



Benthic macroinvertebrate assemblages detect the consequences of a sewage spill: a case study of a South American environmental challenge

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Abstract

Benthic macroinvertebrate assemblages are used to assess anthropogenic stressors and pressures globally—although considerable spatial and temporal variability in those assemblages occur naturally and in their sampling. We evaluated the effects of an untreated domestic sewage spill on the spatial–temporal occurrence, structure and dynamics of benthic macroinvertebrate assemblages, through use of occupancy probability and time series modelling. Our study produced three key results: (1) Site colonization, extirpation, and occupancy probabilities of resistant and sensitive assemblages were not influenced by the predictor variables measured before and after the spill nor between sites. (2) Over time, site occupancy for the proportion of sensitive taxa increased while the proportion of resistant taxa decreased. (3) Artificial substrates reduced natural variability more than Surber sampling of natural substrates, but macroinvertebrate family richness differed over time regardless of sampling device. We conclude that rigorous monitoring and data analyses of benthic bioindicators can be a cost-effective approach for assessing the biotic effects of sewage discharges on neotropical urban streams.

Keywords Eutrophication · Urban streams · Dynamic-occupancy modelling · Sensitive indicators · Resistant indicators · Brazil

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Introduction

Biological communities reflect the effects of multiple anthropogenic chemical, physical, and biological stressors and pressures, as well as natural environmental gradients. However, entire communities are rarely monitored because of costs and limited taxonomic expertise. Instead, taxonomic subsets of communities, i.e., macroinvertebrate or fish assemblages, are assessed. To make such assessments the development and use of analytical tools are essential to assess environmental health, separate natural and anthropogenic disturbance gradients, and to diagnose the specific stressors resulting from anthropogenic disturbances on those assemblages (Jørgensen 2007; Van Sickle and Paulsen 2008). Biological indicators facilitate the interpretation of large amounts of data from ecological units, such as forest fragments and river basins, that are used by researchers, environmental managers, and government employees to develop more effective land use policies in those ecosystems (Hering et al. 2010).

A common concern for environmental management is assessing the effects of untreated or poorly treated sewage discharges. Such discharges can be chronic, resulting in subtle chemical, physical, and biological changes (Moya et al., 2011; Mwedzi et al. 2016) or acute, resulting in fish kills (Haslouer 1979; Lee et al. 2013). Nutrient enrichment or eutrophication is a global issue caused by multiple human activities (Herlihy and Sifneos 2008; USEPA 2016; Stoddard et al. 2016) and its effects are dramatic in South American urban freshwater ecosystems (Merlo et al. 2011; Feio et al. 2015; França et al. 2019). Because it is desirable to predict potential eutrophication impacts and recovery a priori, studies should be capable of detecting the effects of excess nutrients when they are beginning, for assessing the results of pollution mitigation efforts, and for assessing the effects of effluent discharges themselves (Gál et al. 2019; Mor et al. 2019; Edegbene et al. 2020).

Several biological tools have been developed for assessing the effects of sewage discharges. The biological monitoring working party (BMWP) index, based on water-quality tolerance values for biological taxa, was proposed by the Biological Monitoring Working Party (1978) and revised in 1980 (National Water Council 1981) in England. It has been widely adapted (Alba-Tercedor 1996, Spain; Junqueira et al. 2000, Brazil; Roldan 2003, Colombia; Sedeno-Diaz et al. 2012, Costa Rica; Kazanci et al. 2016, Turkey). The BMWP requires only family-level taxonomic precision, which saves time and money, and facilitates monitoring in urban streams (França et al. 2019) where anthropogenic pressures and stressors such as eutrophication are often severe. The family level HBI (Hilsenhoff Biotic Index) was developed for similar reasons

(Hilsenhoff 1988). However, others seeking greater ecological insights have proposed taxonomic diversity and evenness indices (Shannon 1948; Pielou 1966), assemblage structure metrics (Lenat and Penrose 1996), multi-metric indices (Karr and Chu 1999), and functional group indices (Cummins et al. 2005).

Studies of anthropogenic effluents are common in the scientific literature (e.g., Pfeifer and Bennett 2011; Journey et al. 2018; Matej-Lukowicz et al. 2020; Paulsen et al. 2020). However, studies focused on a single taxon or assemblage of interest often interpret the failure to detect that taxon or assemblage as a true absence (MacKenzie et al. 2018). This is problematic given that estimates of true presence or occurrence of a taxon or assemblage can be biased by low detection probabilities (rare members), poor sampling methods, and insufficient sampling effort (Li et al. 2001, 2014; Cao et al. 2002; MacKenzie et al. 2018). Thus, not accounting for imperfect detection may lead to spurious results and misleading conclusions about factors influencing the taxon or assemblage of interest (MacKenzie et al. 2018). Recent studies have focused on detection probability estimation to improve presence or occupancy estimates for individual taxonomic groups (Cortelezzi et al. 2017, 2018, 2020). However, less attention has been given to assemblage inventory studies, especially how anthropogenic disturbances change taxa occurrence or assemblage persistence and resilience.

Occupancy models account for imperfect detection and false absences by modelling taxa detection probability as a function of methodological issues. The occupancy modelling approach is very flexible and can be used not only to model changes in the occupancy state through time at the taxon-level (e.g., Cortelezzi et al. 2017), but also to model those changes at an assemblage level (see MacKenzie et al. 2018; pp. 558–580). Also, this approach allows including taxa that were not detected at any of the study units but that had the probability of being present based on previous studies or taxa distribution maps, which is important to account for imperfect detection and false absences (MacKenzie et al. 2018; p. 564). Thus, such models generate unbiased estimates of such indicators as taxa occupancy or richness (MacKenzie et al. 2003). Also, with occupancy modelling it is possible to evaluate taxa colonization and extirpation probabilities over time as a function of anthropogenic disturbances during that period (MacKenzie et al. 2003). Higher (or unaltered) probability of colonization in a location by assemblages after an anthropogenic impact can indicate greater resilience. On the other hand, lower (or unaltered) probability of extirpation in a location by assemblages after an anthropogenic impact can indicate greater persistence (MacKenzie et al. 2003).

