PHYSICOCHEMICAL AND DIATOM TROPHIC STATE INDEXES: A COMPLEMENTARY APPROACH FOR IMPROVING WATER SUSTAINABILITY IN A HIGH ANDEAN URBAN STREAM

Abstract

Discharge of untreated wastewater into freshwater ecosystems increases nutrient concentrations, causing eutrophication and demanding more intensive monitoring and control activities, particularly in developing areas. Two approaches are commonly used for assessing trophic states of rivers: 1) Physicochemical Trophic State Indexes (TSIs), and 2) Trophic Indexes based on bioindicators, mainly periphytic diatoms (TDIs). Even when these two approaches seem to be very different, they can be complementary under certain circumstances. This is the case with Río Chili (Arequipa, Peru), a shallow regulated river used for multi-purpose activities, but which is highly polluted due to the discharge of municipal wastewater. The present study assessed the suitability of different TSIs and TDIs by processing data from historical water quality registers and recent monitoring, including periphytic diatom sampling. TDIs were compared with TSIs applied to both recent and historical records. Results indicated that TSIs can be easily obtained from measurements of phosphorus concentrations, but they are less sensitive and resulted in a high degree of homogeneity among the classification of trophic conditions along the urban path of the river. Alternatively, TDIs showed higher precision and sensitivity, reporting detailed classifications of the sampling points. TDIs suggested that Río Chili presented conditions that varied from mesotrophic to eutrophic as consequence of wastewater discharges and soil occupation. A routine use of TDIs with occasional assessment by physicochemical TSIs may contribute to water quality sustainability by informing managers of the effects of organic and phosphorus pollution on eutrophication at less cost.

Keywords: Periphytic diatoms, water quality, Río Chili, trophic pollution, trophic state indexes.

1. Introduction

Water quality indexes (WQIs) classify levels of pollution based on a set of frequently monitored parameters that describe the general condition of water. The use of WQIs increases cost-efficiency in water management by optimizing the number of parameters frequently monitored and by producing more understandable results. A number of important indexes have been proposed for the assessment of general water quality, such as Brown's WQI (1970), developed by the USA's National Sanitation Foundation (NSF) and which has been broadly applied with local adaptations throughout the world, including in Brazil, Spain and Colombia (Torres et al., 2009). However, general water quality indexes give minimal insight into trophic pollution and, further, there are an abundance of indexes for assessing eutrophication in lakes while there are few such indexes for classify streams and rivers, and so their uses in these ecosystems is rare (Dodds, 1998).

In the last few decades, several trophic state indexes (TSIs) have been proposed specifically for freshwater streams, which are usually based on one of two different approaches: 1) physiochemical-based TSIs and 2) indexes based on bioindicator organisms, such as diatoms (TDIs) and benthic species. Psychochemical indexes have been more commonly applied in the USA and Latin America, especially for deep and slow flowing rivers in which structuring of a benthic community is not possible (Lampareli, 2004). Bioindicator indexes, on the other hand, have been used in the evaluation of shallow and steeply sloped rivers, where hydrodynamic conditions allow the formation of a benthic community. This approach has been broadly used in Europe, even though important studies have considered diatom indexes in the USA and other countries (Ponader et al., 2005; Potapova et al., 2007).

Diatom assemblages in rivers are a component of periphyton, which comprises a complex microbial community that is attached to organic or inorganic substrates on submerged surfaces (Wetzel 2012). Since periphyton structure at any given location is governed by local water quality, its assessment can be useful for characterizing environmental conditions (Rice et al., 2012) and evaluating spatial and temporal changes. Diatom periphyton also respond quickly to increased nutrient loading, and thus are useful for detecting changes on small spatial or temporal scales, such as the effects of sewage discharge (Jüttner et al., 2010).

Some indexes that are based on the identification and quantification of diatom assemblages (TDIs) are strongly correlated with the physicochemical parameters of the water (Peszek et al., 2015). The most commonly used TDI is Kelly's Trophic Diatom Index, first developed by Kelly and Whitton (1995) for correlating diatom ecology with trophic state. Based on one hundred diatom *taxa,* Kelly's TDI produces numerical values varying from 1 (indicating low nutrient concentrations) to 5 (indicating high nutrient concentrations). Another widely used index is Rott's TDI (1999), which was developed for streams in Austria. Both indexes are included as part of various software packages, such as Omnidia®. Local adaptations of such indexes might be required depending on the particular environmental factors involved and their local variability (Besse-Lototskaya et al*.,* 2011). The development of regional autoecological indexes has been a common practice in many countries, such as in the Netherlands (Van Dam et al., 1994), Germany (Coring, 1999), Spain (Delgado, 2012) and USA (Stancheva et al., 2012).

There are fewer TSIs based on physicochemical assessment than there are TDIs. Based on phosphorus, nitrogen and chlorophyll concentrations, Dodds' (1998) proposed boundaries for trophic states of rivers and streams have been widely used, especially in the USA, and include upper boundaries for P of 25 $\mu g \cdot L^{-1}$ for oligotrophic-mesotrophic systems, and of 75 $\mu g \cdot L^{-1}$ for mesotrophic-eutrophic systems. An index developed for temperate regions (Carlson's TSI) was adapted by Toledo (1998) and Lamparelli (2004) for the evaluation of tropical streams in Brazil, and which are currently widely used (Fernandez-Cunha et al., 2008; Fia et al., 2009; Farage et al., 2010).

Physicochemical and diatom indexes require specific approaches with local statistical adaptations and both possess advantages and disadvantages. Trophic indexes based on physicochemical variables are powerful tools for the assessment of eutrophication by directly measuring changes in nutrient concentrations in water; however, they can be very dependent on the water quality at a specific point in time and give only short-term insights into the real ecological status of an ecosystem. Another disadvantage of physiochemical-based trophic indexes is their requirement for constant and expensive monitoring, which could limit their use by stakeholders in developing countries. However, TDIs require diatom identification, which often depends on sophisticated taxonomic skills that are not commonly available (Dodds et al., 1998). Nonetheless, samples can be easily obtained and preserved, and since a microscope is the only equipment required for analysis, the acquisition of results is likely to be less expensive than physicochemical analyses. Additionally, TDIs can accurately assess the ecological status of an ecosystem since diatom assemblages are less affected by momentary or short-term variation in physicochemical variables.

River basins in the Andean region of southern Peru are characterized by possessing water-holding systems that include series of reservoirs affected by anthropogenic activities that promote eutrophication. The Río Chili Basin (Arequipa, Peru) provides drinking water in the city of Arequipa but experiences the occurrence of cyanotoxins and other effects of trophic pollution and cyanobacteria blooms (DIGESA 2014). Río Chili is a shallow, steeply-sloped river subjected to variation in water quality due to intermittent wastewater discharge and variation in flow rate due to regulation. Consequently, conditions favorable to the formation of periphyton are expected to predominate in the river, making TDIs a useful tool for assessing trophic states.

