.C.I. ITA090018; Sicily, Italy).

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ABSTRACT:Mediterranean river ecosystems have a highly endangered biodiversity;consequently, a monitoring activity is neededto identify the most impacted areas, the risk factors and to evaluate the efficacyof protection and restoring activities. This is particularly true for tributaries that are often overlooked by public agencies, while they are central elements for the protection of biodiversity. According to the Water Framework Directive (W.F.D.) this requires a multidisciplinary approach, linked to the study of the physicochemical, hydromorphological and biological characters of the water environments. In this study the results are reported of a monitoring activity in the Tellesimo Stream, a high quality Sicilian water course, wheretwo fish species included in the I.U.C.N. Red List as Critically Endangered (*Anguilla Anguilla)*and Near-Threatened (*Salmo cettii)* live. According to the WFD multidisciplinary approach, both a biological quality index (I.B.E.), using benthic macroinvertebrates as indicators, and a chemical-microbiological quality index (L.I.M.) were used. The two indexes gave different and sometimes contrasting results. This led to ascertaindifferent environmental risks for this stream: a seasonal (autumn) pollution, detected by I.B.E., probably due to the illegal discharge of olive mills waste water into the karst system, and a lighter but more chronicalteration, detected by L.I.M., probably due to theagricultural and/or small industrialactivities existingin the catchment area. These results highlight the need to use different methods to evaluate stream quality: in fact this allows the identification ofdifferent risk factors that sometimes can act in a very subtle or unseen way.

KEYWORDS:water quality, IBE index, LIM index, macroinvertebrates, WFD, multidisciplinary approach.

I. INTRODUCTION

Mediterranean river ecosystems, whose communities and functional processes are different from temperate rivers, have a highly endangered biodiversity, due to a long history of human disturbances. Feio et al.[1].Consequently, a widespread and constant monitoring of the status of the river waters is greatlyneeded, both to identify the most impacted areas, together with the environmental risk factors, and to evaluate the efficacyof protection and restoring activities.According to the Water Framework Directive (WFD) [2], European aquatic ecosystems need to be protected, their deterioration must be stopped and their status improved: thisrequires a multidisciplinary approach, linked to the study of the physicochemical, hydromorphological and biological characters of the different water environments.

The use of Biotic Indexes, and particularly of benthic macroinvertebrates, for the assessment of the quality of aquatic habitats is widely used in Europe. Metcalfe[3], Rolauffs et al. [4], Keçi et al.[5]. In Italy it is widely utilized the Indice Biotico Esteso (I.B.E.), Ghetti[6] [7], an Italian adaptation of the Trent Biotic Indexand of the subsequent Extended Biotic Index. Woodiwiss[8][9]. The I.B.E. has been used also in other countries, such as Nicaragua, Argentina and Brazil.Fenoglio et al. [10], Rodríguez Capítulo et al. [11], Mugnai et al. [12].

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Sicily, the bigger island in the Mediterraneansea, has an extensive network of wetlands and Sicilian inland waters face many different environmental impactssuch as: water shortages, pollution, degradation of channel morphology and vegetation, urbanization, impassable weirs, introduction of alien fish species and badangling management.Ferrito and Tigano[13], Duchi [14][15][16][17], Mazzola et al.[18].In Sicily the use of benthic macroinvertebrates and of the biological quality indexes for environmental assessment began some years ago (e.g.: Duchi[14], Rizzo and Migliore[19],Duchi[15],Bella et al.[20]) but it does not cover all water courses. Moreover systematic and appropriate biological monitoring plans do not include all the watercourses.This is particularly true for tributaries that are often overlooked by public agencies monitoring activities, although they are central elements for the protection of biodiversity. Clarke et al.[21].

In South-Eastern Sicily, an area with limestone geology with karst topography, there are a number of rivers that, from the top of the Hyblean Plateau, flow to the Strait of Sicily and the Ionian Sea in deep valleys and narrow gorges, locally known as "cave". Privitera and Quercio[22]. One of these is the Tellesimo Stream, which flows in the homonymous valley: one of the area's biodiversity hotspots, designated asSite of Community Importance (S.C.I. ITA090018).AA.VV. [23].Particularly important are the quality of its water and the integrity of its riverbed, also for the maintenance of a fish fauna of significant interestlike: the near threatened trout *Salmo cettii*, the critically endangered European eel (*Anguilla anguilla*) and the freshwater blenny (*Salaria fluviatilis*), which is included in the Bern convention. Duchi [24][14][25]. In relation to that, the Tellesimo Stream was definitely one of the first water courses in Sicily to be investigated by the use of macroinvertebrates as bioindicators. This monitoring was not done by public agencies, but in the context of an activity for the conservation of the *Salmo cettii* trout population. Duchi [14]. However, in 1991the monitoring was carried out only in a single season (spring). But in 1998 an autumn monitoring was done byDuchi and Occhipinti[26]. In both cases the values of the Biotic Indexes indicated a high quality of the water course (Tab. I).

