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Monitoring the biological quality of an urban stream using a learning by doing approach with higher education students.

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ABSTRACT

Monitoring the biological quality of an urban stream using a learning by doing approach with higher education students.

This study assessed the longitudinal and temporal trends in the biological quality of a stream using a learning by doing approach with higher education students under supervision. From 2004 to 2014, benthic macroinvertebrates were collected nine times in five sampling campaigns to calculate the scores of the biotic index IBMWP and of the multi-metric Portuguese index IPtIS. The two indices provided similar information but the scores were more constrained for IPtIS than for IBMWP. The biological quality was moderate along most of the longitudinal profile and time. Scores decreased downstream attaining a poor condition at the two lower reaches. The overall quality of the stream increased temporally from 2004 to 2013, decreasing in 2014 due to the disruption of the benthic macroinvertebrate communities by strong flash floods. In 2004, the exotic gastropod *Potamopyrgus antipodarum* was detected in relatively low abundances but the cumulative density in the stream increased steadily over time, conforming to an invasion curve. This learning by doing approach provided useful information on the spatial and temporal trends in the biological quality of the stream and detected invasion by an alien species. Data collected by higher education students may fill data gaps and complement information gathered by national authorities.

KEYWORDS: benthic macroinvertebrates; biomonitoring; IBMWP; IPtIS; invasion by *Potamopyrgys antipodarum*; peri-urban stream.

RESUMEN

Monitoreo de la calidad biológica de un arroyo urbano utilizando un enfoque de aprender haciendo con estudiantes de educación superior.

Este estudio evaluó las tendencias longitudinales y temporales en la calidad biológica de un arroyo utilizando un enfoque de aprender haciendo con estudiantes de educación superior bajo supervisión. De 2004 a 2014, los macroinvertebrados bénticos *se recolectaron nueve veces en cinco campañas de muestreo para calcular las puntuaciones del índice biótico IBMWP y del índice portugués multimétrico IPtIS. Los dos índices proporcionaron información similar, pero las puntuaciones fueron más limitadas para IPtIS que para IBMWP. La calidad biológica fue moderada a lo largo de la mayor parte del perfil longitudinal y del tiempo. Los puntajes disminuyeron río abajo alcanzando una mala condición en los dos tramos inferiores y la calidad general aumentó temporalmente de 2004 a 2013, diminuyendo en2014 debido a la ruptura de las comunidades de macroinvertebrados bénticos por fuertes inundaciones repentinas. En 2004, el gasterópodo exótico* Potamopyrgus antipodarum*. se detectó en abundancias relativamente bajas, pero la densidad acumulada en el arroyo aumentó constantemente con el tiempo, conforme a una curva de invasión. Este enfoque de aprender haciendo proporcionó información útil sobre las tendencias espaciales y temporales en el estado ecológico del arroyo y detectó la invasión de una especie exótica. Los datos recopilados por estudiantes de educación superior pueden llenar los vacíos de datos y complementar la información recopilada por las autoridades nacionales*

PALABRAS CLAVES: macroinvertebrados bentónicos; biomonitoreo; IBMWP; IPtIS; invasión de Potamopyrgys antipodarum*; arroyo periurbano.*

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INTRODUCTION

Urbanization represents a threat to freshwater ecosystems and their services, such as freshwater supply, flood mitigation, carbon storage, and soil fertility (Eigenbrod et al., 2011). The ecological degradation of urban rivers and streams often shows consistent responses that include increased channel width, pool depth, and scour, high pollutant loads, reduced channel complexity, and an overall loss of sensitive taxa, symptoms that are referred to as the urban stream syndrome (Walsh et al., 2005). Along with the urbanization effects, agricultural land use poses a significant threat to the ecological integrity of rivers worldwide, and particularly in Mediterranean regions (Stefanidis et al., 2016). In small streams, benthic macroinvertebrate metrics are significantly decreased, and community composition altered by cattle density (Braccia & Voshell, 2007) and by agriculture (Egler et al., 2012; Stefanidis et al., 2016). Furthermore, disturbed ecosystems are more prone to invasion by alien species because chronic stress tends to erode ecological resilience, decreasing resistance to invasion over the long term (Meyer et al., 2021). Many freshwaters are already invaded by several alien species (Strayer, 2010), among which the gastropod *Potamopyrgus antipodarum* (J.E. Gray, 1843), native to New Zealand, has spread all over the world except Antarctica (Alonso et al., 2023) but with only nine records in Portugal (Alonso et al., 2019).

The European Water Framework Directive (EWFD) requires member states to assess all their surface waters based on biological elements, including benthic macroinvertebrates, but there are substantial delays in implementing many of the measures planned (Carvalho et al., 2019). In Portugal, lack of data may compromise the fulfillment of the objectives of the EWFD and the country was advised to improve monitoring of surface waters (European Comission, 2019). Data collected by students may increase the sampling network and provide additional data for the implementation of the EWFD in Portugal (Vidal et al., 2021).