The present study assessed and compared the suitability of both physicochemical-based TSIs and TDIs in order to verify their applicability for describing freshwater ecosystems of Andean highlands basins and providing more precise information to water managers. The main hypothesis tested was that TDIs can produce a more accurate classification than TSIs since periphytic diatoms are less dependent to instantaneous variations of physicochemical measurements and they could summarize information in relation to several water quality variables beyond merely nutrients concentrations. For this purpose, historical water quality data were used to stablish the general water quality status along the urban path of the river and to estimate Toledo's, Lamparelli's and Dodds' TSIs based on orthophosphate (PO_4-P) and total phosphorus (Total-P) concentrations. Additionally, diatoms were sampled and identified during the austral winter and spring of 2015 accompanied by a basic monitoring of physicochemical parameters than can be easily repeated given the capacities of local management. Preliminary relationships were established between the structure and dynamics of periphytic diatoms and water quality. Kelly's and Rott's TDIs were estimated and compared with the physicochemical-based TSIs using both historical and recent data.

2. Materials and Methods

The city of Arequipa is located in the Andean Cordillera of the southwestern Peru (16°23′0″S, 71 32′0″W) at a mean elevation of 2300 m. The Río Chili Basin is a subunit of the Quilca-Chili system with a catchment area of 6758.56 km^2 and mean temperatures between 14.6°C (winter) and 17.7°C (summer). The rainy season occurs between December and March with historical averages ranging 63-173 mm. During drought years, precipitation can be less than 45 mm. Humidity ranges between

30% and 70%, with higher values corresponding to the rainy season. The UV index is very high with maximums of U.V 13 during October and November (CRHCQCH, 2013).

Río Chili originates upstream of Arequipa's urban area, and just downstream of the outlet of the Aguadablanca reservoir, at 3600 m.a.s.l. The main uses of Río Chili include providing supply water to the urban area of Arequipa and for economic activities such as irrigation and mining and manufacturing industries. Throughout its course through Arequipa's urban area, Río Chili becomes a primary component of the historical downtown urban landscape. At the end of its urban path, Río Chili receives the Río Tingo, which is also polluted with municipal wastewater and carries the majority of untreated wastewater discharge from Arequipa's sewage system. Some kilometers downstream, Río Chili supplies water for irrigation of crops in the La Joya district.

The local water authority has established eight sampling points for water quality monitoring along the urban path of Río Chili. The first three sampling points are located upstream of the city and downstream of the outlet of the Aguadablanca reservoir, which regulates the flow rates of Río Chili. The points 132Chil4 to 132Chil6 are located within the urban perimeter, while 132Chil7 and 132Chil8 are located downstream of it. Figure 1 shows the general location of the study area, including the sampling points established by the local water authority.

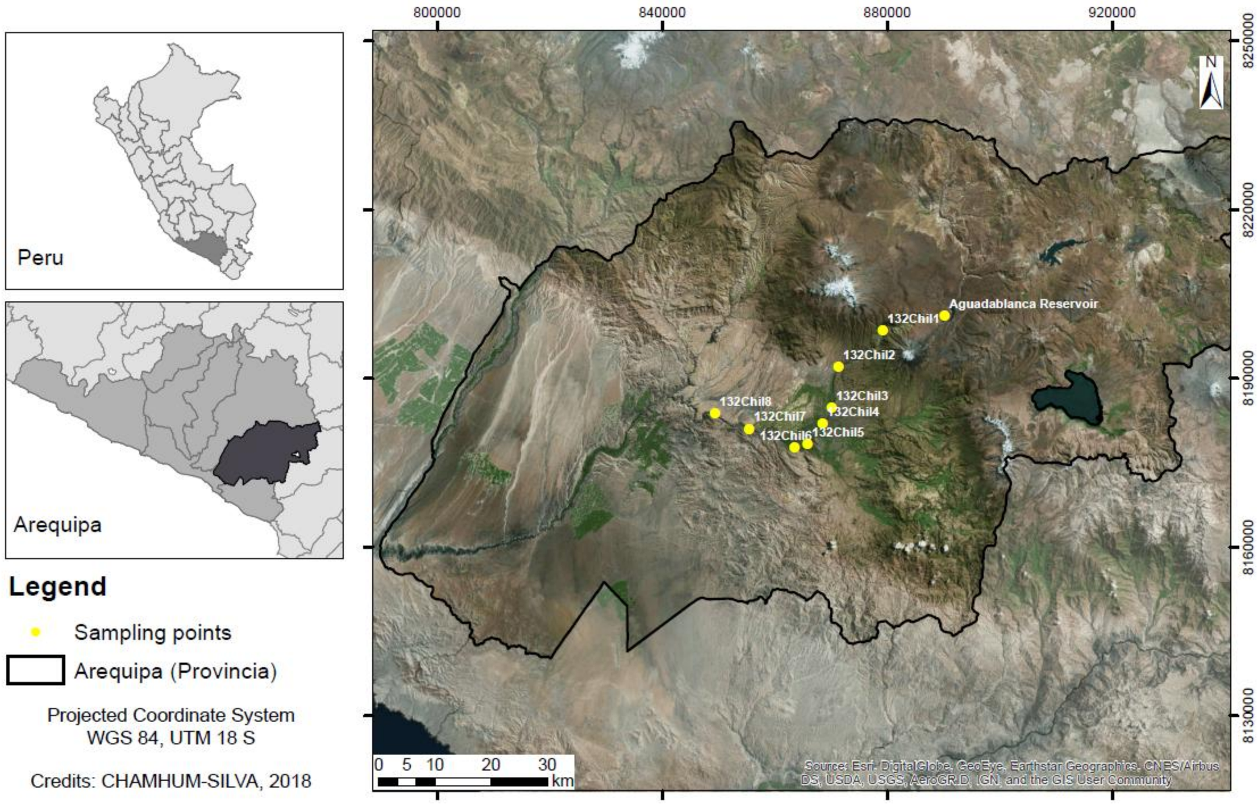

Figure 1 (a) Location of the study area and (b) the distribution of sampling points 132Chil to 132Chil2 along Río Chili, Arequipa, Peru.

To evaluate the general trophic pollution gradient along the urban stretch of Río Chili, available historical records of water quality were assessed, including water temperature (T), flow rate (Q), dissolved oxygen (DO), biochemical oxygen demand (BOD_5), nitrate (NO_3-N), total nitrogen (Total-P), orthophosphates (PO_4-P), total phosphorus (Total-P), conductivity (Cond), Boron (B), Aluminum (Al), Iron (Fe), and main cations including Calcium (Ca), Magnesium (Mg), Sodium (Na) and Potassium (K) . All parameters were assessed by the local water authority following standard methods (Rice et al 2012). The dataset used corresponded to 12 samplings days carried out from 2011 to 2015 with a sampling frequency of 2-4 times per year.

