The Andean Biotic Index (ABI): revised tolerance to pollution values for macroinvertebrate families and index performance evaluation

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Abstract: Score-based biotic indices are widely used to evaluate the water quality of streams and rivers. Few adaptations of these indices have been done for South America because there is a lack of knowledge on macroinvertebrate taxonomy, distribution and tolerance to pollution in the region. Several areas in the Andes are densely populated and there is need for methods to assess the impact of increasing human pressures on aquatic ecosystems. Considering the unique ecological and geographical features of the Andes, macroinvertebrate indices used in other regions must be adapted with caution. Here we present a review of the literature on macroinvertebrate distribution and tolerance to pollution in Andean areas above 2 000masl. Using these data, we propose an Andean Biotic Index (ABI), which is based on the BMWP index. In general, ABI includes fewer macroinvertebrate families than in other regions of the world where the BMWP index has been applied because altitude restricts the distribution of several families. Our review shows that in the high Andes, the tolerance of several macroinvertebrate families to pollution differs from those reported in other areas. We tested the ABI index in two basins in Ecuador and Peru, and compared it to other BMWP adaptations using the reference condition approach. The ABI index is extremely useful for detecting the general impairment of rivers but class quality boundaries should be defined independently for each basin because reference conditions may be different. The ABI is widely used in Ecuador and Peru, with high correlations with land-use pressures in several studies. The ABI index is an integral part of the new multimetric index designed for high Andean streams (IMEERA). Rev. Biol. Trop. 62 (Suppl. 2): 249-273. Epub 2014 April 01.

Key words: Andes, aquatic macroinvertebrates, altitudinal distribution, tolerance to pollution, BMWP adaptations, biomonitoring, water quality.

Aquatic macroinvertebrates are ubiquitous, and their sensitivity to environmental changes makes them good indicators of water condition. Diversity and biotic indices for benthic macroinvertebrate samples are often applied in an attempt to measure river pollution (Giller & Malmqvis, 1998). Score-based biotic indices are one of the most common biomonitoring methods used by water managers to synthesize large amounts of data from environmental monitoring. In these indices, a score is given to taxa (usually family or genera level) according to tolerance to organic pollution, giving highest or lowest scores (depending on the index) to sensitive taxa. These indices synthesize ecological information and the results are more accessible to non-biologists who require data for management purposes (Armitage, Moss, Wright & Furse; 1983). Indices of this kind were developed mainly in Europe (Woodiwiss, 1964; Armitage et al., 1983), South Africa (Chutter, 1972), North America (Hilsenhoff, 1982; 1987) and Australia (Chessman, 1995). One of the most commonly used index is the BMWP (and its derivations), which was developed in 1978 by the Biological Monitoring
Working Party (BMWP) in the United Kingdom (Armitage et al., 1983). This index gives a score to each taxa (mostly families) according to the sensitivity of pollution (mainly organic), being the most sensitive taxa scored with values of 10 and the less sensitive (or more resistant) to pollution a score of 1. It has been adapted to many countries, such as Poland (Czerniawska-Kusza, 2005), Canada (Barton & Metcalfe-Smith, 1992), Thailand (Mustow, 2002) and Spain (Alba-Tercedor & Sanchez-Ortega, 1988; Zamora-Muñoz & Alba-Tercedor, 1996), and modified versions of this last one are currently used in other countries, such as Portugal (Chaves, Costa, Chainho, Costa & Prat, 2006) and Greece (Skoulidakis, Gritzalis & Kouvarda, 2002), as a monitoring tool. Currently, several European countries are considering this index to assess ecological status, as required by the European Water Framework Directive. For example, the IBMWP index has been extensively used as monitoring tool in Spain (e.g. GUADALMED project, www.ub.edu/fem, Alba-Tercedor et al., 2002).

In developing countries, interest in biological monitoring of water bodies has increased in recent years. In the Andean mountain ranges, a number of studies from Colombia, Ecuador, Bolivia, Argentina, Venezuela, and Chile have used macroinvertebrates as biological indicators (Roldán Builes, Trujillo & Suárez, 1973; Zúñiga de Cardozo, Rojas de Hernández & Mosquera, 1997; Domínguez & Fernández, 1998; Posada, Roldán & Ramírez, 2000; Pescador, Hubbard & Zúñiga, 2001; Figueroa, Valdovinos, Araya & Parra, 2003), and several of these studies applied modified versions of the BMWP index (Jacobsen, 1998; Roldán, 1999; Vásconez, 2000; Fernandez, Romero, Vece, Manzo, Nieto & Orce, 2002; Riss, Ospina & Gutierrez, 2002; Leiva, 2004). Colombia and Argentina made their own preliminary adaptations of the index, which with few modifications, has been used in other countries of South America (see review by Prat, Ríos-Touma, Acosta & Rieradevall, 2009). Although, macroinvertebrate families in the neotropics generally receive similar scores of sensitivity to organic pollution relative to families in temperate zones (Jacobsen & Encalada, 1998; Tomanova & Tedesco, 2007), little is known about the autoecology of Andean taxa. Moreover, in Andean areas the altitudinal gradient is very important and likely influences macroinvertebrate presence and resistance to pollution. In addition, considerable differences in basins (e.g. altitudinal limitations, vegetation changes and their effects on different types of river input) have not been taken into account in the various adaptations of the BMWP index to Andean streams, or studies have been performed in small basins without reference sites (Gutierrez, Riss & Ospina, 2004). Therefore, scores obtained with these preliminary adaptations may not properly reflect water quality. Many areas of the Andes are densely populated; as a result, there is an urgent need for methods to assess water quality in these regions in an effective and affordable way. In this regard, the BMWP index is useful because of its simplicity. However, the BMWP index must be adapted in order to take into consideration the appropriate pollution score of each macroinvertebrate family.

Here we present the Andean Biotic Index (ABI) as a method that properly uses the rationale of the BMWP for the evaluation of biological quality of Andean streams, with the main goal of creating an improved tool that uses family scores appropriate for the Andean region. We had two specific aims: First we propose appropriate score values for representative macroinvertebrate families of Northern and Central Andean streams above 2 000 meters. For this, we reviewed the different adaptations of the BMWP index currently being used in Andean regions and also survey published and unpublished data (from “gray literature”) on the sensitivity of macroinvertebrate taxa to pollution in the region. The second aim was to construct and test the performance of the ABI in evaluating high-altitude Andean streams. We applied the ABI index to streams in basins of Ecuador and Peru and compared results with other indices used on the area and to family diversity as well. Finally, we assessed
its performance along a gradient of human impacts. The ABI is already in use as part of the CERA (Calidad Ecológica de Ríos Altoandinos, Ecological Quality of High-Andean rivers; Acosta, Ríos-Touma, Rieradevall & Prat, 2009). However, this is the first time the index is described and tested.

