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## Biological water quality in tropical rivers during dry and rainy seasons: A model-based analysis





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#### ARTICLE INFO

# Keywords: Cuenca River basin Ecological modelling Generalized Linear Models Pollution Ecological assessment Biological water quality Andes

#### ABSTRACT

Recent studies on water quality in tropical rivers indicate substantial differences between seasons. However, investigations on the needs and added value of season-specific models are lacking. Thus, this paper aims to determine the accuracy and relevance of season-specific and season-overarching models to predict biological water quality. Additionally, we investigated the variation of prediction accuracy using sub-datasets from different parts of the Cuenca River basin. This study was accomplished in the rivers that pass through the urban and suburban areas of the city of Cuenca, which is located in the southern Andes of Ecuador. The Andean Biotic Index (ABI) was used as an indicator of biological water quality. Subsequently, models were developed to predict the ABI, with physicochemical and morphological variables as predictors, which were collected in 43 sites during both the dry and the rainy seasons. The predictions were obtained using three kinds of generalized linear models (GLMs): Gaussian, Gamma and Inverse Gaussian. The season-specific models were more accurate than the season-overarching models. Similarly, the predictions of the biological water quality in sites sampled in the urban area were more accurate than the forecasts performed in reference sites. The major variables predicting the ABI during the dry season were five-day biological oxygen demand (BOD<sub>5</sub>), ammonium and orthophosphate, while dissolved oxygen (DO), oxygen saturation (OS), nitrate, total solids proved to be important during the rainy season. The results of this research emphasize the importance of developing season-specific models and the implementation of different key actions for river restoration during both the dry and rainy seasons. The accuracy and the replication of these models could be improved and checked with more data taken from new sampling events. The modelling approach developed in this study can be applied to similar basins in the tropics and reveals that environmental investments need to count on monitoring strategies and data and analyses of the biological water quality variation in dry and rainy seasons, within the context of sustainable development.

#### 1. Introduction

Efforts to restore the good ecological status of water bodies throughout the world have been conducted in urban and suburban areas. These efforts have been developed using various measures such as the implementation of wastewater treatment plants and the application of temporary storage for sewer overflows. However, this approach only focused on the control of the point source releases without considering the impact of diffuse sources such as livestock and agricultural into the river (Erbe et al., 2002). Moreover, the impact on the ecology of running waters was not taken into account before the

implementation of these measures in most cases (Mouton et al., 2009). For this reason, the identification of the main drivers that affect the ecological status of rivers and what impact produced by these drivers is paramount with regard to the implementation of river restoration measures (Holguin-Gonzalez et al., 2013a). Hence, restoration options must be evaluated using scenario analyses before their application to determine their effectiveness in improving river ecology (Palmer et al., 2005).

The assessment of ecological water quality on freshwater bodies has been frequently performed through the monitoring of benthic macroinvertebrates (Jacobsen, 1998; De Pauw et al., 2006; Gabriels et al.,

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2010). These bioindicators respond to both hydromorphological variation and physicochemical changes as a result of human and natural stressors in streams, rivers and their surrounding areas (Cairns and Pratt, 1993; Thorne and Williams, 1997). Furthermore, macroinvertebrates continuously respond to environmental variations over a long period, as opposed to physicochemical samples that reflect the water quality at a particular point in time (Džeroski et al., 2000; De Pauw et al., 2006). Thus, the Biological Monitoring Working Party (BMWP), an index that assesses the surface water quality in England, was developed based on the presence of taxa that are sensitive to organic pollution in water bodies (Armitage et al., 1983). This index has been adapted to tropical countries, such as Brazil (Junqueira and Campos, 1998), Thailand (Mustow, 2002) and Colombia (Roldán Pérez, 2003). The BMWP-Col - an adaptation to the BMWP - has been used in different regions of Colombia and Ecuador (Roldán Pérez, 2003; Álvarez, 2005; Damanik-Ambarita et al., 2016). The BMWP-Col was further updated and adapted to the high altitude regions (i.e. the Andes of Ecuador and Peru), and was designated as the Andean Biotic Index (ABI) (Ríos-Touma et al., 2014).

Predicting the ecological water quality in rivers has frequently been implemented through ecological modelling, using linear relationships between the biotic and abiotic information despite the non-linear behavior of the ecosystem (Džeroski et al., 2000; Forio et al., 2016; Forio et al., 2018). By selecting the most appropriate ones, these models have been recognized as powerful tools of predictive ecology (Holguin-Gonzalez et al., 2013b). They are instruments that can be used in the restoration and conservation of aquatic ecosystems (Mouton et al., 2009). However, biological models in tropical rivers often have been based on season-specific data collections such as in the Philippines (Forio et al., 2018), in Ecuador's coastal region (Damanik-Ambarita et al., 2016) and in Bolivia (Moya et al., 2011). Furthermore, biological studies in tropical countries indicate that there can be substantial differences between seasons, leading to dissimilarities in environmental conditions between the dry and rainy seasons (Arunachalam et al., 1991; Jacobsen and Encalada, 1998; Beauchard et al., 2003; Mereta et al., 2012). However, a lack of model-based comparisons in tropical countries needs to be addressed to answer questions such as "How well do models perform in one season compared to another season?", "What is the benefit of season-overarching models?" and "Is the use of a selective combination of season-specific models more effective than a season-overarching model?".

Ecological models have been developed by using different techniques such as artificial neural networks (ANNs), Bayesian belief networks (BBNs), classification and regression trees (CTs and RTs), genetic algorithms (GAs), generalized linear models (GLMs), and support-vector machines (SVMs) (Goethals, 2005). In particular, the GLMs often have been used to identify variables that could affect the ecology of water bodies (Heino et al., 2007; Wilson et al., 2008; Domisch et al., 2011). This technique is mainly used to predict outcomes with the use of continuous variables (McCullagh, 1984; Zuur et al., 2009). Similarly, it has been demonstrated that GLMs are very efficient in providing the main variables that contribute to obtaining accurate predictions using small datasets (Vayssières et al., 2000).

This research was carried out in the framework of the VLIR-UOS IUC Programme – University of Cuenca and the VLIR-UOS Ecuador Biodiversity Network Project. In this study, new developmental and methodological steps for environmental indication were constructed. For that, we aim to build and to evaluate season-specific and season-overarching models to predict the biological water quality based on the Andean Biotic Index (ABI) in the Cuenca River, a tropical river located in the southern Andes of Ecuador. Furthermore, we analyzed the predictability of sites throughout seasons and between the dry and the rainy season based on simulations. We also included an analysis whether the models can be effectively applied or extrapolated to the main rivers or to their tributaries, as well as be tested to urbanized or to natural sites. In order to link environmental variables with the

biological index ABI, generalized linear models (GLMs) were constructed. The framework and objectives of the current study are presented in Fig. 1. The findings of this study can be applied to scenario analyses for season-specific river restoration based on the key disturbance variables obtained from the modeled indices. As indicated in the framework, the cause deduction (phase two in Fig. 1) is not addressed in this study because this requires additional analysis and simulations, either in the actual rivers or in river water quality models.

#### 2. Materials and methods

#### 2.1. Study area

The study area corresponds to the urban and suburban area of the Cuenca River basin, which is situated in the southern Province of Azuay in the Andes of Ecuador. The Cuenca River is an Andean mountain stream that is part of the Paute Upper Basin. The latter is one of the tributaries of the Santiago River, which is an affluent of the Amazon River. The Cuenca River is located downstream from the city of Cuenca (Fig. 2), and its four main tributaries are the Tarqui, Yanuncay, Tomebamba and Machangara Rivers that run from upstream and through the urban area.

The study area is 223 km², representing 13% of the Cuenca River basin, of which 16% is an urban area (i.e. the city of Cuenca), 73% is a mosaic between pastures and crops, 6% is forest and about 5% consists of lakes, moorland and bare land. The urban area had approximately 382,000 inhabitants in 2017 (INEC, 2010; SENPLADES, 2016). Of the four subcatchments, only the Machangara Basin is regulated all year by the presence of two hydropower dams: the Labrado and the Chanlud, which are located upstream from the city of Cuenca. Two natural reserves are also located upstream from the Cuenca River basin: Cajas National Park and the Machangara-Tomebamba protected forest. Both are water sources for the Tomebamba, Yanuncay and Machangara Rivers.

The mean altitude of the study area is 2655 m a.s.l., which varies from 2335 in the lowest part of the Cuenca River to 2770 m a.s.l. in the Yanuncay tributary. The highest altitudes are 2545, 2695, and 2622 m a.s.l. in the Machangara, Tomebamba and Tarqui Basins, respectively. The average annual rainfall in the study area is approximately 879 mm per year, while the average annual temperature is 16.3 °C (Aereopuerto-Mariscal-Lamar, 2012). There are two seasons during the year: the rainy season, which starts from the middle of February until the beginning of July and from the second half of September until the first two weeks of November, while the rest of the year constitutes the dry season (Fig. A1). The average flow of the Cuenca River measured before its discharge into the Paute River is around  $28\,\text{m}^3\text{·s}^{-1}$  (Cordero Domínguez, 2013). The 95th percentile annual flow, which generally happens during August and September - dry season -, is around 6 m<sup>3</sup>·s<sup>-1</sup>, while the 5th percentile annual flow which is present during the rainy season, is about 71 m<sup>3</sup>·s<sup>-1</sup> (ETAPA-EP, 2018).

#### 2.2. Data collection

The study considered 43 sampling sites located in the city of Cuenca and nearby areas. Of these sites, 27 were sampled during the dry season of July 2015 (Fig. 2A), while 35 sites were sampled during the rainy season of March 2016 (Fig. 2B). Nineteen sites were sampled during both seasons. The samples were collected during the day, from 8:00 am to 5:00 pm. These sampling sites were selected based on two criteria: macro-locations and micro-locations. Macro-locations are river stretches sampled in a basin or sub-basin, while micro-locations are placed to represent critical points like outfalls (Strobl and Robillard, 2008). Application of the macro-locations was in order to understand the state of the rivers from upstream to downstream, for which the location of the sampled sites was determined according to similar contributing tributary subareas (Sharp, 1971) in the Cuenca River basin. In order to

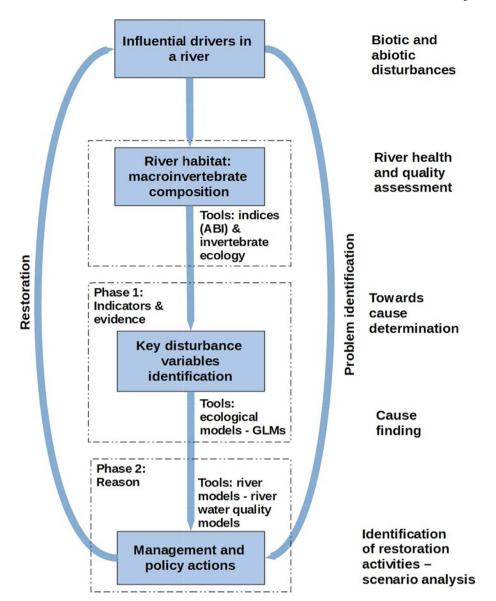


Fig. 1. The applied framework for the restoration of river water quality, of which Phase One was implemented in this study — adapted from Forio et al. (2017)

assess local impacts and critical sites in the rivers, micro-locations were selected upstream and downstream from the outfalls (Ward, 1973; Strobl and Robillard, 2008). Thus, the first sampled sites were located prior to the start of the city's suburban area and situated upstream of the four main tributaries of the Cuenca River. The combined sewer overflow (CSOs) are in operation only during the rainy seasons; thus, additional sites were sampled providing insights into the impact of CSOs. The locations that were sampled in both seasons focus on the water quality variation between them.

At each sampling site, environmental (physical-chemical and hydromorphological) conditions were recorded and biological (macroinvertebrates) samples were collected. In particular, 28 physicochemical, hydraulic and microbiological variables that could influence the biological water quality were measured. Six of these variables were measured directly in the field with two YSI\*6920-V2 (Yellow Spring, OH, USA) multi-parameter probes: water temperature, specific conductivity, dissolved oxygen (DO), turbidity, pH and chlorophyll-a. To obtain the flow in each sampling site, the river cross section was divided into various subsections, in which depth, width and mean stream velocity were measured. The latter variable was determined in 40% of the water depth in each subsection, using a water current meter (Rantz,

1982) Gurley 622A. The discharge was calculated as the sum of the subflows computed in each subsection. Table A1 presents the overview of the chemical data per season and over both seasons, which were measured in the laboratory of Sanitation at the University of Cuenca. Additionally, information was compiled on elevation, land use, river morphology, substrate characteristics, macrophytes, shading on the rivers at each site.