Monitoring period

Diatom samples were collected during August to December of 2015 at the sampling points: 132Chil2 (16° 19'38.67", 71°31'52.77"), 132Chil4 (16° 23' 14.22", 71°32'14.22") and 132Chil6 (16°2707.11", 71°35'41.61") for being representative of the main trophic states of the gradient produced by the urban area of Arequipa: Point 132Chil2 is located upstream of the urban area, 132Chil4 is located after a few kilometers of passage within the city and 132Chil6 is located downstream of the urban area. Anthropogenic activities are present both upstream and downstream of the Aguadablanca reservoir. Sources of pollutants upstream of the Aguadablanca reservoir include trout aquaculture in upstream reservoirs, and agriculture and livestock, whereas downstream, and upstream of 132Chil2, mainly hydroelectric infrastructure is present. Several water intakes are present between 132Chil2 and 132Chil4, including the main derivation channel for the supply of drinking water and for agriculture. Some minor amounts of wastewater are also discharged in the river along this stretch. Between 132Chil4 and 132chil5, the Río Chili receives the Río Tingo and the main sewage line from Arequipa, which discharges 1 m^3/s of untreated municipal wastewater. Institutional and agricultural uses of land predominate between 132Chil2 and 132Chil4, whereas agricultural land uses predominate near 132Chil6.

At each sampling point, three diatom samples were obtained from three different rocks retrieved from the riverbed 15-30 cm below the water surface. Diatoms samples were fixed in loco according to APHA 10300 (Rice et al., 2012), by brushing periphyton off of a measured 5-cm^2 area and rinsing it with formalin (6%) until obtaining a sample of approximately 100 mL. In the laboratory, samples were centrifuged (1500 rpm) and the precipitated solids containing diatoms were treated with H_2O_2 (30%) and heated (200°C) until solids obtained a gray appearance, to digest organic matter, which was later washed with distilled water and centrifuged again. Zrax® high refractive index mounting media was used to fix samples for microscopic observation. Diatom species were identified using the taxonomic references of Krammer et al. (1986, 1988). Relative abundances of each species were calculated by counting up to 400 individuals of the dominant species of each sample. A 1 L water sample was also taken at each sampling point for the analyses of pH (pHmeter Oakton test30®), turbidity (turbidimeter LUTRON ® TU-2016), NO_3^--N (APHA 4500-NO_3-B) and Total-P (APHA 4500-P-E).

Assessment of physicochemical-based Trophic State Indexes

To assess the trophic states at each sampling point along the urban stretch of Río Chili, TSI_L, TSI_T and Dodds' indexes were determined from Total-P and PO_4-P concentrations and classified according to the range of trophic states with boundaries defined by the minimum and maximum values. Lamparelli's and Toledo's trophic indexes (TSI_L and TDI_T for PO_4-P and Total-P- µg/L) were estimated using equations 1, 2a and 2b, respectively. Results were classified by trophic state according to boundaries reported in Lamparelli (2004), Toledo (1990) and Dodds' (1998).

$$TSI_L = 10 \cdot \left(6 - \left(\frac{1.77 - 0.36 \cdot ln\left(\frac{80.32}{P_T}\right)}{\ln(2)}\right)\right) \quad \text{(Eq. 1)}$$

$$TDI_T\ (PO_4\text{-}P) = 10 \cdot \left(6 - \left(\frac{ln\left(\frac{21.67}{PO_4^3 - P}\right)}{\ln(2)}\right)\right) \quad \text{(Eq. 2a)}$$

$$TDI_T\ (\text{Total-P}) = 10 \cdot \left(6 - \left(\frac{ln\left(\frac{80.32}{P_T}\right)}{\ln(2)}\right)\right) \quad \text{(Eq. 2b)}$$

Diatom ecology and indexes

Species richness and Shannon's diversity index (H') were determined for each sampling point using PAST® software. Based on the Zelinka and Marwan (1961) formula, the Trophic Diatom Index of Kelly et al. (2001) was estimated on the basis of relative abundances of species, and nutrient sensitivity (s) and indicator values (v) reported by Kelly et al. (2001) and the *freshwaterecology* database of Schmidt-Kloiberand Hering (2015). Likewise, Rott's Index was estimated in the same manner using the *freshwaterecology* database of Schmidt-Kloiber and Hering (2015); values of *s* and *v* were acquired from the database, while those with missing values were taken from Kelly et al. (2001). Kelly's and Rott's TDIs have been found to be widely relevant in the evaluation of diatom metrics in Europe (Besse-Lototskaya et al., 2008), and their combined use could offer a balanced species-level assessment.

Data analysis

Statistical analyses were run in PAST® software (Hammer et al., 2001). Descriptive statistics and parametric ANOVAS were used to investigate the occurrence of significant differences between sampling points in physicochemical parameters, diatom relative abundancies and trophic indexes. Assumptions of normality and homoscedasticity were checked using Shapiro-Wilk and Levene tests, respectively. When the assumptions were not met, data were log10(x+1) transformed and normality and homoscedasticity tested again. When the ANOVA was significant, a Tukey's test was employed to determine the differences. If transformed data were not normal and homoscedastic, non-parametric Kruskall-Wallis and Dunn's post hoc tests were used to evaluate the occurrence of significant differences between sampling points.

The main variables found to be responsible for describing variability within the historical physicochemical data were identified and selected for further statistical analyses, including principal component (PCA) and Spearman's correlation analyses. The PCA was applied to log10-transformed data in order to investigate the relationships among the main variables responsible for defining the trophic state of each sampling point for historical water quality records. Finally, Canonical Correspondence Analysis (CCA) were used to explore cause-effect relationships between the main diatom species and the physicochemical parameters measured during the monitoring period of 2015.

Spearman's correlations were applied to log-transformed data in order to establish the level of correspondence between selected environmental variables.

3. Results

Historical water quality data

Table 1 provides the main parameters of water quality from historical records for the urban stretch of Río Chili from 2011 to 2015. Significant spatial differences were found for Q (H=14.4, p<0.05), T (F= 4.56, p<0.05), DO (F=4.17, p<0.05), BOD_5 (H 24.6 =, p<0.05), NO_3-N (H=32.27, p<0.05), PO_4-P (H=26.99, p<0.05) and Total-P (H=30.31 p<0.05). The lowest values of BOD_5 and nutrients were found at 132Chil2, which also had the highest values of Q (23.1±15.4 L/s). Conditions associated with high levels of pollution were found in at 132Chil6, characterized by the highest values of BOD_5 (43.9±26.9 mg/L) and Total-P (3.46±3.35mg/L) and the lowest values of DO (5.5±1.2mg/L). Concentrations of NO_3-N exhibited a tendency towards higher values at 132Chil7 and 132Chil8, where organic and phosphorus pollution seemed to be less critical than at 132Chil6 – Table 1. Other parameters also presented longitudinal and significant increases of their concentrations (Cond: H=52.2 p<0.05; B: H=44.82 p<0.05; Ca: H= 47.69 p<0.05; Mg: H=53.42 p<0.05; Na: H =51.38 p<0.05; K: H = 32.14 p<0.05), with the exception of Al (H= 40.086, p>0.05) and Fe (H=6.844, p>0.05).