Tab. I – Results of the first biological monitoring activities in the Tellesimo Stream.

YEAR	SITE	QUALITY INDEX	VALUE	QUALITY CLASS
1991 (spring)	Scala Vacca	E.B.I. (Ghetti, 1986)	11	I
	Urvo Emanuele		10	I
	Pizzo Taverna		11-10	I
1998 (autumn)	Ponte Cipollone	I.B.E. (Ghetti, 1997)	11	I
	Pizzo Taverna		10	I

This study had the aim to check the persistence of such high quality and to implement the monitoring activities according to the WFDapproach, as part of the investigation for the Icthyological Master Plan of the Province of Ragusa (Duchi[16]) with an integrated approach: both through the use of a biological quality index (I.B.E.) and through a chemical and microbiological quality index (L.I.M.). In fact, although the presence of direct discharges in the water course was never observed, it was hypothesized the abusive inletof a source of pollution since there had been reports, done by the volunteer fishing guards of the localgroup of the Italian Federation of Sport Angling (F.I.P.S.A.S.-Ragusa), of signs of probable sources of alteration, such as foams.

II. STUDY AREA - MATERIAL AND METHODS

The Tellesimo Stream, right tributary of the Tellaro River, flows in NE-SW direction for about 15 km in a narrow and deep valley; the riparian vegetation is a wood of*Platanus orientalis* and*Salix sp.*, which is altered in some stretches, where sparse *Salix sp.* trees, dense *Rubus sp.* and cane thicket can be found; the catchment's landscape is a mosaic of *Quercus ilex* wood, reafforestation areas with *Pinus sp.*, garrigue, Mediterranean bush and cultivated land with olive,

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almond and carob trees, wheat, corn and pasture. In this study the quality of the stream was investigated with the use of benthic macroinvertebrates, and also by chemical-microbiological analyses, as required by Italian Law Decree n. 152/1999. The surveys were conducted atfour sites: one near the sources of the stream (Scala Vacca), two in the intermediate section (Urvo Emanuele and Ponte Cipollone), one in the final stretch, just upstream of the confluence with the Tellaro River (Pizzo Taverna). The survey periods were two: Autumn (16-26.11.2004) and late Spring/early Summer (25.05-24.06.2005).Tab. IIshows the main characteristics of the sites in the two sampling periods.

Tab. II. Main characteristics of the sampling sites in the Tellesimo Stream. Data of samples collected in spring are given in italic when different from autumn data.

	SCALA VACCA	URVO EMANUELE	PONTE CIPOLLONE	PIZZO TAVERNA
Latitude	36°56'56" N	36° 55' 45" N	36° 55' 08" N	36°53' 58" N
Longitude	14°51' 03" E	14° 53' 23" E	14° 54' 41" E	14°56'06" E
Altitude (m.a.s.l.)	350	200	184	130
Distancefrom the source (km)	2	8	10,5	15
Rock (%)	0//*10*	0//*20*	0//*15*	0
Boulders (%)	5	20//*30*	5//*10*	5
Cobbles (%)	15//*25*	30//*15*	10	20//*10*
Gravel (%)	25//*30*	25//*10*	25//*20*	15//*20*
Sand (%)	35//*25*	20	40//*20*	25//*40*
Silt (%)	20//*5*	5	20//*25*	35//*15*
Artificial structures	no	no	no	no
Litterretention	high	high	high	high//*moderate*
Anaerobiosisin the bottom	no	no	no	perceivable, localized//*no*
Filamentous bacteria	no	no	no	No
Wetted width (m)	2,7//*2,5*	2,2//*3*	2,3//*1,8*	0,8//*1*
River channel width (m)	7	6,5//*9*	5,5	11
Average depth (cm)	12//*11*	16//*8*	17//*8*	6//*13*
Maximum depth (cm)	15//*20*	22//*15*	22//*12*	10//*25*

Benthic macroinvertebrates where monitored according to the methods by Ghetti [7]. For each sampling station they were collected using a kick net along transects covering the entire channel width, to allow the sampling ofall the different microhabitats in the stream bottom. The sorting of the invertebrates and their identification were carried out at the sampling site, to give a judgment of quality in the field, which enables to verify doubts and in casecarry

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outdeeper investigations. A significant sample of individuals was maintained in 70% alcohol in order to confirm in the laboratory the determination made in the field, through the use of a binocular stereo microscope (5-56X). The animals were determined by means of the identification keys by: Sansoni [27], Tachet et al.[28]and Campaioli et al.[29].