The samples of benthic macroinvertebrates were taken from the river and its tributaries with the kick-sampling procedure. This method is applied by shuffling the feet walking backwards against the current, while holding a standard net (a conical net with a frame size of  $0.20\times0.30\,\mathrm{m}$  and mesh size of  $500\,\mu\mathrm{m}$ , attached to a stick) against the current for five minutes (Gabriels et al., 2010). The sampling was performed in a stretch approximately  $10\text{--}20\,\mathrm{m}$  of length in different aquatic habitats, including bed substrates (stones, sand or mud), macrophytes (floating, submerged, and emerging) and other floating or submerged natural or artificial substrates. In addition to the hand net sampling, macroinvertebrates were also collected manually from stones, leaves and branches. Living animals were sorted in a white tray. Macroinvertebrates were sampled during the dry and rainy season to understand the seasonal effects in their assemblage's composition,

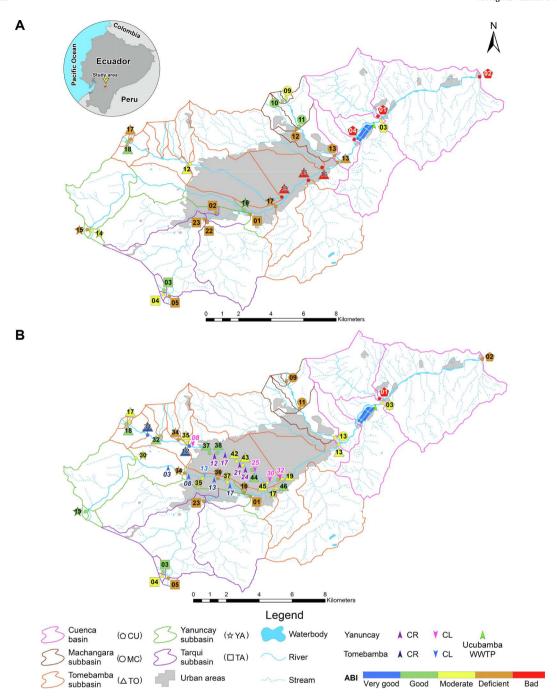


Fig. 2. Sampling sites location with their Andean Biotic Index (ABI) in both seasons: dry season, (B) rainy season. The figures (i.e. pentagon, square, triangle, star, and circle) indicate to what basin each site belongs.

produced either by the animals' life-cycle, by functional feeding group variety or by different environmental characteristics of the streams (Jacobsen and Encalada, 1998). During both seasons, 43 families of macroinvertebrates were found (Tables A2 and A3), identification was done by means of a stereomicroscope (Roldán Pérez, 1988; Álvarez, 2005; Encalada et al., 2011). For a detailed overview of the sampling campaigns and description of the locations, the authors refer to Jerves-Cobo et al. (2018a).

At each sampling site, the Andean Biotic Index (ABI) was calculated to assess the water quality. The ABI was built based on adaptations of the Biological Monitoring Working Party score (BMWP) of South America (Encalada et al., 2011; Ríos-Touma et al., 2014). This index was used since it was shown to be more suitable for the Andes in Ecuador above 2400 m a.s.l. (Jerves-Cobo et al., 2018a). The sensitivity

score ranges from one for very tolerant taxa to 10 for the most sensitive families. The ABI gives a five-water quality classification in function of the sum of sensitivity scores obtained in each location: bad ( $\leq$ 15), deficient (16–35), moderate (36–60), good (61–99), and very good (>99) (Álvarez, 2005; Zúñiga et al., 2009). Fig. 2 shows the ABI score of each sampling site in both seasons by means of a color code.

#### 2.3. Ecological model

An ecological model was developed to determine parameters such as BOD<sub>5</sub>, DO nitrate, nitrite or bank material that could influence the performance of the aforementioned biological index in the study area. For this, the data was fitted by a generalized linear model (GLM), as this modelling technique can deal with the non-linear behavior of the

ecosystem and has frequently been used for ecological related studies (Guisan et al., 2006; Zuur et al., 2009; Forio et al., 2018). The ABI, which was the response variable, takes values that are positive and continuous in nature. Thus, Gaussian, Gamma and Inverse Gaussian distributions are considered as the most appropriate distributions for the response variable (McCullagh, 1984; Zuur et al., 2009; Hardin and Hilbe, 2013). Furthermore, these distributions were also chosen based on the best fitting of the ABI score (response variable) that could be adjusted to either symmetric or skewed distributions. A Gaussian (Normal) distribution is applicable to a wide range of phenomena and its shape is symmetric, while the skewedness of the Gamma distribution depends on their parameters  $\alpha$  and  $\beta_i$ . The Inverse Gaussian is a distribution that is skewed to the right and is frequently used for studying the diffusion process (Forbes et al., 2011).

The GLMs with Gaussian, Gamma and Inverse Gaussian distributions are represented in Eqs. (1)–(3), respectively; where  $E(Y_i)$ , also expressed as  $\mu_i$ , is the mean of the response (or dependent) variable Y, given the regressor  $X_i$  (or independent) variable, i.e. the mean is a function of the regressor.  $\alpha$  and  $\beta_i$  are parameters, referred to as the intercept and the regression coefficient, respectively. The unexplained information is captured by the residuals, which are assumed to be normal, Gamma and Inverse Gaussian distributed, respectively. The variance  $\sigma^2$  in Gaussian GLM is denoted as  $var(Y) = \sigma^2$ , while the variance in Gamma and Inverse Gaussian GLMs are represented as  $var(Y) = \mu^2/\vartheta$  and  $var(Y) = \mu^3/\vartheta$ , respectively, in which  $\vartheta^{-1}$ denotes the shape parameter (Zuur et al., 2009; Lovison et al., 2011; Hardin and Hilbe, 2013).

Gaussian GLM:

$$\underline{\mathbf{E}(\mathbf{Y}_i)} = \underline{\mu}_i = \eta_i = \alpha + \beta_1 \times X_{1i} + \beta_2 \times X_{2i} + \dots + \beta_n \times X_{ni}$$
 (1)

Gamma GLM:

$$\mathbf{\underline{E}(Y_i)} = \underline{\mu_i} = \frac{1}{\eta_i} = \frac{1}{\alpha + \beta_1 \times X_{1i} + \beta_2 \times X_{2i} + \dots + \beta_n \times X_{ni}}$$
(2)

Inverse Gaussian GLM:

$$\mathbf{E}(Y_i) = \underline{\mu}_i = \frac{1}{\sqrt{\eta_i}} = \frac{1}{\sqrt{\alpha + \beta_1 \times X_{1i} + \beta_2 \times X_{2i} + \dots + \beta_n \times X_{ni}}}$$
(3)

#### 2.3.1. Model development and validation

To build the model, five datasets as described in Fig. 3 were fitted with Gaussian, Gamma and Inverse Gaussian GLMs. The first dataset (complete dataset) included all the sites (i.e. 43 cases) that were sampled in both seasons in the study area. The second dataset (withoutoutliers dataset) contained the previous dataset, but without the influence of an (outlier) data point (i.e. one site). The influence observation (data point) was obtained using two techniques: Cook's distance and the hat elements. Cook's distance is a measure of the change in the regression coefficients, which reveals the most influential cases that affect the regression equation. Whereas, the hat elements identify points that do not necessarily affect the regression coefficients. However, this technique could be useful in the identification of isolated points (Stevens, 1984). The third dataset (main-rivers dataset) comprised the locations sampled in both seasons solely in the main four rivers and not in their tributaries (i.e. 29 cases). The fourth dataset (dryseason dataset) consisted of all the sites sampled during the dry season (i.e. 26 cases) and finally, the last dataset (rainy-season dataset) involved all locations taken during the rainy season (i.e. 35 cases). For the construction of the models, 12 variables were removed before analysis due to missing values, which are present in Table 1. A total of 18 variables were considered during model development of each dataset (Table 1). The bank material surveyed (Jerves-Cobo et al., 2018a) was classified as bedrock, boulder, cobble, pebble, gravel, sand, silt and clay. It entered the models as an ordinal categorical variable with values from one to seven. Prior to the model development, a correlation analysis was performed between the ABI and the response variables and

between the response variables alone. The correlation coefficients obtained were lower than 0.8, which indicated that no strong correlations were detected and no redundant variables were included (Booth et al., 1994; Hering et al., 2006; Forio et al., 2018). The only variable discarded was temperature, which was highly correlated with DO (-0.9). Moreover, the correlation coefficient between ABI and oxygen saturation and between variables such as BOD5 and DO, Orthophosphates and  $BOD_{5}$ , was in the range of  $\pm$  0.6. In this regard, Zuur et al. (2009) indicated a value of  $\pm$  0.6 is not large enough to exclude any variable. Thus, all 18 variables were included during the construction of the models. The statistical differences of ABI scores and the 18 aforementioned variables were performed between the data collected at different seasons (i.e. 19 sites). For that, two tests were used, the two tailed paired Student t-test when the explanatory variables or response variable (ABI) were normally distributed; otherwise the paired Wilcoxon test was applied (Demšar, 2006). Both tests were performed at the significance level of 5%. The normal distribution of the variables was analyzed by means of the Shapiro-Wilk test (Thode, 2002), while the homogeneity of their variance was checked using the Levene test (Anderson, 2006).

During the construction and validation of the GLMs, the three-fold cross-validation procedure was implemented (Goethals, 2005). For this, each dataset was randomly stratified according to the response variable (i.e. the ABI) and then partitioned into three equal sub-datasets resulting in three groups with a similar quantity of cases for each ABI class (Everaert et al., 2013; Forio et al., 2016). Subsequently, two sub-datasets were used for training the model while the third was used for testing (validation) (Fig. 3A and B). This procedure was repeated, so that all tree folds were used once for validation. In total, 15 training sets (i.e. based on two sub-datasets) were used for model development. Each training set was fitted with the three different types of GLMs indicated in Eqs. (1)–(3).

#### 2.3.2. Variable selection

To identify which explanatory variables have an influence on the biological index ABI, a stepwise backward selection procedure was implemented. The least important input variables that had the highest p-value were consecutively eliminated. However, the previously removed variables were introduced again whenever they improve the model performance, that is, when the lowest Akaike information criterion (AIC) is attained (Gabriels et al., 2007; Jerves-Cobo et al., 2017). This is due to the fact that the AIC measures goodness-of-fit as well as the complexity of a model. When its value is low, GLMs tend to better fit the data, minimizing the number of parameters in the model, because a model is penalized for having too many parameters (Agresti and Kateri, 2011). The selection of the abiotic variables was accomplished with the help of the command stepAIC available in the MASS package in R (Venables and Ripley, 2002). The final model had the lowest AIC. However, based on the AIC value, one model was accepted when all or all minus one of its explanatory variables had p-values < 0.05. In the case of the model that had one explanatory variable with a p-value higher than 0.05, the model was accepted when the p-value of this explanatory variable was < 0.10. All statistical tests were performed at the 5% significance level.

#### 2.3.3. Model selection and evaluation

From the models constructed in each dataset, the model that had the highest pseudo  $R^2$  was preliminarily chosen. The pseudo  $R^2$  was calculated based on the training set and their testing set (i.e. two-third subdatasets used as a training set and the last sub-dataset was used as a testing set). The pseudo  $R^2$  is a likelihood ratio index in a generalized regression model that is analogous with the  $R^2$ , which issued in multiple linear regression techniques. The value of pseudo  $R^2$  gives a measurement of how well-observed outcomes are replicated by the model. Its value is calculated based on the Kullback-Leibler divergence (Cameron and Windmeijer, 1997). Similarly, the goodness-of-fit of each model



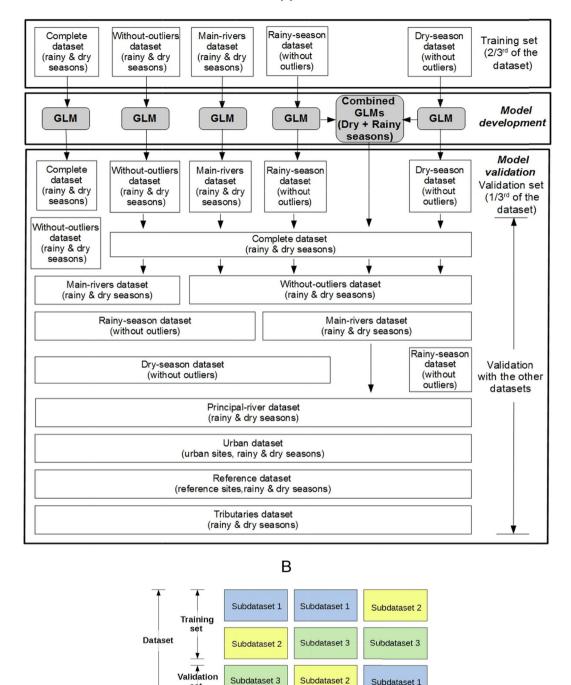


Fig. 3. Model development and validation scheme: (A) General schema of the models construction and (B) three-fold cross-validation procedure. The GLM represents generalized linear models.

set

Subdataset 1

Table 1 Variables included and removed for the construction of the ecological models.

Action	Variables
Included variables	Nitrate-N, nitrite-N, ammonium-N, five-day biological oxygen demand (BOD <sub>5</sub> ), dissolved oxygen (DO), oxygen saturation (OS), fecal coliforms –which was measured as most probable number (MPN) and analyzed on a logarithmic scale–, orthophosphate-P, chloride, total solids (TS), turbidity, flow velocity, water temperature, pH, conductivity, alkalinity, true color and bank material
Removed variables	Chemical oxygen demand (COD), chlorophyll-a, iron, nickel, tannins + lignins, copper, aluminum, silicon, chrome, manganese and fluorides

was also assessed with the residual plots (Zuur et al., 2009). Residual plots determine if the data meets the assumptions of the model such as homoscedasticity and normality. In case the assumptions were violated, the model was not withheld.