Universities play an important role in creating environments that increase the persistence of students in science. Learning by doing activities improve student performance (Freeman et al., 2014; Deslauriers et al., 2019) and undergraduate students engaged in early research activities often demonstrate greater persistence in science degree programs and careers (Graham et al., 2013). At Coimbra Agriculture School, all students carry out a learning by doing final degree project in an area related to their field of studies. Students engage in science practices, including planning, and carrying out investigations, analyzing and interpreting data, constructing explanations and writing a report. From 2004 to 2014, four students assessed the biological quality of Ribeira de Covões, a small peri-urban stream not monitored under the EWFD which flows through the campus. The objectives of this work are to assess (1) if data collected and handled by different students following the same protocol could provide a long-term monitoring of the biological quality of Ribeira de Covões, and (2) if the abundance of *P. antipodarum* in the stream conformed to the impact curve described by Haubrock et al. (2022).

events increasing in frequency over the years (Ferreira et al., 2020) which result in streambed and bank scouring.

In the area of Coimbra Agriculture School, the stream has been historically used to water the agricultural fields and pastures for cattle and horses, and somewhere in the past (there is no record of when) the channel was straightened and redirected. Although many of the constructed walls are now in ruins, there are still a few reaches with constructed banks and in the non-channelized reaches, channel morphology shows increased cross-section due to deepening. Along most of the longitudinal profile, riparian vegetation is highly disturbed, and in some cases almost inexistent, further contributing to this effect. In 2004, total suspended solids (13-282 mg/L), copper (0.04-0.41 mg/L), iron (0.30-15.98 mg/L), and ammonia (0.02-2.80 mg/L $N\text{-}NH4^+$) of the water were higher than the legal limits (Dias et al., 2006).

Table 1 Characteristics of the study sites, physical and chemical parameters of the water (average ± 95%CL of the sampling dates) and respective number of observations (N) during the study period. *Características de los sitios de estudio, parámetros físicos y químicos del agua (promedio ± 95% CL de las fechas de muestreo) y respectivo número de observaciones (N) durante el período de estudio.*

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Distance from source (m)	750	1550	2190	2680	3470	3900	4200
Altitude (m)	149	100	80	50	22	21	20
Stream order	1	3	3	4	4	4	4
Temperature (◦C)	13.3±2.5	15.2±3.3	16.2±3.1	13.6±1.7	14.0±2.0	13.4±2.6	13.9±3.0
Dissolved oxygen (mg/L)	9.6±1.5	9.7±3.3	7.8±3.4	9.6±1.7	9.8±2.4	10.6±1.7	13.8±2.3
Conductivity (mS/cm)	231±19	362±150	409±85	418±101	431±96	469±67	452±112
pH	6.9±0.25	7.6±0.25	7.5±0.15	7.6±0.19	7.9±0.29	7.8±0.62	8.4±0.34
N	5	4	5	8	9	5	3

MATERIAL AND METHODS

Study site

The study was conducted in Coimbra, Central Portugal (40°12′41″ N, 8°25′45″ W), an area with a humid Mediterranean climate with dry summers and rainy winters, an average annual temperature of +15ºC and a total rainfall of 980 mm, with strong seasonal and inter-annual variability (Ferreira et al., 2011).

Ribeira de Covões (Fig. 1) is a 4th order peri-urban stream with a 3.4% gradient which flows 4.5 km along small, urbanized areas, enters Coimbra Agriculture School, and drains into the Mondego River. It has a basin of 6.2 km^2 with an average slope of 9° but including steep slopes of up to 46° (Ferreira et al., 2016). Land-use in the basin comprises 56% forest, 40% urban and 4% agricultural area (Ferreira et al., 2020), the latter mostly within the area of Coimbra Agriculture School. As a result of the morphological attributes of the basin, of the increasing urbanization pressure and road construction, and of soil hydrophobicity due to eucalypt plantations which increase overland flow (Ferreira et al., 2013) the stream presents a flashy hydrograph with flood

The seven sampling sites were distributed along the longitudinal profile of the stream (750 m to 4200 m from the source), the first three in upper reaches mainly influenced by urban development, road construction, and impervious surfaces and the last four within the campus of Coimbra Agriculture School, mainly influenced by channelization and agricultural activities (Fig. 1). The sites were sampled at least four times between March 2004 and April 2014 and at least two times