In this study, we used a set of analytical and ecological tools to assess the effects of a sewage spill on benthic macroinvertebrates in a southeastern Brazil stream.

To do so, we had the following three objectives: (1) Through time-series analyses, we sought to determine which of several commonly used sampling methods and benthic macroinvertebrate indicators were most sensitive for detecting the effects of the spill. We predicted that the BMWP and multimetric indices (MMIs) would best detect the spill effects because the BMWP was designed for assessing pollution and MMIs incorporate multiple components of assemblage structure, composition and function. In addition, we predicted that passive colonization samplers would be less variable than Surber samplers but that Surber sampling would yield greater numbers of individuals and taxa, because colonization samplers minimize habitat variability. (2) We wanted to determine the effects of an ~6-month sewage spill on the spatial-temporal occurrence of benthic macroinvertebrate assemblages. We expected higher occupancy of the resistant assemblage than the sensitive assemblage at the spill site when compared to the upstream site and that occupancy would be influenced by the predictor variables measured at the sites. (3) Finally, we wanted to evaluate the degree to which the type of sampling method (passive versus active) and season influenced detection probability of the resistant and sensitive assemblages. We expected that Surber samples would have greater detection probability, because they sample more habitat types. We also expected that the detection probability would be lower in the rainy season, because of freshets and increased macroinvertebrate drift.

Methods

Study area

From 2006 to 2015, we sampled two sites in Guritá stream, which is located near the city of Ouro Branco at the south end of the Espinhaço Range, in a transitional area between the Cerrado and Atlantic Forest biomes, southeastern Brazil. The landscape is dominated by grasslands, pastures, and forest fragments of different sizes. The climate type is mesothermic (Cwb) according to Köppen's classification with dry winters, wet summers, and mild air temperatures (20.7 °C average annual air temperature, 1200 mm average annual precipitation).

The two sites were on the same second-order stream (3 km apart), inside forest fragments at an altitude of 900 m a.s.l. and the stream was >90% shaded by riparian vegetation. Our reference site (P1; 20° 32' 55.1"S, 43° 43' 08.7"W) was located upstream of a peri-urban area and had >90% natural cover and no human pressures in its catchment. Our spill site (P2; 20° 31' 43.2"S, 43° 45' 47.1"W) was located downstream of that area and normally received treated industrial effluents and some urban run-off, but was upstream of Ouro Branco's treated sewage discharge point (Fig. 1). Regarding the physico-chemical characteristics (Table S3), the water at the spill site was enriched when compared to the reference site: both total N: 0.7 (\pm 2.3) mg L⁻¹ versus 0.1 (\pm 0.1) mg L⁻¹; total P: 329.3 (\pm 259.3) μ g L⁻¹ versus 10.0 (\pm 14.7) μ g L⁻¹ were higher. The sites had similar mean widths (2.5 \pm 0.4 m), depths (0.35 \pm 0.15 m) and current velocities (0.2 \pm 0.1 m s⁻¹ versus 0.5 \pm 0.2 m s⁻¹). Although

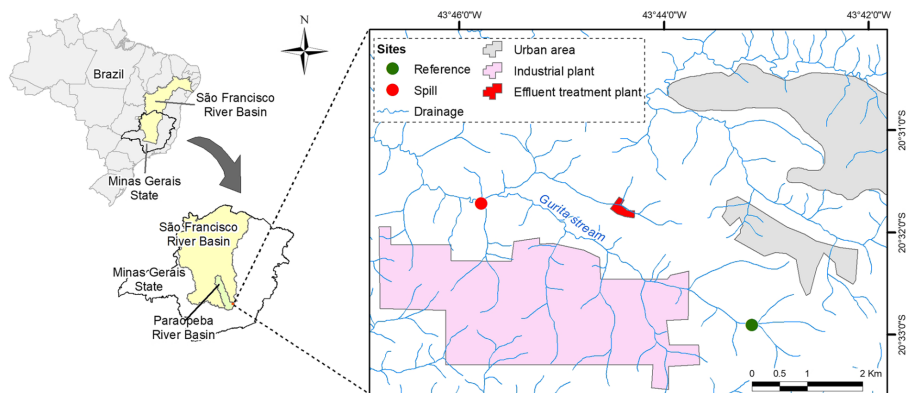


Fig. 1 Study location

normally the spill site would receive treated sewage, an untreated sewage spill occurred from November 2008 to April 2009 because of a broken pipe at this spill site (Fig. 1).

Environmental sampling

We sampled both water quality and physical habitat conditions. To assess physical habitat structure at each site we used a qualitative habitat index (Callisto et al. 2002) adapted from US-EPA (1987) and Hannaford et al. (1997). It was based on 22 variables incorporating instream physical habitat complexity, hydromorphological features, and riparian and sub-basin conditions (Table S1). We also measured several water quality measures recommended by Brasil (2005) for assessing urban waters. We measured pH and conductivity ($\mu\text{S}/\text{cm}$) through use of a multimeter (YSI Multiprobe). Water samples were placed in a cooler and taken to the laboratory where dissolved oxygen (mg/L^{-1}), total alkalinity ($\text{mEq}/\text{L CO}_2$), and total phosphorus (mg/L^{-1}) were determined via standard methods (APHA 1998) (see Table S3). As a preliminary analysis, we ran a time-interrupted series analysis (McDowall et al. 2019), using the sewage spill as the interrupting variable. Among these, only the qualitative habitat index was significantly altered by the spill, suggesting that the physical habitat structure was significantly altered by the spill. These analyses were made using the *its* analysis package (English 2019) in R (R Core Team 2015).

