

A COMPARISON BETWEEN BIOMONITORING METHODS FOR THE ANALYSIS OF MACROBENTHIC INVERTEBRATE COMMUNITIES IN DIFFERENT RIVER TYPES OF FRIULI VENEZIA GIULIA

Marco BERTOLI, Marzia AZZONI & Elisabetta PIZZUL

Department of Life Sciences, University of Trieste, I-34127 Trieste, I-34127 Trieste, Via L. Giorgieri 10, Italy
E-mail: pizzul@units.it

ABSTRACT

According to the Framework Water Directive, 2000/60/EC, macrobenthic invertebrates are very important as bioindicators for the definition of the Ecological Status of lotic systems. In Italy, different collection methods are requested for the application of biotic indices in wadeable and non-wadeable rivers: a proportionally distributed multi-habitat sampling (MH) and the use of artificial substrates (AS), respectively. This work was performed to compare the results obtained with both methods in three different lotic environments: an alpine stream, a high plain river and a spring fed channel. Data obtained from the application of the two different techniques have led to a good overlapping of results for all analysed watercourses, even though some macrobenthic invertebrate taxa showed selectivity in the artificial substrate colonization. No significant differences were found even among the metrics of the STAR_ICMi index, provided by the D.M. 260/2010 for the assessment of the watercourses ecological status in Italy.

Key words: macrobenthic invertebrates, freshwater ecosystems, North-east Italy, multi-habitat sampling, artificial substrates

DIFFERENTI STRATEGIE DI MONITORAGGIO PER LO STUDIO DELLE COMUNITÀ MACROZOOBENTONICHE IN DIVERSE TIPOLOGIE FLUVIALI DEL FRIULI VENEZIA GIULIA

SINTESI

I macroinvertebrati bentonici sono bioindicatori di fondamentale importanza nella definizione dello stato ecologico delle acque lotiche, ai sensi della Direttiva 2000/60/CE. In territorio italiano gli indici biotici, previsti dal D.M. 260/2010, riportano metodiche di monitoraggio diverse per i fiumi guadabili e per quelli non guadabili, prevedendo nel primo caso un campionamento multi-habitat proporzionale (MH) e nel secondo caso l'impiego di substrati artificiali (SA). Per questo studio, è stato ritenuto interessante effettuare un confronto tra tali metodiche di raccolta del macrozoobenthos, utilizzando entrambe in tre diverse tipologie fluviali: un corso d'acqua montano, uno di pianura ed uno di risorgiva. I dati ottenuti dopo l'applicazione delle due tecniche di campionamento hanno portato a risultati concordanti in tutte le tipologie fluviali analizzate, benché sia stata osservata una certa selettività da parte di alcuni taxa nella colonizzazione dei substrati artificiali. Inoltre, non sono state rilevate differenze significative tra i valori delle metriche che compongono l'indice STAR_ICMi, previsto dal D.M. 260/2010, portando a concludere che le due metodiche conducono a risultati ampiamente confrontabili tra loro.

Parole chiave: macroinvertebrati bentonici, ecosistemi d'acqua dolce, Nord Est Italia, campionamento multi-habitat proporzionale, campionamento con substrati artificiali

INTRODUCTION

The wadeable rivers can be crossed easily and all riverbed parts and micro-habitats are easily reachable in all seasons, except during periods of high-water and flooding. In these cases, the sampling protocol used for macrobenthic invertebrates collection is based on a multi-habitat procedure, originally proposed in the United States for the "Rapid Bio-assessment Protocol" (Barbour *et al.*, 1999) and successively adopted to develop methods which can fit the requirements of the Water Framework Directive 2000/60/EC (Directive 2000/60/EC). The basic principles of this method were tested during the European AQEM project (Integrated Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macro-invertebrates) (Buffagni *et al.*, 2001; Hering *et al.*, 2004) and the multi-habitat technique was then applied during the STAR project (STANDARDisation of River Classifications). The principal rule foresees the collection of samples in proportion to the number of different micro-habitats observed in a river (both biotic and abiotic substrates) whose presence must be previously determined. Three different monitoring types (surveillance, operational and investigative) are indicated by the Water Framework Directive 2000/60/EC, depending of the desired information level and requiring both a different number of sub-samples and a different taxonomical identification level for the collected taxa (Buffagni & Erba, 2007). In the wadeable watercourses, the Surber