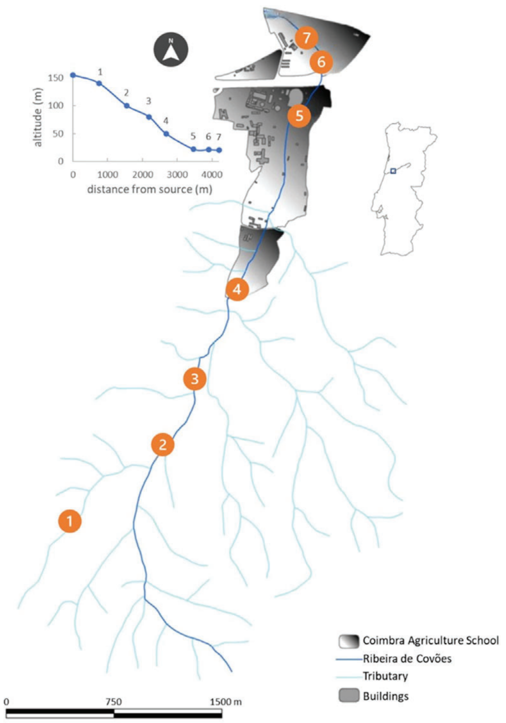

Figure 1 Drainage basin of Ribeira de Covões, showing the seven sampling sites (modified from Dias et al., 2006). The inset shows the longitudinal profile. Sites 1 to 3 were mainly affected by urban development, road construction, and impervious surfaces, while sites 4 to 7 were mainly affected by channelization, agricultural and animal production activities. *Cuenca de Ribeira de Covões, mostrando los siete sitios de muestreo (modificado de Dias et al., 2006). El recuadro muestra el perfil longitudinal. Los sitios 1 a 3 fueron afectados principalmente por desarrollo urbano, construcción de caminos y superficies impermeables, mientras que los sitios 4 a 7 fueron afectados principalmente por actividades de canalización, agricultura y producción animal.*

in each season except Summer (Table S1, see supplementary information available at https://www.limnetica.net/en/limnetica). The physical and chemical parameters measured during the study period show increasing conductivity and pH values along the longitudinal profile (Table 1).

Macroinvertebrate sampling and identification

Benthic macroinvertebrates were collected nine times in five sampling campaigns from January 2004 to April 2014. Sampling followed the standardized approach of Jáimez-Cuéllar et al. (2002), similar to the methodology later adopted by Portugal in the scope of the European Water Framework Directive (INAG, 2008), but using a standard Surber net (0.09 m^2, 500 µm mesh size) instead of a hand-net. Six benthic samples were collected from the microhabitats in each sampling site, taken to the laboratory in an ice-chest, washed through a 0.5 mm sieve, divided in fractions to facilitate sorting, and the macroinvertebrates from all fractions were collected and stored in 75% alcohol.

Macroinvertebrates were identified to the lowest practicable taxonomic level using the taxonomic key of Tachet et al. (2000), but for the purpose of quality assessment the level of taxonomic resolution was uniformized as follows: phylum in the case of Nematoda, subclass in the case of Collembola, and family in all other groups. The macroinvertebrates were classified into functional feeding groups (collector-gatherers, collector-filterers, shredders, scrapers, and predators) according to Merritt and Cummins (1996). Data from the six samples was pooled to obtain a composite sample representative of each site on each sampling day.

Sampling and processing of the macroinvertebrates was carried out by each student as part of their learning by doing activity. Sampling was always carried out in the presence of the supervisor to assure the same methodology and effort. The students were trained for macroinvertebrate identification, which was also closely supervised. This allowed comparison of the data sets collected and drawing valid inferences from the data.

Community characterization and evaluation of biological quality

The community was characterized in terms of proportion of functional feeding groups, density (number of individuals/m^2), percentage of insect taxa, diversity (Shannon-Wiener H'), and Pielou's evenness (E), where p_i is the proportion of individuals in each taxon and S is the number of taxa:

$$H' = -\sum_{i=1}^{i=S} p_i \times \ln(p_i) \qquad E = \frac{H'}{\ln(S)}$$

The biological quality of the stream was evaluated with the biotic index IBMWP and the multi-metric index IPtIS. IBMWP, the Iberian biological monitoring working party, is an indicator of species sensitivity calculated as the sum of the score of intolerance to pollution (1: very tolerant to 10: very intolerant) of reference taxa and distinguishes five ecological status classes: very good (≥101), good (61-100), moderate (36-60), poor (16-35), and bad (≤15) (Jáimez-Cuéllar et al., 2002). The IPtIS, the multi-metric index developed for the Portuguese running waters in the scope of the European Water Framework Directive, is derived from the common intercalibration index STAR-ICMi (Feio et al., 2014) and assesses general degradation impacts on benthic macroinvertebrates. It is based on four metrics (INAG, 2009): (1) number of taxa, the total number of taxa identified to the levels described above; (2) EPT, the number of families of the orders Ephemeroptera; Plecoptera and Trichoptera; (3) IASPT, the Iberian average score per taxon, calculated as the value of IBMWP divided by the number of indicator taxa; and (4) selected EPTCD, the sum of the abundances of families Chloroperlidae, Nemouridae, Leuctridae, Leptophlebiidae, Ephemerellidae, Philopotamidae, Limnephilidae, Psychomyiidae, Sericostomatidae, Elmidae, Dryopidae, and Athericidae. Before calculation, the value obtained for each metric is divided by the reference value of the pristine condition for streams and rivers in littoral Central Portugal: number of taxa = 20, EPT = 8, IASPT-2 = 3.6, and $\log_{10}$(selected EPTCD+1) = 2.57, to obtain a type-specific normalized value (INAG, 2009). This value is used to calculate the ecological quality ratio (EQR), which varies between 1 and 0 and distinguishes five quality classes: excellent (EQR 0.980.75), good (EQR 0.740.57), moderate (EQR 0.560.38), poor (EQR 0.370.20), and bad (EQR ≤0.19):