MATERIALS AND METHODS

Study area: The Andean ranges extend along western South America, from south Venezuela to Argentina (Tierra de Fuego). Gansser (1973) divided the Andean range into three regions: northern Andes, from Venezuela to the Huancabamba depression (in Peru); central Andes, from Huancabamba to 46°S in Argentina, transversal at the latitude of the Golfo de Penas; and southern Andes to Tierra de Fuego (see also Corvalán, 1990; Gregory-Wodzicki, 2000; Lavenu, 2006). Our target area included the northern Andes from the Venezuelan Andes to the Altiplano in the central Andes (Fig. 1). Maximum geomorphological complexity of the northern Andes is reached in Colombia, where they are divided into three main ranges: the western, central and eastern Cordilleras, separated by the sedimentary basins of the Cauca and Magdalena rivers (Kattan, Franco, Rojas & Morales, 2004). In Ecuador, the Andes are divided into the eastern and western ranges. The latter divides the Pacific and Atlantic slopes (Ulloa & Jorgensen, 2004). The southern limits of the Eastern Cordillera are in northern Peru, at the Huancabamba depression, in an area where the chain is bisected approximately at 6°S. This area forms a barrier that divides biogeographically the Andes in two regions (Myers, 2000). In Peru, the Andes include three mountain ranges: the western, central and eastern ranges (ONERN, 1970). The Altiplano Subdomain extends from 15°S in Peru to 24°S in Bolivia and includes lake Titicaca (Allmendinger, Jordan, Kay & Isacks, 1997; Gregory-Wodzicki, 2000). The Andean ranges have steep gradients on their Pacific slopes (Pringle, Scatena, Paaby-Hansen & Nuñez-Ferrera, 2000), resulting in rivers that are relatively short compared to other zones of Latin America. The Andean region includes basins that lie in mountain ranges and in the endorheic inter-Andean basins. We focused our bibliographic review on macroinvertebrate assemblages on fluvial systems (study area) from 2000masl to the highlands (more than 4500m or below the perpetual glaciers), extending from latitude 24°S (limit of the Altiplano Subdomain) to the northern section of the Andes (Fig. 1).

Revision of pollution tolerance for Andean macroinvertebrate families: We reviewed more than 500 documents, including scientific publications of indexed journals and Latin American scientific journals (locally indexed). In addition, we reviewed gray literature in the form of university theses (BS, MSc and PhD), seminar communications, and technical reports available from international meetings, local agencies and the web, many of them available only at governmental offices. This literature included species descriptions, ecological studies, and reports on monitoring and environmental impact. To make the final selection of taxa for our adaptation of the BMWP index, we focused on studies that included macroinvertebrate taxa listed together with data on water quality from the Andean regions of Colombia, Ecuador, Peru and Bolivia (more than 70 studies). In few cases, we did not find information on pollution tolerance for some families. In these cases we used the original score and European adaptations for comparison purposes. We focused the bibliographic search to our target area, but for comparison we included data from adaptations of similar indices used in Chile that sometimes are applied in lower altitude Andean regions.

ABI construction and testing: We constructed the ABI index using the same rationale as the original BMWP index. A score was assigned to each family (according to the review of literature made previously) and the total sum is the ABI score. Dividing this value for the total number of taxa found at one site, the Andean Average Score per Taxon
(AASPT) may be obtained. We used two basins to develop the index. These basins flow to the Pacific Ocean and are located in the Ecuadorian and Peruvian Andes. In Ecuador, we evaluated 45 sample sites located between 2,200 to 3,800 masl, in the upper Esmeraldas river basin, corresponding to the upper Guayllabamba river sub-basin. In Peru, we sampled 40 sites at the upper Cañete river basin, between 2,500 to 4,500 masl. Sites were located at both Páramos (and Punas) and Andean forest. A detailed description of the basins and sampling sites of Peru can be found in Acosta et al. (2009).

We use the reference condition approach (Reynoldson, Norris, Resh, Day & Rosenberg, 1997) to delimit the boundaries of quality

Fig. 1. Andean Mountain Range. Latitude 24° S marks the southern limit of our bibliographic research, and the north limit was at the end of the Andes in Venezuela. Latitude 6° S marks the area of the Huancabamba depression; 15°S marks the beginning of the Altiplano Sub domain (Map by Pau Fortuño, Universitat de Barcelona).
classes. In this approach, the score “very good” is identified according to different criteria that enable us to discard sites that are altered by human activities as targets for water quality objectives. Given the limited information available on the region, we developed a simple method (adapted from Chaves et al., 2006) that allowed us to test whether a site is a potential reference. Evaluations were conducted using a fact-sheet that includes four groups of characteristics, assessing human impacts at: basin, hydrology, reach and site levels. This method is a summation that provides a reference index ranging from 0 to 120, where values lower than 100 indicate that the site is not a good reference. This method and the validation of reference conditions in our study basins are described in Acosta et al. (2009). Applying the reference index to both basins, they found that 60% and 77% of the studied sites in Ecuador and Peru, respectively, could be considered as reference sites. We included all sites (reference and impaired) in our macroinvertebrate survey to validate ABI index. However, several sites, especially in the Cañete Basin, were excluded due to mining sewage impacts, which have dramatic effects on the macroinvertebrate fauna. The ABI and BMWP are indices developed to assess the effects of organic pollution and riparian alteration, and cannot be used to evaluate mining effects on streams. As a consequence, data from the studied basins is skewed to the reference communities.

**Macroinvertebrate sampling:** We sampled the macroinvertebrate assemblages and environmental characteristics on summer of 2003 and 2004, collecting a total of 45 sample sites in Ecuador and 42 sampling sites in Peru. Following Guadalmed sampling method, we sampled the macroinvertebrate assemblage using a D-net (Alba-Tercedor et al., 2002, Bonada et al., 2002) in all habitats available (see also Acosta et al., 2009). Multihabitat sampling is required to apply this type of index (Alba-Tercedor & Sanchez-Ortega, 1988). Samples were preserved in formaldehyde 10%, transported to the laboratory, and examined under the stereoscope. Samples were sorted and macroinvertebrates identified to family level and counted to establish the relative abundance of taxa.

**Definition of quality boundaries:** Five quality classes were defined: excellent, good, moderate, poor and bad conditions, following the indications of the Water Framework Directive (WFD 2000). The threshold between classes for each index was defined for each basin (Ecuador or Peru) independently following a similar methodology of Barbour et al. (1996; 1999) and Alba-Tercedor et al. (2002). We used the 25th percentile of the reference site values to define the boundary between excellent and good conditions. Following to the WFD (D.O.C.E., 2000; Alba-Tercedor et al., 2002) and considering that ABI is an adaptation of the BMWP, which has an exponential behavior in response to impact gradients (Munne & Prat, 2009), class boundaries were defined at 61% (between moderate and good), 36% (between moderate and poor), and 15% (between poor and bad) of the 25th percentile of the index value at reference sites.

**ABI relationship with environmental variables:** In order to assess the performance of the ABI (validation of the proposed score values), we performed a Principal Components Analysis (PCA) using Primer 6 (United Kingdom). We included environmental parameters measured at each station (Appendix 1), including the index of riparian habitat (QBR-And), the physical habitat index (IHF) and the reference condition value (numerical value that resumes alteration at basin, hydrology, reach, and site) (see Acosta et al., 2009 for detailed description of all indices). Also the following water characteristics were included: nitrates (an indicator of eutrophication), conductivity, pH, and dissolved oxygen. We chose the PCA component that explained most of the environmental variability and described environmental parameters closely related to this component (Appendix 1). Finally, we related this component to the ABI index to assess responses to
environmental impairment. We used pooled data to maximize the number of impaired sites (that were fewest in the Cañete basin in Peru) and to have the widest range of conditions, and also for the Guayllabamba basin individually because it showed a wider set of conditions. We did not perform a separated analysis for Peru due to the low number of impacted sites.

RESULTS

Delimiting a high Andean fauna: In the analysis of published data, we found four versions of the BMWP currently in use in our target region: the original BMWP, the adaptation for the Iberian Peninsula (IBMWP), the adaptation for Antioquia, Colombia (BMWPA), and an adaptation for Chile (CHBMWP). These versions include up to 111 macroinvertebrate taxa, including some that do not occur in the neotropical region (e.g., Nemouridae) or occur only at low elevations (below 2000 masl). Therefore, a first step was to exclude taxa not reported for our target region (Table 1). We excluded 52% of the families in the original BMWP, 44% of those in the IBMWP, 22% for the BMWPA, and 29% of the families in the CHBMWP.