Macroinvertebrate sampling

We collected macroinvertebrates in three ways from both stream sites. We used Surber samplers in both the dry and wet seasons, but for differing numbers of months because of logistical constraints, for a total of 63 site-visits from 2006 to 2015. The three Surber samples (30×30 cm, 250 μm mesh) taken in each site-visit were individually returned to the lab for processing (totaling 378 Surber samples). In addition, from 2008 to 2015 in 38 dry season months, we employed two different artificial substrates (38 composite samples for each). We secured 25 pebble-filled mesh bags ($20 \text{ cm} \times 30 \text{ cm}$, 2 cm mesh size) each containing approximately 25 pebbles (~ 2 kg) into the stream bed. We also secured 200 g of hollow plastic cylinders (hereafter, curlers) in each of 25 mesh bags ($20 \text{ cm} \times 30 \text{ cm}$, 2 cm mesh size) into the stream bed. Both artificial substrates were incubated for 30 days prior to recovery. In each of the five dry season months (April to August) the contents of each bag and the bag itself were separately placed in a container and returned to the laboratory. The two types of artificial substrates were not used in the wet season because of their high probability of loss and difficulty of retrieval during bank-full flows (water discharge and water depth often increased over 20 times in the rainy season compared to the dry season).

In the laboratory, all three types of samples were emptied separately into a sorting tray. The pebbles and curlers were cleaned with fresh water and a small paint brush and the bags were washed with water. Both the sample bag and individual pebbles and curlers were examined for invertebrates before being removed from the sorting tray. Likewise, the Surber samples were placed in a sorting tray. The contents of the sorting tray (water and substrate) were then rinsed through a sieve (250 μm mesh), and all invertebrates were removed with forceps and placed into glass vials, containing 70% ethanol for later identification. Specimens were identified to family under a stereomicroscope through use of taxonomic keys (Pérez 1988; Merritt and Cummins 1996; Fernández and Domínguez 2001; Costa et al. 2006; Mugnai et al. 2010). Family-level identification is considered an efficient, easy-to-use, reliable rapid assessment method for researchers with insufficient taxonomic knowledge to monitor tropical streams that have high macroinvertebrate diversity (Godoy et al. 2019; Ligeiro et al. 2020).

The benthic macroinvertebrates were classified into resistant or sensitive families, based on Junqueira et al. (2000). Families scored from 1 to 3 were classified as resistant, those scored 8 to 10 were classified as sensitive, and the rest were classified as tolerant. For this study we focused on resistant and sensitive taxa because they are expected to have a clearer response to environmental changes (Rosenberg and Resh 1993; Karr and Chu 1999; Stoddard et al. 2008).

Data analyses

Because seasonal disturbances can have a pivotal role in shaping lotic ecosystem processes and structure (Linares et al. 2013; Müller et al. 2016), we ran preliminary analyses to assess its effects. We first evaluated which of the 27 benthic macroinvertebrate indicators for the 63 site-visits collected by Surber sampling showed significant difference between dry and wet seasons (Table S2). We checked for seasonal influence to decide whether we should include dry and wet periods as an additional predictor variable in subsequent analyses and thus, avoid biased estimates and potential misleading conclusions. To assess seasonal effects, we used R (R Core Team 2015) to run generalized linear models (GLM's) with a Poisson or Gaussian distribution, depending on the response variable (the R script is available in Table S3). Then, we tested model significance by an Analysis of Deviance. We chose four different types of widely used indicators (e.g., Cairns and Pratt 1993; Thorne and Williams 1997; Linares et al. 2019). (1) Taxonomic composition variables included taxa richness, EPT (Ephemeroptera, Plecoptera, Trichoptera) richness, % EPT, EPT abundance, % Chironomidae, Chironomidae abundance, % chironomids + oligochaetes, chironomid + oligochaete abundance, and BMWP (Junqueira et al. 2010). (2) Assemblage

structure indicators included total abundance, Jaccard evenness, Pielou evenness, and Shannon–Wiener diversity. (3) Multimetric indices included those of Ferreira et al. (2011), Macedo et al. (2016), and Silva et al. (2017), each of which had been developed for Minas Gerais streams. (4) Functional composition indicators consisted of % collector-gatherers, collector-gatherer abundance, % scrapers to shredders + collectors, % filtering collectors to gathering-collectors, % scrapers + filtering-collectors to shredders + gathering-collectors, % shredders to filtering + gathering-collectors, and % predators, as recommended by Cummins et al. (2005). We found significant differences between dry and wet seasons, especially in variables affected by abundance (Table S2). Total abundance, collector-gatherer abundance, % collector-gatherers, EPT abundance, Chironomidae abundance, and chironomid + oligochaete abundance were significantly higher in the dry season than in the wet season ($p < 0.05$). Therefore, we accounted for seasonal influence in subsequent analyses.

To test which metrics were most capable of detecting the biological effects of the spill through time (objective 1), we ran a time-interrupted series analysis (McDowall et al. 2019). To do so we used each of the 27 biological metrics (see Table 1) as a response variable and the November 2008 to April 2009 spill as the interrupting variable. We ran the analysis each time for the three different sampling methods (63 Surber site visits; 38 curler and pebble site visits) and added Sampling Site as a covariate. Then, we estimated the proportion of variation of each response variable (i.e., effect size; η^2) that was explained by the interrupting variable by dividing the sum of squares of the predictor variable (i.e., the interrupting variable) by the total sum of squares of each time-interrupted series analysis. It resulted in the proportion of variation of each response variable that was explained by the spill presence. These analyses were made using the *its* analysis package (English 2019) in R (R Core Team 2015).

To evaluate the effects of an ~6-month spill on the spatial–temporal occurrence (or occupancy) of benthic macroinvertebrate assemblages (objective 2), we used a multi-season occupancy approach (MacKenzie et al. 2003). This approach allowed us to model changes in site occupancy of the two assemblages (resistant or sensitive) between sampling seasons as a function of environmental site (upstream or spill) conditions. Then, to evaluate whether changes in site occupancy were likely to occur among months, we defined sampling ‘season’ as a month (MacKenzie et al. 2003).