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Table 1. Historical water quality of Río Chili over eight sampling point used for monitoring by peruvian water authorities based in physical and chemical variables (*Mean ±Standard deviation, (N): number of data,* T: temperature, Q: flow rate, DO: dissolved oxygen, BOD_5: biochemical oxygen demand, Total-N: total nitrogen, NO_3-N: nitrate, PO_4-P: orthophosphate, Total-P: total phosphorus, Ca: Calcium, Mg: Magnesium, Na: Sodium, K: Potassium)

Parameter		Sampling Point							
		132Chil1	132Chil2	132Chil3	132Chil4	132Chil5	132Chil6	132Chil7	132Chil8
T	°C	12.8±2.0 (7)	14.1±2.7 (8)	14.9±0.5 (4)	15.9±1.8 (12)	18.1±1.9 (8)	17.8±1.7 (12)	18.2±3.4 (12)	18.5±1.9 (12)
Q	L/s	23.6±14.4 (7)	25.1±15.4 (6)	8.8±0.9 (4)	14.6±14.0 (10)	15.4±16.6 (7)	17.3±14.9 (9)	17.6±14.9 (9)	11.4±9.9 (7)
pH	-	7.3-8.6 (7)	7.6-8.2 (8)	7.1-8.8 (4)	7.6-8.8 (12)	6.3-8.0 (8)	7.5-7.9 (12)	6.8-8.4 (12)	7.3-9.0 (12)
DO	mg/L	8.3±1.5 (7)	8.2±1.0 (8)	8.4±1.7 (4)	7.9±0.7 (12)	6.4±1.2 (8)	5.5±1.2 (12)	7.0±1.2 (12)	7.4±1.0 (12)
BOD_5	mg/L	5.4±1.3 (8)	4.9±1.6 (8)	5.5±0.9 (4)	5.1±3.0 (12)	39.7±32.2 (8)	43.9±26.9 (12)	17.5±10.6 (11)	11.4±7.1 (11)
Total-N	mg/L	12.3±12.8 (3)	2.7±2.2 (4)	11.0±6.2 (4)	5.1±5.1 (8)	13.3±8.6 (4)	17.9±6.7 (7)	14.7±10.0 (8)	16.0±11.4 (8)
NO_3-N	mg/L	0.16±0.11 (6)	0.22±0.18 (7)	0.64±0.19 (4)	0.46±0.28 (11)	0.38±0.41 (8)	0.65±0.27 (7)	2.72±3.63 (12)	4.25±2.94 (12)
PO_4-P	mg/L	0.05±0.02 (6)	0.08±0.06 (6)	0.06±0.01 (4)	0.12±0.10 (12)	1.52±1.19 (8)	1.98±1.46 (12)	1.48±1.13 (12)	0.86±0.48 (12)
Total-P	mg/L	0.49±0.19 (7)	0.51±0.15 (8)	0.46±0.24 (4)	0.32±0.24 (12)	3.09±2.05 (8)	3.46±3.35 (12)	2.33±2.83 (12)	1.90±1.14 (12)
Cond	µS/cm^2	182.9±45.2 (7)	183.9±30.6 (8)	272.8±52.3 (4)	259.4±103.1 (12)	498.8±98.8 (8)	591.7±190.8 (11)	623.3±176.1 (12)	668.1±180.1 (1@)
Al	mg/L	2.9±4.0 (7)	2.9±3.3 (8)	0.8±0.2 (4)	6.9±12.3 (12)	2.7±2.8 (8)	2.0±2.6 (11)	2.0±2.1 (12)	2.2±4.3 (12)
Fe	mg/L	2.2±3.0 (7)	2.2±2.6 (8)	0.2±0.2 (4)	3.2±5.2 (12)	2.2±2.1 (8)	1.6±1.9 (11)	1.7±1.5 (12)	1.9±3.3 (12)
B	mg/L	0.14±0.06 (7)	0.15±0.06 (7)	0.18±0.08 (3)	0.20±0.10 (12)	0.35±0.15 (8)	0.52±0.19 (11)	0.66±0.25 (12)	0.69±0.24 (12)
Ca	mg/L	13.1±2.2 (7)	13.1±1.7 (7)	16.4±3.2 (4)	19.5±13.7 (12)	23.7±5.1 (8)	30.3±8.1 (12)	37.4±9.7 (15)	42.2±10.8 (12)
Mg	mg/L	4.4±1.0 (7)	4.9±0.6 (7)	6.2±1.0 (4)	6.8±2.8 (12)	9.9±2.6 (8)	13.4±3.4 (12)	16.2±4.4 (12)	17.6±4.5 (12)
Na	mg/L	14.7±4.3 (6)	15.3±3.6 (7)	19.2±3.7 (3)	21.7±7.9 (12)	46.2±14.8 (8)	61.7±18.0 (11)	67.7±20.3 (12)	69.8±18.9 (11)
K	mg/L	3.9±0.9 (3)	4.3±0.6 (4)	4.0±0.6 (4)	5.1±2.4 (9)	10.0±1.2 (5)	11.6±3.2 (8)	10.6±2.0 (9)	9.8±1.6 (9)
Sampling Period		2011-2013	2011-2014	2011-2012	2011-2015	2011-2014	2011-2015	2011-2015	2011-2015

The BOD_5, Na, conductivity and Total-P were important variables to describe the system conditions, with loading coefficients of 0.44, 0.39, 0.34 and 0.30, respectively. The Axis 1 and 2 explained a 45.2% and 19.3% of the general variation, respectively. In the first component, Na, conductivity, Mg, B, Ca, BOD_5, PO_4-P, Total-P and K were strongly correlated (>0.70) and in the PC2, Al, Fe and Q presented strong correlations. There were also moderate and significant correlations between BOD_5 and PO_4-P (r_s -0.50, $p<0.05$), BOD_5 and Total-P (r_s 0.69, $p<0.05$), corroborating that those variables were the most important for describing the effects of the municipal wastewater discharges and the urban area of Arequipa.