Among non-insect taxa, we considered Turbellaria at class level, as Jacobsen & Encalada (1998) did, because although Planariidae and Dugesiidae were reported in South America, most studies in the area only provide the presence of the class. Also, other identifications are erroneous, mixing Dugesiidae genera inside the Planariidae family (Roldán, 1996). Similarly, the Hirudinea class was taken as a whole because of a lack of taxonomic information, although in the literature Glossiphoniidae was the most reported family. Mollusca included 13 freshwater families (Alvarenga & Ricci, 1981; Paraense, 1981) in South America. Of those Sphaeriidae, Planorbidae, Lymnaeidae, Physidae, Hydrobiidae and Ancylidae are the only families that have been reported in Andean areas above 2000 masl (Posada et al., 2000; Carrera & Gunkel, 2003; Jacobsen, 2004) and were the only ones included in our index. Benthic Crustacea reported in Andean areas includes: Ostracoda and Hyalellidae

| Order or taxonomic group | Number of families in South America | Number of families in High Andes (2000 m asl) | Reference |
|--------------------------|------------------------------------|-----------------------------------------------|-----------|
| Turbellaria              | 10a(?)                             | ?                                             | Ringuelet, 1981 |
| Hirudinea                | 7                                  | 7                                             | Gavrilov, 1981; Marchese, 2009 |
| Oligochaeta              | 9                                  | ?                                             | Paraense, 1981; Cuezzo, 2009 |
| Mollusca Gastropoda      | 13                                 | ?6b                                           | Alvarenga & Ricci 1981; Ituarte, 2009 |
| Mollusca Bivalvia        | 4                                  | ?                                             | Peralta, 2001; Peralta & Grosso, 2009. |
| Amphipoda                | 5                                  | 1                                             | Rosso de Ferradás & Fernandez, 2001; 2009 |
| Hydracarina (Acari)      | 22                                 | ?                                             | Dominguez et al., 2011; 2009 |
| Ephemeroptera            | 14                                 | 4                                             | Paulson, 2012; von Ellenrieder & R. Garrison, 2009 |
| Plecoptera               | 6                                  | 2                                             | Romero, 2001; Froehlich, 2009 |
| Heteroptera              | 16                                 | 6                                             | Alvárez & Roldán, 1983; Jacobsen, 2004; Mazzucconi et al., 2009 |
| Trichoptera              | 21                                 | 13                                            | Angrisano & Korob, 2001; Angrisano & Sganga, 2009 |
| Lepidoptera              | 8                                  | 1                                             | Romero & Navarro, 2009 |
| Coleoptera               | 29                                 | 11                                            | Archangelsky et al., 2009 |
| Diptera                  | 26                                 | 17                                            | Lizarralde de Grosso, 2001; 2009 |

a Including interstitial microturbellarians.

b According to the information compiled in the present document.
(Amphipoda) (Vásconez, 2000; Ríos-Touma, 2004; Ríos-Touma & Prat, 2004; Acosta & Prat, 2011). *Hyalella* is the only freshwater Hyalellidae genera present in South America (Peralta, 2001), but we kept this taxon as a family for the index. Although Acari includes 22 benthonic freshwater families reported for the continent (Rosso de Ferradás & Fernandez, 2001; 2009), the Hydracarina group was taken as a whole, as in the IBMWP index system (Alba-Tercedor & Sánchez-Ortega, 1988), because usually ecological studies do not include identifications for families or genera and therefore the environmental tolerance would be difficult to define.

With respect to insects, for Ephemeroptera we first excluded the families not found in Peru, Ecuador and Colombia using the checklist in Dominguez, Hubbard, Pescador, Molinari & Nieto (2011). Of the families reported, we excluded: Caenidae, Euthyplociidae and Polyembriocidae because they are limited to elevations below 2000 masl, being more frequent in the Amazonian and Andean foothills (Jacobsen, 2003; Monaghan et al., 2004; Jacobsen, 2004). The Odonata reported in South America (Paulson, 2012) includes 18 families, 14 in the tropical Andean regions, mainly in the Amazon and foothill region of the mountain range. Only the families Coenagrionidae, Calopterygidae, Polyphoridae, Aeshnidae, Gomphidae and Libellulidae have been reported in the Andes (Roback, 1980b; Monaghan et al., 2000; Posada et al., 2000; Jacobsen, 2003; Jacobsen, 2004). Of the six families of Plecoptera in South America (Romero, 2001; Froehlich, 2009), only two (Perlidae and Grippoptyrgidae) occur in tropical Andean region (Illies, 1964; Roback, 1980a; Jacobsen, 2003; Jacobsen, 2004). Of the six families of Plecoptera in South America (Romero, 2001; Froehlich, 2009), only two (Perlidae and Grippoptyrgidae) occur in tropical Andean region (Illies, 1964; Roback, 1980a; Jacobsen, 2003; Jacobsen, 2004). Of these, we excluded Limnichidae and Luthrochidae because there is a lack of information on distribution and tolerance to pollution.

The Dipterans of South America include 26 families (Lizarralde de Grosso, 2001; 2009), 17 of which have been included in indices. Of these families, we excluded Rhagionidae, as it has not been reported in Andean highlands. Although Limoniidae are not distinguished from Tipulidae in some publications on the neotropics (Roback & Coffman, 1983; Jacobsen, 2003; Jacobsen, 2004), we differentiated these two groups because they are separate families (Zoological Records; Tachet, 2000) and recent published information of pollution tolerance have been provided for these taxa as separate families (Rios-Touma, 2004; Villamarín, 2012).

**Tolerance to pollution:** In general, we maintained scores that did not change among the different BMWP indices available and also those that were supported by autecological information from Andean areas (Table 2). Families in Turbellaria, Hirudinea, Oligochaeta, and
### TABLE 2
Comparative table of BMWP and the different adaptations vs. the proposed index ABI (Andean Biotic Index)