The I.B.E. values were then calculated according to the use of a table with two entries: one horizontally with the taxonmost sensitive to pollution and one vertically with the total number of taxa observed. This leads to values that vary basically from 1 (strongly altered environments) to 12 (high quality environments). The I.B.E. values are then grouped into 5 quality classes, from I (clean environment) to V (heavily polluted or altered environment).

In addition to I.B.E. another index was calculated: the L.I.M., based on a series of chemical and microbiological parameters, which have specific scores (Tab. III).

Tab. III. Parameters and scores used for the calculation of the L.I.M. Index

Parameter	Level 1	Level 2	Level 3	Level 4	Level 5
100-Dissolved O_2 (% saturation)	≤ 10	≤ 20	≤ 30	≤ 50	> 50
BOD_5 (O_2 mg/L)	< 2,5	≤4	≤8	≤ 15	> 15
COD (O_2 mg/L)	<5	≤ 10	≤ 15	≤ 25	> 25
NH_4 (mg/L)	< 0,03	≤ 0,10	≤ 0,50	≤ 1,50	> 1,50
NO_3 (mg/L)	< 0,3	≤ 1,5	≤ 5,0	≤ 10,0	> 10,0
Total P (mg/L)	< 0,07	≤ 0,15	≤ 0,30	≤ 0,60	> 0,60
Escherichia coli (CFU/100 mL)	< 100	≤ 1000	≤ 5000	≤ 20.000	> 20.000
Scores	80	40	20	10	5
L.I.M. values	480-560	240-475	120-235	60-115	< 60

L.I.M. values can vary from a minimum of < 60 to a maximum of 560 and are grouped in 5 levels, level 1 beeing the best.

Chemical/microbiological analyses were performed on water samples collected on the same sites and at the same time of the investigation of benthic macroinvertebrates. The water samples were transported to the laboratory in sterilecontainers held in a coolbox.The laboratory analyses were carried out according to standard methods provided by the Italian Decree 152/99. The chemical analyses were carried out by A.R.P.A.-D.A.P.Ragusa, the microbiological analyses by the Laboratory of Public Health of the A.S.P.7 of Ragusa.

The water temperature and conductivity were measured on site using the portable electronic probe Hanna Instruments HI 98311.

III. RESULTS

Tab.IV shows the results with regard to the quality monitoring by benthic macroinvertebrates.

Tab. IV. Values of the I.B.E. quality index.

Sampling Site	AUTUMN	Scala Vacca	Urvu Emanuele	Ponte Cipollone	Pizzo Taverna	SPRING-SUMMER	Scala Vacca	Urvu Emanuele	Ponte Cipollone	Pizzo Taverna
I.B.E.		12	4	10-9	5		11	10	10	10-9
Quality Class		I	IV	I-II	IV		I	I	I	I-II

The I.B.E. showed, in some areas of the stream, a heavy fall of water quality in the autumn, with a recovery, at times limited, in the period late spring/early summer. In fact, the middle and lower sections of the river did not achieve the high quality values typical of this stream.

Tab. V reports the chemical-microbiological data.

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Tab. V. Chemical and microbiological data at the sampling stations of the Tellesimo Stream.

	pH	Temperature	Conductivity	NO_3	NH_4	P	DixolvedO_2	O_2(saturation)	BOD_5	COD	*Escherichia coli*
		°C	µS/cm	mg/L	mg/L	mg/L	mg/L	%	mg/L	mg/L	u.f.c./ 100 cc
AUTUMN			□								
Scala Vacca	7,31	15,2	705	0,95	0	0	8,91	87	2,11	8,5	-
Urvo Emanuele	7,64	16,1	680	0,75	0,07	0,06	8,86	88	4,16	9,5	98
Monte Cipollone	7,45	17,7	607	0,18	0	0	9,52	99	4,71	9,8	18
Pizzo Taverna	7,38	15,4	569	0,8	0	0	10,4	102	6,3	10,4	240
SPRING-SUMMER											
Scala Vacca	7,45	14,3	636	2,1	0	0	8,9	85	9,8	14,6	-
Urvo Emanuele	7,51	17	568	0,81	0	0	8,7	93	8,2	14,6	200
Monte Cipollone	7,58	18,4	560	0,74	0	0	8,9	95	8,2	16	500
Pizzo Taverna	7,8	18,1	566	0,81	0	0,13	8,6	90	9,4	16,8	0

Tab.VI shows the scores, the values of the L.I.M. and the quality level of the different sampling sites. With regard to the site Scala Vacca, for which the data of *Escherichia coli* were missing, the minimum and maximum scores were inserted: in both cases, while changing the score the quality leveldid not change.