The initial occupancy probability (Ψ_i) was the probability that a site was occupied by an assemblage in the first month (MacKenzie et al. 2003). The dynamic processes driving changes in assemblage occupancy at a site between consecutive months are its colonization and extirpation. Colonization (γ) is the probability that an unoccupied site in a given month t is occupied by families of an assemblage in month

$t + 1$. Extirpation (ϵ) is the probability that a site occupied in month t is unoccupied by members of an assemblage in month $t + 1$ (MacKenzie et al. 2003). Among-month persistence is the probability of the site being occupied by members of an assemblage in successive months; thus, persistence is the complement of extirpation probability ($1 - \epsilon$) (MacKenzie et al. 2003). The occupancy probability estimates for the other months can be derived by the following formula from MacKenzie et al. (2003):

$$\Psi_{t+1} = \Psi_t(1 - \epsilon_t) + (1 - \Psi_t)\gamma_t \quad (1)$$

This approach also allowed us to account for imperfect detection when estimating the occupancy-related parameters by modelling detection probability and thus, to evaluate the degree to which the type of sampling method (passive versus active) and season influenced detection probability of the resistant and sensitive assemblages (objective 3). Detection (p) is the probability of detecting members of an assemblage at a site i within each month, given the site is occupied by these members.

We used a subset of the water quality variables recommended by Brasil (2005) for assessing pollution as predictor variables in our models. For screening these variables, we first tested for correlation among our predictor variables and found that only pH and alkalinity were highly correlated ($|r| > 0.70$; Stoddard et al. 2008; Dormann et al. 2013; Table S5). Because alkalinity and pH were correlated, we retained pH because it is known to limit taxa persistence and is more easily, accurately and precisely measured than alkalinity. Therefore, we considered monthly measurements (during each visit) of conductivity, pH, dissolved oxygen, total phosphorus and physical habitat structure. We then evaluated the degree to which predictor variables influenced initial occupancy, colonization, and extirpation probabilities of the resistant and sensitive assemblages.

The predictor variables were modelled by using their first month values for initial occupancy, and then their changes between month t and month $t + 1$ for modelling colonization and extirpation parameters. For example, both latter parameters were modelled for pH as the result of the pH change between month t and month $t + 1$ (i.e., $\Delta\text{pH} = \text{pH}_t - \text{pH}_{t+1}$). Finally, we modelled detection probability as a function of our three different sampling methods (Surber, curlers, pebbles) and as a function of the dry and wet periods because higher dry season abundances may have increased detection probability.

We assessed detections for 63 Surber-sampled months and 38 passive-sampled months to determine detection histories for each taxon in each month. We had a limited number of sites (2) and, therefore, we could not model changes in the occupancy state through time for any family separately. Also, we were especially interested in evaluating the degree

to which changes in the predictor variables influenced colonization, extirpation, and occupancy probabilities of the resistant and sensitive assemblages (i.e., assemblage-level change) in the months following the spill. Therefore, we used macroinvertebrate family as an ‘assemblage subunit’ for each site (MacKenzie et al. 2018; pp. 559–560). Specifically, we recorded whether a family member of a resistant or sensitive assemblage was detected (1) or not (0) at either the upstream or spill site for each method in each month. This approach allowed us to model the parameters of interest by considering the site-level variables that changed among months and thereby compare assemblage-level site occupancy among months and between sites.

We also added families that were not detected by us, but that had the potential to be present in our sites according to previous studies in the region (Abelho et al. 2010; Boyero et al. 2011). Thus, we added three and eight families in the resistant and sensitive family list, respectively (see Table S4). This strategy allowed us to account for possible false absence families and to estimate the proportion of families from a list of known size that could occupy the sites during the study (MacKenzie et al. 2018).

We employed the multi-season occupancy model (MacKenzie et al. 2003) in Program MARK (White and Burnham 1999) for analyzing the data separately for the resistant and sensitive assemblages, but used month instead of season. We built 18 models for each assemblage consisting of our *a priori* hypothesis for initial occupancy (Ψ_i), colonization (γ), extirpation (ϵ), and detection (p) probabilities. The intercept-only model structure or the null model [$\Psi_i(.) \epsilon(.) \gamma(.) p(.)$], which implies that no predictor variables influenced the model parameters of interest, was also included in each of the model sets. Using Akaike’s Information Criterion adjusted for small sample size (AICc), we considered models with $\Delta\text{AICc} \leq 2$ as the most likely to be supported by our data (Burnham and Anderson 2002). We used the maximum likelihood methods incorporated in Program MARK to obtain estimates of occupancy, colonization, extirpation and detection of the resistant and sensitive macroinvertebrate assemblages among months and sites (MacKenzie et al. 2003). For example, an encounter history with only two subsequent months, such as $h_1 = 101,000$, means that a family present at the site in the first month was detected by the first and third sampling method, but not detected by the second method. Between months, the family was not extirpated but was never detected in the second month, or was extirpated. That mathematically is as follows:

$$\text{Probability}(h_1 = 101000) = \psi_i p_{1,1} (1 - p_{1,2}) p_{1,3} \times \left\{ (1 - \epsilon_1) \prod_{j=1}^3 (1 - p_{2,j}) + \epsilon_1 \right\} \quad (2)$$

Then, as proposed by MacKenzie et al. (2003), the model likelihood was calculated according to the equation below, which considered the encounter histories of all families in either the resistant or sensitive assemblage during the 63 months, and where the parameter values that maximize the likelihood could be obtained by the following:

$$L(\psi_i, \epsilon, \gamma, p | h_1, \dots, h_N) = \prod_{i=1}^N \text{Probability}(h_i) \quad (3)$$