To choose the most appropriate model for each dataset, the predicted ABI values were transformed into a categorical variable based on the water quality classification as elaborated in Section 2.2. The categorical class obtained from the predicted ABI values was compared with the categorical class observed in the field. The accuracy of the predicted categorical classes was evaluated using the testing datasets with two measurements obtained from the confusion matrix: the correctly classified instances (CCI) and the Cohen's Kappa statistic (κ). This matrix identifies true positive (TP), false positive (FP), false negative (FN) and true negative (TN) cases. The CCI is calculated as the sum of the diagonal (i.e., TP + TN) divided by the sum of all values (i.e., TP + FP + TN + FN) (Kohavi and Provost, 1998). The value of CCI is expressed in percentage and it ranges from 0 to 100%, where a value of 100% means that the accuracy of the model is the highest (Fukuda et al., 2011). The Cohen's Kappa statistic (κ) (Eq. (4)) is a derived statistic that measures the proportion of possible cases of correct predictions (TP and TN) by a model after accounting for chance predictions (Cohen, 1960; Goethals et al., 2007; Jerves-Cobo et al., 2018b). According to Goethals et al. (2007), models are considered good when the Kappa statistic is higher than 0.4 and the CCI is at least 70% (Goethals, 2005). The models that comply with these criteria were chosen. However, in some datasets no model reached the aforementioned thresholds. Therefore, selection in these cases was done with the models achieving the highest Kappa statistic and CCI.

$$Kappa(\kappa) = \frac{(TP + TN) - \frac{(TP + FN)(TP + FP) + (FP + TN)(FN + TN)}{n}}{n - \frac{(TP + FN)(TP + FP) + (FP + TN)(FN + TN)}{n}}$$
(4)

Each chosen model had its own explanatory variables and its own coefficients. However, the model whose variables were most closely related to organic pollution was adopted. Because of this, the final model could be tested in a scenario analysis, under new improvements in the wastewater management in the city of Cuenca and its surrounding areas.

The final selected models from each dataset were validated with other datasets to check their accuracy (CCI) in different conditions. These datasets were featured with or without outliers in one or both seasons (Fig. 3A). In addition, these models were validated with subdatasets consisting of sites from the Tomebamba River (principal-river dataset), the urban sites (urban dataset), the reference sites (reference dataset) and the tributaries (tributaries dataset) (Fig. 3A). Finally, the models obtained during the dry and rainy seasons were combined and selectively validated according to the season in which the sites were sampled (Fig. 3A). The sites included in each dataset are presented in Table 2 and Fig. 2.

#### 3. Results

#### 3.1. Variable differences between seasons and influential points analysis

In sampling sites measured during dry and rainy seasons, the total number of taxa, the Shannon-Wiener index (SWD), water temperature, pH, DO, total solids and Nitrite-N were significantly different, according to the paired Student *t*-test and paired Wilcoxon test (Table A4). However, turbidity, oxygen saturation, alkalinity, chloride, ammonium and flow velocity did not show differences between seasons.

Site Ta02 of the total dataset was found to be a highly influential point based on both Cook's distance and hat elements tests when Gamma and Inverse Gaussian distributions were applied (Fig. A2A–D). According to the Cook distance, this highly influential point would affect the regression coefficients of the equations obtained from the aforementioned distributions (Stevens, 1984). However, no influential

point was detected for Gaussian distribution (Fig. A2E and F). Despite this, the site Ta02 was excluded from the other datasets. The Ta02 site registered high concentrations of organic pollutants and nutrients that could sway its detection as a highly influential point. The detection of influential points was also applied to the remaining training datasets (i.e. without-outliers, main-rivers, dry-season and rainy-season datasets), in which no further sites were excluded (Figs. A3–A6). Furthermore, the Ta01 site was detected as an influential point during dry season in the main-rivers dataset, when the three GLMs (Gaussian, Gamma and Inverse Gaussian) were applied, but its influence was marginal (Fig. A4).

#### 3.2. Season-specific models

In this section and forward, we only present the models that were performing with the highest  $\kappa$  and CCI values during the validation process. The season-specific models indicated that different variables were associated with the ABI during each season. Thus, during the dry season, BOD $_5$  and orthophosphate were the best predictors of the ABI (Tables 3, 4 and A5). Gamma and Inverse Gaussian GLMs predicted the ABI better than the Guassian GLM. However, results suggest that Inverse Gaussian was the best performing GLM.

The models obtained to predict the ABI during the rainy season (Tables 3 and A6) displayed oxygen saturation (OS) as the only common explanatory variable. Whereas nitrate, nitrite, DO, total solids and bank material were found as predictors in two of the four models (Table A6). Ammonium was included in one of the four models. Gaussian and Inverse Gaussian GLMs had good accuracy for the ABI prediction in one of the three validation datasets (Table A6).

The accuracy of the models obtained during the dry season was higher than the model developed for the rainy season. This was based on the three-fold cross-validation results obtained with the evaluation metrics: pseudo  $R^2$ , CCI and  $\kappa$  (Table 5). The higher concentration of organic pollutants and nutrients could have positively influenced the prediction of the ABI during the dry season. Fig. 4 presents the comparison between the observed and the predicted ABI classes, which were obtained from the models, during both seasons. The residual plots of the models obtained in each season can be seen in Fig. A7.

#### 3.3. Season-overarching models

This section summarizes the results obtained for the models trained with complete, without-outliers, and the main-rivers datasets. These models were developed to forecast the ABI class during any season. Additionally, we compared the accuracy of the season-overarching models with the combination of season-specific models, which were selectively applied according to the season in which the sites were sampled.

Orthophosphate, DO and oxygen saturation were the common predictors of the models developed to calculate the ABI (Table 4) trained with the complete, without-outliers and main-rivers datasets. The Gamma and Gaussian GLMs provided the most accurate predictions for the aforementioned datasets. The residual plots and scale location of the models developed from the three datasets are presented in Fig. A8.

In addition, ammonium, DO and OS were selected in two of the four models developed using the complete dataset (Table A7), while, BOD<sub>5</sub>, DO and OS were the explanatory variables in two of the models obtained for the without-outliers dataset (Table A8). In the main-rivers dataset, orthophosphate was the common predictor variable for the ABI in the four constructed models, while DO and OS were predictor variables for the ABI in three of the four constructed models (Table A9). Figs. 5A, A9A and B show the deviation of observed values and the predicted values with the complete, the without-outliers and the mainrivers datasets, respectively. Consequently, the season-overarching models shown as predictors, a mixture of variables from both dry and rainy season models. Based on the three-fold cross-validation results

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**Table 2**Sites included in each dataset (i.e. complete, without-outliers, main-rivers, tributaries, urban and reference sites).

Tome-bumble sub-bumble season   Seaso	Sites	Complete da	ataset	Without-out	tliers	Main-rivers		Principal-riv	ver	Tributaries		Urban sites	Reference sites
CUIO   X		Dry season	•	Dry season	•	Dry season	•	Dry season	•	Dry season	•	_	
CU02	Tomeb	amba sub-bas	in										
CUOS X X X X X X X X X X X X X X X X X X X	CU01	X	X	X	X					X	X	X	
CUO	CU02	X			X				X				
TO12			X		X		X		X				
TOTO													
TO16 X													
TO10			X		X	X	X	X	X				
TOTO    X													
TO19												X	
TO19										X	X		
TO31													X
TO32		X		X		X		X					
TO34							X		X				
TO35													
TO37													
T038         X         X         X         X         X         X         X         TO42         X </td <td></td> <td>X</td> <td></td> <td></td>											X		
TO42													
TO43													
TO44													
T045													
Machangara Sub-basin													
Machangara Sub-basin           MC09         X													
MC10	TO46		X		X		X		X			X	
MC10	Macha	ngara Sub-bas	sin										
MC11         X	MC09	X	X	X	X	X	X						X
MC12 X	MC10	X		X						X			X
MC13 X X X X X X X X X X X X X X X X X X X	MC11	X	X	X	X	X	X					X	
Yanuncay Sub-basin       YA14       X       X       X       X         YA15       X       X       X       X       X       X         YA16       X       X       X       X       X       X       X       Y	MC12	X		X						X		X	
YA14       X       X       X         YA15       X       X       X       X         YA16       X       X       X       X         YA17       X       X       X       X         YA30       X       X       X       X         YA34       X       X       X       X         YA35       X       X       X       X         YA36       X       X       X       X         YA37       X       X       X       X         Tarqui Sub-basin       Tarqui Sub-basin       X       X       X         TA02       X       X       X       X         TA03       X       X       X       X         TA04       X       X       X       X         TA05       X       X       X       X         TA02       X       X       X       X         X       X       X       X       X	MC13	X	X	X	X	X	X					X	
YA15       X       X       X       X       X       X       YA16       X       X       X       X       X       X       X       X       YA17       X       X       X       X       X       X       X       X       YA17       X       X       X       X       X       X       X       YA17       X       X       X       X       X       YA17       X	Yanun	cay Sub-basin											
YA16       X       X       X       X       X       X       YA       YA<	YA14	X		X						X			X
YA17       X       X       X       X       X       X       YA30       X       X       X       X       YA34       X       X       X       X       X       X       YA35       X       X       X       X       X       X       X       YA36       X       X       X       X       X       X       YA37       X       X       X       X       X       X       X       X       YA37       X <td>YA15</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>X</td>	YA15	X	X	X	X	X	X						X
YA30       X       X       X       X       YA34       X       X       X       X       YA       YA<	YA16	X	X	X	X	X	X					X	
YA34       X       X       X       X       X       YA	YA17	X	X	X	X	X	X					X	
YA35       X       X       X       X       X       YA	YA30		X		X		X					X	
YA36       X	YA34		X		X		X					X	
YA37         X         X         X         X           Tarqui Sub-basin         TA01         X	YA35		X		X		X					X	
Tarqui Sub-basin  TA01 X X X X X X X X X X X X X X X X X X X	YA36		X		X		X					X	
TA01     X     X     X     X       TA02     X     X     X       TA03     X     X     X     X       TA04     X     X     X     X       TA05     X     X     X     X       TA22     X     X     X     X	YA37		X		X		X					X	
TA02     X       TA03     X     X     X     X       TA04     X     X     X     X       TA05     X     X     X     X       TA22     X     X     X     X	Tarqui	Sub-basin											
TA02     X       TA03     X     X     X     X       TA04     X     X     X     X       TA05     X     X     X     X       TA22     X     X     X     X	TA01	X	X	X	X	X	X					X	
TA04 X X X X X X X X X TA05 X X X X X X X X X X X X X X X X X X X	TA02	X								X		X	
TA04 X X X X X X X X X X TA05 X X X X X X X X X X X X X X X X X X X	TA03	X	X	X	X					X	X		X
TA22 X X X	TA04	X	X	X	X					X			
TA22 X X X	TA05	X	X	X	X	X	X						
TA23 X X X X X X X X X	TA22	X		X						X		X	
	TA23	X	X	X	X	X	X					X	

obtained with the evaluation metrics: pseudo  $R^2$ , CCI and  $\kappa$  (Table 5), the models obtained with the main-rivers dataset, showed a lower accuracy in comparison with the models obtained with the complete and without-outliers datasets.

Based on the CCI and  $\kappa$ , the models trained with the different datasets were validated with the other datasets (Fig. 3). The best performing models (CCI  $\sim$  70% and  $\kappa$  > 0.4) were the selective combination of season-specific models validated with the principal-river dataset. Furthermore, the selective validation of the combination of season-specific models had good accuracy (CCI > 60% and  $\kappa$  > 0.4), when they were validated with the without-outliers and the dry-season datasets as well as the urban and the tributaries datasets. The CCI and the  $\kappa$  were similar when the season-specific models were validated with the complete and without-outliers datasets, obtaining a slightly higher accuracy for the without-outliers dataset. When the rainy season models were validated with the principal-river (Tomebamba) dataset, the CCI and  $\kappa$  was over 60% and 0.4, respectively. The models trained with the complete dataset showed marginally higher accuracy than the

models developed from the without-outliers dataset. In general, the season-specific models performed better than the overarching models. Thus, the selective validation of the combination of the models developed from the specific-season datasets had points closer to the bisector, indicating that the predicted values are deviating to a lesser extent from the observed values (Fig. 5B). By contrast, the lowest accuracy was noted when the reference dataset was analyzed with the other datasets. A low accuracy also resulted when a season-specific model was validated with the dataset from a different season. Table 6 presents the validation results of models developed from the different datasets.

#### 4. Discussion

4.1. Biological and environmental differences between seasons in tropical rivers

One of the strengths of our study design is the comparison between the combinations of season-specific models with season-overarching

 Table 3

 The best-performing season-specific models.