| Order          | Family          | BMWP¹ | IBMWP² | BMWPA³ | CHBMWP⁴ | ABI | Bibliographic references of pollution tolerance |
|----------------|-----------------|-------|--------|--------|---------|-----|-------------------------------------------------|
| Turbellaria    |                 | 5     | 5      | 5      | 5       | 5   | Jacobsen, 1998; Vásconez, 2000; Ríos & Prat, 2004 |
| Hirudinea      |                 | 3     | 3      | 3      | 3       | 3   |                                                   |
| Oligochaeta    |                 | 1     | 1      | 1      | 1       | 1   |                                                   |
| Gasteropoda    | Ancylidae       | 6     | 6      | 6      | 6       | 6   |                                                   |
|                | Physidae        | 3     | 3      | 3      | 3       | 3   |                                                   |
|                | Hydrobiidae     | 3     | 3      | 3      | 3       | 3   |                                                   |
|                | Limnaeidae      | 3     | 3      | 3      | 3       | 3   |                                                   |
|                | Planorbidae     | 3     | 3      | 3      | 3       | 3   |                                                   |
| Bivalvia       | Sphaeriidae     | 3     | 3      | 3      | 3       | 3   |                                                   |
| Amphipoda      | Hyalellidae     | 8     | 6      | 6      | 6       | 6   | Viña-Vizcaíno & Ramírez-Gonzáles, 1997; Jacobsen, 1998; Ríos & Prat, 2004 |
| Ostracoda      |                 | 3     |        | 3      |         | 3   | Ríos-Touma & Prat, 2004                          |
| Hydracarina    |                 | 4     | 4      | 4      |         |     |                                                   |
| Ephemeroptera  | Baetidae        | 4     | 4      | 8      | 4       | 4   | Roldán, 1980; Jacobsen, 1998; Viña-Vizcaíno & Ramírez-Gonzáles, 1997; Zúñiga de Cardoso et al., 1997; Ríos & Prat, 2004 |
|                | Leptophlebiidae | 10    | 10     | 10     | 10      | 10  |                                                   |
|                | Leptophyidae    | 8     | 8      | 8      | 8       | 8   |                                                   |
|                | Oligoneuridae   | 5     | 10     | 10     | 10      | 10  | Roldán, 1980; Zúñiga de Cardoso et al., 1997     |
| Odonata        | Aeshnidae       | 8     | 8      | 8      | 8       | 8   | Arango & Roldán, 1983                           |
|                | Gomphidae       | 8     | 8      | 10     | 8       | 8   |                                                   |
|                | Libellulidae    | 8     | 8      | 8      | 8       | 8   | Arango & Roldán, 1983                           |
|                | Coenagroniidae  | 6     | 6      | 6      | 6       | 6   |                                                   |
|                | Calopterygidae  | 8     | 8      | 7      | 8       | 8   |                                                   |
|                | Polythoridae    | 10    |        |        |         |      |                                                   |
| Plecoptera     | Perlidae        | 10    | 10     | 10     | 10      | 10  | Turcotte & Harper, 1982; Jacobsen, 1998; Vásconez, 2000 |
|                | Griopterygidae  | 10    |        |        |         |      |                                                   |
| Heteroptera    | Velidae         | 3     |        |        |         | 5   | Alvarez & Roldán, 1983                          |
|                | Gerridae        | 5     | 3      | 3      | 3       | 3   | Alvarez & Roldán, 1983                          |
|                | Corixidae       | 5     | 3      | 7      | 3       | 3   | Alvarez & Roldán, 1983                          |
|                | Notonectidae    | 5     | 3      | 5      | 3       | 3   | Alvarez & Roldán, 1983                          |
|                | Belostomatidae  | 4     | 4      | 4      |         | 4   |                                                   |
|                | Naucoridae      | 5     | 3      | 4      |         | 5   |                                                   |
| Trichoptera    | Helicopsychida  | 10    |        |        |         | 10  | Ballesteros et al., 1997; Jacobsen, 1998         |
|                | Calamoceratidae | 10    | 10     | 10     | 10      | 10  |                                                   |
|                | Odontoceridae   | 10    | 10     | 10     | 10      | 10  |                                                   |
|                | Leptoceridae    | 10    | 10     | 8      | 10      | 10  | Ballesteros et al., 1997; Viña-Vizcaíno & Ramírez-Gonzáles, 1997; Jacobsen, 1998 |
|                | Polycentropodida| 7     | 10     | 8      | 7       | 8   | Correa et al., 1981; Ballesteros et al., 1997   |
|                | Hydroptilidae   | 6     | 6      | 8      | 6       | 6   | Flint, 1991                                     |
|                | Xiphocentronidae| 8     |        |        |         | 8   | Roldán et al., 1992                             |
|                | Hydrobiosidae   | 8     | 7      | 8      | 8       | 8   | Ballesteros et al., 1997; Jacobsen, 1998        |
|                | Glossosomatidae | 8     | 7      | 8      | 7       | 7   | Viña-Vizcaíno & Ramírez-Gonzáles, 1997; Jacobsen, 1998 |
|                | Hydropsychidae  | 5     | 5      | 5      | 5       | 5   |                                                   |
|                | Anomalopsychida | 10    |        |        |         |      | Jacobson, 1998; Holzenthal & Flint, 1995       |
| Lepidoptera    | Pyralidae       | 4     | 4      | 4      |         | 4   |                                                   |
Mollusca were assigned the same scores they received in available adaptations of the BMWP index. Moreover, these values are consistent with the presence of these taxa in a wide range of water conditions (Machado et al., 1997; Viña-Vizcaíno & Ramírez-Gonzáles, 1997; Jacobsen & Encalada, 1998; Vásconez, 2000; Ríos-Touma, 2004; Ríos-Touma & Prat, 2004).

Hyalellidae is found in a wide variety of habitats, shows diverse feeding strategies (Peralta, 2001; Acosta & Prat, 2011), and is resistant to certain types of organic pollution (Jacobsen & Encalada, 1998). Therefore, we used the score for Gammaridae (6) from the index developed by Armitage et al. (1983), which is consistent with the frequent presence of this family in reference to mildly impaired streams. For Ostracoda (3) and Hydracarina (4) we also used the original IBMWP index value, because the pattern found in the literature was consistent with the presence of these taxa in more impaired streams.

### TABLE 2 (Continued)

| Order     | Family                  | BMWP<sup>1</sup> | IBMWP<sup>2</sup> | BMWPA<sup>3</sup> | CHBMWP<sup>4</sup> | ABI | Bibliographic references of pollution tolerance |
|-----------|-------------------------|-------------------|-------------------|-------------------|-------------------|-----|-----------------------------------------------|
| Coleoptera| Ptilodactylidae         | 10                | 5                 | 6                 | 3                 | 3   | Viña-Vizcaíno & Ramírez-Gonzáles, 1997       |
|           | Lampyridae              | 10                | 5                 | 6                 | 5                 | 5   |                                               |
|           | Psephenidae             | 10                | 4                 | 3                 | 3                 | 3   |                                               |
|           | Sciridae (Helodidae)    | 5                  | 7                 | 3                 | 3                 | 3   |                                               |
|           | Staphyliniidae          | 6                  | 3                 | 3                 | 3                 | 3   |                                               |
|           | Elmidae                 | 5                  | 6                 | 5                 | 5                 | 5   |                                               |
|           | Dryopidae               | 5                  | 6                 | 5                 | 5                 | 5   |                                               |
|           | Gyrinidae               | 5                  | 3                 | 3                 | 3                 | 3   |                                               |
|           | Dytsicidae              | 5                  | 3                 | 3                 | 3                 | 3   |                                               |
|           | Hydrophilidae           | 3                  | 3                 | 3                 | 3                 | 3   |                                               |
|           | Hydracaridae            | 5                  | 5                 | 5                 | 5                 | 5   |                                               |
| Diptera   | Blepharoceridae         | 10                 | 10                | 10                | 10                | 10  | Viña-Vizcaíno & Ramírez-Gonzáles, 1997       |
|           | Simuliidae              | 5                  | 5                 | 8                 | 5                 | 5   | Jacobsen, 1998; Ríos & Prat, 2004          |
|           | Tabaniidae              | 5                  | 4                 | 4                 | 4                 | 4   |                                               |
|           | Tipulidae               | 5                  | 4                 | 5                 | 5                 | 5   |                                               |
|           | Limoniidae              | 4                  | 4                 | 4                 | 4                 | 4   |                                               |
|           | Ceratopogonidae         | 4                  | 4                 | 4                 | 4                 | 4   |                                               |
|           | Dixiidae                | 4                  | 4                 | 4                 | 4                 | 4   |                                               |
|           | Psychodidae             | 4                  | 4                 | 4                 | 4                 | 4   | Machado et al., 1997; Jacobsen, 1998; Vásconez, 2000; Ríos & Prat, 2004 |
|           | Dolichopodidae          | 4                  | 4                 | 4                 | 4                 | 4   |                                               |
|           | Stratiosyidae           | 4                  | 4                 | 4                 | 4                 | 4   |                                               |
|           | Empididae               | 4                  | 4                 | 4                 | 4                 | 4   |                                               |
|           | Chironomidae            | 2                  | 2                 | 2                 | 2                 | 2   | Jacobsen, 1998                              |
|           | Culicidae               | 2                  | 2                 | 2                 | 2                 | 2   |                                               |
|           | Musciidae               | 4                  | 2                 | 2                 | 2                 | 2   |                                               |
|           | Ephydridae              | 2                  | 2                 | 2                 | 2                 | 2   |                                               |
|           | Athericidae             | 10                 | 10                | 10                | 10                | 10  |                                               |
|           | Syrphidae               | 1                  | 1                 | 1                 | 1                 | 1   |                                               |