Results

We detected a total of nine resistant families, seven at the reference site and nine at the spill site; conversely, we detected five sensitive families at the reference site and two at the spill site (see Table S4). Sampling method affected which biological variables were most capable of distinguishing differences in macroinvertebrate assemblages resulting from the spill (Table 1). No differences were observed for most metrics: only 7.44% of the metrics for Surber, 29.62% for curlers and 18.51% for pebbles showed significant results. Surber sampling was effective for family richness and the BMWP index. Both curlers and pebbles captured the discontinuity for shredders/collectors and % scrapers + collectors. Curlers also distinguished discontinuity in total abundance and % resistant assemblage abundance. Pebbles distinguished discontinuity in the BMWP index and EPT richness. In other words, two methods detected discontinuity in family richness, shredders/collectors, % scrapers and filtering-collectors, BMWP index, and EPT richness. The interrupted time series analyses also confirmed the results of our preliminary analysis, showing that abundance variables based on Surber sampling varied significantly between dry and wet seasons (Table S2).

Contrary to our prediction, no predictor variable that we measured influenced the occupancy-related parameters for the resistant or sensitive assemblages (Table 2). Thus, the variables that we measured did not influence the colonization, extirpation or occupancy probabilities of those assemblages between sites and the sewage spill did not influence those model parameters months after the initial spill. However, the estimates of the occupancy-related parameters of those assemblages differed (Fig. 2). Also, and contrary to our prediction, the proportion of families

Table 1 Interrupted time series analysis results for all variables using the three sampling methods

Variable	Surber			Curlers			Pebbles		
	F	Pr(<F)	ES	F	Pr(<F)	ES	F	Pr(<F)	ES
Scrapers/Shredders + Collectors	0.19	0.66	0.003	0.29	0.59	0.004	0.31	0.58	0.004
Filtering-collectors/Gathering-collectors	1.52	0.22	0.006	0.47	0.00	0.35	0.46	0.01	0.08
Shredders/Collectors	0.42	0.52	0.02	47.51	0.49	0.006	6.66	0.50	0.006
% Scrapers + Collectors	0.64	0.43	0.009	48.37	0.00	0.35	6.179	0.02	0.08
% Predators	0.00	0.96	>0.001	0.88	0.35	0.01	0.08	0.78	0.001
MMI (Ferreira et al. 2011)	0.65	0.42	0.008	1.23	0.27	0.01	1.54	0.22	0.02
MMI (Macedo et al. 2016)	0.14	0.71	0.002	0.31	0.578	0.003	0.59	0.45	0.006
MMI (Silva et al. 2017)	1.67	0.20	0.02	0.58	0.45	0.007	0.63	0.43	0.005
Abundance	1.06	0.31	0.01	12.81	0.00	0.15	2.04	0.16	0.03
Family Richness	7.32	0.01	0.08	2.95	0.09*	0.02	3.96	0.05*	0.05
H' Shannon–Wiener	1.26	0.27	0.02	0.11	0.74	0.001	0.20	0.65	0.002
J' evenness	0.06	0.80	>0.001	0.05	0.82	>0.001	0.00	0.97	>0.001
P' evenness	0.15	0.70	0.002	0.03	0.87	>0.001	0.00	0.97	>0.001
BMWP	5.74	0.02	0.07	2.12	0.15	0.02	5.83	0.02	0.07
% Collector-gatherers	0.11	0.74	0.002	3.07	0.08*	0.04	1.31	0.26	0.02
Collector-gatherer abundance	0.89	0.35	0.01	1.37	0.25	0.02	0.09	0.77	0.001
% Chironomidae	0.54	0.47	0.007	2.04	0.16	0.03	0.21	0.64	0.003
Chironomidae abundance	0.92	0.34	0.01	1.40	0.24	0.02	0.11	0.74	0.001
% EPT	0.017	0.90	>0.001	0.23	0.63	0.003	0.43	0.52	0.005
EPT abundance	0.58	0.455	0.007	0.22	0.64	0.003	1.54	0.22	0.02
EPT richness	0.62	0.43	0.007	3.64	0.06*	0.03	4.35	0.04	0.04
% Chironomids + Oligochaetes	0.18	0.67	0.002	2.98	0.09*	0.04	1.03	0.31	0.01
Chironomid + Oligochaete abundance	0.89	0.35	0.01	1.35	0.25	0.02	0.09	0.77	0.001
% Abundance Resistant	0.01	0.94	>0.001	4.97	0.03	0.06	0.94	0.34	0.01
% Richness Resistant	0.87	0.35	0.01	0.07	0.79	>0.001	1.90	0.17	0.02
% Abundance Sensitive	0.41	0.52	0.005	0.58	0.45	0.006	0.65	0.42	0.008
% Richness Sensitive	0.66	0.42	0.007	0.32	0.58	0.002	0.01	0.91	>0.001

Effect Size (ES; η^2) was calculated for all variables

Bold indicates $p < 0.05$, * indicates $p < 0.1$

that occupied the sites increased for the sensitive assemblage but decreased for the resistant assemblage. The colonization and extirpation probabilities for the resistant assemblage were 0.04 (SE = 0.01) and 0.13 (SE = 0.02), respectively, leading to a slightly decreasing occupancy probability (Fig. 2). On the other hand, the colonization and extirpation probabilities of the sensitive assemblage were 0.004 (SE = 0.002) and 0.03 (SE = 0.01), respectively, leading to a slightly increasing occupancy probability for the sensitive assemblage (Fig. 2). Also, the proportion of families that occupied the sites during our study was very low compared to the regional list of resistant and sensitive families. The average occupancy probability was 0.24 (range = 0.23–0.32) for the resistant assemblage and 0.11 (range = 0.08–0.13) for the sensitive assemblage.