$$\text{IPtIS EQR} = \text{number of taxa} \times 0.4 + \text{EPT} \times 0.2 + (\text{IASPT-2}) \times 0.2 + \log_{10}(\text{selected EPTDC} + 1) \times 0.2$$

Because the number and identity of the sites differed among sampling campaigns, data was averaged and the 95% confidence limits of the mean were calculated to provide the longitudinal, the seasonal, and the temporal trends, as follows. The longitudinal trend was the average of sampling dates for each site, the seasonal trend was the average of the sites sampled within each meteorological season: autumn (September 1 to November 30), winter (December 1 to February 29), and spring (March 1 to May 3), and the temporal trend was the average of the sites sampled on each sampling campaign. Values were considered different whenever the 95% intervals did not overlap.

Potamopyrgus antipodarum abundance

The assessment of the abundance of the gastropod *P. antipodarum* along time in the stream followed the general procedures described in Haubrock et al. (2022). Data sets containing ≥4 observations were selected for analysis (this excluded site 7), resulting in a time series containing on average 5.83 ± 1.99 95% CL sampling occasions. The average density of the sites sampled on each date (except site 7) was used to calculate the cumulative density in the stream, and simple linear regression was used to test if time significantly predicted those values. This analysis was carried out with the software STATISTICA 10.0 with the level of significance set at 0.05.

RESULTS

Benthic macroinvertebrate community

The macroinvertebrate community was dominated by two taxa, which constituted 77% of all individuals across sites and sampling occasions: Oligochaeta (47%), mainly represented by family Naididae (30%), and the gastropods Hydrobiidae (30%), represented by *P. antipodarum* (Fig. 2A-C).

The relative abundance of these two taxa changed along the longitudinal profile, distinguishing the upper reaches, where Hydrobiidae were dominant, from the lower reaches, where Oligochaeta were dominant (Fig. 2A). Seasonally, Oligochaeta increased, while Hydrobiidae decreased, from autumn to spring (Fig. 2B), resulting in oscillations in the relative abundance

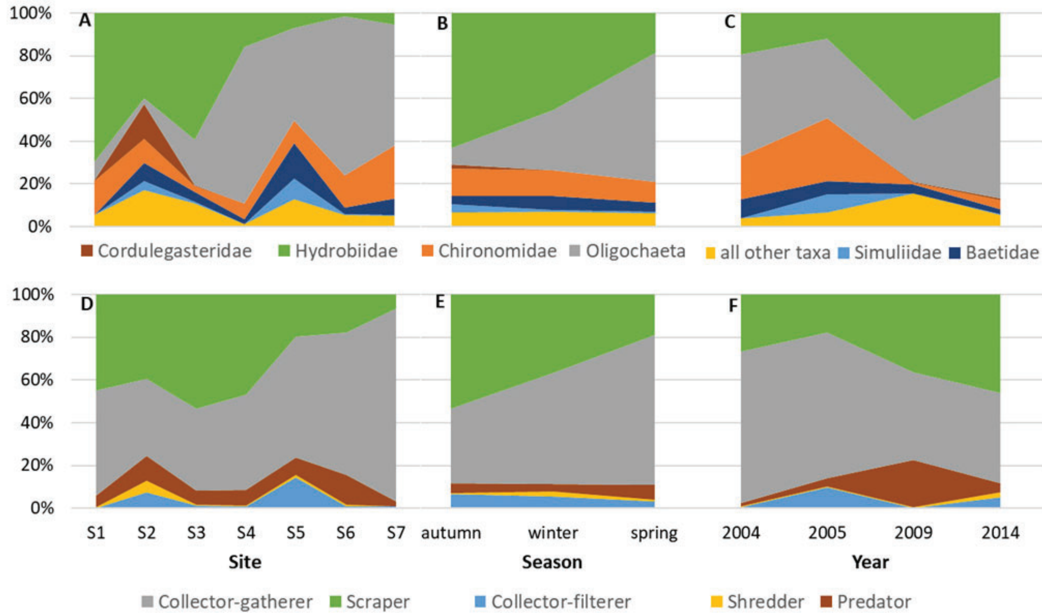

Figure 2 A-C Relative abundance of the six most abundant taxa, representing ≥10% of total abundance in at least one site, and D F Proportion of functional feeding groups. AD Longitudinal variation, BE Seasonal variation, and CF Temporal variation of the benthic macroinvertebrate community of Ribeira de Covões during the study period. *A-C Abundancia relativa de los seis taxones más abundantes, que representan ≥10% de la abundancia total en al menos un sitio, y D-F Proporción de grupos funcionales de alimentación. AD Variación longitudinal, BE Variación estacional y CF Variación temporal de la comunidad de macroinvertebrados bentónicos de Ribeira de Covões durante el período de estudio.*