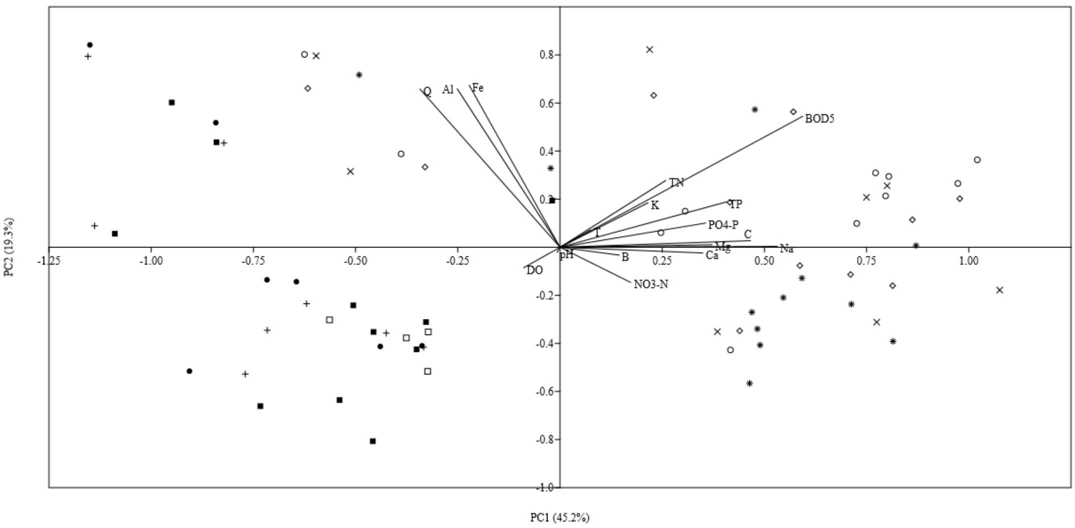

Figure 2. PCA ordination for the historical water quality data of Río Chili (2011-2014). (Q: flow rate, T: temperature, DO: dissolved oxygen, NO_3-N: nitrate concentration, PO_4-P: phosphate concentration, C: Conductivity, TN: Total Nitrogen, TP= Total-P. Chemical elements are represented by Al, C, Ca, Fe, K, Mg and Na. ● = 132Chil1, (+) = 132Chil2, □ = 132Chil3, ■ = 132Chil4, x = 132Chil5, O = 132Chil6, ◊ = 132Chil7, * = 132Chil8.

The scores and classifications of trophic states obtained for each sampling point are presented in Table 2. The indexes TSI_L and TSI_T (Total-P) classified all sampling points as hypertrophic or mesotrophic to hypertrophic or eutrophic to hypertrophic, respectively. The TSI_T (PO_4-P) index classified almost all points as eutrophic to hypertrophic. Dodds' index (which is directly related to the concentration of Total-P in µg/L) suggested conditions from mesotrophic to eutrophic and eutrophic among all sampling points.

Table 2. Physicochemical Trophic State Index (TSI) for historical water quality data of Río Chili (2011-2014) *U: Ultraoligotrophic; O: Oligotrophic; M: Mesotrophic; E: Eutrophic; H: Hypertrophic*

Sampling Point	TSI_L	TSI_T (Total-P)	TSI_T(PO_4-P)	Dodds (1998)
132Chil1	74-87 (H)	53-89 (M-H)	68-82 (E-H)	38-600 (M-E)
132Chil2	80-87 (H)	68-89 (E-H)	68-93 (E-H)	42-600 (M-E)
132Chil3	74-87 (H)	53-89 (M-H)	68-75 (E-H)	50-600 (M-E)
132Chil4	75-87 (H)	57-89 (E-H)	68-98 (E-H)	63-600 (M-E)
132Chil5	11-38 (H)	88-100 (H)	68-131 (E-H)	700-750 (E)
132Chil6	87-103 (H)	89-133 (H)	51-138 (M-H)	600-12700 (E)
132Chil7	80 -102 (H)	68-130 (E-H)	68-134 (E-H)	137-10078 (E)
132Chil8	85-97 (H)	84-117 (H)	68-124 (E-H)	412-4085 (E)

Physicochemical monitoring and trophic index estimation

The monitoring period experienced no rainfall, and the temperatures ranged from 9.3 to 23°C (SENAMHI Meteorological Station 47520, SPQU). Results for the monitored physicochemical parameters are presented in Table 3. Turbidity ($H=14.32$ $p<0.05$) and concentrations of NO_3-N ($F=21.41$ $p<0.05$) and Total-P ($H=21.62$ $p<0.05$) at 132Chil6 were significantly higher than at 132Chil2 and 132Chil3. Mean flow rates measured at 132Chil1 during the monitoring were significantly lower than those from the historical records. Scores for the trophic indexes exhibited a tendency for a longitudinal increase, but the resulting classifications of trophic states were the same for the three sampling points (eutrophic for TSI_T and Dodds's TSI and hypertrophic for TSI_L).

Table 3. Physicochemical parameters and TSIs for Río Chili measured during the diatom sampling campaigns at 132Chil2, 132Chil4 and 132Chil6

Sampling Point	132Chil2	132Chil4	132Chil6
$N\text{-}NO_3^-$ (mg/L)	0.85±0.60	1.57±0.87	5.85±2.92
Total-P (mg/L)	0.45±0.22	0.54±0.10	5.20±2.04
Turbidity (UNT)	6.42±2.11	6.21±3.48	68.16±34.746
pH	7.54±0.47	7.80±0.44	7.26±0.42
TSI_L	84-92, H	87-90, H	93-102, H
TSI_T (Total-P)	56-59, E	57-59, E	59-60, E
Dodds's TSI	2192-1053, E	4310-7755, E	1214-7180, E

(*) A flow rate of 10.01±0.01L/s was discharged from Aguadablanca Reservoir (132Chil1) during the monitoring period

Diatom identification and metrics

Diatom assemblages collected from the three sampling points during the monitoring of 2015 possessed a total of 42 different species. The genera *Nitzschia*, *Navicula* and *Achnanthes* possessed 12, 4 and 3 species, respectively. Other genera identified were *Diatoma, Gomphonema, Fragilaria* and *Planothidium*. One species was identified for *Eolimna*, *Coocconeis*, *Rhoicosphenia*, *Gomphoneis*, *Asterionella*, *Encyonema*, *Cyclotella*, *Reimeria*, *Mellosira*, *Surirella*, *Synedra*, *Epithemia* and *Cymbella*.

Major significance was given to variation in ecological indicator species, which were also the most dominant species. Each sampling point possessed different dominances of ecological indicator species (Figure 3). At 132Chil2, *Nitzschia inconspicua* was predominant during the winter while *Cocconeis placentula* dominated during spring. At 132Chil4, *Navicula lanceolate* and *Nitzschia*

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inconspicua were the most abundant during spring, while *Cocconeis plancentula* dominated during winter. At 132Chil6, *Eolimna subminuscula*, *Nitzschia* sp1, *Nitzschia* sp2 and *N. capitellata* dominated throughout the entire sampling period with no seasonal variation, while other species exhibited an inferior presence.

