The evaluation of ecological status in a Large Portuguese River using Macroinvertebrates Assemblages

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Abstract
Macroinvertebrate communities are extensively used for water quality assessment due to their great variety of taxonomic and functional feeding groups, which provides them advantages as indicators of the ecological status of aquatic ecosystems. The implementation of the Water Framework Directive in Europe led to an extensive use of biomonitoring programs in order to access the water quality of streams and rivers. Still, the established Portuguese sampling protocol for aquatic macroinvertebrates does not differentiate small wadeable streams and large nonwadeable rivers. The development of better standardized sampling methods and the identification of more suitable metrics and indexes are extremely important to obtain an accurate ecological status of rivers. Given the importance of the correct evaluation of ecological status in aquatic ecosystems management, our main objectives are: (1) to identify the vulnerability of the established sampling protocol and selected metrics to our sampling sites and (2) to present a review of the different studies which have been carried out during the last years in large rivers and that could be applied on further bioassessment programs in Portuguese large rivers, both feasible and cost effective.

Keywords
Ecological status; large rivers; macroinvertebrates; multimetric indexes

INTRODUCTION

Macroinvertebrates have a significant importance in the study of running water ecosystems, particularly concerning the linkage between their community structure and environmental variables such as organic pollution, where they were initially applied as indicators in streams and rivers in Europe (Kolkwitz and Marsson, 1909). Macroinvertebrate communities are frequently used in water quality assessment of small streams and rivers, being well-known indicators of water quality due to the great range of taxonomic and functional feeding groups, but also due to their vulnerability to different stressors as residents of riverbed sediments.

The implementation of the Water Framework Directive (WFD 2000/60/CE) in Europe led to an extensive use of biomonitoring programs in order to access the water quality of streams and rivers, and the correct measures to apply in case of a quality status below "Good". The latest River Basin Management Plans will enclose the results from several monitoring studies throughout the entire country, during the last couple of years. Nevertheless, the lack of suitable protocols can restrain the correct assessment of the effects of environmental stressors on larger rivers. The particularities of non-wadeable systems have been largely disregarded in Portugal and the established Portuguese sampling protocol for aquatic macroinvertebrates (INAG, 2008) does not differentiate small wadeable streams from large nonwadeable rivers. As wadeable streams are abundant and relatively easy to sample when compared to large rivers, the main effort has been focused on ecosystems bioassessment of smaller systems (e.g. Barbour *et al*., 1999). On the other hand, wadeable stream sampling protocols may be inappropriate to sample large rivers because of their depth and distance from shore. In most of the cases, even more experienced teams face difficulties and the samplings are conducted along the river margin in shallow areas, when easily reachable, or from a boat when there is no access.

Nevertheless, the development of suitable bioassessment protocols for non-wadeable streams and rivers have been studied in other countries (e.g. Blocksom and Johnson, 2006; Flotemersch *et al*., 2006a, b). In most of the cases, the accuracy of the assessment based on macroinvertebrates in large rivers can be increased by an adaptation of the sampling methodology procedures and by the identification of more suitable metrics and indexes.

Given the importance of the correct evaluation of ecological status in aquatic ecosystems management, our main objectives are: (1) to identify the vulnerability of the established metrics to a large river in the South of Portugal and (2) to present a review of the different studies which have been carried out during the last years in large rivers and that could be applied on further bioassessment programs in Portuguese large rivers, both feasible and cost effective.

METHODS

Study area

We used macroinvertebrate data from several sites sampled in the Guadiana River basin during the years 2005 and 2009. Sites were selected by the National Water Institute (INAG), considering both the longitudinal gradient of Guadiana River in Portuguese territory and its accessibility. From all the sites sampled in Guadiana basin during the monitoring programs (INAG-DQA and ARH), only a total of 12 sites were sampled in the main Guadiana River (Figure 1). The Guadiana River basin encloses a total surface of 66800 km^2, of which 55220 km^2 (83%) in Spain and 11580 km^2 (17%) in Portugal. It is the fourth biggest hydrographic basin of the Iberian Peninsula. Guadiana River flows through 810 km, from Ruidera in Spain at a 1700 m of altitude, to Vila Real de Santo António, where it reaches the sea (Garcia, 2003). In Portugal the river has a total length of 260 km, with sections of exposed rifles and rapids and sections with restricted flow associated with dams, which include the Alqueva dam, the largest reservoir in Europe.