of the taxa along time (Fig. 2C). The high abundance of Oligochaeta and Hydrobiidae resulted in a community dominated by collector-gatherers and scrapers (Fig. 2D-F). Collector-gatherers increased while scrapers decreased, downstream (Fig. 2D) and from autumn to spring (Fig. 2E). Scrapers increased, while collector-gatherers decreased, along time (Fig. 2F).

Although insects constituted only 37% of all macroinvertebrates, insect taxa were 61% of all richness (Fig. 3A-C). The percentage of insect taxa was higher in the upper reaches and decreased downstream, with higher values at sites 2 and 3 than at site 6 (Fig. 3A) and was stable across seasons and along time (Fig. 2BC). Due to the very high localized abundances of Naididade and Hydrobiidae, density oscillated along the longitudinal profile with very broad confidence intervals, being higher at site 6 than at site 7 (Fig. 3D) and in spring than in winter (Fig. 3E). Community evenness fluctuated in opposite direction to density, due to dominance by either Naididae or Hydrobiidae (Fig. 3EF).

Biological quality

Both indices provided similar information, although the values of IPtIS were in general more constrained and presented narrower confidence intervals than the values of the IBMWP (Fig. 4). IPtIS classified sites 1-3 and 5 with moderate and sites 4, 6-7 with poor quality (Fig. 4A). IBMWP classified site 3 with good, sites 1, 2, 4-5 with moderate and sites 6-7 with poor quality (Fig. 4D). Seasonally, the values were highest in autumn but always in the class of moderate quality (Fig. 4BE). Temporally, the values tended to increase until 2013, decreasing afterwards, but only IPtIS distinguished an improvement in the biological quality from 2004 to 2005 and a deterioration in 2014 (Fig. 4CF).

The metrics of the IPtIS (Fig. S1, see supplementary information, available at https://www.limnetica.net/en/limnetica) were all much lower than the reference values and in general followed the same trend of decreasing along the longitudinal profile with highest values in autumn.

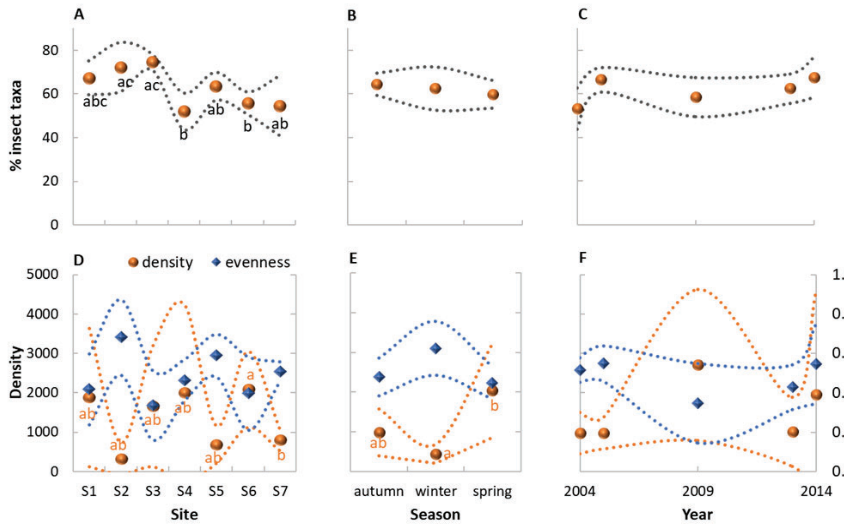

Figure 3 A C Percentage of insect taxa, D F Density (number of individuals/m^2) and Pielou's evenness of the benthic macroinvertebrate community of Ribeira de Covões during the study period. Values are averages (dots) and 95% confidence intervals (dotted lines). AD Longitudinal variation, BE Seasonal variation, and CF Temporal variation. Different lower-case letters identify values of percentage insect taxa and density with non-overlapping confidence intervals. *A-C Porcentaje de taxones de insectos, D-F Densidad (número de individuos m-2) y uniformidad de Pielou de la comunidad de macroinvertebrados bentónicos de Ribeira de Covões durante el período de estudio. Los valores son promedios (puntos) e intervalos de confianza del 95 % (líneas de puntos). AD Variación longitudinal, BE Variación estacional y CF Variación temporal. Diferentes letras minúsculas identifican valores de porcentaje de taxones de insectos y densidad con intervalos de confianza que no se superponen.*