net is the appropriate sampler for the multi-habitat procedure. However, there are some river types where a representative sample cannot be collected due to different reasons such as high water depth, elevated current speed, dispersal of microhabitats over wide areas, different riverbank types (Buffagni *et al.*, 2007). In Italy, the D.M. 260/2010 recommends the protocol of Buffagni *et al.* (2007) to collect macrobenthic invertebrates in non-wadeable watercourses, using Hester-Dendy modified hardboard artificial substrates (AS) (Cairns & Dickson, 1971; Battezzato, 1994; Buffagni *et al.*, 2000). This method can be applied to different watercourse types, as well as big rivers, spring fed watercourses, channels and brooks with sharply sloped banks and/or high water depth. This study investigates the macrobenthic invertebrate communities of three different lotic environments using both multi-habitat and artificial substrates sampling techniques.

Our aim was to verify if the monitoring performed with artificial substrates could give results comparable with those obtained from a multi-habitat approach, even though the AS are applied in a single micro-habitat.

MATERIAL AND METHODS

Study area

Three different watercourse types were monitored: a mountain stream, a high plain river and a spring fed channel (Fig. 1). The sampling sites were chosen con-

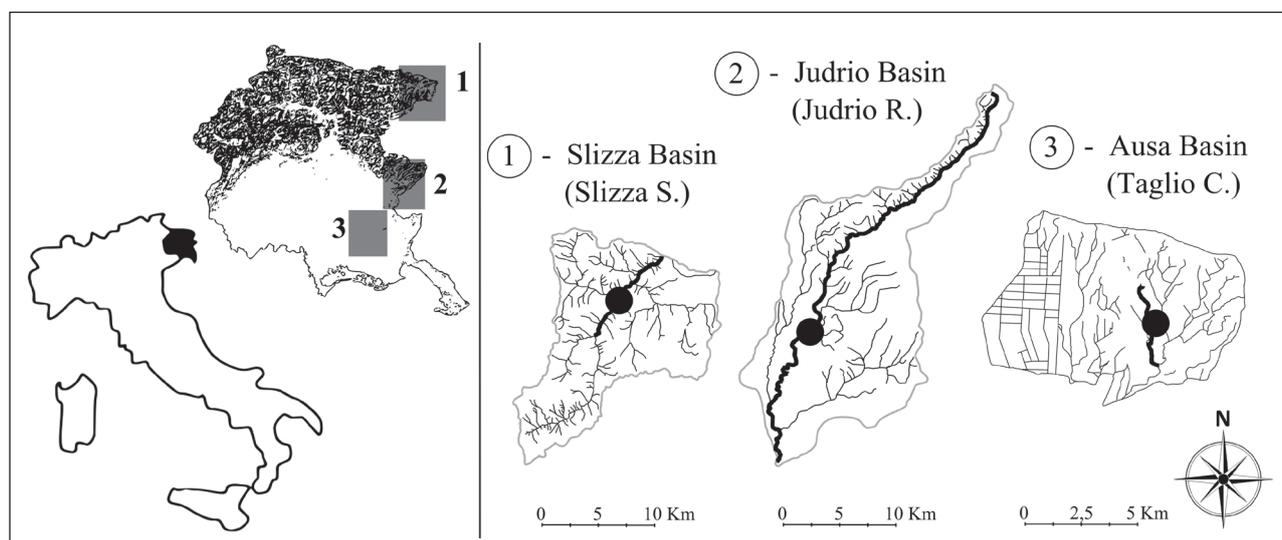


Fig. 1: Study areas and basins including the monitored sites placed in three different watercourses types (UTM coordinates): site 1: N 33T 5149448.84 – E 391576.81; site 2: N 33T 5092992.04 – E 380051.86; site 3: N 33T 5078066.48 – E 370729.18) (www.regione.fvg.it, modified).