The detection probability of the resistant assemblage was higher for Surber sampling ($p = 0.73$; SE = 0.04) than curlers ($p = 0.64$; SE = 0.04) or pebbles ($p = 0.59$; SE = 0.04), but did not differ substantially between the dry and wet periods. Conversely, the detection probability of the sensitive assemblage was not influenced by any of the three sampling methods, but it did differ between the dry and wet periods. Nonetheless, the null (i.e., intercept only) model was also supported by our data, meaning that the dry and wet season effect on detection probability of the sensitive assemblage was weak. In fact, the model-averaged estimates of detection probability of the sensitive assemblage between the seasons were very similar: 0.24 (SE = 0.05) and 0.26 (SE = 0.03) for the wet and dry seasons, respectively.

Table 2 Model selection results for initial occupancy (Ψ_i), colonization (γ), extirpation (ϵ), and detection (p) probabilities of resistant and sensitive aquatic macroinvertebrates

Model	AICc	Δ AICc	AICc Weights	Num. Par	Deviance
Resistant taxa					
$\{\Psi_i(.)\epsilon(.)\gamma(.)p(\text{Method})\}$	1393.94	0.00	0.45	6	1381.89
$\{\Psi_i(P)\epsilon(P)\gamma(P)p(\text{Method})\}$	1396.06	2.12	0.16	9	1377.94
$\{\Psi_i(C)(C)\gamma(C)p(\text{Method})\}$	1397.33	3.39	0.08	9	1379.21
$\{\Psi_i(\text{DO})\epsilon(\text{DO})\gamma(\text{DO})p(\text{Method})\}$	1397.67	3.73	0.07	9	1379.55
$\{\Psi_i(.)\epsilon(.)\gamma(.)p(.)\}$	1398.25	4.31	0.05	4	1390.22
$\{\Psi_i(\text{PHS})\epsilon(\text{PHS})\gamma(\text{PHS})p(\text{Method})\}$	1398.34	4.40	0.05	9	1380.22
$\{\Psi_i(\text{PH})\epsilon(\text{PH})\gamma(\text{PH})p(\text{Method})\}$	1398.70	4.76	0.04	9	1380.58
$\{\Psi_i(.)\epsilon(.)\gamma(.)p(\text{Season})\}$	1399.40	5.45	0.03	5	1389.36
$\{\Psi_i(P)\epsilon(P)\gamma(P)p(.)\}$	1400.86	6.92	0.01	7	1386.79
$\{\Psi_i(P)\epsilon(P)\gamma(P)p(\text{Season})\}$	1401.71	7.77	0.01	8	1385.62
$\{\Psi_i(C)\epsilon(C)\gamma(C)p(.)\}$	1401.76	7.82	0.01	7	1387.69
$\{\Psi_i(\text{DO})\epsilon(\text{DO})\gamma(\text{DO})p(.)\}$	1401.85	7.91	0.01	7	1387.77
$\{\Psi_i(C)\epsilon(C)\gamma(C)p(\text{Season})\}$	1402.78	8.84	0.01	8	1386.68
$\{\Psi_i(\text{PHS})\epsilon(\text{PHS})\gamma(\text{PHS})p(.)\}$	1402.86	8.92	0.01	7	1388.78
$\{\Psi_i(\text{DO})\epsilon(\text{DO})\gamma(\text{DO})p(\text{Season})\}$	1403.01	9.06	0.00	8	1386.91
$\{\Psi_i(\text{PH})\epsilon(\text{PH})\gamma(\text{PH})p(.)\}$	1403.34	9.40	0.00	7	1389.26
$\{\Psi_i(\text{PHS})\epsilon(\text{PHS})\gamma(\text{PHS})p(\text{Season})\}$	1403.95	10.01	0.00	8	1387.85
$\{\Psi_i(\text{PH})\epsilon(\text{PH})\gamma(\text{PH})p(\text{Season})\}$	1404.15	10.21	0.00	8	1388.05
Sensitive taxa					
$\{\Psi_i(.)\epsilon(.)\gamma(.)p(.)\}$	515.27	0.00	0.35	4	507.25
$\{\Psi_i(.)\epsilon(.)\gamma(.)p(\text{Season})\}$	516.89	1.62	0.16	5	506.85
$\{\Psi_i(\text{PHS})\epsilon(\text{PHS})\gamma(\text{PHS})p(\text{Season})\}$	518.47	3.20	0.07	8	502.38
$\{\Psi_i(C)\epsilon(C)\gamma(C)p(\text{Season})\}$	518.67	3.40	0.06	8	502.58
$\{\Psi_i(\text{PH})\epsilon(\text{PH})\gamma(\text{PH})p(\text{Season})\}$	518.69	3.42	0.06	8	502.61
$\{\Psi_i(.)\epsilon(.)\gamma(.)p(\text{Method})\}$	519.03	3.76	0.05	6	506.98
$\{\Psi_i(\text{DO})\epsilon(\text{DO})\gamma(\text{DO})p(\text{Season})\}$	519.09	3.82	0.05	8	503.01
$\{\Psi_i(P)\epsilon(P)\gamma(P)p(\text{Method})\}$	520.07	4.79	0.03	9	501.96
$\{\Psi_i(P)\epsilon(P)\gamma(P)p(.)\}$	520.27	5.00	0.03	7	506.20
$\{\Psi_i(\text{PH})\epsilon(\text{PH})\gamma(\text{PH})p(\text{Method})\}$	520.89	5.62	0.02	9	502.78
$\{\Psi_i(\text{PHS})\epsilon(\text{PHS})\gamma(\text{PHS})p(.)\}$	521.18	5.91	0.02	7	507.11
$\{\Psi_i(C)\epsilon(C)\gamma(C)p(\text{Method})\}$	521.24	5.97	0.02	9	503.13
$\{\Psi_i(P)\epsilon(P)\gamma(P)p(\text{Season})\}$	521.30	6.03	0.02	8	505.21
$\{\Psi_i(\text{DO})\epsilon(\text{DO})\gamma(\text{DO})p(\text{Method})\}$	521.50	6.23	0.02	9	503.39
$\{\Psi_i(\text{PH})\epsilon(\text{PH})\gamma(\text{PH})p(.)\}$	521.90	6.63	0.01	7	507.83
$\{\Psi_i(C)\epsilon(C)\gamma(C)p(.)\}$	522.23	6.96	0.01	7	508.16
$\{\Psi_i(\text{DO})\epsilon(\text{DO})\gamma(\text{DO})p(.)\}$	522.61	7.33	0.01	7	508.54
$\{\Psi_i(\text{PHS})\epsilon(\text{PHS})\gamma(\text{PHS})p(\text{Method})\}$	524.28	9.01	0.00	9	506.17