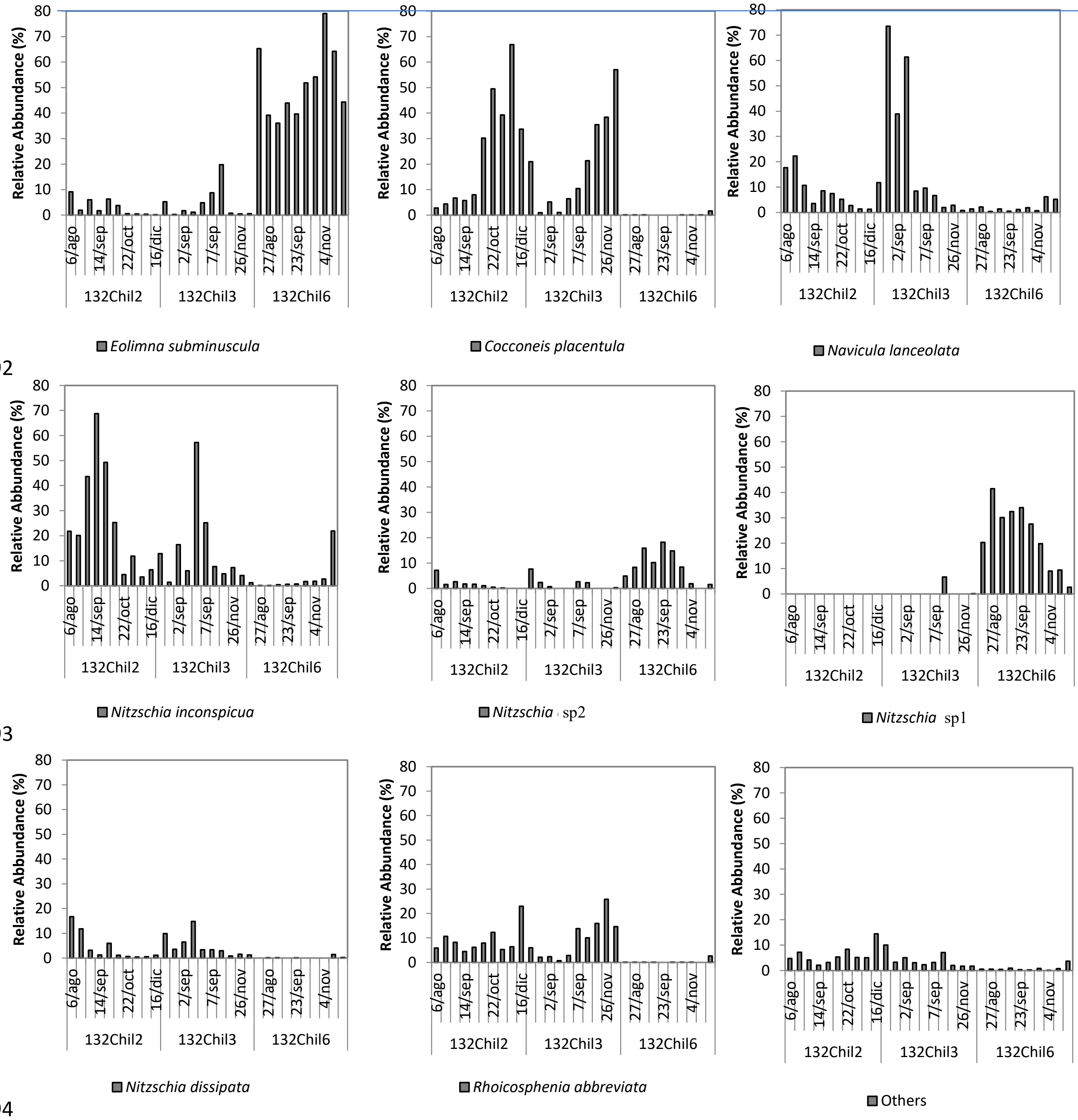

Figure 3. Spatial-temporal distribution of main diatoms along the urban gradient of Río Chili.

The CCA (Figure 4) showed that the occurrence of *Nitzschia* sp1*, Eolimna subminuscula* and *Nitzschia* sp2 were likely correlated with high values of Total-P (Spearman r_s 0.49, 0.63 and 0.85 respectively, p<0.05). *Eolimna subminuscula* and *Nitzschia sp*. were also highly correlated with high concentrations of nitrate (r_s 0.55, 0.73, p<0.05) and turbidity (r_s 0.52, 0.76, p<0.05), whereas all the other species seemed to have higher abundancies in less polluted environments with slightly higher pH, such as those found at 132Chil2. In fact, *Navicula Lanceolata*, *Nitzschia inconspicua*, *Nitzschia dissipata* and others were strongly correlated with low values of Total-P (r_s -0.58, -0.72, -0.61, -0.62 respectively, p<0.05) and nitrate (r_s -0.64, -0.58, -0.69, -0.68 respectively, p<0.05) and turbidity (r_s -0.70, -0.69, -0.76, 0.52, 0.76, p<0.05).

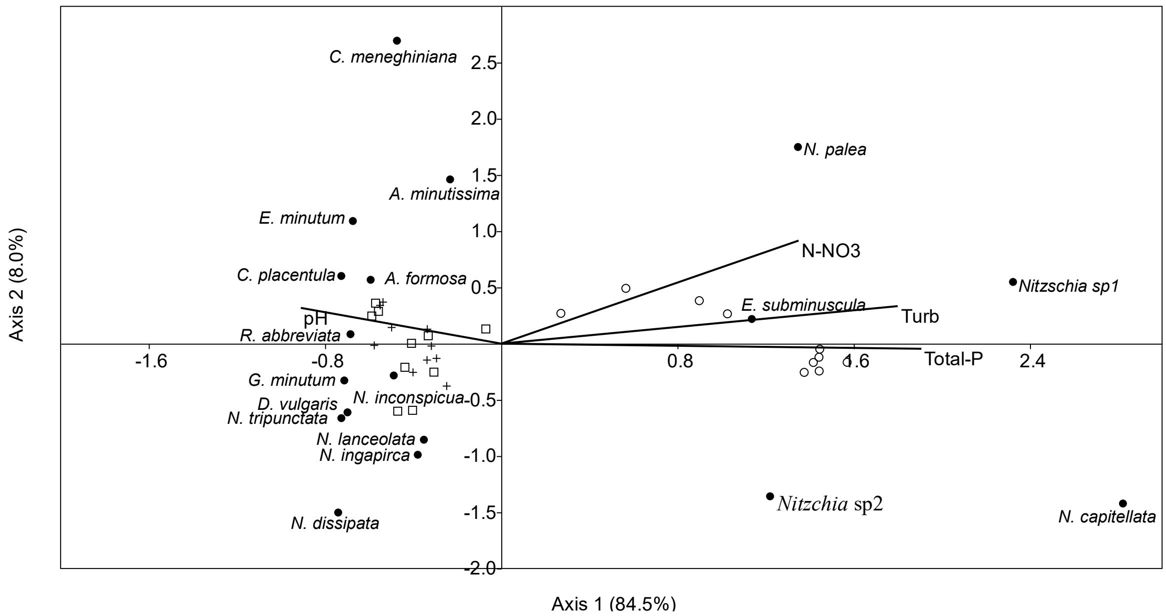

Figure 4. Triplot of Canonical Correlation Analysis (CCA) to analyze the relationship between the main diatom indicator species of trophic pollution found and environmental factors in the urban stretch of Chili River (TP = Total-P, + = 132Chil2, ■ = 132Chil4, O = 132Chil6).