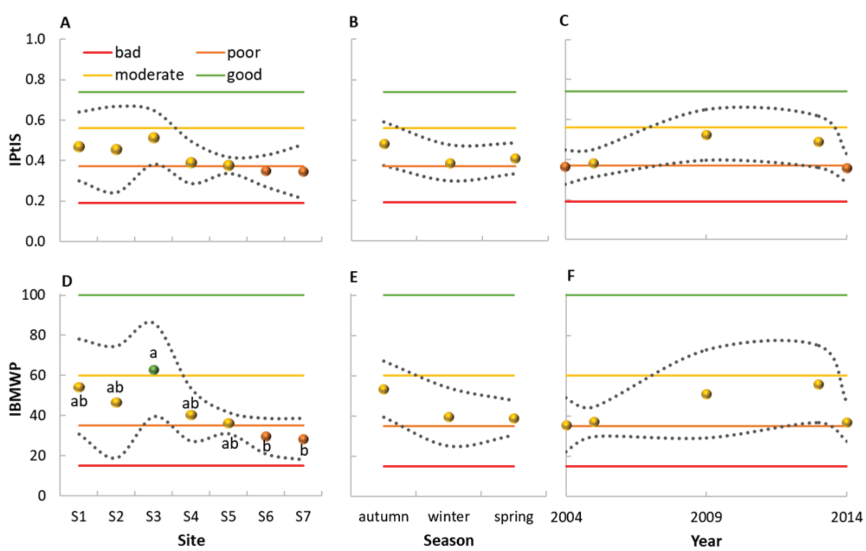

Figure 4 Biological quality of Ribeira de Covões during the study period A C IPtIS index and D F IBMWP index. Values are averages (dots) and 95% confidence intervals (dotted lines). AD Longitudinal variation, BE Seasonal variation, and CF Temporal variation. The horizontal full lines represent the ecological status classes. Different lower-case letters identify values with non-overlapping confidence intervals. *Estado ecológico de la Ribeira de Covões durante el período de estudio Índice A-C índice IPtIS y D-F índice IBMWP. Los valores son promedios (puntos) e intervalos de confianza del 95 % (líneas de puntos). AD Variación longitudinal, BE Variación estacional y CF Variación temporal. Las líneas completas horizontales representan las clases de estado ecológico. Diferentes letras minúsculas identifican valores con intervalos de confianza que no se superponen.*

Potamopyrgus antipodarum abundance

The density of *P. antipodarum* in the stream (mean ± 95% CL) varied considerably among sites (505 individuals/m^2 ± 468) and sampling dates (431 individuals/m ± 327), reaching higher values at site 1 (1247 individuals/m^2 ± 1887) and site 3 (1252 individuals/m^2 ± 1610) than at the others (maximum 318 individuals/m^2 ± 129 at site 4). The cumulative density in the stream increased steadily during the study period, from a global average across sites of 328 in January 2004, to 2100 in May 2009, and 3778 in April 2014 (Fig. 5). In the regression model, the overall population strongly adhered to a linear fit (R^2 = 0.99, $F_{1,7}$ =1301, $p < 0.00001$) with an estimated mean initial size of N_0 = 372 individuals/m^2 ± 150 (95% CL) and a monthly rate of increase of 28 individuals/m^2 ± 1.8 (95% CL).

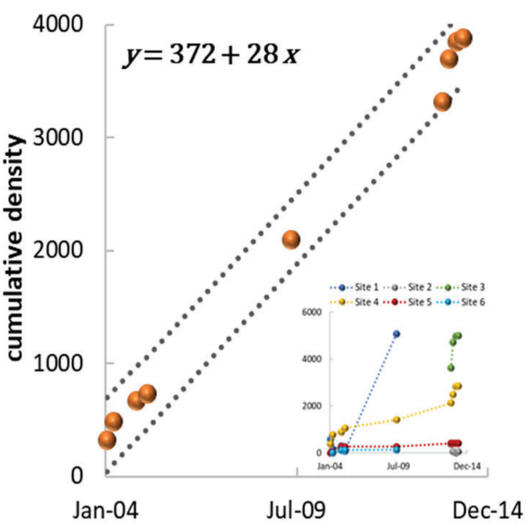

Figure 5 Cumulative density of the gastropod *Potamopyrgus antipodarum* in Ribeira de Covões from January 2004 to April 2014, calculated from the average density across sites. Values are cumulative densities ± 95% prediction interval of linear regression. The inset shows cumulative density at each of the sites 1 to 6. *Densidad acumulada del gasterópodo Hydrobiidae en Ribeira de Covões desde enero de 2004 hasta abril de 2014, calculada a partir de la densidad media entre sitios. Los valores son densidades acumulativas ± 95 % del intervalo de predicción de la regresión lineal. El recuadro muestra la densidad acumulada en cada uno de los sitios 1 a 6.*