Explanatory variables	Regression parameters				Season-specific mod	lels	
			Gamm	season a model: 2a3.gamma		Inverse G	ny-season aussian model: gaussian
		Coefficient	Std. Error	p-Values	Coefficient	Std. Error	p-Values
	A	1.3E-02	3.1E-03	1.0E-03	-725.5	195.9	1.0E-03
Nitrate	B1				85.3	26.6	3.0E - 03
Nitrite	B2				-1100.7	319.9	2.0E - 03
Ammonium	В3	-4.2E-02	1.7E - 02	2.5E - 02			
BOD <sub>5</sub>	B4	5.2E - 03	1.3E - 03	1.0E - 03			
DO	B5				-42.8	19.7	3.9E - 02
Oxygen Saturation	В6				10.9	2.8	1.0E - 03
Orthophosphate	В8	1.4E - 01	4.7E - 02	9.0E - 03			
Total solids	B10				-0.1	0.1	3.3E - 02
Bank material	B18				10.6	4.1	1.6E - 02
Training subset (2/3)							
AIC:		140.6			314.0		
Pseudo R <sup>2</sup> :		0.7			0.6		
CCI:		72.2%			68.6%		
κ:		0.6			0.5		
Validation subset (1/3)							
Pseudo R <sup>2</sup> :		0.4			0.3		
CCI:		62.5%			72.7%		
κ:		0.4			0.6		

**Table 4**Best-performing season-overarching models.

Explanatory variables	Regression Parameters				Mode	els for both sea	ason			
		G	mplete dataset amma model 3fcv2a3T.gam		G	ut-outliers dat amma model 3fcv2a3.gamm		(	ain-rivers data Gaussian mode m3fcv1a2.gau	1:
		Coefficient	Std. Error	p-Values	Coefficient	Std. Error	p-Values	Coefficient	Std. Error	p-Values
	A	3.0E-01	8.1E-02	< 0.01	2.2E-01	7.8E-02	0.01	-764.7	243.8	< 0.01
Ammonium	В3	-9.4E-03	2.3E - 03	< 0.01						
BOD <sub>5</sub>	B4				1.5E - 03	7.2E - 04	0.04			
DO	B5	1.8E - 02	5.9E - 03	0.01	2.0E - 02	5.7E - 03	< 0.01	-30.7	13.5	0.03
Oxygen saturation	В6	-4.1E-03	1.0E - 03	< 0.01	-3.6E-03	9.7E - 04	< 0.01	10.5	2.7	< 0.01
Orthophosphate	B8	6.7E - 02	2.3E - 02	0.01	3.3E - 02	1.8E - 02	0.07	-88.6	40.9	0.04
Training subset (2/3)										
AIC:			353.6			347.4			273.4	
Pseudo R <sup>2</sup> :			0.5			0.6			0.5	
CCI:			48.8%			53.7%			53.3%	
κ			0.3			0.3			0.3	
Validation subset (1/3)										
Pseudo R <sup>2</sup> :			0.5			0.6			0.4	
CCI (%):			61.9%			60.0%			57.1%	
κ			0.5			0.4			0.4	

**Table 5** Three-fold cross-validation results of the models with different datasets. Mean and stand deviation of pseudo  $R^2$ , CCI and  $\kappa$ .

Dataset	Pseu	do R <sup>2</sup>	± sd	CC	CI ±	sd		κ±	sd
Dry-season	0.35	±	0.30	42.3%	±	16.6%	0.21	±	0.20
Rainy-season	0.24	±	0.24	44.9%	$\pm$	10.4%	0.24	$\pm$	0.24
Complete	0.29	±	0.17	44.1%	$\pm$	10.1%	0.22	$\pm$	0.13
Without-outliers	0.33	±	0.24	44.6%	$\pm$	11.4%	0.22	$\pm$	0.16
Main-rivers	0.18	$\pm$	0.12	39.8%	$\pm$	12.1%	0.16	$\pm$	0.15

models in tropical places. With respect to the modelling of water quality in tropical rivers during the dry and rainy seasons, Maillard and Santos (2008) conducted a study in Brazil and found that some of the

physicochemical variables used in the season-specific models had the opposite behavior between seasons. However, these researchers constructed physicochemical water quality models, in which they analyzed neither the results of a possible season-overarching model, nor the modelling of the biological status of the rivers. In another study, Jacobsen (1998) discovered that there was a variation of macroinvertebrate assemblage between the dry and rainy seasons at the high altitude tropical environment.

The  $BOD_5$ , orthophosphate, and ammonium mainly predicted the ABI in the Cuenca River basin during the dry season. The increase of  $BOD_5$ , orthophosphate and ammonium along the rivers during dry season in the study area was possibly due to three causes: (1) sewage discharges; (2) some sewage overflows whose operational level was incorrectly calibrated; and (3) diffuse pollution from runoff originated

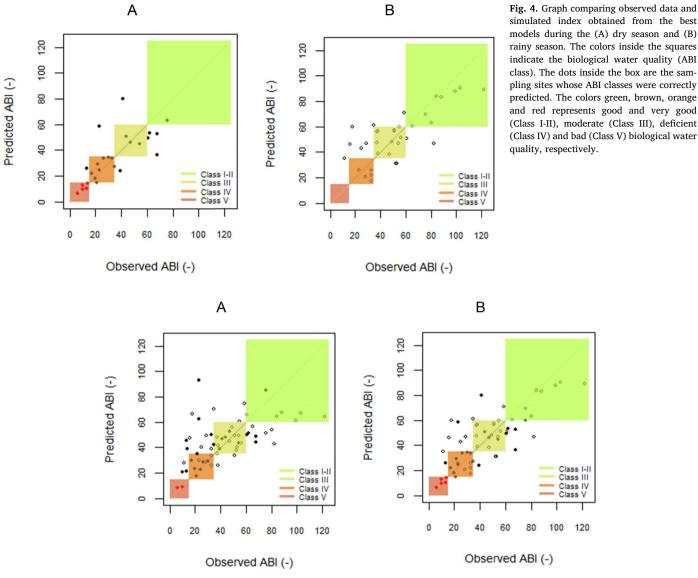


Fig. 5. Graph comparing simulated index and field data for models developed from: (A) the complete dataset, and (B) selective validation of the combination of the best models for the dry and the rainy seasons. The filled dots and the empty dots are the sampling sites taken during dry and rainy seasons respectively.

by irrigation of agriculture and grassland areas in the suburban areas of the city (Jerves-Cobo et al., 2018a). Thus, the concentration of these pollutants was higher during the dry season, affecting the presence of macroinvertebrates and consequently the biological index ABI. Macroinvertebrates per se are affected by oxygen saturation and not by pollutants such as orthophosphate, BOD<sub>5</sub> and ammonium (Jacobsen, 1998; Beyene et al., 2009). However, the depletion of oxygen concentration is influenced by these pollutants (Hynes, 1960), which were higher correlated to the oxygen concentration during the dry season than the rainy season. These results agreed with the findings of other studies carried out in the high altitude of the Andes in Ecuador (Jacobsen, 1998; Burneo and Gunkel, 2003; Jerves-Cobo et al., 2017). These authors indicated that the number of macroinvertebrate species was reduced by the increase of organic pollutants and phosphates. Similarly, the BOD5 and nutrient concentrations were negatively associated with most taxa, both in tropical and higher latitude streams (Schleiter et al., 1999; Beyene et al., 2009; Friberg et al., 2010).

During the rainy season, the ABI was on average higher than that obtained during the dry season. The effect of organic pollution during

the rainy season was diminished by a greater dilution of the wastewater (Jacobsen, 1998), resulting in the selection of variables such as oxygen saturation (OS), dissolved oxygen (DO), nitrite, nitrate and total solids as predictors of the ABI. During the rainy season, the percentage of OS, as well as the concentration of TS and nitrite, was higher. The DO concentration in most sites of the main rivers was close to the saturation level during both the dry and rainy seasons, with higher values during the dry season. Moreover, in both seasons the values of the DO were higher in the sites located upstream than the downstream sites, which was the opposite with the concentrations of BOD<sub>5</sub> and nutrients (Jerves-Cobo et al., 2018a). Similar findings concerning the DO in both seasons were found by Ríos-Touma et al. (2011) in the northern Andes of Ecuador. Additionally, the flow velocity and turbulence were also greater during the rainy season aiding the maintenance of relatively high oxygen saturation (Jacobsen, 1998; Jerves-Cobo et al., 2017). However, the oxygen saturation level is relatively low for 2500 m a.s.l. regions, in comparison with low altitude regions whose average values for the dry and rainy seasons, respectively, are of 8.1 mg·l<sup>-1</sup> and 7.9 mg.l<sup>-1</sup>. The average value of DO, obtained during the sampling

Table 6 Independent validation of the models developed from the different datasets (complete, without-outliers, main-rivers, dry-season and rainy-season datasets). Mean and standard deviation of CCI and  $\kappa$  were derived after the application of three-fold cross-validation. Values in bold represent the best-performing models: CCI > 60% and  $\kappa > 0.4$ .

				Model		
Validation sets	Comple	te dataset	Without-outl	iers dataset	Ma	in-rivers dataset
	CCI ± sd	κ ± sd	CCI ± sd	κ ± sd	CCI ± sd	κ ± sd
Complete dataset	54.0 ± 5.0	0.4 ± 0.1	52.0 ± 4.6	$0.3 \pm 0.1$	51.2 ± 0.8	$0.3 \pm 0$
Without-outliers	$54.9 \pm 5.1$	$0.4 \pm 0.1$	$51.6 \pm 5.1$	$0.3 \pm 0.1$	$52.1 \pm 0.8$	$0.3 \pm 0$
Main-rivers	$54.6 \pm 4.9$	$0.3 \pm 0.1$	$51.7 \pm 6$	$0.3 \pm 0.1$	$50.6 \pm 1.1$	$0.3 \pm 0$
Dry-season	$50.0 \pm 4.4$	$0.3 \pm 0.1$	$48.1 \pm 5$	$0.3 \pm 0.1$	$50.0 \pm 3.1$	$0.3 \pm 0$
Rainy-season	$58.6 \pm 6.8$	$0.4 \pm 0.1$	$54.3 \pm 7.7$	$0.3 \pm 0.1$	$53.6 \pm 1.4$	$0.3 \pm 0$
Principal-river	$58.3 \pm 2.4$	$0.4 \pm 0$	$58.3 \pm 8.1$	$0.4 \pm 0.1$	$58.3 \pm 4.6$	$0.4 \pm 0.1$
(Tomebamba River)						
Urban sites	$55.7 \pm 6.0$	$0.4 \pm 0.1$	$52.8 \pm 5.4$	$0.3 \pm 0.1$	$52.8 \pm 1.1$	$0.4 \pm 0$
Reference sites	$37.5 \pm 8.3$	$0.1 \pm 0.1$	$39.6 \pm 8$	$0.1 \pm 0.1$	$35.4 \pm 4.2$	$0 \pm 0.1$
Tributaries	$52.8 \pm 7.2$	$0.4 \pm 0.1$	$52.8 \pm 3.2$	$0.4 \pm 0$	$52.8 \pm 3.2$	$0.4 \pm 0$
		Mo	odel		Selecti	ve validation of the
Validation sets	Dry-seas	on dataset	Rainy-seaso	on dataset	Combination of dr	y season + rainy season models
	CCI ± sd	κ ± sd	CCI ± sd	κ ± sd	CCI ± sd	κ ± sd
Complete dataset	45.6 ± 2	0.2 ± 0	47.6 ± 4.7	0.3 ± 0.1	59.3 ± 3.6	0.4 ± 0.1
Without-outliers	$46.3 \pm 2.1$	$0.2 \pm 0$	$48.4 \pm 4.7$	$0.3 \pm 0.1$	$60.3 \pm 3.6$	$0.5 \pm 0.1$
Main-rivers	$46.6 \pm 4.8$	$0.2 \pm 0.1$	$48.3 \pm 4.7$	$0.3 \pm 0.1$	$58 \pm 2.9$	$0.4 \pm 0$
Dry-season	$60.6 \pm 4.8$	$0.5 \pm 0.1$	$37.5 \pm 8.5$	$0.2 \pm 0.1$	$60.6 \pm 4.8$	$0.5 \pm 0.1$
Rainy-season	$35.7 \pm 5.5$	$0 \pm 0.1$	$56.4 \pm 6.3$	$0.4 \pm 0.1$	$56.4 \pm 6.3$	$0.4 \pm 0.1$
Principal-river (Tomebamba River)	$36.9 \pm 2.4$	$0.1 \pm 0$	$60.7 \pm 4.6$	$0.4 \pm 0.1$	$69.1 \pm 6.2$	$0.6 \pm 0.1$
Urban sites	$50 \pm 1.9$	$0.3 \pm 0$	$50 \pm 12.5$	$0.3 \pm 0.1$	$65.3 \pm 5$	$0.5 \pm 0.1$
Reference sites	$20.8 \pm 4.8$	$-0.1 \pm 0.1$	$33.3 \pm 17.4$	$0 \pm 0.2$	$33.3 \pm 6.8$	$0 \pm 0.1$
Tributaries	$43.1 \pm 2.8$	$0.2 \pm 0$	$45.8 \pm 7.9$	$0.3 \pm 0.1$	$62.5 \pm 9.5$	$0.5~\pm~0.1$

campaigns was  $7.3 \,\mathrm{mg.l^{-1}}$  and  $7.6 \,\mathrm{mg.l^{-1}}$  for the dry and rainy seasons, respectively. In this regard, Jacobsen (1998) pointed out that the oxygen concentration of saturated water decreases with increased altitude. Moreover, the author revealed that in the high Andes region with oxygen saturation between 80 and 90%, many Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa almost disappeared completely. Hence, macroinvertebrates from the tropical highland Andes streams may be particularly sensitive to the diminishing oxygen levels by organic pollution. The depletion of DO in the rivers prevails during the aerobic conversion of organic pollutants (Hynes, 1960) by bacterial decomposition (Rauch et al., 1998). However, the higher water temperature measured during the rainy season could have had a more significant influence on the DO than the lower concentration of the organic pollutants. Thus, the dissolved oxygen saturation decreased on average around 6% due to the higher water temperature during the rainy season, while the DO diminished on average around 5% in main rivers, possibly due to the higher water temperature and the lower organic pollutant concentrations. It is important to note that the day and night cycles of the DO and OS were not analyzed in this study. According to Wilcock et al. (1978), when the water temperature increases at the same partial pressure, the solubility of oxygen decreases leading to a reduced DO concentration. Whereas oxygen saturation (OS) is related to water temperature and atmospheric pressure variation, diminishing with the increase of temperature and with a drop of atmospheric pressure (Mortimer, 1981). Oxygen saturation and the dissolved oxygen (DO) have also been established as key variables that explain the presence of macroinvertebrates in the Ecuadorian Andes (Jacobsen and Marín, 2008).