1. (England) (Armitage et al., 1983).
2. (Iberian Peninsula) (Alba-Tercedor & Sánchez-Ortega, 1988).
3. (Antioquia, Colombia) (Roldán, 1999).
4. (Chile) (Figueroa, 2004).
Within Ephemeroptera, Leptophlebiidae was given the same score (10) as in all the indices analyzed, because we did not find this family under impaired conditions. For Leptohyphidae, we used the score (7) reported by Roldán (1999), as the family is present in slightly polluted waters (e.g. Roldán, 1980; Roldán, 1996; Zúñiga de Cardoso et al., 1997; Viña-Vizcaíno & Ramírez-Gonzáles, 1997). In contrast, to some studies, we maintained Oligoneuriidae with a high score (10) because this family is reported only in clean waters (e.g. Roldán, 1996; Zúñiga de Cardoso, 1997; Ríos-Touma, 2004). Although Roldan (1999) assigned a score of 8 to Baetidae, we used a value of, 4 as in the original BMWP index, as this family is commonly found in polluted waters (e.g. Roldán, 1980; Viña-Vizcaíno & Ramírez-Gonzáles, 1997; Zúñiga de Cardoso et al., 1997; Jacobsen & Encalada, 1998; Ríos & Prat, 2004).

Information found on Gomphidae (8), Coenagrionidae (6) and Calopterygidae (8) was consistent with the original scores assigned by Armitage et al. (1983). For Aeshnidae and Libellulidae we used the scores given by Roldán (1999) because these groups show a higher tolerance to pollution in Andean streams (Álvarez & Roldán, 1983). The BMWPA adaptation was the only index to include the Polythoridae family and this was the only Odonata family to achieve a maximal score. In our adaptation, we kept this score for this family as larvae is found in clean mountain rivers (Bick & Bick, 1985; Acosta, 2003; Sanchez-Herrera & Realpe, 2010).

For Plecoptera, we maintained the maximal score reported, because these families are found only in clean sites above 2000m asl (Ríos-Touma, 2004; Acosta, 2005; Acosta et al., 2009). For most Heteroptera families we used a score of 5, as they show similar resistance to moderately polluted waters (Álvarez & Roldán, 1983). For Naucoridae, Notonectidae, and Corixidae the score (5) used was that same as that reported in Armitage et al. (1983), because with the ability of live in moderately impaired streams mainly due to their semi-aquatic life. For the Belostomatidae we applied a score of 4, given by Roldán (1999) and Figueroa (2004) for its better resistance to pollution than other heteropterans.

Regarding Trichoptera, in our adaptation, Calamoceratidae and Odontoceridae were kept at the highest value. Hydroptilidae (6), Hydropsychidae (5), Philopotamidae (8), and Limnephilidae (7) maintained the scores reported in the original BMWP index. We also used the scores reported by Roldán (1999) for Helicopsychidae, Leptoceridae, Polyceratopodidae, Xiphocentronidae, Hydrobiosidae and Glossosomatidae, because of their concordance with the literature (e.g., Correa, Machado & Roldán, 1981; Flint, 1991; Ballesteros, Zúñiga de Cardoso & Rojas de Hernández, 1997; Viña-Vizcaíno & Ramírez-Gonzáles, 1997; Jacobsen & Encalada, 1998). On the other hand, Anamolopsidea maintained the maximum score assigned by Figueroa (2004), which is also consistent with data reported by Jacobsen & Encalada (1998), Holzenthal & Flint (1995) and Holzenthal & Ríos-Touma (2012). For the Lepidoptera, Crambidae, we maintained the scores assigned in the IBMWP index and in the Antioquia index, although there is a lack of information on the resistance of this family to pollution.

There is little data on water pollution tolerance for Coleoptera in South America, and most data is associated with species descriptions (e.g., Gustafson & Short, 2010; Perkins, 2011). Ptilodactylidae, Lampyridae, Hydraenidae, and Psephenidae are abundant in the Andes and absent from European indices. Therefore, they were assigned a score of 5, which is the maximum value for Coleoptera families that are usually semi-aquatic and have respiratory adaptations that make them less vulnerable to water quality. Elmidae, Dryopidae, and Hydrophilidae maintained the same values as in the indices analyzed due to the dominance of semi-aquatic life cycles (e.g., Hansen, 1991). Staphylinidae was scored 3, because this family shows adaptations that make it less responsive to water quality (Merrit & Cummins, 1996). The same applies to
Dytiscidae and Gyrinidae, which maintained the scores reported in the other BMWP adaptations for South America. This is also supported by information on the presence under strong organic pollution and their role as decomposers of animal tissues in Andean streams (Barrios & Wolf, 2011).

Most adaptations of the BMWP index to South America have similar scores for dipteran families. Given the limited information available, we mostly used the same scores. We changed scores for two families reported by Roldán (1999), because they were not consistent with the information on Andean polluted waters (Machado et al., 1997; Viña-Vizcaino & Ramírez-Gonzáles, 1997; Jacobsen & Encalada, 1998; Ríos & Prat, 2004). Simuliidae, and particularly Psychodidae, had lower scores in our adaptation because they may be present on low water quality streams, especially the latter, which was present under highly toxic concentrations of pollutants in Andean rivers (Machado et al., 1997; Vásconez, 2000; Jacobsen & Encalada, 1998; Ríos-Touma, 2004).

Application of the ABI to Andean rivers: The threshold between quality classes defined for each basin through the quartile method (Table 3), showed higher minimum and maximum values for ABI in all reference sites, which allowed easier differentiation of the excellent and good quality classes compared to BMWP and CHBMWP. ABI was the index that arrived to the highest scores for reference sites, meaning more information (families) included.

We found a naturally lower family richness in reference sites of Cañete basin compared to the Guayllabamba basin. The limit between excellent and good classes in Guayllabamba basin was 96; in the Cañete basin it was 74. The final quality values for all sites and all metrics and indices were highly correlated (Spearman correlation p<0.05), showing that all indices were providing similar information, but these similarities were caused by the extreme classes (excellent or bad) with important differences in intermediate quality classes (Table 3). Also,
the Guayllamamba basin had more sites with moderate to bad quality classes (35% of sites), which made the differences clearer between quality classes than at the Cañete basin that did not have sites with poor and bad quality classes.

For the pooled data, the first component of the PCA explained 61.4% of the environmental variation. The main contributions were a positive relation of conductivity with the first component and a negative correlation with the QBR index in the second component (Table 4). Also, we found a positive relation of the 2nd component with nitrates and temperature and a negative relation with the reference score. These components had a highly significant inverse Pearson correlation (r= -0.53, -0.45, respectively p<0.001) with the ABI, indicating that higher ABI scores were found at sites with better riparian and chemical quality at the site. The same analysis only for Ecuadorian sites (than included a wider set of impairment conditions than Peru) showed even a stronger correlation with the first component of PCA, that for this basin explained up to 69% of the environmental variation (Fig. 2). The second component also showed a strong positive relation with nitrates, showing a possible effect of eutrophication at lower ABI values. Although IHF was not strongly represented in any of the two PCA components, at Guayabamba sites it has a positive relationship with ABI (Pearson correlation=0.7).