Sl. 1: Območje raziskovanja, vključno z lokalitetami v treh različnih vodnih telesih (UTM koordinate): lokaliteta 1: N 33T 5149448,84 – E 391576,81; lokaliteta 2: N 33T 5092992,04 – E 380051,86; lokaliteta 3: N 33T 5078066,48 – E 370729,18) (www.regione.fvg.it, prirejeno)

sidering the possibility to apply both methods (multi-habitat sampling and artificial substrates) in relation to riverbed characteristics, especially water depth and flow velocities. In the mountain area, we have chosen the Slizza stream whose basin is located in the most north-eastern part of Friuli Venezia Giulia and it is included in the Danube basin. The Slizza stream originates from the confluence of two creeks (Rio del Lago Inferiore from the Lake Predil and the Rio Freddo) and flows in a Northeast direction, first crossing the Italian town of Tarvisio then entering Austrian territory, until it flows into the Gail River. This stream exhibits a torrential regime, high current velocity, and great flow rate variation, with strong flood events followed by marked low water levels. At the site, during sampling operations, the mean width of stream bed was 9.0 m and the bottom substrate was mainly constituted by rocks and boulders, even though gravel and pebbles were observed.

In the plains area, the second study site was placed on the Judrio River, included in the Isonzo Basin. It originates from the springs of the Colvarat Mount and flows with a torrential regime near the boundaries between Italy and Slovenia. The Judrio River then runs along the hills near the eastern portion of Friuli Venezia Giulia and finally along the lowland with wide meanders, until it flows into the Torre Stream (Autorità di Bacino, 2010). The mean width of the stream bed was 12.0 m and the bottom substrate mainly consisted of gravel and pebbles. Coarse particulate organic matter was also observed. The last sampling site was placed in the Taglio Channel, a spring fed watercourse included in the Ausa Basin. The mean width was 10.0 m and the bottom substrate was mainly covered by vegetation (submerged macrophytes) or consisted of fine gravel.

Sampling design

At each site, two sampling campaigns were conducted, using both multihabitat protocol (MH) and artificial substrates (AS). Collection activities were performed seasonally during the late spring (from June to the beginning of July) and the autumn (from November to December) of the year 2012.

The MH samplings were carried out following an operational protocol using appropriate Surber nets for the specific monitored Hydro-ecoregions (HER) (Buffagni & Erba, 2007), covering 1 m² sampling area for the Slizza site, placed in the HER 02 (Calcareous southern Alps and Dolomites) and 0.5 m² for the Judrio and Taglio sites, placed in the HER 06 (Po Plain). A 50 m length stream longitudinal section, representative of each monitored watercourse, was generally considered for each sampling site. Sorting operations and taxonomical identification were performed mainly on field, but some individuals belonging to the orders Diptera and Plecoptera and to the class Oligochaeta were later identified in the laboratory due to the small size of the organisms.

Taxonomical identification was conducted to the lowest possible level, and at least to the family level, for the application of the STAR_ICMi index (Buffagni *et al.*, 2008). The sampling operations with AS were conducted using Hester-Dendy substrates (Buffagni *et al.*, 2007). According to the sampling protocols indicated for non-wadeable streams, the AS were built with ten hardboard plates separated by rubber rings and groups of five AS were chosen as sample unit, with a total area of 0.5 m². Two sampling units were placed in the monitored watercourses along non-wadeable sections, and suspended at 0.5-1.5 m water depth by ropes secured to trees or to artificial structures and secured to the bottom using bricks. The AS were collected after 30 days of submersions: the plates were cleaned from organisms and other material and the resulting samples were sieved with a 500 µm sieve. Sorting and taxonomical identification were conducted as for the MH samples, which were collected during the same day.