In the main rivers, during the rainy season, the concentration of

ammonium was a little lower than that measured during the dry season, while nitrite registered a little higher value in its concentrations. In this regard, during the rainy season ammonium has been related to the wash-out from the sewer systems and discharged by means of CSOs (Holzer and Krebs, 1998). The concentration of nitrite, a pollutant measured in a very low range and expressed, as µg N/L, is apparently associated with the ammonium concentrations and higher flows resulting in higher stream velocities during the rainy season. In this regard, it is known that increased levels of nitrite can be found in fastflowing aerobic small streams, a characteristic of Andean mountain rivers, in which ammonium oxidation via nitrification is responsible for the higher concentrations of this pollutant (Kelso et al., 1999; Philips et al., 2002). Moreover, runoff from the livestock areas that originated during rainy events could contribute to the diffuse fluxes of organic pollutants (Fernandez de Cordova and González, 2012; Jerves-Cobo et al., 2018a). The total solids (TS), which are the sum of dissolved solids plus suspended solids and settled solids, were similar in both seasons, with a concentration during dry seasons at approximately 75% of that registered during the rainy season. The increase of TS in rivers is attributable to multiple factors such as runoff, relief, lithology, rainfall pattern, vegetation, and basin size (Meybeck et al., 2003). In the study area, the TS may have been transported to the rivers from combined sewage outfalls (CSOs) from urban areas that discharge wastewater and runoff during rainfalls. Moreover, the runoff from rural areas, which is produced both by irrigation during the dry season and by rains during the rainy season, may have moved the total solids (TS) to the rivers. In this regard, it has been found that suspended solids influence the community structure of macroinvertebrates in streams (Gray and Ward, 1982; Doeg and Milledge, 1991).

#### 4.2. Season-specific and season-overarching models

Our results demonstrate that GLMs can be used to select physicochemical variables that best predict the biological water quality based on the ABI index. However, in most models,  $\Psi$  was lower than 0.4 and CCI was lower than 70%, indicating that only a few models were reliable. The accuracy of the models could also have been affected by the transformation of a continuous response variable ABI, into a categorical variable.

The selective combination of season-specific models that were applied according to the season in which the sites were sampled, displayed a higher accuracy than the season-overarching models. Thus, the season-specific models showed higher values in CCI and  $\kappa$ , at least 5% and 10%, respectively. This could be due to the fact that the main variables predicting the ABI were different between seasons. Similarly, the obtained models developed for the dry season were more precise and stable than the models developed for the rainy season. Thus, during the dry season, the ABI was mainly predicted by orthophosphate, ammonium, and BOD<sub>5</sub>. In this regard, Jacobsen (1998) found that the biotic indices BMWP (Biological Monitoring Working Party) and ASPT (Average Score Per Taxon) were closely related to the dissolved oxygen and phosphate concentrations in the high Andes region during the dry season. Additionally, Holguin-Gonzalez et al. (2013b) established that the Trichoptera presence was mostly related to the BOD5. Consequently, these sensitive taxa prefer water with low concentrations of BOD<sub>5</sub>. Yet, the concentration of organic pollutants decreased during the rainy season. Thus, the ABI was primarily associated with variables such as OS, DO, nitrite and total solids during the rainy season. Similar outcomes were described by Jacobsen (1998), who found a weaker correlation between the biotic indices and organic pollutants during the rainy period. Furthermore, during the rainy season, the models revealed the influence of morphological variables such as bank material. In this regard, Burneo and Gunkel (2003) found in a study developed in the northern Andes of Ecuador that bank material, a morphological characteristic, affected invertebrate communities. By contrast, the presence of macrophytes and the effect of shading were not relevant; probably they were limited in the sampled sites (Jerves-Cobo et al., 2018a). The validation results obtained from the rainy dataset were quite stable as indicated by the standard deviation of.  ${}^{c}\Psi$  with a variation between 0 and  $\Psi$  10% of the mean  $\kappa$ . (Table 6). However, models developed for the rainy season showed a higher variation of explanatory variables in three-fold cross-validation than the models developed for the dry season (Tables A5 and A6).

As previously mentioned, the predictors of the season-specific models for ABI varied between seasons. Thus, during dry season, the ABI was mainly predicted by orthophosphate and the BOD5, while variables such as OS, DO, nitrite, nitrate and total solids primarily influenced the prediction of the ABI during the rainy season. The application of the season-specific models developed for dry season showed a low accuracy when they were validated with the rainy-season dataset, and vice versa. In this regard, McCullagh (1984) revealed that a model developed with a particular dataset is not able to incorporate the inevitable changes given in another dataset that was collected under different conditions. In contrast, the season-overarching models had the following predictors: orthophosphate, BOD5, DO and OS. This demonstrates that a mixture of predictors from both the dry and rainy season models influenced the season-overarching models. Variables such as nitrite, nitrate and total solids had less weight in the prediction of the ABI in the season-overarching models. Both the mixture and loss of predictors in the season-overarching models have likely caused their lower accuracy in comparison to the season-specific models. However, when the season-specific models were validated with the different season dataset, the accuracy of the season-overarching models was better.

#### 4.3. Model development

The only correlated variable that was eliminated prior to model construction was temperature. Assuming that this research included multi-collinearity, in this regard, Shmueli (2010) pointed out that multi-collinearity is not damning because the prediction performance of the models was not affected. Consequently, either for supposed possible inclusion of the correlated variables or for their exclusion, the forecast capacity of the models was not altered.

During the construction of the models, we compared the results obtained from the datasets both with and without outliers. An outlier is a result from either an influence observation or a measurement error (Rousseeuw and Leroy, 2005). In this research, we classified the influence observations as outliers that were registered in the polluted tributaries of the main rivers. The models constructed in this study indicated that both CCI and  $\kappa$ . varied on average  $\pm$  2%. However, the models developed using these two datasets had few explanatory variables that were different (Tables A7 and A8). This would imply that outliers affected the selection of explanatory variables, but not the accuracy of the models. In this regard, Chatterjee and Hadi (1986) pointed out that outliers can affect the linear regression models, either concerning the lack of fit, the selection of explanatory variables, or both.

Gamma and Inverse Gaussian distributions demonstrated a better accuracy in the prediction of the ABI over all the datasets used in this research. This could be because most of the data was collected in urban and suburban areas, where the biological water quality varied principally between moderate and bad, particularly during the dry season. Therefore, the data were skewed, resulting in the suitability of the aforementioned GLMs (Folks and Chhikara, 1978; Attrill et al., 1996). However, the Gaussian GLM performed well during the rainy season. This was likely due to the Gaussian distribution of biological water quality classes that varied mainly between good and deficient; consequently, the data were less skewed.

The datasets used in this research were relatively small, ranging from 27 to 62 samples. According to Stockwell and Peterson (2002) and Vaughan and Ormerod (2005), the application of a large sample size higher than 100 observations - allows models to obtain better accuracy in their prediction. However, Stockwell and Peterson (2002) concluded that the accuracy of samples was close to maximum when the size was higher than 50 observations while the accuracy diminishes to 90% when the sample size was 10 observations. Furthermore, Vayssières et al. (2000) indicated that the results obtained from small datasets were more efficient with GLMs, than with non-parametric techniques such as generalized additive models or classification trees. Similarly, biological indices from a small dataset have been modeled with GLMs, demonstrating a stable goodness-of-fit in their prediction (Holguin-Gonzalez et al., 2013a; Damanik-Ambarita et al., 2016). Moreover, to avoid possible overfitting in the linear models that could be produced with small datasets, we follow the recommendations given by Shmueli (2010). Thus, (1) for the construction of the models the cross-validation procedure was performed; and (2) the similarity of the accuracy of the models, measured as pseudo-R2, obtained within the training and validation datasets were checked. Furthermore, the outliers that could produce overfitting were deleted before the model construction (Zuur

The water quality of rivers is not static. Its variation is related to the frequency of discharges from anthropogenic sources, and the presence of substances transported by runoff from rainfall and irrigation.

Consequently, river water quality displays a dynamic variation over time. Similarly, with regard to macroinvertebrates, a taxon could be recorded absent in a site although it was present as it was not detected (Elith and Leathwick, 2009). Additionally, in habitats, it is possible that some taxa may not be present due to migration patterns, seasonal variation or unstable habitats (Jacobsen and Encalada, 1998). However, this water quality was assessed with discrete sampling campaigns with one sampling event per season. Consequently, the models were constructed with information from these sampling campaigns, which offered insight mainly into the impact generated from natural and anthropogenic sources.

An ecological model is an important tool that can be applied in river restoration. However, ecological models have two limitations: the models are constructed with information of a specific area, and the models are developed with datasets whose variables have a definite range of values. Consequently, the use of ecological models is restricted to the area for which they were generated (Forio et al., 2016; Jerves-Cobo et al., 2018b). Furthermore, the accuracy of these statistical models can be altered if their range of values for each variable is different than that which was obtained in their construction (McCullagh, 1984).

In this research, numerous hypothesis tests that included many environmental variables, model selection procedures and a biological index were performed. However, no stage corrections for multiplicity were developed. This implies that the presence of more false positives, which could be obtained with the applied individual statistical hypothesis test at 5%, is possible. Potential approaches that could be used to correct the p-values in a multiplicity analysis could be the false discovery rate (FDR) or the family-wise error rate (FWER) at 5%. However, these procedures are rarely applied in ecological studies (Forio, 2017). For this reason, this correction was not implemented in this research.

In this study, despite the limited number of samples, the use of the GLMs, the three cross-validation procedure and the standardized method applied during the collection of samples, all contributed to the reliability and accuracy of the models showing relevant ecological patterns. However, a future update to the ecological models constructed in this research is recommended. This update could be developed with future data collected from new sampling campaigns or with information from a monitoring program. Furthermore, this new information could help to check for the replication of the current results and to enhance the accuracy of the current models.

#### 4.4. Main rivers and tributaries, and their impacts from urbanization

The expansion of sewage interceptors and the combined sewage network in the city of Cuenca and the Ucubamba Wastewater Treatment Plant (U-WWTP) (ETAPA-EP, 2007) have improved the water quality of the rivers (Fernandez de Cordova and González, 2012). However, degradation of the water quality is still occurring because of pollution point sources such as industrial discharges, combined sewage overflows (CSOs), surface water outfall (SWO), overflow before the U-WWTP and fluxes of organic pollution from an extensive livestock area (Jerves-Cobo et al., 2018a). For these reasons, we analyzed the accuracy of the developed models to assess their applicability in improving

#### Appendix A

water quality status and their effectiveness in river management. In this way, the constructed models displayed the lowest accuracy when applied to the sub-dataset of the reference sites, wherein sites were classified as having only good and very good biological water quality. Possibly, this low accuracy was because the models were mainly constructed with information collected in disturbed sites, located in urban and suburban areas. In this regard, McCullagh (1984) indicated that a model provides good predictions according to the range of conditions used in its development. Furthermore, the complexity of the biological interactions is not incorporated into the models, which could also contribute to reduced accuracy. Conversely, the selective combination of the season-specific models that were applied according to the season in which the sites were sampled, appeared to be more suitable for their validation with the sub-datasets of the main-rivers, the principal-river and tributaries, obtaining the highest accuracy with the principal-river sub-dataset. In this regard, the combination of the season-specific models could be used to support the results of possible improvements for the main rivers (Tomebamba, Machangara, Yanuncay and Tarqui Rivers), the principal river (Tomebamba), or their tributaries. These improvements could be tested in a scenario analysis wherein possible actions can be simulated in order to effectively restore the rivers.