DISCUSSION
Here we reviewed most of the information available for benthic freshwater macroinvertebrates in Andean areas, with emphasis on their resistance to pollution, in order to propose an adaptation of the BMWP index for the Andes. Although there have been recent important advances in the taxonomy of South American aquatic invertebrates (e.g., Fernandez & Dominguez, 2001; Dominguez & Fernandez, 2009) and for some Latin American countries (e.g., Hanson, Springer & Ramirez, 2010), most information is still limited to unpublished “gray literature” (Pringle, 2000; Pringle et al., 2000) and studies that classify macroinvertebrates to family level only. A considerable part of the information included in the present work is focused on the analysis of technical reports, conference summaries, and local scientific publications. We also examined taxonomical descriptions from journals of restricted distribution, often not available in developing countries.

### TABLE 4
Scores for the first and second PCA components for environmental parameters in the upper Guayllabamba basin (Ecuador) and pooled data from Ecuador and Peru

| Variables       | Pooled data | Ecuador |
|-----------------|-------------|---------|
|                 | PCA 1 | PCA 2 | PCA 1 | PCA 2 |
| Oxygen          | -0.064 | -0.135 | -0.088 | 0.016 |
| Conductivity    | **0.955** | -0.27 | **0.878** | **-0.454** |
| pH              | 0.041 | -0.035 | 0.027 | -0.031 |
| Nitrates        | 0.139 | **0.51** | 0.284 | **0.648** |
| Temperature     | 0.139 | **0.331** | 0.156 | 0.087 |
| Reference Score | -0.099 | -0.26 | -0.163 | **-0.338** |
| IHF             | -0.1 | -0.007 | -0.138 | -0.191 |
| QBR             | -0.156 | **-0.686** | -0.264 | **-0.463** |
| % Cumulative Variation explained | 61.4 | 76.9 | 69.6 | 79.5 |
| Eigenvalue      | 0.693 | 0.175 | 0.883 | 0.126 |
| Correlation with ABI | **-0.53** | **-0.45** | **-0.76** | **-0.36** |

Eigenvalues, % of variation and correlation with ABI provided.
Although the distribution of several families in the study area is still incomplete, we obtained enough information to make a selection of taxa commonly found in the high Andes, which is appropriate for a biotic index (Table 1). Ecuador was the only country well studied, as Jacobsen, Schultz & Encalada (1997) and Jacobsen (2003; 2004) performed a thorough revision of the distribution of macroinvertebrate families. Our review is now adding information on macroinvertebrate families present in the Andean regions of Colombia (Arango & Roldán, 1983; Álvarez & Roldán, 1983; Zúñiga de Cardoso et al., 1997; Posada et al., 2000) Peru (Roback et al., 1980, Roback & Coffman, 1983; Acosta, 2001; 2005) and Bolivia (Illies, 1964; 1967; Roback et al., 1980; Roback & Coffman, 1983; Rocabado & Wasson, 1999).

We consider that we included enough information at family level to design the first version of the ABI index. This level of taxonomic resolution has demonstrated to be effective in bioassessments of water quality. In some cases family works better than genus level (Bailey et al., 2001) providing the same information at a lower effort and cost (Chessman et al., 2007). However, further studies are required to validate their tolerance to pollution, especially for other Andean areas that remain unexplored.

The autecology of the different families, their response to basin alterations and their resistance to pollution in the Andes is largely unknown. In addition, these high altitude areas have lower water oxygen contents and tolerance to pollution may differ from that reported for the same families in the lowlands or in mountains of Europe (Jacobsen, Rostgaard & Vásconez, 2003). Although we based our report on recent autecological studies of macroinvertebrates in the area (Table 2), the scores assigned to each family should be used with caution until more information is available, especially for Coleoptera. In addition to elevation, tolerance to pollution can vary depending on the type of contamination. Studies on the effects of pollutants and ecotoxicology have focused mainly on temperate areas and pollutant behavior may differ between tropical Andean freshwaters and temperate ecosystems (Lacher & Goldstein, 1997; Wishart, Davies,
Boon & Pringle, 2000). Here we addressed mainly organic pollution, but mining activities are a considerable source of pollutants to water ecosystems in south Ecuador, Peru, and Bolivia (Pringle et al., 2000). It has been estimated that approximately 5 000 tons of mercury have been deposited in forest and urban environments in Latin America since the onset of the new gold expansion (Veiga, 1997 in UNEP 2000) and there is a lack of information on the effect of this kind of pollution on freshwater communities. At present, we do not know whether the current ABI values are representative of different kinds of disturbances and for this reason their application in some cases may produce misleading information on water quality. In Mediterranean regions, studies have reported that macroinvertebrates show distinct levels of resistance to different pollutants and that the final richness of a community does not necessarily reflect the ecological status of the river (Marqués, Martínez-Conde & Rovira, 2003). In contrast, other studies report that the IBMWP scores are a useful tool to monitor waters receiving coal mine drainage (García-Criado, Tomé, Vega & Antolín, 1999) and that this index varies not only with organic pollution but also with habitat heterogeneity and mine pollution (Solà, 2004). In this regard, ABI scores showed certain sensitivity to mining pollution in Andean streams (Ordóñez-Arizaga, 2011b; Villamarín, 2012), but additional detailed studies are still needed.

Freshwater Andean ecosystems are also greatly affected by suspended solids and the excessive use of agrochemicals. Macroinvertebrate assemblages are good indicators of suspended solid impacts in the Bolivian Andes due to road construction (Fossati, Wasson, Héry, Salinas & Marín, 2001). Contrastingly, the effects of agrochemicals, particularly pesticides, on freshwater communities in these areas have not been widely studied (Pringle et al., 2000). Given that agrochemicals are a common source of pollution, the effects of these compounds on freshwater communities (UNEP, 2000) should be taken into account in the adaptation of a biotic index. Although the ABI shows some sensitivity to agricultural alterations (Ordóñez-Arizaga, 2011a; Bragado-Quero, 2011), we recommend that in these cases the index should be used very carefully because the effects have not been deeply investigated (especially for pesticides).

We found the ABI to be a good representation of the environmental status of rivers, especially when studies include reference and impacted sites, and boundaries among classes are accurately assigned (e.g., Table 3). Although our sites presented physicochemical degradation, physical habitat impacts were not evident. Therefore, it is not surprising to find strong relationships between the ABI and conductivity, nitrates, reference condition and QBR-, but to with the IHF. Removing the Peruvian reference sites results in a strong correlation between ABI and IHF, thus an effective implementation of the ABI index requires the applications of the reference condition approach. In each basin, reference values may be different indicating that thresholds among quality classes might be different (as shown for Ecuador and Peru, Table 3). The absolute value of the index is not representative of water quality; it should be compared against reference conditions values. With this approach, the application of the ABI at two Andean sub-basins in Ecuador showed a strong correlation with changes in land use (Ordoñez, 2011a). Also, a recent detailed study of the relationship of environmental factors and macroinvertebrates in high altitude Andean streams, showed also that the ABI is an strong indicator of the ecological quality of streams (with significant diminishing when impairment increases), and an important part of a new multimetric index for Andean streams (IMEERA Index by Villamarín, Rieradevall, Paul, Barbour & Prat, 2013). However the relationship at basin level of multiple stressors and the aquatic biota should be better investigated in the Andes.