In addition, values of chemical and physical water parameters (temperature, °C; pH; conductivity, µS cm⁻¹; dissolved oxygen concentration, mg l⁻¹) were monthly registered with field meters (Hanna Instruments, Padova, Italia) and the water depth (cm) was also monitored using a graduated rod. During the sampling operations, substrate composition and vegetation bottom cover were registered, as requested for the application of the MH protocol (Buffagni & Erba, 2007).

Data analysis

All ecological data were initially processed using Microsoft Excel 2007. A graph describing the macrobenthic invertebrate community structure was produced with the same software. The Sørensen index (Sørensen, 1948) was used for comparing the two sampling methods. Calculations were made considering the family taxonomical level, as usually considered for the application of the STAR_ICMi index (Buffagni *et al.*, 2008). In addition, the non-parametric Multi-Dimensional Scaling (N-MDS) and the one-way PERMANOVA (999 permutations) (Anderson, 2001), both based on the Bray-Curtis similarity matrix, were performed to assess differences between the two methods, using density data (ind m⁻²) of all families collected with MH and AS techniques. The STAR_ICMi metrics were used as community descriptors because they provide information based on taxa tolerance (ASPT), organisms abundance (Log₁₀(Sel_EPTD+1) and 1-GOLD) and biodiversity (Total Families Number, EPT Families Number and Shannon-Wiener index). Differences among metrics values calculated both from the MH and AS datasets were investigated with the non-parametric Wilcoxon paired-sample test. All data were previously log(x+1) transformed, and normality of datasets was assessed using the Kolmogorov-Smirnov test with software STATISTICA 7.1. The same software was used for the Wilcoxon paired-sample test, while N-MDS and

Tab. 1: Seasonal mean values and standard deviations (\pm S.D.) of chemical and physical water parameters measured during the monitoring period.

Tab. 1: Povprečne vrednosti po sezonah in standardna deviacija (\pm S.D.) kemijskih in fizikalnih parametrov, izmerjenih v obdobju monitoringa

		Depth (cm)	Dissolved oxygen (mg l ⁻¹)	Temperature (°C)	pH	Conductivity (µS cm ⁻¹)
Slizza S.	Spring	42.48 \pm 1.55	9.69 \pm 0.22	9.53 \pm 0.59	8.52 \pm 0.37	339.67 \pm 64.59
	Autumn	54.71 \pm 10.77	9.92 \pm 0.29	7.48 \pm 2.14	8.57 \pm 0.22	256.75 \pm 96.15
Judrio R.	Spring	55.57 \pm 6.98	7.61 \pm 0.22	21.97 \pm 0.75	8.19 \pm 0.27	462.00 \pm 51.97
	Autumn	69.82 \pm 20.64	9.31 \pm 1.02	11.85 \pm 5.19	8.33 \pm 0.16	542.25 \pm 130.93
Taglio C.	Spring	101.04 \pm 4.97	6.60 \pm 0.60	16.20 \pm 2.10	7.80 \pm 0.13	652.00 \pm 61.73
	Autumn	103.67 \pm 2.06	8.91 \pm 1.19	14.75 \pm 1.71	8.08 \pm 0.31	597.00 \pm 50.00

one-way PERMANOVA were performed using the PAST 3 application (Hammer et al., 2001).

RESULTS

Seasonal mean values of main chemical and physical parameters for all sampling sites are given in Table 1. The full list of the collected taxa is given in Table 2.

Macroinvertebrates community structure observed both with MH and AS sampling techniques during the two monitoring seasons are shown in Figure 2. With each sampling technique in the Slizza spring 19 taxa were collected. Nearly all (96 %) collected organisms were assigned to the class Hexapoda, but the order Ephemeroptera was the more abundant taxon in the MH

samples while in the AS samples the Diptera Chironomidae were dominant. Unfortunately, the autumn AS sample was lost due to a strong flood event so that the comparison between methods was precluded for those seasonal samples. The low number of organisms (179 individuals, belonging to the Ephemeroptera order) collected in the MH fall samples was probably a consequence of this flood event.