DISCUSSION

Period of sampling

Streams in temperate, and particularly in Mediterranean areas, often exhibit strong seasonal variability in hydrology, water chemistry and habitat availability (Boulton & Lake, 1992a,b) which in turn affect community structure of benthic macroinvertebrates (Townsend et al., 1997). Leunda et al. (2009) found a significant seasonal effect on the values of IBMWP and IASPT, and Álvarez-Cabria et al. (2010) found that IBMWP and the ICMi (the multi-metric European index equivalent to the IPtIS) were highest in autumn. Given that four of the nine samplings occurred in spring, time of sampling could potentially affect the obtained scores. However, only IASPT-2 was higher in autumn than spring, the 95% CI of the other metrics overlapped showing that seasons were overall similar. Thus, the period of sampling is unlikely to confound interpretation of the obtained results (Stark & Phillips, 2009).

Sampling method

At the site scale, number of taxa has been shown to constantly increase with increasing number of samples (Silva et al., 2016). The standard protocols for IBMWP and IPtIS demand sampling with a hand-net (width 0.25 m) over a length of one meter, corresponding roughly to an area of 0.25 m^2 per sample and 1.5 m^2 for the composite sample of the six microhabitats per site. The samples were collected with a Surber net, with a total area of 0.56 m^2 for the composite sample. Given that richness contributes to the scores of the indices, the lower sampling effort could have contributed to the overall low values obtained. However, impacted streams may require less sampling effort than reference or taxa-rich streams (Paller et al., 2020, James et al., 2022). Moreover, while comparing Surber and hand-net samplers, Burrial & Ocharan (2007) found similar results in the total number of taxa, EPT taxa, and IBMWP taxa, and it is thus possible that the different sampling methodology did not affect the results obtained.

Spatial and temporal trends of the ecological status

Multi-metric indices combine several aspects of ecosystem functioning and of ecological integrity into a holistic evaluation (Gabriels et al., 2010). The IPtIS combines richness and diversity metrics (number of taxa and Pielou's evenness), sensitivity metrics (IASPT, which in turn is based on IBMWP), and a mixture of these two (EPT taxa and abundance of selected EPTCD taxa). The geomorphological, physical, and chemical characteristics of streams change from source to mouth (Vannote et al., 1980) affecting taxa richness and taxonomic composition. Even in near pristine systems, alterations in canopy cover, current velocity and substrate particle size, water temperature, and conductivity, promote alterations in taxonomic composition of the benthic macroinvertebrate communities resulting in a decrease in taxa-dependent scores, such as the IBMWP and the IASPT indices, along the longitudinal profile (Leunda et al., 2009). The decrease of the scores from the upper to the lower reaches was thus a consequence of the natural variation combined with the cumulative effects of the several sources of perturbation along the longitudinal profile.

One of the major objectives of water monitoring efforts is to provide a temporal trend of the ecological status of running waters. The lower reaches of the stream were mainly affected by ancient channelization and leaching from agricultural fields and animal production, but these effects decreased along time due to the implementation of wastewater treatment plants at Coimbra Agriculture School. By 2013-2014 there was no direct release of animal production effluents into the stream. However, although there was an improvement in quality from 2005 to 2013, in 2014 the scores decreased. On the other hand, the upper reaches of the stream were mainly affected by urbanization and road construction (Ferreira et al., 2020), more intense between 2007 and 2013 with an increase in impervious surfaces from 12.8% to 17.2% (Ferreira et al., 2016). Thus, a decrease in quality of the upper reaches may have counterbalanced an improvement at the lower reaches. Furthermore, the winter in 2013-2014 was very rainy, with precipitation in the basin reaching peak values of 61.5 mm/day. The high precipitation and the increase in impervious surfaces resulted in flash floods and in the collapse of the bridge at site 3 in December 2013. Across the four sites sampled on this campaign, density and richness of the benthic macroinvertebrates decreased after the flood, with density recovering in April 2014 due to the enormous abundance of oligochaetes. Flash floods reduce abundance, biomass, richness, diversity, and evenness of the macroinvertebrate communities due to scouring of unstable sediments, and the effects may last 12 months, with oligochaetes and dipterans (mainly representing tolerant organisms) recovering faster than intolerant taxa (Molles, 1985). Moreover, communities of forested streams recover faster than those of agricultural streams (Li et al., 2012, Chattopadhyay et al., 2021). Thus, the decline in quality observed in the last sampling campaign was probably the result of a disrupted benthic macroinvertebrate community.