The geographical distribution of water pollution in the Andes is now dominated by flows from large metropolitan areas (UNEP, 2000). Large cities such as Bogotá, Medellín, Quito, Cuenca, La Paz, Cochabamba, Mérida,
Arequipa and Cuzco are located in the Andes and population pressure, and its consequent water requirements, is increasing in these regions. Only 5% of the sewage water of the region is treated and the pollution of superficial and underground waters is becoming a controversial issue (UNEP, 2002), mainly because there is a lack of an administrative model that assure equity and environmental sustainability of the water supply (Pirez, 2000). The pressure on aquatic ecosystems is further exacerbated by the fact that Andean glaciers are threatened by climate change (Bradley, Vuille, Díaz & Vergara, 2006). Overall, aquatic resources will decrease in the future as demand for water increases. Tools like the ABI index, the CERA protocol (Acosta et al., 2009), and multimetric indices (e.g. the IMEERA, Villamarin et al., 2013) are of increasing importance as they provide reliable and rapid results for water management institutions, from an ecosystem point of view. Moreover, the ABI index is currently the most used, with success, in the Paute basin in Ecuador (Ordoñez, 2011b).

Our study provides a basis for future studies and for the implementation of methods of ecological assessment of river water quality, as those currently used in Europe, Australia and North America. To develop these methods, exercises of method standardization for water and biota sampling, collecting, sorting and analyzing are necessary. These methods should be applied in a wide range of polluted and unpolluted sites around the Andes. Another future task in monitoring in the region is the definition of reference conditions for different types of rivers following, for example, the Water Framework Directive guidelines. Although some preliminary research has been done (Ríos-Touma, 2004; Acosta, 2005; Acosta et al., 2009; Ordóñez-Arizaga, 2011b; Villamarín 2012), there is a need to standardize sampling and data interpretation to obtain a large set of data from different river types. A further step in this research is the construction of a multimetric index (e.g., IMEERA, Villamarin et al., 2013) that uses the reference condition approach and provides another tool for biomonitoring. The ABI index is one of the most important components of this multimetric index, therefore, the explanations of family scores provided here are also important for the users of the IMEERA multimetric index.

The participation of management institutions, universities, local and international specialists, and civil society is important for the success of monitoring activities using a biological index like the ABI, and its usefulness in management and conservation policies. We encourage ABI users to provide feedback, comments, suggestions and results to the authors, in order to increase the knowledge and adjust the index accordingly.

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RESUMEN

Los índices bióticos basados en puntuación son ampliamente utilizados para evaluar la calidad del agua de los arroyos y ríos. Varias áreas de los Andes están densamente pobladas y hay necesidad de métodos para
evaluar el impacto de la creciente presión humana sobre los ecosistemas acuáticos. Dadas las características ecológicas y geográficas únicas de los Andes, los índices de macroinvertebrados utilizados en otras regiones deben adaptarse con cautela. Aquí se presenta una revisión de la literatura sobre distribución de macroinvertebrados y la tolerancia a la contaminación en las zonas andinas por encima de 2000msnm. Usando estos datos, se propone un Índice Biológico Andino (ABI), que se basa en el índice de BMWP. En general, ABI incluye un menor número de familias de macroinvertebrados que en otras regiones del mundo donde se ha aplicado el índice BMWP porque la altitud restringe la distribución de varias de ellas. Nuestra revisión muestra que la tolerancia de varias familias a la contaminación en los ríos altoandinos difiere de lo reportado en otras áreas. Probamos el índice ABI en dos cuencas en Ecuador y Perú, y comparamos con otras adaptaciones BMWP utilizando el enfoque de condición de referencia. Nuestros resultados muestran que el índice de ABI es extremadamente útil para detectar el deterioro general de los ríos, pero que los límites entre las clases de calidad deben ser definidos independientemente para cada cuenca debido a que las condiciones de referencia pueden ser diferentes. El ABI es ampliamente utilizado en Ecuador y Perú, y es parte integral del nuevo índice multimétrico diseñado para corrientes altas andinas (IMEERA).

**Palabras clave:** Andes, macroinvertebrados acuáticos, distribución altitudinal, tolerancia a la contaminación, adaptaciones del BMWP, biomonitoro, calidad del agua.

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### APPENDIX 1

Characteristics of studied sites in the Upper Guayllabamba basin in Ecuador and the Cañete Basin in Peru

| Site   | Country | Basin            | Altitude m asl | Reference Score | IHF | QBR | Oxygen (mg/l) | Conductivity (µS/cm) | pH   | Nitrates (mg/l) | Temperature (°C) | Order |
|--------|---------|------------------|----------------|-----------------|-----|-----|--------------|----------------------|------|-----------------|------------------|-------|
| CA01   | Peru    | Cañete_headwater | 4 396          | 107             | 46  | 86  | 7.3          | 70                   | 6.83 | 0.62            | 7                | 2     |
| CA02   | Peru    | Cañete_headwater | 4 425          | 107             | 43  | 86  | 7.7          | 100                  | 7.1  | 1.24            | 4                | 1     |
| CA03   | Peru    | Cañete_headwater | 4 352          | 107             | 41  | 100 | 7.6          | 200                  | 7.6  | 0.62            | 8.5              | 1     |
| CA04   | Peru    | Cañete_headwater | 4 309          | 107             | 46  | 86  | 6.8          | 130                  | 7.2  | 0.62            | 10               | 2     |
| CA05   | Peru    | Cañete_headwater | 4 276          | 113             | 36  | 100 | 6.4          | 170                  | 8.21 | 0.62            | 11.5             | 2     |
| CA06   | Peru    | Cañete_headwater | 3 913          | 113             | 41  | 100 | 7.6          | 440                  | 8.1  | 0.62            | 9.5              | 3     |
| CA07   | Peru    | Cañete_headwater | 3 935          | 107             | 41  | 73  | 7.2          | 560                  | 7.7  | 1.24            | 10.5             | 4     |
| CA08   | Peru    | Cañete_headwater | 4 065          | 107             | 45  | 93  | 7.5          | 570                  | 7.5  | 0.62            | 10.5             | 1     |
| CA09   | Peru    | Cañete_headwater | 4 065          | 107             | 47  | 86  | 7.4          | 570                  | 7.4  | 0.62            | 10               | 1     |
| CA10   | Peru    | Cañete_headwater | 3 906          | 107             | 36  | 86  | 7.2          | 480                  | 7.5  | 0.62            | 11.5             | 2     |
| CA11   | Peru    | Cañete_headwater | 3 912          | 107             | 31  | 86  | 6.9          | 270                  | 8.01 | 1.24            | 11.3             | 1     |
| CA12   | Peru    | Cañete_headwater | 3 537          | 104             | 31  | 60  | 7.5          | 470                  | 8.12 | 0.62            | 10               | 4     |
| CA13   | Peru    | Cañete_headwater | 3 450          | 102             | 29  | 55  | 7.5          | 440                  | 8.3  | 0.62            | 10.5             | 4     |
| CA14   | Peru    | Cañete_headwater | 3 400          | 110             | 26  | 70  | 9.6          | 470                  | 8.12 | 0.62            | 11               | 4     |
| CA15   | Peru    | Miraflores       | 4 117          | 113             | 52  | 85  | 7.4          | 60                   | 6.9  | 0.62            | 9.2              | 2     |
| CA16   | Peru    | Miraflores       | 4 061          | 113             | 52  | 85  | 7.3          | 150                  | 7.2  | 1.24            | 8.7              | 2     |
| CA17   | Peru    | Miraflores       | 3 300          | 112             | 65  | 95  | 7            | 430                  | 8.04 | 0.62            | 10.2             | 3     |
| CA18   | Peru    | Alis             | 3 900          | 105             | 49  | 100 | 7.4          | 360                  | 8.2  | 0.62            | 6                | 2     |
| CA19   | Peru    | Alis             | 3 900          | 105             | 42  | 86  | 7            | 580                  | 8.03 | 0.62            | 6                | 2     |
| CA20   | Peru    | Alis             | 3 850          | 105             | 51  | 100 | 7.4          | 500                  | 8.05 | 0.62            | 7                | 3     |
| CA21   | Peru    | Alis             | 3 650          | 111             | 48  | 86  | 7.5          | 500                  | 8.1  | 0.62            | 7.4              | 3     |
| CA22   | Peru    | Lamos            | 3 736          | 108             | 33  | 55  | 6.2          | 290                  | 7.2  | 0.62            | 9.2              | 1     |
| CA23   | Peru    | Lamos            | 3 600          | 104             | 37  | 75  | 7.2          | 230                  | 7.8  | 0.62            | 8                | 1     |
| CA24   | Peru    | Lamos            | 3 050          | 110             | 68  | 85  | 8.8          | 290                  | 8.15 | 0.62            | 13               | 3     |
| CA25   | Peru    | Morro            | 2 800          | 114             | 38  | 60  | 7.3          | 540                  | 8.1  | 0.62            | 10               | 5     |
| CA26   | Peru    | Huantan          | 3 726          | 108             | 47  | 65  | 7.25         | 230                  | 7.5  | 0.62            | 7.5              | 4     |
| CA27   | Peru    | Huantan          | 3 350          | 104             | 52  | 75  | 8.2          | 50                   | 6.9  | 0.62            | 6                | 2     |
| CA28   | Peru    | Huantan          | 3 140          | 104             | 55  | 55  | 7.6          | 110                  | 7.2  | 1.86            | 8                | 2     |
| CA29   | Peru    | Huantan          | 3 126          | 100             | 45  | 40  | 7.1          | 250                  | 8.11 | 0.62            | 12.4             | 4     |
| CA30   | Peru    | Tupe             | 2 800          | 108             | 79  | 75  | 7.3          | 150                  | 6.7  | 0.62            | 11               | 3     |
| CA31   | Peru    | Tupe             | 2 580          | 112             | 47  | 70  | 6.9          | 150                  | 8.12 | 0.62            | 12.5             | 1     |
| CA32   | Peru    | Lincha           | 3 497          | 104             | 78  | 65  | 6.21         | 1250                 | 8.6  | 0.62            | 12.5             | 4     |
| CA33   | Peru    | Lincha           | 3 208          | 104             | 76  | 65  | 6.4          | 840                  | 8.7  | 0.62            | 12.6             | 4     |
| CA34   | Peru    | Lincha           | 2 553          | 104             | 74  | 65  | 6.7          | 680                  | 8    | 0.62            | 13.2             | 4     |
| CA35   | Peru    | Lincha           | 2 563          | 108             | 87  | 65  | 8.1          | 90                   | 7.4  | 0.62            | 10.8             | 2     |
| CA36   | Peru    | Yauyos           | 2 946          | 90              | 67  | 15  | 7.96         | 87.5                 | 7.1  | 0.29            | 6.8              | 3     |
### APPENDIX 1 (Continued)