Field sampling methods

The selected sites (n=12; Figure 1) were sampled following the Portuguese macroinvertebrate sampling method, as described in INAG (2008). Briefly, the sampling was performed in a 50 m reach and in each site were performed six sweeps, each one of 1 m in length, using a quadrangular dipnet (500 µm mesh). The sweeps were divided according to the existing habitats (e.g. sand, macrophytes) and were collected by kicking the substrate by feet and hand. Samples were all preserved in the field with formaldehyde. As all the sites had an unknown depth, the sampling was performed only along the shoreline. The sampling is similar to the method used in shallow water-shoreline sampling, described by Flotemersch *et al.* (2006b). In each site environmental parameters such as the temperature (°C), conductivity (µS/cm), dissolved oxygen (DO, mg/l) and pH were measured *in situ* using a Troll® 9500 multiparameter instrument. Water samples were additionally collected for further chemical analyses for general chemical elements and specific pollutants (see physic-chemical parameters list in INAG, 2009).

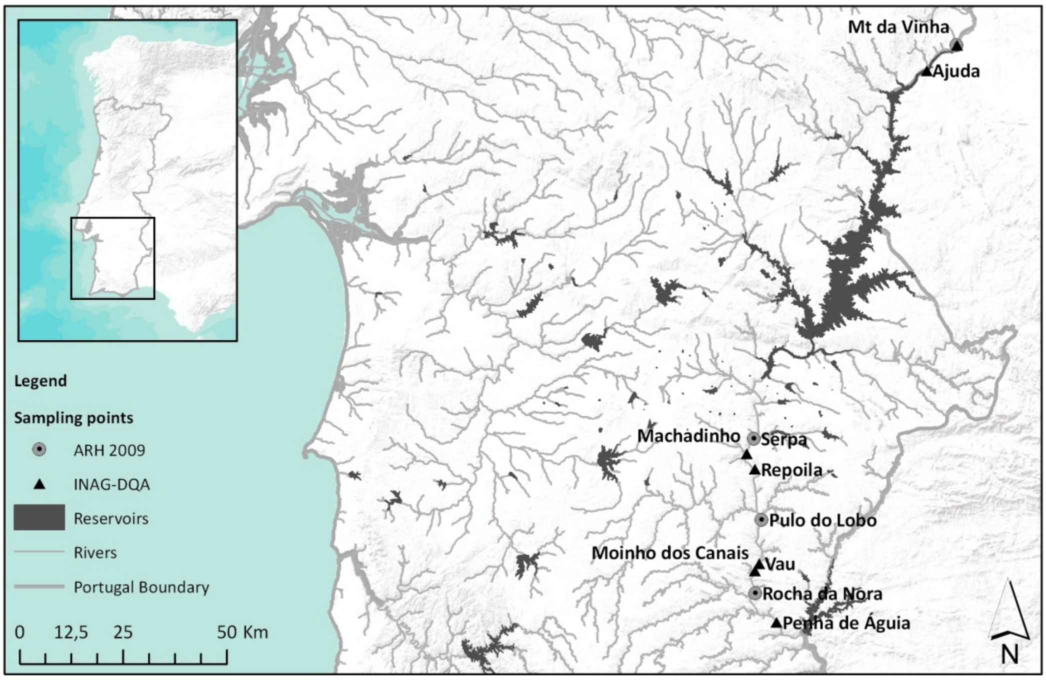

Figure 1. Sites sampled in the Guadiana River during 2005 (INAG-DQA monitoring) and 2009 (ARH monitoring).

Laboratory processing

Samples were washed with current water in a 500 µm-mesh sieve to remove preservative and fine sediment. After washing, the samples were transferred to a marked gridded pan. Five squared grids were randomly selected and all the organisms were sorted. If the five initial grids did not count up to 700 individuals an extra grid was taken repeatedly, until the 700 individuals were reached (INAG, 2008). Samples collected in 2005 were identified to the lowest taxonomic level. Samples collected in 2009/2010 were identified until the family level, except for Oligochaeta, which stood at the class level (INAG, 2008). Although all organisms were identified to the lowest practical taxonomic level in the first monitoring program, the sampling protocol, published in 2008, referred the family level as the lowest level needed. Given that, the last monitoring programmes established family as the lowest taxonomic level to be identified.