Ψ_i , γ and ϵ modelled as functions of phosphorus (P), conductivity (C), dissolved oxygen (DO), pH, and physical habitat structure (PHS). p modelled as function of method type (Method: Surber, Curbers, Pebbles) and dry and wet seasons

Discussion

Sampling methods and metrics

The results of our preliminary analyses showed that abundance-related metrics varied significantly between dry and wet seasons (Table S2), with significantly higher values in the dry season. Therefore, we recommend that future neotropical biomonitoring projects invest less on rainy and dry

season sampling (Feio et al. 2015; Fierro et al. In Press). Instead, we recommend that available resources be used to sample more sites and more stations over more years to detect spatial and temporal trends and reduce sampling variance (Larsen et al. 2004; Hughes and Peck 2008; Callisto et al. 2019). Furthermore, because of lower accessibility of some sites during the wet season and crew safety, some sites are best sampled in the dry season (Hughes and Peck 2008). Besides, during the end of the dry season, most insect

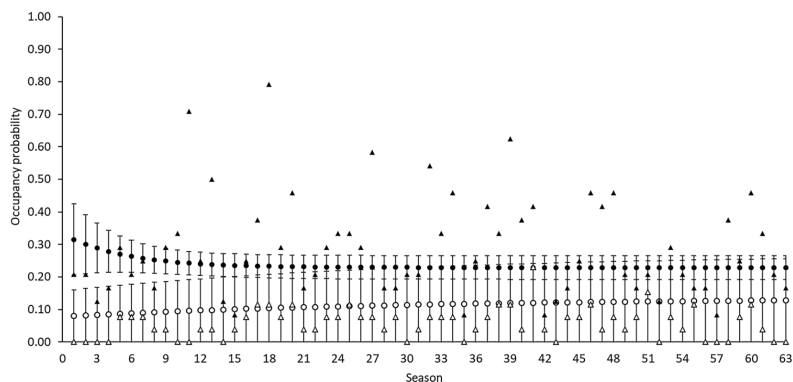


Fig. 2 Probability of occupancy (\pm SE) of the resistant (black dots) and sensitive (white dots) assemblages of aquatic macroinvertebrates sampled in 63 months (2006–2015). The sewage spill occurred from November 2008 to April 2009 (month or season 13–16). The occupancy probability of each assemblage is from the most parsimonious model of Table 2 and the black and white triangles are the naïve occupancy for the resistant and sensitive assemblages, respec-

tively. The naïve occupancy did not account for imperfect detection and related to the number of families of each assemblage that we observed at each site in each month divided by the total number of families of each assemblage. Because differences between sites were not important, we calculated the naïve occupancy for each assemblage by averaging the naïve occupancy between sites

taxa are close to emerging, meaning that individuals will be larger and easier to detect and identify (Martins et al. 2017; Agra et al. 2018; Castro et al. 2018, 2020; Ligeiro et al. 2013). Last, during the rainy season, macroinvertebrate drift dislodges many organisms downstream (Callisto and Goulart 2005; Castro et al. 2013a, b), resulting in lower abundances, whereas the lower dry season flows are more likely to be impaired by pollution discharges.

Our predictions from objective 1 were only partially supported (Table 1). None of the three MMIs detected differences in macroinvertebrate assemblages resulting from the sewage spill as predicted, but the single metrics (BMWP, shredders/collectors, % scrapers + collectors and EPT richness) detected differences for two sampling methods. Family richness detected differences for all three sampling methods, whereas abundance, % collector-gatherers, % chironomids + oligochaete and % resistant taxa abundance did so for one method. Family richness was the most successful metric and it is a widely used indicator alone and in MMIs (Ruaro et al. 2020). EPT richness was responsive with two sampling devices. Stoddard et al. (2008) found that EPT richness was an effective indicator of disturbance in seven of nine USA ecoregions. The curlers and pebbles employed only in the dry months detected discontinuity in shredders/collectors and % scrapers + collectors. Thus, we assume that focusing on season and artificial substrates removes some natural variability from macroinvertebrate sampling, thereby improving the usefulness of functional group indicators. However,

Ligeiro et al. (2020) found that single-habitat sampling (leaf packs) reduced macroinvertebrate assemblage responsiveness along a disturbance gradient.

There are advantages and disadvantages to using artificial substrate samplers. Letovsky et al. (2012) found that artificial substrate sampling produced lower abundances, species richness, evenness, and diversity. Nonetheless, Guild et al. (2014) recommended artificial substrates for sampling water quality impacts on aquatic macroinvertebrates. However, they also reported that artificial substrate samplers were biased toward colonizing taxa. In addition, artificial substrate samplers require at least two site visits and are susceptible to anthropogenic and natural disturbances (freshets, droughts) if not carefully placed. For both artificial substrates that we used, we found significant differences in metrics that serve as proxies for ecosystem functions. Artificial substrate samplers have been reported to provide results more sensitive to disturbances in water quality than Surber samplers (Letovsky et al. 2012; Guild et al. 2014). Our results corroborate this, suggesting that the artificial substrates provided data sensitive enough to detect water quality differences caused by the sewage spill that would have been missed by Surber sampling. Although sampling device choice depends on the study objectives, logistics, and costs (Hughes and Peck 2008), we recommend using artificial substrates rather than Surber samplers for assessing sewage effects on benthic macroinvertebrates. Besides, we specifically recommend that routine biomonitoring programs,

either by State Agencies or Private Companies, should include the use of artificial substrates in their monitoring.