The Judrio River community showed a higher biodiversity (number of taxa) than the Slizza spring (Fig. 2). Using the MH sampling allowed collection of 27 taxa while in the AS 22 taxa were identified. The MH samples were dominated by Hexapoda, especially by Diptera Chironomidae in spring and Ephemeroptera Caenidae (genus *Caenis*) in autumn. In this latter season *Caenis* was the most abundant taxon also in the AS samples, but in spring the class Ostracoda was dominant. Finally, 41 taxa were identified in the MH spring samples and 19 in the autumnal samples collected at the Taglio site: Oligochaeta Lumbriculidae and Crustacea Asellidae were the most abundant taxa in spring and in autumn respectively. Asellidae was also the dominant among 34 taxa in the AS spring samples while Trichoptera Hydropsychidae showed the highest abundance in the AS autumnal samples, when 37 taxa were identified.

The application of the Sørensen index provided values ranging between 0.72 and 0.97, indicating a comparable community composition observed in samples collected using the two sampling techniques. The N-MDS applied on the two biotic datasets, obtained from the densities of all the observed families, also showed a good comparability of the two methods (Fig. 3) hence appearing consistent with the results of the Sørensen index. In addition, the one-way PERMANOVA did not show significant differences between communities due to the sampling method ($F = 0.714, p > 0.05$). Finally, also the STAR_ICMi metrics values calculated from the MH and AS biotic datasets did not show significant differences between the two sampling approaches (Wil-

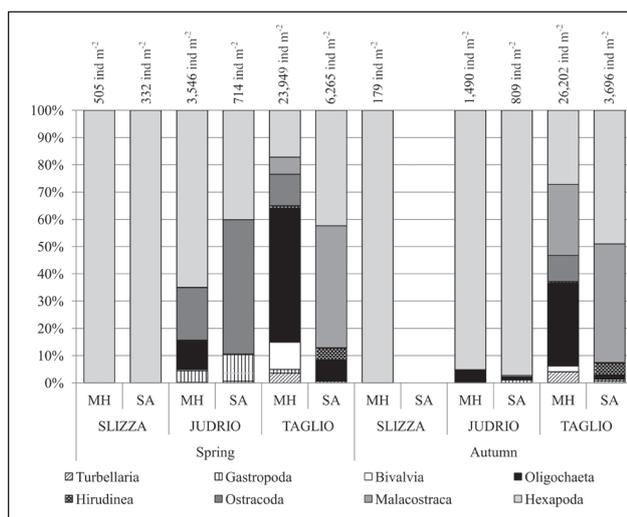


Fig. 2: Occurrences of the main taxa observed in the MH and AS samples during the two monitoring seasons. Sl. 2: Pojavljanje glavnih taksonov v vzorcih MH in AS v dveh spremljanih sezonah

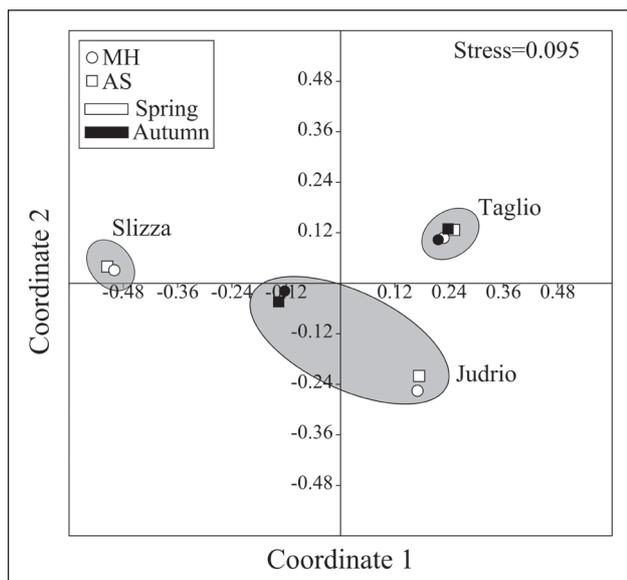


Fig. 3: N-MDS applied to the biotic datasets obtained from the two sampling techniques (MH and AS).