Data analyses

It was calculated the Portuguese multimetric index (*Índice Português de Invertebrados Sul*), $IPtI_s$, the established index used in the ecological classification using macroinvertebrates, in the south of Portugal (INAG, 2009; Table 1).

Table 1. Description of the metrics included in the Portuguese multimetric index, $IPtI_s$, and respective weighting coefficients (INAG, 2009).

Index/ Metrics	Description
$IPtI_S$	N.º taxa x 0.4 + EPT x 0.2 + (IASPT – 2) x 0.2 + Log (Sel. EPTCD+1) x 0.2
Number of taxa	Total number of existent taxa
IASPT	IBMWP/ Number of taxa punctuated by IBMWP
EPT	Number of families of the orders Ephemeroptera, Plecoptera and Trichoptera
Log (Sel. EPTCD+1)	Log_{10} 1 + Σ abundance of the families Chloroperlidae, Nemouridae, Leuctridae, Leptophlebiidae, Ephemerellidae, Philopotamidae, Elmidae, Limnephilidae, Psychomyiidae, Dryopidae, Sericostomatidae and Athericidae

RESULTS AND DISCUSSION

The results from the Portuguese index ($IPtI_s$) provided the classification of the different sites in terms of biological quality status (Table 2). Overall, the obtained results showed the low diversity of macroinvertebrates in all sites (low number of taxa), with the lack of more sensitive taxa such as Ephemeroptera, Plecoptera and Trichoptera (low EPT and Log(Sel EPTCD+1) values). The composition and abundance of the macroinvertebrates assemblages reflected the sampling along the shoreline, typically more homogenous in terms of habitat diversity, when compared with most wadeable streams. The particularities of Guadiana River in terms of drainage area, give rise to its proposal as a heavily modified water body (HMWB). Still, all the boundaries used to classify each site based on the $IPtI_s$ index were the ones considered to river basins up to 100 km^2 drainage area (S1>100 km^2; INAG, 2009), as there is no data adapted to this type of stream yet.

Table 2. Results of the multimetric Portuguese index (IPtIs) and each individual metrics. Last column shows the macroinvertebrate classification based on the quality boundaries established for IPtIs index (INAG, 2009).

Site name	Sampling year	EPT taxa	Number taxa	IASPT-2	Log (Sel EPTCD+1)	$IPtI_s$*	Classification
Guadiana-Caia	2005	2	8	1.75	0.22	0.32	Poor
Machadinho	2005	2	8	1.88	0.00	0.31	Poor
Mértola azenha	2005	2	5	1.00	0.00	0.18	Bad
Moinho dos Canais	2005	0	6	1.20	0.00	0.17	Bad
Monte da Vinha	2009	1	10	1.56	0.00	0.31	Poor
Penha de Águia	2005	0	4	0.25	0.00	0.09	Bad
Pulo do Lobo	2009	2	15	1.17	0.00	0.40	Poor
Pulo do Lobo	2010	1	3	0.33	0.00	0.10	Bad
Repoila	2005	2	10	2.33	0.00	0.36	Poor
Rocha da Nora	2009	2	11	1.80	0.22	0.36	Poor
Serpa	2005	0	6	0.83	0.00	0.17	Bad
Serpa	2009	2	10	1.60	0.00	0.33	Poor
Vau	2005	6	11	2.70	1.07	0.58	Moderate

Only one sampling site (Vau) had a “Moderate” classification, the overall site classification varied between “Poor” and “Bad” (Table 2). The Portuguese multimetric index was developed to wadeable streams and rivers and has been used in the last years to classify the macroinvertebrate communities with high precision and efficiency. Metrics that compose the index allow the integration of composition and abundance, requested by the WFD, and at the same time, they permit the separation of a degradation gradient and differentiate water quality classes. Currently, the need of reviewing the water quality of different water bodies has exposed the weakness of the classification. In the south of Portugal, the research panel responsible for the classification of the Guadiana river basin decided to not consider the results from macroinvertebrates monitoring in the biological classification of all sites sampled in Guadiana River (Biological Elements column, marked with asterisk; Table 3), as their classification did not disclose the correct existing conditions. Despite of the importance of biological elements in the assigning the water bodies ecological status, the discrepancy of the classification based on different elements led to the need to base the classification only on physico-chemical parameters (Table 3). Furthermore, it exposed the need to adapt the evaluation methods, not only the sampling procedures but also the identification level and fitting metrics.