Invasion by *Potamopyrgus antipodarum*

One of the most striking patterns observed was the invasion of the stream by *P. antipodarum* (Hydrobiidae). Although the family had already been recorded during 2004 and 2005, the density increased enormously in 2009 at site 1, reaching more than 5000 individuals/m and constituting 95% of all macroinvertebrates collected. In 2013, density at site 3 was also very high (3600 individuals/m), constituting 91% of all individuals. At sites 1 to 3, the species represented 40 to 70% of all individuals collected. Even though the cumulative abundances calculated are an underestimation because sampling was irregular through the years, the trend conforms to the invasion curve described by Haubrock et al. (2022). The species establishes easily due to highly competitive ability at early stages of succession provided by the fast parthenogenic reproduction and the capacity to escape native predators and parasites, spreads fast by active and passive dispersal, and thrives in human-altered ecosystems across the world due to tolerance to a wide range of physical and chemical factors (Alonso & Castro-Díez, 2008).

P. antipodarum can colonize all substrates, including silt and sand (Richards et al., 2001) and is tolerant to organic pollution and nutrient enrichment (Alonso & Camargo, 2003). However, abundance decreased downstream with decreasing quality, with the rate of increase at sites 1 and 3 (1144 and 1001 individuals/year) much faster than the rate of increase at sites 4 (169 individuals/year) and 5 (29 individuals/year). In another Mediterranean stream, Múrria et al. (2008) found that abundance of *P. antipodarum* was higher at sites with coarse substrate and intermediate impaired conditions and that flow velocity and the effluent from a wastewater treatment plant decreased their abundance. The observations in Mediterranean streams (this study and Múrria et al., 2008) contrast with observations of increased abundances downstream of a wastewater treatment plant and a negative influence of substrate coarseness on *P. antipodarum* in other regions within the Iberian Peninsula (Álvarez-Cabria et al., 2010). The environmental variability characteristic of Mediterranean streams, namely the floods and droughts (Gasith & Resh, 1999) and the heat waves (Mouthon & Daufresne, 2015) has been hypothesized to facilitate invasion by creating niche space (Yu et al., 2020), but it seems that *P. antipodarum* cannot simultaneously cope with Mediterranean environmental fluctuations, fine substrate, and disturbance conditions.

Until 2015, there were only 9 assessments of *P. antipodarum* in Portugal (Alonso et al., 2019). The results of this study contribute new information on the presence and the trend of increase of *P. antipodarum*. The species was first detected in the stream in 2003 at very low abundances (personal observation) and cumulative abundance increased steadily from 2004 to 2014. However, the highest density found is low when compared to top values found elsewhere, for instance 18 424 individuals/m^2 in Poland (Krodkiewska et al., 2021), 35 018 individuals/m^2 in a Spanish Mediterranean stream (Múrria et al., 2008), or 98 300 individuals/m^2 in Northwest Spain (Rólan, 2004). Given the low top density and the continuous increase in cumulative density, by 2014 the population was most probably still growing. At the European level, the dynamics of invasion follow a logistic model, with N_0 = 325, an inflection point of 22.7 years, and a carrying capacity of 43 426 individuals per sample (Haubrock et al., 2022). The cumulative abundance of *P. antipodarum* at Ribeira de Covões during the 10 years perfectly fits the predictions of Haubrock et al. (2022). With the linear regression, N_0 was 372 individuals/m^2 with a rate of increase of 336 individuals/year. However, if fitted an exponential model (R^2=0.98), the rate of increase would be 0.21 individuals/year a value lower to the one obtained across Europe, but with a bigger initial population size (N_0 = 490). If these values are applied to a logistic curve with the carrying capacity of Haubrock et al. (2022), it would take 60 years (January 2064) after the first record to achieve carrying capacity, strikingly fitting the predictions of Haubrock et al. (2022).

Small high education projects for long-term biomonitoring

The engagement of students in early research activities increases confidence and motivation, spurring academic success and persistence in higher education (Graham et al., 2013). Throughout the years, academic staff often supervise several of these small research projects, whose results may not be shared as scientific knowledge because they cover a narrow spatial, seasonal, and/or temporal scale. The results of this study show that long-term data, pooled from several small individual student projects following the same protocol and supervised by an experienced professional, may provide a valuable source to inform local resource management strategies, thus filling observational voids within water quality monitoring. Moreover, the analysis of the data collected also allowed detecting and following the dynamics of colonization by the alien species *P. antipodarum*, constituting a valuable source of scientific information. The participation of students in data collection and analysis has also the advantages of lowering monitoring costs and increasing data coverage. Furthermore, because students already have background scientific knowledge, follow a protocol, and are supervised by an experienced professional scientist, collected data is reliable thus allowing reproducibility of the studies.

In conclusion, the analysis of data sets collected by students at different sites of this small catchment over 10 years proved useful to detect spatial, seasonal, and temporal trends in the biological quality of the stream. Moreover, it also provided valuable information regarding the dynamics of invasion of the stream by the exotic gastropod *P. antipodarum*. Small scale student projects can be a valuable source of information for monitoring freshwaters.

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