Sl. 3: N-MDS (nemetrično večvrstično lestvičenje) biot-skih nizov podatkov, dobljenih iz obeh tehnik vzorčenja (MH in AS)

coxon paired-sample test: at least $p > 0.05$ for all comparisons) (Tab. 3).

DISCUSSIONS AND CONCLUSIONS

In the present study, the values obtained using the Sørensen index and those resulting from the comparison of the STAR_ICMi metrics values have shown that the macrobenthic invertebrate communities monitored

with the multi-habitat sampling method (MH) and with Artificial Substrate samplers (AS) were not significantly different regardless of the lotic system here investigated. This is in contrast with the results of other investigations, where the Artificial Substrates did not allow a collection of representative samples of the whole macrobenthic community (Rosenberg & Resh, 1993; Braioni, 2001; Buffagni *et al.*, 2007). This has been attributed to a single microhabitat being investigated and to the selectivity of the artificial substrate for some macrobenthic taxa that could lead to an underestimation of the global community richness.

Different occurrences due to the sampling methodology observed in the present investigation for some taxa at the same site, agree with previous studies (Genoni & Strada, 2000) also showing that the artificial substrate technique allows abundant colonization by Diptera Chironomidae and Trichoptera Hydropsychidae, which are very competitive and very able to colonize new substrates (Hall, 1982; Hemphill & Cooper, 1983; Braioni, 2001). Contrary to habits shown by more tolerant taxa, Valenty & Fisher (2012) reported that Ephemeroptera, Plecoptera and Trichoptera (which can influence one of the STAR_ICMi metrics) show negative preferences for newly built substrates, probably due to roughness of plates, residual oils or leaching toxins, but we have not observed similar tendencies in our samples. Occurrences of Oligochaeta and Bivalvia families were generally lower than in the multihabitat samples. As suggested by Buffagni *et al.* (2007) this is probably due to the poor swimming ability of such organisms which are disadvantaged in the colonization of substrates placed in the water column. On the other hand, in the Judrio River the occurrence of Gastropoda in the AS samples was higher than in the MH ones due to the scarce presence of macrophytes on the substrates. In fact, the vegetation cover

Tab. 3: Values of the STAR_ICMi metrics and results of the non-parametric Wilcoxon paired-sample test.

Tab. 3: Vrednosti metrik indeksa STAR_ICMi in rezultati ne-parametričnega Wilcoxonovega testa parnih vzorcev

Metrics	Spring						Autumn						Wilcoxon paired-sample test	
	Slizza		Judrio		Taglio		Slizza		Judrio		Taglio		W	p-level
	MH	AS	MH	AS	MH	AS	MH	AS	MH	AS	MH	AS		
ASPT	7	7	5.16	5.39	3.54	3.64	7.86	-	5.26	5.06	4	4.7	8	0.273
Log10 (Sel_EPTD+1)	2.24	1.65	1.45	1.06	2.02	2.46	2.05	-	1.23	1.18	2.05	1.51	12	0.224
1-GOLD	0.85	0.58	0.55	0.71	0.21	0.31	0.92	-	0.78	0.65	0.43	0.73	9	0.685
Total families	15	14	23	20	34	28	8	-	16	20	29	32	6	0.787
ETP families	9	8	8	7	4	4	7	-	7	7	4	7	3	1.000
Shannon-Wiener index	2.05	2.07	2.31	1.82	1.88	1.3	1.46	-	1.29	1.61	1.87	1.71	11	0.345

in this site was lower than the threshold value of 10% required for the operational sampling protocol applied in this study. The results of the N-MDS application showed good comparability between biotic datasets obtained from the two sampling techniques. This suggests that both sampling methods could lead to similar and reliable descriptions of the macrobenthic invertebrate communities. In the Slizza stream, high occurrences were registered for Ephemeroptera, Plecoptera and Trichoptera which are related to high water oxygen concentrations, low water temperature and coarse substrates (mainly macro- and megalithal). In the Judrio sampling sites, the most abundant taxa were Ephemeroptera, Odonata, Coleoptera and some Gastropods genera (*Physa*, *Planorbis* and *Gyraulus*) which appeared more related to a fine substrate (micro- and mesolithal), slightly higher trophic levels and presence of coarse particulate organic matter. Finally, in the Taglio channel the most abundant