Tab. VI. Scores assigned, L.I.M. Index values and quality level atthe sampling stations of the Tellesimo Stream.

	100-Dissolved O_2 (% saturation)	BOD_5	COD	NH_4	NO_3	P	*Escherichia coli*	L.I.M	Quality Level
AUTUMN									
Scala Vacca	40	80	40	80	40	80	5-80	365-440	2
Urvo Emanuele	40	40	40	40	40	80	80	360	2
Monte Cipollone	80	20	40	80	80	80	80	460	2
Pizzo Taverna	80	20	40	80	40	80	40	380	2
SPRING-SUMMER									
Scala Vacca	40	10	20	80	20	80	5-80	255-330	2
Urvo Emanuele	80	20	20	80	40	80	40	360	2
Monte Cipollone	80	20	10	80	40	80	40	350	2
Pizzo Taverna	80	10	10	80	40	40	80	340	2

The chemical and microbiological investigations, unlike those relating to macrobenthic fauna, did not giveany highly negative results, but auniform class quality for all sites for both seasons. Looking at the scores it can be noted on the

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other hand a certain tendency to the deterioration in a seasonal opposite direction to that indicated by macrobenthic fauna, in particular with regard tothe BOD_5, COD and Nitric nitrogen in Spring.

IV. DISCUSSION AND CONCLUSIONS

The monitoring activities showed a diversity of information provided by the two indices, I.B.E. and L.I.M. In fact, the I.B.E.revealeda significant drop in quality in the middle and lower stretches of the stream in autumn; after that the macrobenthic community showed evidence ofa recovery in late spring/earlysummer (6-7 months later), never reaching the high values typical for this river. In addition, in the more downstream site (Pizzo Taverna) a first I.B.E. class was not reached. This photograph was not reflected by that produced by the L.I.M. index which on the whole showed a more uniform evaluation, with a slightly worse response for the higher quality sites identified by I.B.E. and a considerably better quality for the worse quality sites according to the I.B.E., with a general trend, from the seasonal point of view, in a direction opposite to that detected by benthic macroinvertebrates.

The investigations carried out and the comparison between the two indices let believe that there has been a kind of 'point' and/or seasonal pollution in the water course, which notoriously is not always detected by chemical analysis, which must take place when the pollution is still present, while the macrobenthos keeps the traces of alteration for longer times, thus allowing to locate sources of alteration happened prior to the date of sampling. In fact one of the main advantages of the quality evaluation through biotic indices, compared to the chemical investigations, is the ability to identify occasional or even momentary pollution sources. Moss[30].

It is therefore highly likely that the alteration is due to the presence of 'point' and non-constant pollution discharges, in the autumn period. Given the geographical position of the watercourse (flowing in a valley often narrow and steep and quite far from towns) it has then been hypothesized the presence of pollutants arrived to the stream through a discharge into the groundwater. The area where the Tellesimo valley is located is within the olive oil producing area called I.G.P. Monti Iblei. Anna Catania[31], Bracco et al.[32]. Given the time (autumn), the heavy pollution and the presence of several olive mills active in the area in that period, it is highlylikely that the origin of this alteration was due to the discharge of olive oil mill wastewaters, whose heavy bad effect on macrobenthic communities has been already highlighted in other areas of Southern Italy. Mancini et al.[33]. This discharge probably would have occurred directly and illegally into the groundwater, so it could have reached the stream through the karst system.

On the other hand the chemical and bacteriological investigations showed the presence of stress factors for the water course, probably to be linked with agricultural and/or small industrialactivities in the stream catchment, which probably instill in the system a chronic source of alteration that, however, did not significantly alter the macrobenthic community.

The investigations carried outshow how important it is to include in the institutional quality monitoring also streams considered (wrongly) less important but actually of great natural and scientific interest. It was also shown that the alteration sources are sometimes present in a subtle or unseen way: in this case not through a direct discharge into the stream but through a probable discharge into the groundwater, a fact highly impactful given the karst nature of the territory. This highlights on the one hand the importance of working togetherwith volunteers who control the territory (in this case the fishing guards of F.I.P.S.A.S.) and on the other hand the importance of using different complementary evaluation systems.In this study a biological and chemical-microbiological index were used, but other indexes, such as the Fluvial Functioning Index (F.F.I.) Negri et al.[34], could be used in the future in the same stream to evaluate the overall naturalness of the river channel.

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