Table 3. Results from Biological, Physico-chemical and Hydromorphological elements and resultant ecological status, for each sampled site and sampling season.

Site name	Year	Biological Elements	Physico-chemical Elements	Hydromorphological Elements	Ecological status	Water body description
Guadiana-Caia	2005	Poor*	Moderate	Good or under	Moderate	PT07GUA1428I2 (downstream Caia reservoir and Badajoz dam)
Monte da Vinha	2009	Poor*	Moderate	-	Moderate	
Serpa	2009	Poor*	Good and above	Good or under	Good and above	PT07GUA1530 (downstream Alqueva reservoir)
Serpa	2005	Bad*	Moderate and under	Good or under	Moderate and under	
Pulo do Lobo	2009	Poor*	Good and above	-	Good and above	PT07GUA1588 (downstream Alqueva and Enxoé reservoirs)
Penha de Águia	2005	Bad*	Moderate and under	Good or under	Moderate and under	
Mértola azenha	2005	Bad*	Moderate and under	Good or under	Moderate and under	
Moinho dos Canais	2005	Bad*	Good and above	Good or under	Good and above	
Repoila	2005	Poor*	Moderate and under	Excellent	Moderate and under	
Machadinho	2005	Poor*	Moderate and under	Good or under	Moderate and under	
Rocha da Nora	2005	Poor*	Good and above	-	Good and above	
Vau	2005	Good	Good and above	Excellent	Good and above	

CONCLUSIONS AND ASSESSMENT PROTOCOLS PROPOSALS

As we know from literature, biological communities change with stream size, habitat type and quality. As a result, assemblages adapted to deeper and wider streams, with limited canopy cover, are more likely to occur downstream, in higher order reaches (Vannote *et al.*, 1980). Therefore, communities in large rivers are assumed to be very different from those in smaller systems (Flotemersch *et al.*, 2006a, b).

Though the implemented Portuguese sampling protocol for macroinvertebrates is omissive concerning large rivers, the sampling performed along the shoreline in the wadeable shore zone is completely accepted as it may be the most productive and diverse zone for benthic invertebrates (Wetzel, 2001). Even though the wadeable shore zone only accounts for a small proportion of the entire river channel, it has the greatest light penetration for benthic algae and aquatic macrophytes (Flotemersch *et al.*, 2006b). Nevertheless, the restrictive classification of biological elements alerted to the fact that the applied method might not be enough adequate. There is a need to develop a consistent bioassessment protocol for further assessment of biological status in Portuguese large rivers, both feasible and cost effective. Given that, the existing habitat assessment and benthic sampling methods should be improved in order to cover a large assortment of disturbance gradients. Considering all the studies already published concerning large rivers bioassessment, we propose further methods to add the national sampling method (Table 4).

Several authors suggested that the best approach to assess benthic macroinvertebrate assemblages of large rivers is to apply multiple sampling methods (Bartsch *et al.*, 1998; Flotemersch *et al.*, 2006a), in order to effectively sample all components of a macroinvertebrates assemblage in aquatic ecosystems. However the problem does not rely only in adjusted assessment protocols; there is a need to develop adapted metrics and indexes. Multimetric index is the most common method of data analysis that combines several biological variables into a single index. As metrics translate the predictable variation of biota with the increase of human influence (Barbour *et al.*, 1999), the use of multiple metrics maximizes the available information of functions and processes of aquatic communities.

Table 4. A resume of large rivers macroinvertebrate sampling approaches (based on Flotemersch *et al.*, 2006b).