taxa were Oligochaeta, Diptera, Turbellaria, Hirudinea Bivalvia and Gastropoda. The community structure in this site appeared related to the less coarse bottom composition, high presence of submerged vegetation (which covers a great section of the river bed) and slightly lower water oxygen levels which seemed consistent with the impact of human presence in the area (*i.e.* a fish farm and agricultural activities).

In conclusion, our results have shown that the Hester-Dendy artificial substrates could lead to results which can be comparable to those obtained with multi-habitat sampling in different lotic environments. As reported by Calpcott *et al.* (2012) this instrument allows quantitative sampling of macrobenthic invertebrates in the non-wadeable watercourses to be performed and could be applied to many different habitats even though the risk of loss and/or damages and the potential selectivity of some taxa could be a limit of the technique.

PRIMERJAVA METOD BIOMONITORINGA ZA ANALIZO MAKROBENTOŠKIH SKUPNOSTI NEVRETEČARJEV V RAZLIČNIH REČNIH TIPIH FURLANIJE - JULIJSKE KRAJINE

Marco BERTOLI, Marzia AZZONI & Elisabetta PIZZUL

Department of Life Sciences, University of Trieste, I-34127 Trieste, I-34127 Trieste, Via L. Giorgieri 10, Italy
E-mail: pizzul@units.it

POVZETEK

Evropska vodna direktiva (2000/60/ES) opredeljuje makrobentoške nevretečarje kot zelo pomembne pokazatelje ekološkega stanja lotičnih sistemov. V Italiji uporabljajo različne metode vzorčevanja, ki jih potrebujejo za aplikacijo biotskih indeksov v prehodnih in neprehodnih rekah, kot sta proporcionalno razširjeno vzorčevanje multi-habitatov (MH) in uporaba umetnih substratov (AS). Namen tega prispevka je primerjava rezultatov, dobljenih z obema metodama v treh različnih lotičnih okoljih (planinski potok, reka na planoti in izvirski kanal). Podatki obeh metod so pokazali dobro prekrivanje vseh raziskanih lotičnih sistemov, čeprav je bila pri nekaterih taksonomskih skupinah opažena selektivnost v naseljevanju umetnih substratov. Tudi med metrikami uporabljenega indeksa STAR_ICMi, ki ga je priporočila odredba ministrstva (D.M. 260/2010) za ovrednotenje ekološkega stanja vodnih teles v Italiji, ni bilo značilnih razlik.

Ključne besede: makrobentoški nevretečarji, sladkovodni ekosistemi, severovzhodna Italija, multihabitatno vzorčevanje, umetni substrati