#### 5. Conclusions

Three main conclusions could be drawn from this modelling study. First, the season-specific models were more reliable than the seasonoverarching model. Second, the prediction of the biological water quality was more accurate at sites sampled in the urban area than at the reference sites indicating the difficulty in predicting at more biodiverse sites. Finally, the main predictors of the Andean Biotic Index (ABI) varied between seasons. The five-day biological oxygen demand (BOD<sub>5</sub>), orthophosphate and ammonium were the main explanatory variables during the dry season, while oxygen saturation (OS), dissolved oxygen (DO), nitrate and total solids were the major variables during the rainy season. Consequently, the development of seasonspecific models seems relevant as a basis to establish key actions for river restoration. The constructed models could be improved with information from new sampling campaigns. The modelling approach developed in this study can be applied and/or extrapolated to similar basins in tropical countries.

#### Acknowledgements

This research was executed in the context of the VLIR-UOS IUC Programme – University of Cuenca and the VLIR Ecuador Biodiversity Network project. Marie Anne Eurie Forio receives financial support from the special research fund of Ghent University. The authors would also like to extend their gratitude for the collaboration with the Universidad Técnica del Norte, Universidad del Azuay and Universidad Politécnica Salesiana. Similarly, appreciation is extended to the Water Supply and Wastewater Management Municipal Company ETAPA – EP, Electro Generator of the Austro Company – ELECAUSTRO S. A. and the Ecuadorian Environmental Ministry.

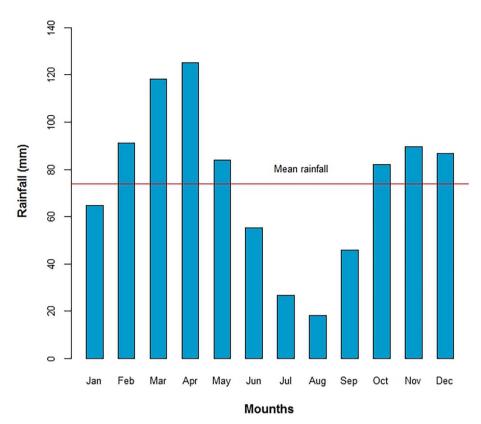


Fig. A1. Histogram of monthly average rainfall in the city of Cuenca (1990–2012).

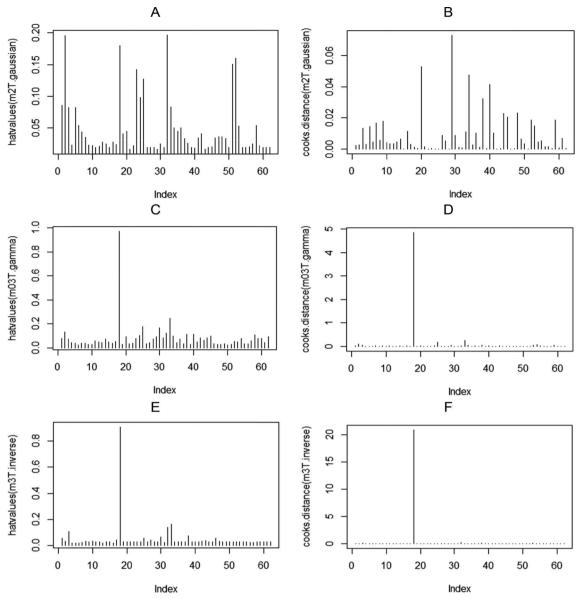


Fig. A2. Plots of influential points with the complete dataset, using Cook's distance and hat elements with different kind of GLM: (A and B) Gaussian, (C and D) Gamma, and (E – F) Inverse Gaussian.

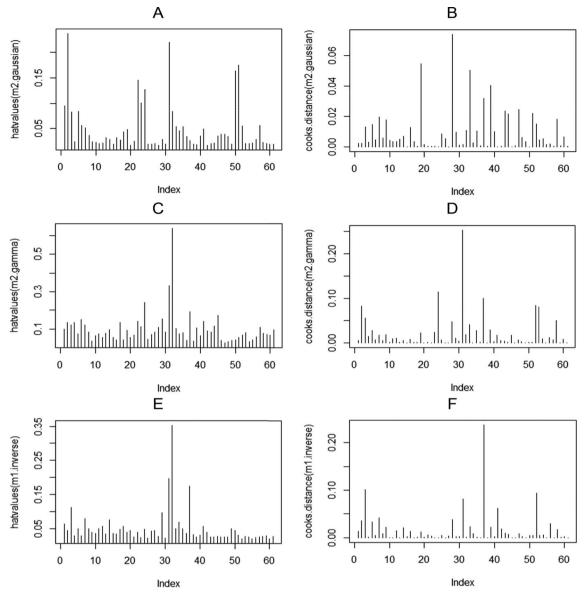


Fig. A3. Plots of influetial points with the without-outliers (Ta02) dataset, using Cook's distance and hat elements with different kind of GLM: (A – B) Gaussian, (C – D) Gamma, and (E – F) Inverse Gaussian.

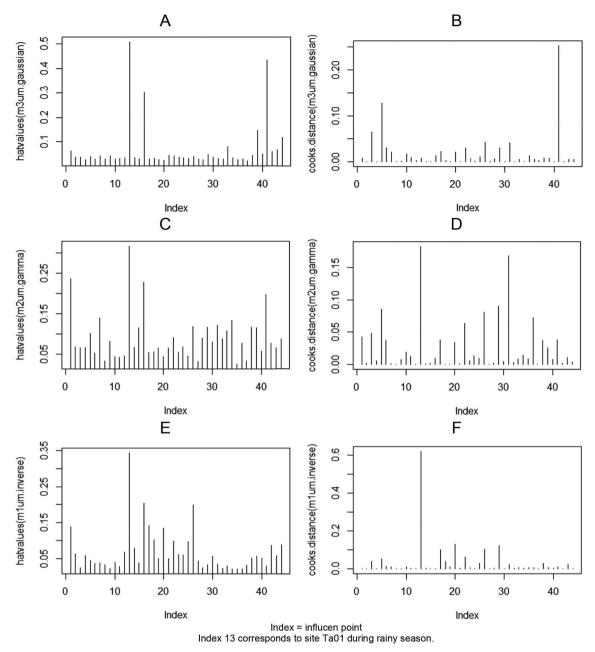


Fig. A4. Plots of influential points with the main-rivers dataset, using Cook's distance and hat elements with different kind of GLM: (A – B) Gaussian, (C – D) Gamma, and (E – F) Inverse Gaussian.

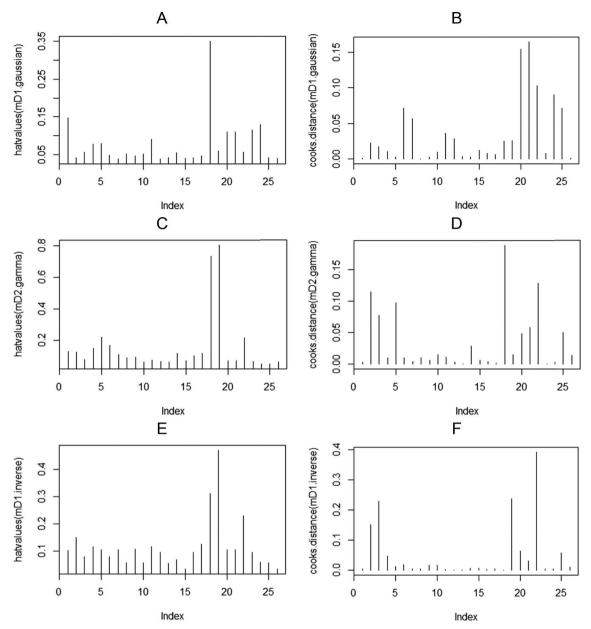


Fig. A5. Plots of influential points with the dry-season dataset, using Cook's distance and hat elements with different kind of GLM: (A – B) Gaussian, (C – D) Gamma, and (E – F) Inverse Gaussian.

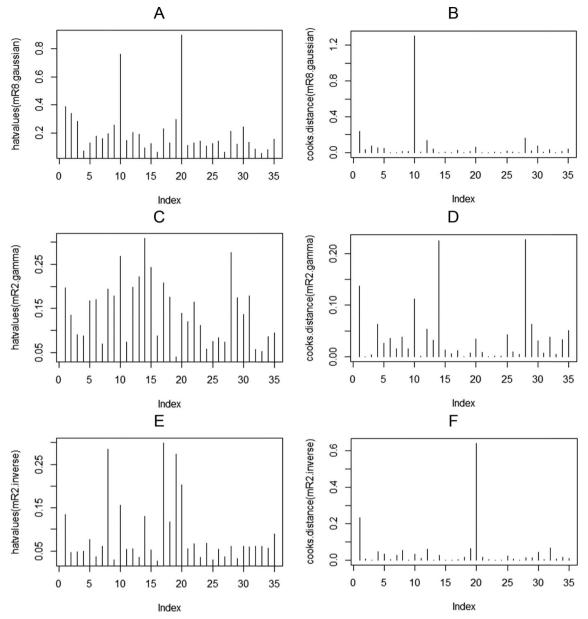


Fig. A6. Plots of influential points with the rainy-season dataset, using Cook's distance and hat elements with different kind of GLM: (A - B) Gaussian, (C - D) Gamma, and (E - F) Inverse Gaussian.

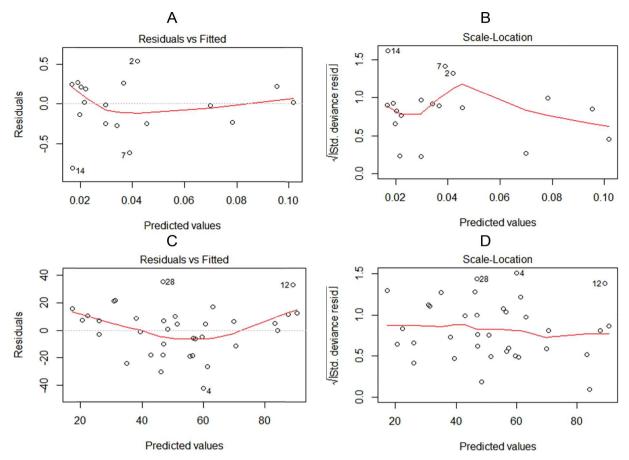


Fig. A7. Plots of Residual vs. Fitted of the best models: (A) Dry season, (B) Rainy season, and plots of Scale-Location of the best models: (C) Dry season, (D) Rainy season.

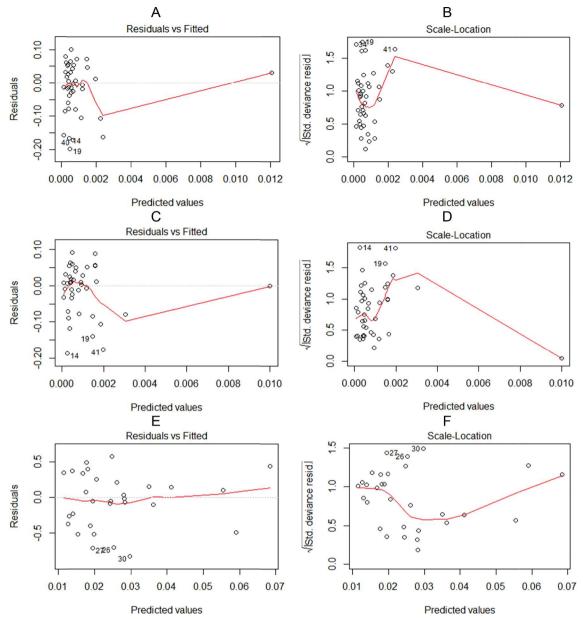


Fig. A8. Plots of Residual vs. Fitted of the best models: (A) with the complete dataset, (B) with the without-outliers dataset, (C) with the main-rivers dataset, and plots of Scale-Location of the best models: (D) with the complete dataset, (E) the without-outliers dataset, and (F) with the main-rivers dataset.

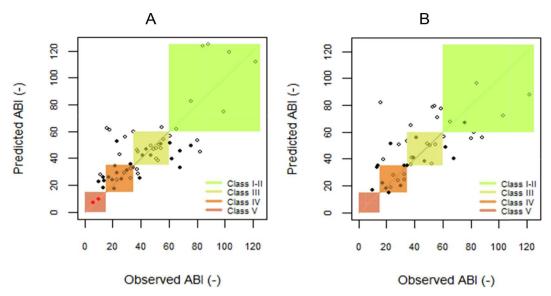


Fig. A9. Graph comparing simulated index and field data for models developed from: (A) the without-outlier dataset, and (B) the main-rivers dataset. The filled dots and the empty dots are the sampling sites taken during dry and rainy seasons respectively.

Table A1
Summary of the physical, chemical and microbiological data collected in the study area based on 43 samples in 2015 and 2016.