Although the sewage spill did not affect the long-term occupancy of the families measured by the occupancy models, its effects did disrupt several metrics, such as taxa richness, which is analogous to occupancy probability here. Occupancy can be viewed as the proportion of families within each of the assemblages that occupied the sites throughout the 10 years of sampling. However, we are unsure if the effects revealed by the time-interrupted series analysis resulted from anthropogenic disturbances or natural disturbances (freshets, droughts, fires) that were not considered in this analysis. Also, the effects on total family richness of imperfect detection, colonization, and extirpation of the families during this study and others deserves further investigation. In addition, detection differences indicate the need for rigorous site-scale sampling (Li et al. 2001, 2014; Cao et al. 2002; Hughes and Peck 2008) and sample processing (Li et al. 2014; Ligeiro et al. 2020) of macroinvertebrates.

Occupancy probabilities

Our assessment revealed that site colonization, extirpation, and occupancy probabilities of resistant and sensitive assemblages were not influenced by the predictor variables measured before and after a sewage spill nor between sites, contrary to what we predicted (objective 2). However, the Surber sampler was more efficient in detecting the resistant assemblage than the curlers or pebbles as predicted (objective 3). This can be explained by the association of the most abundant families of this category, Chironomidae and Tubificidae, with soft substrates (White 2014; Corbi and Trivinho-Strixino 2017). Therefore, these families would have had more difficulty in colonizing the hard-artificial substrates. A possible explanation is the lower amounts of fine particulate organic matter (FPOM) retention on the artificial substrates compared to the streambed because resistant taxa are collector-gatherers that feed on FPOM (Cummins et al. 2005; Callisto and Graça 2013; Canning et al. 2019). However, the probability of detecting the sensitive assemblage did not differ among methods, even though this probability was slightly higher in the dry period. Although the seasonal effect on detection of sensitive families was weak, our strength of inference was hampered by the fact that we sampled only two stream sites.

The overall persistence ($1 - \epsilon$) of benthic macroinvertebrates was higher for the sensitive assemblage than for the resistant assemblage contrary to our prediction (objective 2), which means that the probability of the sites being occupied in successive months was higher for the former than the latter. On the other hand, the site colonization probability of both assemblages remained unaltered months after the sewage spill, which indicates their resilience. These results

suggest recovery, if not a slight improvement, of the general environmental quality of our sites during our 10-year study. Although we observed a slight increase in occupancy probability of the sensitive assemblage, the proportion of families that occupied the two sites during the study was very low compared to our known list of resistant and sensitive families, being an average occupancy probability of 0.24 (range: 0.23–0.32) for the former and 0.11 (range: 0.08–0.13) for the latter. This means that ~76% and 89% of the potential resistant and sensitive families, respectively, were not present in the two sites. One might conclude that this indicates the overall poor quality of the sites. More likely, those low proportions of families occupying the two sites compared to those occurring in the basin may simply reflect the high beta richness of Cerrado streams (Ligeiro et al. 2010) and the fact that our two sites cannot represent all families occurring in an entire river basin (Callisto et al. 2019; Castro et al. 2020; Ligeiro et al. 2010). That high beta richness is also a function of numerous uncommon or rare families (see Table S4). Although far from attaining an ideal state given its other stressors, the apparent improvement of site P2 following the spill indicates the natural recovery potential of peri-urban streams (Yeakley et al. 2014; França et al. 2019).

Biomonitoring recommendations

South American nations lack environmental laws and regulations that support the biomonitoring of freshwaters through use of standard methods and indicators (Buss et al. 2015). Therefore, South American countries also lack standard protocols for conducting cost-effective biomonitoring of sewage discharges—despite their widespread occurrence and ecological impacts. For example, 40% of Brazilian cities and 100 million citizens have no sewage collection or treatment (www.ibge.gov.br). However, the Brazilian Congress recently approved a New Regulatory Framework for Basic Sanitation ("Novo Marco Regulatório do Saneamento Básico"; Law Project 4162/2019). Its goal is universal water supply and sewage treatment by 31 December 2033. It encourages investments in, and improvement of, water quality indicators and services by attracting private companies to invest 700 billion Brazilian reais (113 billion USA dollars).

Benthic macroinvertebrates are the most studied assemblages in stream communities to understand the effects of urbanization (Gál et al. 2019). Although our study was based on only two sites monitored for 10 years, our results help lay the foundations for future studies that use macroinvertebrate assemblages for water quality assessment, taking into account parameters such as colonization, extinction and detection. Based on our results, we recommend biomonitoring sewage effects nationally only in the dry season, using pebble-filled mesh bags, and employing family richness, BMWP, EPT richness, shredders/collectors, and %

scrapers + collectors as indicators (Buss et al. 2015; Cummins et al. 2005; Fierro et al. 2021). We also favor monitoring multiple stream sites upstream and downstream of the sewage discharge to better detect the effects of those discharges and to help implement their mitigation as described in Yoder et al. (2019). Such a set of standard methods employed by differing institutions would also facilitate data exchanges and more comprehensive assessments than are possible by single institutions (Mulvey et al. 2009; USEPA 2016; Callisto et al. 2019; Paulsen et al. 2020).

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Author contributions MC, RLM, MSL, and RMH contributed to the conceptualization, analytical methods, formal analyses, writing, reviewing and editing.

Declarations

Conflict of interest RMH is affiliated with the company, Amnis Opes Institute. The views expressed in this paper are those of the authors only and do not reflect the views of any of their employers, nor did they conduct this work in any governmental or commercial capacity and this work is not a product of their employers. We declare no financial or personal relationships with other people or organizations that could inappropriately influence this manuscript or our work in general, and we received no outside financial support for the preparation of the article. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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