REFERENCES

- Anderson, M. J. (2001):** A new method for a non-parametric multivariate analysis of variance. *Austral Ecol.*, 26, 32-46.
- Autorità di Bacino dell'Adige, Autorità di Bacino dell'Alto Adriatico (2010):** Piano di gestione dei Bacini Idrografici delle Alpi orientali. Adottato con delibera dei Comitati Istituzionali dell'Autorità di Bacino dell'Adige e dell'Alto Adriatico in seduta comune in data 24 febbraio 2010. Bacino del Fiume Isonzo, 13, 203 pp.
- Barbour, M. T., J. Gerristen, B. D. Snyder & J. B. Stribling (1999):** Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C., 339 pp.
- Battegazzore, M. (1994):** Procedure di campionamento del macrobenthos per la valutazione della qualità dei corsi d'acqua. Rapporto interno IRSA - Gruppo Metodi Biologici.
- Braioni, M. G. (2001):** Analisi biologiche-ecologiche in alcune aree campione fluviali dell'Adige. Autorità di Bacino Nazionale dell'Adige & Università di Padova, Padova, 71 pp.
- Buffagni, A. & S. Erba (2007):** Macroinvertebrati acquatici e Direttiva 2000/60/CE (WFD) - Parte A: Metodo di campionamento per fiumi guadabili. Not. Metod. Analitici IRSA - CNR, 1, 2-27.
- Buffagni, A., A. Pieri, F. Bordin & L. Galbiati (2000):** Comunità macrobentoniche del Fiume Po - Parte I: Taxa rinvenuti e integrità delle comunità degli Efemerotteri. *Quad. Ist. Ric. Acque*, 113, 175-225.
- Buffagni, A., J. L. Kemp, S. Erba, C. Belfiore, D. Hering & O. Moog (2001):** A Europe wide system for assessing the quality of rivers using macroinvertebrates: the AQEM project and its importance for southern Europe (white special emphasis in Italy). *J. Limnol.*, 60 (suppl. 1), 39-48.
- Buffagni, A., E. Moruzzi, C. Belfiore, F. Bordin, M. Cambiagli, S. Erba, L. Galbiati & R. Pagnotta (2007):** Macroinvertebrati acquatici e Direttiva 2000/60/CE (WFD) - Parte D: metodo di campionamento per i fiumi non guadabili. Not. Metod. Analitici IRSA - CNR, 1, 69-93.
- Buffagni, A., S. Erba & R. Pagnotta (2008):** Direttiva 2000/60/CE (WFD) - Condizioni di riferimento per fiumi e laghi - Classificazione dei fiumi sulla base dei macroinvertebrati acquatici. Not. Metod. Analitici IRSA - CNR, Numero speciale 2008, p. 24-46.
- Cairns, J. Jr. & K. L. Dickson (1971):** A simple method for the biological assessment of the effects of waste discharges on aquatic bottom-dwelling organism. *J. Water Pollut. Control Fed.*, 43, 755-772.
- Clapcott, J., M. Pingram & K. J. Collier (2012):** Review of functional and macroinvertebrate sampling methods for non-wadeable rivers. Report No. 2222. Cawthron Institute, Nelson, 65 pp.
- Directive 2000/60/EC** of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *OJ L 327*, 22.12.2000, p. 1-73.
- D.M. 260/2010:** Regolamento recante i criteri tecnici per la classificazione dello stato dei corpi idrici superficiali, per la modifica delle norme tecniche del decreto legislativo 3 aprile 2006, n.152, recante norme in materia ambientale, predisposto ai sensi dell'art. 75, comma 3, del medesimo decreto legislativo. *Gazzetta Ufficiale* n. 31/L del 07 febbraio 2011, Supplemento Ordinario n. 30.
- Genoni, P. & L. Strada (2000):** Confronto tra metodi di prelievo per l'analisi quantitative del macrobenthos. *Biologia Ambientale*, 14, 17-22.
- Hall, T. J. (1982):** Colonizing macroinvertebrates in the Upper Mississippi River with a comparison of basket and multiplate samplers. *Freshw. Biol.*, 12, 211-215.
- Hammer, Ø., D. A. T. Harper & P. D. Ryan (2001):** PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electronica*, 4, 1-9.
- Hemphill, N. & S. D. Cooper (1983):** The effect of physical disturbance on the relative abundances of two filter-feeding insects in a small stream. *Oecologia*, 58, 378-382.
- Hering, D., C. Meier, C. Rawer-Jost, C. K. Feld, R. Biss, A. Zenker, A. Sundermann, S. Lohse & J. Bömer (2004):** Assessing streams in Germany with benthic invertebrates: selection of candidate metrics. *Limnologica*, 34, 398-415.
- Rosenberg, D. M. & V. H. Resh (1993):** Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall, New York, 488 pp.
- Sörensen, T. (1948):** A method for establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analyses of the vegetation on Danish commons. *K. danske vidensk. Selsk. Skr. Biol. Skr.*, 5, 1-34.
- Valenty, J. & S. J. Fisher (2012):** Effect of previous use and processing technique on performance of multiplate Hester-Dendy samplers. *Freshw. Sci.*, 31, 78-82.