Parameter	Units	Mean Value		Standard Deviation	Min Value	Max Value	Median Value	Mean Dry Season	Mean Rainy Season
Temperature	°C	13.9	±	2.0	10.4	19.8	13.9	13.2	14.4
Specific conductivity	μS·cm <sup>-1</sup>	135.7	±	194.4	29.0	1396.5	90.9	177.6	103.4
pH		7.5	±	0.4	6.6	8.8	7.6	7.7	7.5
Turbidity	NTU	27.3	±	33.4	0.8	187.0	11.8	30.5	24.8
Chlorophyll-a	$\mu g \cdot L^{-1}$	9.7	±	7.3	2.4	29.3	6.9	9.7	-
Dissolved oxygen (DO)	mg·L <sup>-1</sup>	7.5	$\pm$	1.3	0.7	8.5	7.7	7.3	7.6
Dissolved oxygen saturation (DO_Sat)	%	97.0	$\pm$	15.1	9.6	104.4	100.7	92.3	100.6
Biochemical oxygen demand 5 day (BOD <sub>5</sub> )	mg·L <sup>-1</sup>	11.6	±	49.9	0.8	384.0	2.4	22.2	3.4
Chemical oxygen demand (COD)	$mg\cdot L^{-1}$	98.2	<u>+</u>	195.8	7.9	1036.8	53.8	98.2	-
True color (color)	HU	58.9	<u>+</u>	55.0	12.0	293.0	40.5	57.2	60.3
Alcalinity	$mg\cdot L^{-1}$ CaCO <sub>3</sub>	47.0	±	39.6	16.2	209.4	35.9	57.8	38.7
Phenophftaleina	$mg\cdot L^{-1}$ CaCO <sub>3</sub>	0.8	<u>+</u>	3.2	BDL	17.4	0.0	0.6	0.9
Total Hardness	$mg\cdot L^{-1}$ CaCO <sub>3</sub>	55.7	±	55.6	16.6	421.2	45.2	60.8	51.8
Ca++	mg·L <sup>-1</sup>	16.9	±	16.2	3.8	119.8	13.8	17.5	16.5
Mg++	mg·L <sup>-1</sup>	1.5	<u>±</u>	2.2	BDL	9.4	0.5	0.0	2.6
Chloride	$mg\cdot L^{-1}$	10.1	<u>±</u>	13.6	3.2	95.3	6.1	13.3	7.7
Ortophosphate	mg·L <sup>-1</sup>	0.2	<u>+</u>	0.4	BDL	2.2	0.0	0.3	0.1
Total phosphorus	$mg\cdot L^{-1}$	1.2	<u>+</u>	1.3	BDL	5.4	0.6	0.9	1.4
Ammonium-N	$mg\cdot L^{-1}$	1.0	<u>+</u>	4.0	BDL	26.4	0.1	2.1	0.2
Nitrate-N	$mg\cdot L^{-1}$	0.3	<u>+</u>	0.2	BDL	1.7	0.3	0.3	0.3
Nitrite-N <sup>1</sup>	μg·L <sup>-1</sup>	28	<u>+</u>	54	BDL	365	12	38	21
Total Solids	mg·L <sup>-1</sup>	155.1	±	144.9	29.0	998.0	105.5	176.4	138.7
Total coliforms	$MPN.100mL^{-1}$	4.1E + 05	±	3.5E + 01	1.4E + 03	4.3E + 10	1.9E + 05	8.4E + 05	2.3E + 05
	$CFU.100mL^{-1}$	7.5E + 04	<u>±</u>	1.7E + 01	3.9E + 02	8.4E + 09	5.6E + 04	1.2E + 05	5.4E + 04
Fecal coliforms	$MPN.100mL^{-1}$	1.3E + 05	<u>+</u>	3.1E + 01	4.9E + 02	9.2E + 09	8.6E + 04	2.0E + 05	9.4E + 04
	$CFU.100mL^{-1}$	3.1E + 04	<u>+</u>	1.7E + 01	1.1E + 02	4.9E + 08	2.2E + 04	3.6E + 04	2.9E + 04
Mean stream width	m	13.8	$\pm$	8.9	0.9	30.5	13.2	12.6	14.6
Mean depth	m	0.6	<u>±</u>	0.4	0.1	1.6	0.6	0.5	0.6
Flow velocity	$m \cdot s^{-1}$	1.1	<u>±</u>	0.5	0.2	2.0	1.1	1.0	1.2
Flow	m3·s <sup>-1</sup>	13.4	±	16.7	0.0	86.7	9.9	14.6	12.2

 $<sup>^1</sup>$ Nitrite-N is expressed as  $\mu g L^{-1}$ . For its determination the following APHA 4500-NO<sub>2</sub> colorimetric method with a detection value of  $2 \mu g L^{-1}$  was used. Descriptive statistics of physicochemical and microbiological variables are given as mean values  $\pm$  standard deviations, minimums and maximums. NTU = Nephelometric turbidity units; HU = Hazen units; MPN = Most probable number; CFU = Colony-forming unit. BDL = Below Detection Limit.

Bolded numbers mean the concentration difference between main organic pollutants.

 Table A2

 Taxa present in sampling sites in the Tomebamba River during dry and rainy season.

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l'	vit) AB	Sensitiv Score: A	,	1	2	2	3	3	3	3	3	4	4	4	4	4	4	5	2	5	5	5	5	2	9	9	9	9	9	7	7	∞ (	∞ ο	2	2 5	2 6	101	10	10	-				<u>`</u> ↑ ₽	= Taxa present	il water	:
		Site -> Taxa	Water quality ->	Tubificidae	Chironomidae	Muscidae	Hydrophilidae	Lymnaeidae	Physidae	Psychodidae	Sphaeriidae	Acari	Baetidae	Ceratopogonidae	Empididae	Pyralidae	Tabanidae	Elmidae	Hydropsychidae	Limoniidae	Psephenidae	Scirtidae	Simuliidae	Tipulidae	Aeshnidae	Coenagrionidae	Hyallelidae	Hydroptilidae	Limnephilidae	Glossosomatidae	Leptohyphidae	Hydrobioscidae	Leptoceridae	Ploshorogridos	Colomogoratidae	Grinonfervaidae	Heliconsychidae	l entophlehiidae	Perlidae	Dugosiidao	Glossiphoniidae	Limbricidae	Stenonevchidae	Total taxa ner site →	e loca raya pol l	Biological water quality classification	

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 Table A3

 Taxa present in sampling sites in the Machangara, Yanuncay and Tarqui Rivers during dry and rainy season (from upstream to downstream).

	_			Ма	chang	ara R	liver							Ya	nunca	ay Riv	er er										Tarqu	i Rive	r					φ
	Sensitivity Score :ABI Encalda 2011		Dr	y Seas	son		Raiı	าy Sea	ason		Dry S	eason				R	Rainy S	Seaso	n					Dry	/ Seas	son				Raiı	ny Sea	ason		Sampling sites with the same taxa in the three rivers
	sitiv e:/	0	)	1	2	3	Я	R	R	5	1	9		Я	Я	К	Я	В	Ж	В	Я	-5	1	3	3	-	~		22	~	А	В	~	gs sa se t rs
Site ->	ens	MC09	MC10	MC11	MC12	MC13	MC09R	MC11R	MC13R	YA15	YA14	YA16	YA17	YA15R	YA30R	YA34R	YA35R	YA36R	YA37R	YA16R	YA17R	TA05	TA04	TA03	TA23	TA22	TA02	TA01	TA05R	TA04R	TA03R	TA23R	TA01R	is at the list
	So	ž	Ĭ	ž	ž	ž	Σ	MC	MO	7	>	>	≻	ΥA	X	ΥA	Ϋ́	ΥA	Ϋ́	ΥA	ΥA	1	1	-	-	1	1	1		¥	_ ₹	ΤA	. ≰ !	a it l
Taxa		0	0	0	4	4				4	0	0	_	0						4	0	_	0	0	4	4	4	4	4	0				SS ≪ SS
Water quality ->	4	3	2	2	4	4	4	4	3	4	3	2	4	2	3	4	3	4	3	4	3	4	3	2	4	4	4	4	4	3	2	4	4	
Tubificidae Chironomidae	2	p	-	р	р	p	р -	р	р	-	-	p	p	р	-	p	-	р	p	p	p	р	р	р -	p	р	р	р	<u>р</u>	-	р	р	р	26 29
Muscidae	2	<u>р</u>	p p	p p	p p	р -	-	<u>р</u>	р	<u>р</u>	р -	<u>р</u>	<u>р</u>	р -	p p	<u>р</u>	р -	<u>р</u>	р	<u>р</u>	p p	р -	р <u>-</u>	-	р -	р	<u>р</u>	<u>р</u>	-	р -	<u>р</u>	р -	р -	8
Dytiscidae	3	-	Р	_ P	- P	-	-		р -	-		-	-	q	- -	-	_	-	р -	-	р -		-	-	-	p p	-		-	-	-	-		2
Hydrophilidae	3	-	Ë	-		-			-	-		-	-	- P		-		-	p	-	-			-	-	_ P	-		-	-	р	-	-	2
Lymnaeidae	3	-		-	-			-	-	-	-	-	-	-		-	p -	-	- -	-	_		g	-	_		_		-	-	<u>Р</u>	-	-	2
Physidae	3	-	n	g	n	-	_	n	-	-	-	р	-	р	р	р	р	р	р	р	р	р	р		р	р			g	р	_	р	р	21
Psychodidae	3	-	D	<u>-</u>	g	р	р	- -	q	_	_	- -	р	- -	-	<u>-</u>	р	p	р	p	р	-	р	_	-	_ P	р	_	-	-	_	р	р	15
Sphaeriidae	3	-	-	-	- -	-	-	-	_ P	-	-	-	<u>-</u>	-	-	-	D D	р	-	-	р	р	р	-	-	-	_ P	-	р	р	-	р	р	9
Acari	4	-	-	-	-	-	-	g	-	-	g	р	-	g	-	-	-	<u>-</u>	-	-	-		-	g	-	_	-	-	-	-	-	<u>-</u>	-	5
Baetidae	4	р	р	р	р	р	р	р	р	р	р	р	р	р	р	р	р	р	р	р	р	р	р	р	р	р	р	р	-	-	р	-	-	28
Ceratopogonidae	4	-	-	р	-	-	-	-	р	р	р	р	р	р	-	-	-	р	р	-	-	-	-	р	-	-	р	-	-	-	р	-	-	12
Dolichopodidae	4	-	-	-	-	-	-	-	р	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Empididae	4	-	р	р	-	-	-	-	-	-	-	р	-	р	-	-	-	-	р	-	-	-	р	-	-	-	-	р	р	р	-	-	-	9
Pyralidae	4	-	-	р	-	-	-	-	-		-		-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-		-	1
Tabanidae	4		р	-	-	-	-	-	-	-	-	-	•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	•	-	-	-	•	1
Elmidae	5	р	р	р	•	-	-	р	р	р	р	р	р	р	р		-	•	-		-	•	•	р	-	-	р	-	•	р	р	р	-	16
Hydropsychidae	5	-	-	-	-	-	р	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Limoniidae	5	-	р	р	-	р	-	р	р	-	-	р	-	р	р	-	-	-	-	-	р	-	р	р	-	-	р	-	-	р	р	-	р	15
Scirtidae	5	-	р	-	-	-	-	-	-	-	р	-	-	р	р	-	-	-	-	р	-	-	-	-	-	-	-	-	-	-	р	-	-	6
Simuliidae	5	р	р	-	р	-	р	р	р	р	р	р	-	р	-	-	р	р	р	р	р	р	р	р	-	-	-	-	р	р	р	р	-	22
Tipulidae	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	р	-	-	-	-	-	-	-	р	-	-	-	-	2
Aeshnidae	6	-	р	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	р	-	-	р	-	-	-	-	3
Coenagrionidae	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	р	-	-	-	-	-	-	-	-	-	-	-	1
Hyallelidae	6	-	р	-	р	-	-	-	-	р	-	р	р	-	р	р	р	-	р	р	р	р	р	-	р	-	-	р	р	р	р	р	р	20
Hydroptilidae	6	р	р	-	-	-	-	р	р	-	-	-	-	-	-	-	-	-	р	-	р	-	-	-	р	-	-	-	-	р	р	-	-	9
Limnephilidae	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Leptohyphidae	7	-	-	-	-	-	-	-	-	р	р	р	-	р	р	-	р	-	-	-	р	-	р	р	-	-	-	-	-	-	р	-	-	10
Hydrobioscidae Leptoceridae	8 8	р -	p	р -	-	р -	-	-	<u>р</u>	-	р -	р	-	-	-	-	-	-	-	-	-	-	р -	<u>р</u>	-	-	-	-	-	р -	р -	-	-	11 0
Polycentropodidae	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<u>-</u> р	-	-	-	-	-	-	р	-	-	2
Blepharoceridae	10	g	-	g	-	-	_	-	-	-	-	g	 D	g	р	-	g	-	р	-	_			- -	_		_		-	_	р	-	-	9
Calamoceratidae	10	_ P	HĒ	- -		-	-			-		- -	<u>-</u>	р	р	-	μ -	-	- P	-			-			_	-		H -	-	р	-		3
Gripopterygidae	10	-	HĒ	g	-	-	-	-		-		-	-	- P	<u>р</u>	-	_	-	-	-	-			-	-	-	-		-	-	Р -	-	-	1
Helicopsychidae	10	-	Ħ	_ P	-	-	-	-	-	-	-	-	-	q		-	_	-		-	-			-	_		-		-	-		-	-	1
Leptophlebiidae	10		<b>-</b>			-	-			-		-	-	- -	-	-	g	-	-					р	_	_			-	-		-	-	2
Perlidae	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	р	-	-	1
Dugesiidae	-	-	-	-	q	-	-	q	-	-	-	-	-	-	-	-	р	q	-	р	р	g	g	_	р	р	g	р	р	-	р	р	р	16
Glossiphoniidae	-	-	-	-	р	-	-	-	-	-	q	-	-	-	-	-	-	-	-	р	р	-	-	-	р	-	-	р	р	р	-	р	р	10
Lumbricidae	-	р	р	р	-	р	-	-	р	р	-	р	р	р	р	р	р	р	р	р	р	р	р	-	-	-	р	-	-	р	р	-	р	22
Total taxa per point $\rightarrow$ 9 16 14 10 7 5 10 13 8 10 15 9 17 12 6 13 10 14 11 16 10 15 11 8 8 9 7 10 12 19 10 10													i																					
p = Taxa presen						-	-								-															-				
Biological water qua	ality classi	fication	<del>)</del>	1	Very :	good	2	Go	ood	3	Mod	erate	4	Defi	cient	5	Bad																	