For the ecological metrics and diatom indexes (Table 4), significant differences were found in species richness (F= 15.29 p<0.05) and the Shannon diversity index (F= 16.6 p<0.05), with 132Chil6 having values lower than those of 132Chil2 and 132Chil4. Kelly's TDI scores did not exhibit any significant differences among sampling points (TDI's F= 5.91 p<0.05, WMS's F= 5.01 p<0.05) and classified 132Chil2 and 132Chil4 as meso-eutrophic to eu-hypertrophic and 132Chil6 as completely eu-hypertrophic. Rott's TDI scores presented significant differences between sampling points (F= 5.92 15 p<0.05) and differentiated 132Chil2 and 132Chil4 from 132Chil6 by classifying the first two as meso-eutrophic to eutrophic and 132Chil6 as eutrophic. A moderated and significant correlation was found between Rott's TDI and Total-P (r_s 0.61, p<0.05) and low and significant positive correlations between Rott´s TDI and nitrate and turbidity (r_s 0.15, 0.10, p<0.05) were also evidenced. Kelly's scores were not significantly correlated with any physicochemical variable. Additionally, the percentage of motile species was also assessed, but no significant differences were found in global results (F = 2.47 p>0.05), but there was a tendency for higher percentages at 132Chil6.

Table 4. Resulting ecological indices for the urban stretch of Río Chili (Arequipa, Peru).

Parameter	132Chil2	132Chil4	132Chil6
Richness	23.8±2.3	21.9±2.7	16.7±3.4
Shannon (H')	2.0± 0.3	2.0 ± 0.4	1.4±0.3
TDI	78.8±12.6	81.2±12.1	90.1±3.7
TDI's WMS	4.2±0.5	4.3±0.5	4.7±0.2
	Meso-eutrophic to Eu-hypertrophic	Meso-eutrophic to Eu-hypertrophic	Hypertrophic
Rott	52.9 ± 4.9	55.9±4.8	64.0±1.8
	Meso-eutrophic to Eutrophic	Meso-eutrophic to Eutrophic	Eutrophic
% Motile species	14.4 ± 12.6	31.3±28.8	25.4±10.6

4. Discussion

Historical data provided information of water quality and trophic gradients in the urban stretch of Rio Chili, despite missing data and irregular frequencies of samplings. A general situation of deteriorated trophic states, even upstream of the urban area of Arequipa (132Chil1 and 132Chil2), was demonstrated through Total-P concentrations from both historical and recent records, which exceeded the Peruvian water quality standard of 0.15 mg/L (MINAM, 2015). In fact, this river is formed by the outlet of Aguadablanca reservoir, which had previously been reported as a hypertrophic system with mean Total-P concentrations of 0.12 mg/L (CRHCQCH, 2013). Thus, the present study documented that the sampling points located upstream the urban area of Arequipa (132Chil1 to 132Chil3) are already suffering the effects of anthropogenic influences occurring in the watershed, such as trout aquaculture, agriculture and livestock. The situation downstream of the urban area is even more critical, since both historical data and recent sampling revealed longitudinal increases of pollution resulting from municipal wastewater discharge.

Historical data also provided information about main cations (Ca, Na, K, Mg) and conductivity, which showed to have an influence over the general behavior of water quality in the urban stretch of the river since significant longitudinal increases were detected. This may be related with soil laundering during rainy seasons, mainly by the natural short vegetal covering predominating in the watershed (Mohammed et al, 2017). However, conductivity and cations concentrations ranged below limits of Peruvian water quality standard (MINAM 2015) and no significant ecological effects are expected since concentrations were relatively low when compared with values influencing diatoms ecology (Mangadze et al., 2017). Conversely, Al and Fe presented some concentrations exceeding local standards, but they seem to have no importance, as was suggested by the PCA (Figure 2).

Total-P showed a more significant influence and exhibited a major importance in characterizing the ecological condition of the Río Chili, with nitrogen and other physicochemical variables related to carbon and nutrients pollution playing a secondary role, as it has been described for other (hyper) eutrophic aquatic environments (Giani et al. 2005; Figueredo et al., 2016a). The increasing Total-P concentrations along the river is probably a consequence of wastewater discharge and the expansion of the urban area of Arequipa (CRHCQCH, 2013), since human activities often result in a large addition of P to waterbodies (Carpenter et al., 1998). High concentrations of phosphorus in water may increase the rates of primary production and decomposition, which causes a depletion of dissolved oxygen (Correll 1998).

These tendencies were reinforced by our data, since BOD_5 was also an important variable for characterizing the system and was strongly related to Total-P and PO_4-P concentrations. Furthermore, OD depletion in Río Chili was probably intensified by increasing temperature from upstream to downstream, which decreases gas solubility in water and increases metabolic rates (Cox, 2003).

Dodds' and TSI_T (Total-P) indexes were useful in describing changes in water quality along the course of Río Chili. Their scores demonstrated a longitudinal gradient of eutrophication, with a maximum pollution level occurring at 132Chil6, according to both historical and recent data. The high degree of homogeneity among the classifications of the samples by TSI_L and TSI_T (PO_4-P) indicates that these indexes were less sensitive at detecting spatial-temporal variation, and thus were not suitable for evaluating the effects of human actions in the region. The low sensitivity of TSI_T (PO_4-P) could be expected since PO_4-P has less influence in driving eutrophication than Total-P, as shown by previous researchers (Correll 1998; Figueredo et al., 2014; Figueredo et al., 2016b). On the other hand, the wide variations of TSI_L and TSI_T (PO_4-P) suggest the needing of local adaptation those index that improve their sensitivity for describing the environmental gradient in this Andean stream.

The monitoring period of 2015 experienced temperatures typical of Peruvian summer and spring, with mild values due to elevation and small seasonal variation. Dodds' and TSI_T (Total-P) indexes were applied to evaluating the data collected from the monitoring period of 2015, and classified 132Chil2, 132Chil4 and 132Chil6 as eutrophic. This increase in trophic levels compared to historical records, especially for 132Chil2 and 132Chil4, may be due to lower flow rates of the discharge from Agua Blanca, which decreases the dilution of pollutants discharged along the entire course of the river.