Method type	Designation	Brief description	Advantages	Disadvantages
Active (requires only one visit for sample collection)	Deep water: main channel sampling (Klemm *et al.*, 1990)	Sampling from a boat using a dredge or bottom grab sampling devices (Klemm *et al.*, 1990)	- Effective in sampling deepwater habitats and organisms that burrow in soft sediments (e.g. oligochaetes) - Requires little training and collect standardized, quantitative samples	- Usually operate "blind" due to turbidity, with little knowledge of sampled substrate; - Organisms are often washout as the dredge is lifted and removed from water; - Some dredges are ineffective at hard substrates and can be blocked by debris; - Some dredges are heavy and difficult to manage, specially with significant flow rates
	Snag sampling (e.g. Angradi, 2006; Johnson *et al.*, 2004; Merrit *et al.*, 2005)	Sampling woody debris or "snags" (>10cm Ø)	- Snags are natural and stable, have been recognized as some of the most productive invertebrate habitats of large rivers (e.g. Merrit *et al.*, 2005) - Shallow shoreline habitats are easily to observe and to sample proportionally; - Dip-net methods can be used to sample several habitats type, both stable (e.g. woody debris) or unstable (e.g. sand)	- Snags irregular size and shape often make it difficult to standardized the sampled area; - The length that the snag has been inside the water and the colonization period are unknown
Passive (requires more than one visit for sample collection)	Artificial substrates (e.g. Klemm *et al.*, 1990; Rosenberg and Resh, 1982)	Devices made of natural/artificial materials that are placed in the water for a predetermined period and depth for colonization	- Effective in sampling shallow or deep water habitats; - Allow quantitative collection from sites that cannot be sampled using other conventional methods; - Reduces sampling variability associated with the operator	- Measure colonization rather than resident assemblages; - Placement of sampler units can skew results (e.g. current velocity); - Potential damage of artificial substrates due to high flows or vandalism.

For instance, it is well documented that as conditions degrade, the abundance of tolerant organism (density) increases, whereas the number of intolerant taxa (richness) decreases. Our results have shown a clear dominance of more tolerant taxa in opposition to a clear lack of intolerant taxa as it can be perceived by the low number of EPT taxa. The best approach might have into account the dominant organisms and their sensibility to different stressors. Literature refers that Chironomids can be an important freshwater indicator (e.g. Saether, 1975), as the larvae of some species are sensitive to particular forms of pollution, whereas others are rather tolerant. Because the larvae often feed on the dead plant and animal debris in aquatic sediments, they are exposed to all contaminants in the organic matter they consume (Carew *et al*., 2007). Large numbers of pollution-tolerant chironomids are often indicative of poor water quality. Some Chironomids species and their sensitivity to eutrophic conditions have already been used to develop a trophic status classification of lakes (oligotrophic, mesotrophic and eutrophic; e.g. Saether, 1975; Langdon *et al*., 2006). Chironomid surveys with pupal exuviae could offer a low-cost method, as they can be collected easily from shoreline. Oligochaetes, especially the Naididae and Tubificidae families, have also been used in the assessment of water quality because of their capacity of increase in number with increasing organic matter (Lin and Yo, 2008; Verdonschot, 1989). Recent studies have identified the linkages between nutrients and macroinvertebrates. Smith *et al.* (2007) established thresholds for oligotrophic, mesotrophic, eutrophic conditions and defined optimal nutrient intervals for 164 macroinvertebrate taxa based on total phosphorus and nitrates. Wang *et al.* (2007) found significant correlations between nitrogen and phosphorus concentrations and percent and number of Ephemeroptera, Plecoptera and Trichoptera (EPT number), the Hilsenhoff biotic index, and mean tolerance values.

FINAL REMARKS

Water quality assessment, as a tool in large rivers water management, should embrace the largest possible environmental elements, both biological and chemical. Since macroinvertebrates are extremely important bioindicators of aquatic ecosystem conditions, they should not be disregarded or neglected.

The use of multimetric indexes for benthic macroinvertebrates have been broadly developed for many regions and are commonly used in the biological assessment of aquatic resources quality. Therefore, there is a need to establish practical and reliable protocols for measuring ecological integrity, designed specifically for large river systems, logistically feasible and cost effective to support bioassessment programs efficiently.

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