**Table A4**Results of the variables difference between seasons.

Parameter	Norma	lity tests	1	Paired student te	st	1	Paired wilcoxon	test
	Levene test	Shapiro test	Two sided	Greater	during	Two sided	Greate	r during
				rainy season	dry season		rainy season	dry season
Biological parameters								
Andean Biotic Index (ABI)	0.59	0.99	0.10	0.05	0.95			
Total number of taxa	0.28	0.80	0.04	0.02	0.98			
Ephemeroptera - Plecoptera - Trichoptera (EPT) taxa	0.06	0.02				0.33	0.17	0.85
Number of sensitive taxa NST	0.82	0.31	0.08	0.04	0.96			
Shannon-Wiener index (SWD)	0.86	0.45	0.01	< 0.01	1.00			
Mean Tolerance Sore (MTS)	0.72	0.74	0.62	0.31	0.69			
Physicochemical parameters								
Temperature	1.00	0.39	< 0.01	< 0.01	1.00			
Conductivity	0.67	< 0.01				0.36	0.18	0.83
pH	0.33	0.47	< 0.01	1.00	< 0.01			
Turbidity	0.26	0.63	0.20	0.10	0.90			
Dissolved oxygen (DO)	0.94	0.29	< 0.01	1.00	< 0.01			
Oxygen saturation (OS)	0.96	0.85	0.20	0.10	0.90			
Five-day biological oxygen demand (BOD <sub>5</sub> )	0.69	0.01				0.37	0.19	0.82
True color (color)	0.19	0.05	0.11	0.06	0.94			
Alkalinity	0.75	0.14	0.39	0.81	0.20			
Chloride	0.87	0.01				0.23	0.12	0.89
Orthophosphate	0.41	< 0.01				0.51	0.25	0.76
Ammonium-N	0.81	0.05				0.79	0.62	0.40
Nitrate-N	0.40	< 0.01				0.12	0.06	0.94
Nitrite-N	0.10	0.04				< 0.01	< 0.01	1.00
Total solids	0.24	0.30	0.03	0.02	0.99			
Log Fecal Coliforms (MPN/100 mL)	0.79	0.54	0.31	0.15	0.85			
Flow velocity	0.65	0.94	0.70	0.35	0.65			

Bolded numbers have a p-value < 0.05.

**Table A5**Best model outcomes: dry season.

Explanatory variables	Regression Parameters			Models obtained	l from the dry-s	eason dataset (wit	thout outliers)		
		Inverse Gau	ete data sssian model: inverse	Fol Inverse Gau mD9.3fcv1			d-2 ssian model: a3.gamma	Gamma	d-3 model: 2a3.gamma
		Coefficient	p-Values	Coefficient	p-Values	Coefficient	p-Values	Coefficient	p-Values
	A	1.9E-04	0.15	-4.4E-04	0.09	1.6E-02	< 0.01	1.3E-02	< 0.01
Nitrate	B1								
Nitrite	B2								
Ammonium	В3					-2.6E-02	0.04	-4.2E-02	0.03
BOD <sub>5</sub>	B4	1.7E - 04	< 0.01	1.9E - 04	0.06	3.4E - 03	0.04	5.2E - 03	< 0.01
DO	B5								
Oxygen saturation	В6								
Log Fecal Coliforms	В7								
Orthophosphate	B8	4.9E - 03	< 0.01			1.2E - 01	0.02	1.4E - 01	< 0.01
Chloride	В9			1.2E - 04	0.02				
Total solids	B10								
Turbidity	B11								
Velocity	B12								
pH	B13								
Conductivity	B14								
Alkalinity	B15								
True color	B16								
Bank material	B17								
AIC:		198.2		126.8		134.4		140.6	
Training subset (2/3)									
Pseudo R <sup>2</sup> :		0.7		0.7		0.5		0.7	
CCI:		57.7%		58.8%		52.9%		72.2%	
κ:		0.4		0.4		0.4		0.6	
Validation subset (1/3)									
Pseudo R <sup>2</sup> :				9.4E - 04		0.8		0.4	
CCI:				44.4%		55.6%		62.5%	
κ:				0.25		0.4		0.4	

**Table A6**Best model outcomes: rainy season.

Explanatory variables	Regression parameters			Models obtai	ned from the ra	iny-season dataset	(without outlier	rs)	
		Complet Gaussian mR8.ga	model		d-1 n model a2. gaussian	Fol Gaussia mR07.3fcv1	n model	Inverse Gau	ld-3 issian model 2a3. inverse
		Coefficient	p-Values	Coefficient	p-Values	Coefficient	p-Values	Coefficient	p-Values
	A	-725.5	< 0.01	-641.7	0.01	-865.4	0.01	8.7E-03	< 0.01
Nitrate	B1	85.3	< 0.01			68.9	0.02		
Nitrite	B2	-1100.7	< 0.01			-6.4E-01	0.07		
Ammonium	В3			-32.8	0.08				
BOD <sub>5</sub>	B4								
DO	B5	-42.8	0.04			-52.4	0.04		
Oxygen saturation	В6	10.9	< 0.01	7.1	0.03	13.1	0.01	-8.3E-05	< 0.01
Log Fecal Coliforms	В7								
Orthophosphate	B8								
Chloride	В9								
Total solids	B10	-0.1	0.03			-1.2E-01	0.04		
Turbidity	B11			-0.5	0.03				
Velocity	B12			-27.8	0.04				
pH	B13								
Conductivity	B14								
Alkalinity	B15								
True color	B16								
Bank material	B17	10.6	0.02	12.6	0.03				
AIC:		314.0		218.2		202.8		206.7	
Training subset (2/3)									
Pseudo R <sup>2</sup> :		0.6		0.6		0.6		0.3	
CCI:		66.7%		62.5%		56.5%		47.8%	
κ:		0.5		0.4		0.3		0.2	
Validation subset (1/3)									
Pseudo R <sup>2</sup> :		0.3		0.1		0.4		0.6	
CCI:		72.7%		63.6%		50.0%		50.0%	
κ:		0.6		0.5		0.3		0.3	

**Table A7**Best model outcomes with the complete dataset.

Explanatory variables	Regression Parameters	Models obtained from both season with the complete dataset								
		Complete data Gamma model: m03T.gamma		Fold-1 Gamma model m2.3fcv1a2T.gamma		Fold-2 Inverse Gaussian model m5T.3fcv1a3T.inverse		Fold-3 Gamma model m03.3fcv2a3T.gamma		
		Coefficient	p-Values	Coefficient	p-Values	Coefficient	p-Values	Coefficient	p-Values	
	A	2.0E-01	0.08	-1.29E-02	0.09	2.01E-04	< 0.01	3.0E - 01	< 0.01	
Nitrate	B1									
Nitrite	B2									
Ammonium	В3	-7.4E-03	< 0.01					-9.4E-03	< 0.01	
BOD <sub>5</sub>	B4					-2.3E-05	< 0.01			
DO	B5	1.2E - 02	< 0.01					1.8E - 02	0.01	
Oxygen saturation	В6	-2.7E-03	< 0.01					-4.1E-03	< 0.01	
Log Fecal Coliforms	В7									
Orthophosphate	В8	7.5E - 02	< 0.01			8.4E - 03	< 0.01	6.7E - 02	0.01	
Chloride	В9			3.4E - 03	< 0.01					
Total solids	B10									
Turbidity	B11									
Velocity	B12			1.1E - 02	0.02					
pH	B13			-1.29E-02	0.09	2.01E - 04	< 0.01			
Conductivity	B14									
Alkalinity	B15									
True color	B16									
Bank material	B17									
AIC:		527.0		356.1		353.9		353.6		
Training subset (2/3)										
Pseudo R <sup>2</sup> :		0.5		0.5		0.5		0.5		
CCI:		53%		61.9%		43.9%		48.8%		
κ:		0.3		0.5		0.2		0.3		
Validation subset (1/3)										
Pseudo R <sup>2</sup> :				0.1		0.3		0.5		
CCI:				40.0%		47.6%		61.9%		
κ:				0.2		0.3		0.5		

**Table A8**Best model outcomes without-outliers dataset.

Explanatory variables	Regression parameters	Models obtained from both season without-outliers dataset								
		Complete data Gamma model m2.gamma		Fold-1 Gamma model m3.3fcv1a2.gamma		Fold-2 Inverse Gaussian model m2.3fcv1a3.inverse		Fold-3 Gamma model m1.3fcv2a3.gamma		
		Coefficient	p-Values	Coefficient	p-Values	Coefficient	p-Values	Coefficient	p-Values	
	A	1.4E-01	0.02	-1.2E-02	0.11	1.5E-04	0.06	2.2E-01	0.01	
Nitrate	B1									
Nitrite	B2									
Ammonium	В3	-1.3E-02	0.03							
BOD <sub>5</sub>	B4	2.1E - 03	< 0.01					1.5E - 03	0.04	
DO	B5	1.4E - 02	< 0.01					2.0E - 02	< 0.01	
Oxygen saturation	В6	-2.3E-03	< 0.01					-3.6E-03	< 0.01	
Log Fecal Coliforms	B7									
Orthophosphate	B8	6.1E - 02	< 0.01			8.1E - 03	< 0.01	3.3E - 02	0.07	
Chloride	В9			3.4E - 03	< 0.01					
Total solids	B10									
Turbidity	B11									
Velocity	B12			1.1E - 02	0.03					
pH	B13									
Conductivity	B14									
Alkalinity	B15									
True color	B16									
Bank material	B17									
AIC:		513.7		347.6		343.9		347.4		
Training subset (2/3)										
Pseudo R <sup>2</sup> :		0.6		0.6		0.5		0.6		
CCI:		57.4%		63.4%		45.0%		53.7%		
κ:		0.4		0.5		0.2		0.3		
Validation subset (1/3)										
Pseudo R <sup>2</sup> :				0.1		0.3		0.6		
CCI:				45.0%		52.4%		60.0%		
κ:				0.2		0.3		0.4		

**Table A9**Best model outcomes with the main-rivers dataset.

Explanatory variables	Regression parameters	Models obtained from both season with the main-rivers dataset								
		Complete data Gamma model: m6um.gamma		Fold-1 Gaussian model: m2um3fcv1a2.gaussian		Fold-2 Gamma model: m13um3fcv1a3.gamma		Fold-3 Gaussian model m13um3fcv.gaussian23		
		Coefficient	p-Values	Coefficient	p-Values	Coefficient	p-Values	Coefficient	p-Values	
	A	2.6E-01	0.02	-764.7	< 0.01	2.4E-01	0.01	117.5	< 0.01	
Nitrate	B1							-84.6	0.05	
Nitrite	B2									
Ammonium	В3									
BOD <sub>5</sub>	B4									
DO	B5	1.2E - 02	0.02	-30.7	0.03	1.3E - 02	0.06			
Oxygen saturation	В6	-3.4E-03	0.02	10.5	< 0.01	-3.2E-03	0.01			
Log Fecal Coliforms	B7									
Orthophosphate	B8	9.5E - 02	< 0.01	-88.6	0.04	1.2E + 01	< 0.01	264.1	0.06	
Chloride	В9									
Total solids	B10									
Turbidity	B11							-9.2E-01	< 0.01	
Velocity	B12									
pH	B13									
Conductivity	B14							-4.7E-01	0.03	
Alkalinity	B15									
True color	B16									
Bank material	B17									
AIC:		378.5		273.4		247.0		254.3		
Training subset (2/3)										
Pseudo R <sup>2</sup> :		0.5		0.5		0.5		0.5		
CCI:		52.3%		53.3%		55.2%		62.1%		
κ:		0.3		0.3		0.4		0.5		
Validation subset (1/3)										
Pseudo R <sup>2</sup> :				0.4		0.1		0.1		
CCI:				57.1%		40.0%		40.0%		
κ:				0.4		0.1		0.2		

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