Diatom species identified in Río Chili were indicative of trophic pollution. At 132Chil2 and 132Chil4 there was a dominance of *Navicula lanceolata* and *Cocconeis placentula*, which are indicative of moderate levels of organic pollution (Kelly et al., 2001). These species altered dominance between winter and spring, probably due to increases in temperatures creating unfavorable conditions for *Navicula lanceolata,* which is well adapted to cold-waters (Spaulding et al. 2010). Additionally, organic pollution at these two points does not appear to have reached an extreme level because non-resistant species like *Achnanthes minutissima* and *Encyonema minutum* (Kelly et al., 2005; Oliva et al., 2005; Ponader et al., 2007) were detected. The higher abundances of *Nitzschia inconspicua* observed when higher Total-P concentrations were recorded can be explained by its known resistance to P pollution (Kelly. et al., 2001; Kelly et al., 2015). *Eolimna subminuscula* was previously identified as tolerant to organic pollution related to domestic and industrial discharges (Salomoni et al., 2006), and tolerant to eutrophic conditions with low DO concentrations (Van Dam et al., 1994). These attributes probably explain the dominance of this species at 132Chil6, which possessed high concentrations of organic matter and high levels of trophic pollution. The summary of the community results suggests that the above-mentioned species could be used as indicators of water quality for Río Chili.

The CCA analysis (Figure 5) illustrated the relationships between indicator species and the organic and trophic pollution levels detected at each sampling point. The relationship of *Nitzschia lanceolata* and *Nitzschia inconspicua* with mid-to-low conditions of turbidity and Total-P at 132Chil2 and 132chil4 was confirmed, whereas *E. subminuscula* and *Nitzschia* sp. were related to high levels of organic and trophic pollution at 132Chil6. The Shannon index (H') indicated greater diversity at 132Chil2 and 132Chil4 when compared to 132Chil6, where higher loads of organic pollutants resulted in decreased diatom biodiversity because of losses of species that were non-resistant to pollution. This result has been commonly observed in streams exposed to pollution (Jüttner et al., 2003; Moresco and Rodrigues, 2014).

Kelly's and Rott's indexes reinforced the evidence of increasing eutrophication from upstream to downstream. These indexes classified 132Chil2, 132Chi4 and 132Chil6 as meso-eutrophic to eu-hypertrophic and 132Chil6 as eu-hypertrophic. Since none of the species with more than 1% of relative abundancy were previously reported as unreliable for trophic classification, based on Kelly's or Rott's TDI (Besse-Lototskaya et al., 2008), these indexes are expected to be a good fit to the dynamics of diatom assemblages in Río Chili. Rott's index was more closely correlated with Total-P than Kelly's TDI, indicating a higher specificity for assessing trophic states in Río Chili. Kelly's TDI is highly influenced by organic pollution, which causes an increase in abundance of motile species. Thus, this index gives an important insight into the contribution of organic pollution to eutrophication (Kelly, 1997), but is less specific in detecting longitudinal phosphorus increases. However, the combined application of both indexes in the urban stretch of Río Chili suggests that increases in eutrophication and P concentrations were associated with organic pollution occurring from upstream to downstream, as previously evidenced by the significant positive correlation between BOD_5 and Total-P.

Results from diatom ecological indicators and TDIs corresponded more closely to the trophic state classifications of TSIs applied to historical records than to the classifications of the results of TSIs for 2015, probably due to the lesser dependence of TDIs on momentary, short-term water quality variation, such as those caused by increases or decreases in flow. Lower flow rates discharged from Aguadablanca reservoir during 2015 may have caused momentary increases in Total-P concentrations that do not necessarily have an ecological impact. Limitations of physicochemical TSIs have been reported in terms of producing estimations dependent on water quality at the time of sampling, and which may not correspond to the real ecological status of the system (Padisák et al., 1993), and so they should always be analyzed and interpreted as part of a set of water physicochemical and ecological indicators. Furthermore, since significant correlations were found between Rott´s TDI and Total-P, nitrate and turbidity, this index seemed to be able of summarizing information in relation to several water quality variables beyond merely nutrients concentrations.

Results obtained in the present study also suggest that diatom indexes can be successfully applied in high Andean environments, especially in streams with similar characteristics to Chili River (*i.e* altitude ranging up to 3000 m.a.s.l, slopes up to 10% and water temperatures around 10-25 °C). Thus, our results reinforce previous reports indicating the suitability of diatom indexes in assessing eutrophication in streams of the Southern Hemisphere (Bellinger et al., 2006; Moresco and Rodriguez, 2014), although local investigations into the sensitivity of diatoms and the need for adaptations to the classification boundaries of trophic states are always required.

Finally, a sustainable monitoring program for trophic pollution in Río Chili, and similar Andean basins polluted with municipal wastewater, may include a routine monitoring of diatom assemblages complemented with sporadic evaluation of TSIs. The sampling and preservation of samples for physicochemical analyses are often more complex and expensive. Using TDIs implies a lower cost and greater flexibility for water authorities in assessing water quality and making quick decisions, given that trained taxonomists are locally available. The TSIs may only need to be assessed a few times a year for detecting variation in physicochemical water quality. A monitoring program with these characteristics will allow gathering more and more frequent information without considerable additional costs.

5. Conclusions

Diatom species were good indicators of trophic pollution in the Río Chili. *Nitzschia* spp, *Eolimna subminuscula* were correlated with high values of Total-P whereas *Navicula Lanceolata*, *Nitzschia inconspicua*, *Nitzschia dissipata* were highly correlated with low to moderate concentrations of Total-P, which was identified as the master variable driving trophic states.

Dodds' Index, TSI_T (Total-P), and Rott's and Kelly's TDIs performed well in describing the trophic levels of Río Chili and proved to be suitable for evaluating ecological impacts derived from the anthropogenic effects of the urban area of Arequipa. These indexes also coincided in detecting a longitudinal increase in trophic levels caused by discharge of municipal wastewater and urban occupation. In Río Chili, TSI_T (Total-P) and Dodds' Index should always be employed as part of water quality assessment in order to prevent estimations dependent entirely on momentary conditions. Periodical evaluation of TSIs may help in controlling general physicochemical water quality, whereas TDIs are more suitable for frequent monitoring. Rott's index may be used for trophic state assessments whereas Kelly's TDI is valuable for its generalist approach. When applied together with Rott's index, Kelly's TDI was also important in indicating the contribution of organic pollution to eutrophication. A sustainable monitoring program for trophic pollution in high Andean basins, such as Río Chili, should be based on routine assessments of trophic assemblages complemented with monitoring of physicochemical parameters.

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