| Site  | Country | Basin | Altitude m asl | Reference Score | IHF | QBR | Oxygen (mg/l) | Conductivity (μS/cm) | pH  | Nitrates (mg/l) | Temperature (°C) | Order |
|-------|---------|-------|----------------|-----------------|-----|-----|---------------|----------------------|-----|----------------|------------------|-------|
| CA37  | Peru    | Yauyos| 2 881          | 90              | 49  | 40  | 6.66          | 126.4                | 7   | 0.63           | 10.7            | 3     |
| CA38  | Peru    | Laraos| 3 223          | 96              | 49  | 15  | 7.16          | 206.1                | 8.2 | 0.41           | 12.6            | 3     |
| CA39  | Peru    | Alis  | 3 542          | 84              | 38  | 0   | 7.35          | 249.5                | 7.6 | 0             | 8.3             | 4     |
| CA40  | Peru    | Alis  | 3 420          | 90              | 42  | 60  | 7.59          | 353.1                | 8.3 | 0             | 8.3             | 4     |
| CA41  | Peru    | Alis  | 3 218          | 90              | 51  | 10  | 5.71          | 470                  | 8   | 0.2            | 12              | 4     |
| CA42  | Peru    | Miraflores | 3 642       | 94              | 58  | 45  | 5.84          | 288.7                | 7.5 | 0.47           | 13.5            | 3     |
| SP1   | Ecuador | San Pedro | 2 305        | 74              | 50  | 20  | 7.18          | 797                  | 7.96| 1.59           | 18              | 4     |
| SP2   | Ecuador | San Pedro | 2 386        | 70              | 61  | 20  | 7.52          | 533                  | 7.82| 1.74           | 3.8             | 4     |
| SP3   | Ecuador | San Pedro | 2 450        | 64              | 45  | 0   | 6.95          | 585                  | 8.19| 1.06           | 13.6            | 3     |
| SP4   | Ecuador | San Pedro | 2 460        | 70              | 63  | 20  | 7.06          | 695                  | 8.46| 1.32           | 14.2            | 3     |
| SP5   | Ecuador | San Pedro | 2 631        | 72              | 70  | 25  | 8.58          | 756                  | 8.59| 1.22           | 15.6            | 3     |
| SP6   | Ecuador | San Pedro | 2 825        | 76              | 54  | 25  | 7.2           | 771                  | 6.73| 1.12           | 15.1            | 3     |
| SP7   | Ecuador | San Pedro | 2 935        | 82              | 63  | 30  | 7.5           | 500                  | 7.44| 1.67           | 13.6            | 3     |
| M1    | Ecuador | Machangara | 3 190        | 92              | 58  | 25  | 8.32          | 118.9                | 7.51| 0.5            | 10.1            | 1     |
| M2    | Ecuador | Machangara | 2 799        | 72              | 56  | 20  | 0.51          | 703                  | 7.26| 1.09           | 18.9            | 3     |
| M3    | Ecuador | Machangara | 2 589        | 84              | 54  | 20  | 6.02          | 665                  | 8.02| 1.09           | 17.3            | 3     |
| M4(SP9)| Ecuador | Machangara | 2 142        | 82              | 52  | 20  | 4.58          | 466                  | 7.96| 1.69           | 17.1            | 4     |
| M5(SP8)| Ecuador | Machangara | 1 932        | 76              | 57  | 25  | 5.44          | 443                  | 7.92| 2.48           | 17.8            | 5     |
| M6    | Ecuador | Guayllabamba  | 1 542        | 86              | 55  | 25  | 5.44          | 504                  | 7.96| 3.08           | 19.4            | 5     |
| M7    | Ecuador | Machangara | 2 382        | 78              | 57  | 25  | 2.4           | 675                  | 6.52| 0.05           | 23              | 3     |
| 1.1 SP| Ecuador | San Pedro | 3 621        | 120             | 85  | 90  | 11.59         | 152.37               | 8   | 0.02           | 10.54           | 1     |
| 1.2 SP| Ecuador | San Pedro | 3 618        | 120             | 87  | 100 | 6.44          | 194                  | 7.52| 0.36           | 8.71            | 1     |
| 1.3 SP| Ecuador | San Pedro | 3 595        | 116             | 79  | 90  | 6.38          | 351                  | 8.03| 0.03           | 9.22            | 1     |
| 1.4 SP| Ecuador | San Pedro | 3 610        | 110             | 77  | 85  | 6.15          | 124.67               | 7.68| 0.03           | 10.77           | 1     |
| 2.1 SP| Ecuador | San Pedro | 3 192        | 106             | 90  | 80  | 6.3           | 140.33               | 2.43| 1.23           | 10.17           | 2     |
| 2.3 SP| Ecuador | San Pedro | 3 199        | 110             | 90  | 80  | 5.71          | 60.23                | 6.93| 0.79           | 9.48            | 1     |
| 2.4 SP| Ecuador | San Pedro | 3 333        | 110             | 90  | 85  | 6.11          | 88.67                | 7.04| 0.05           | 8.32            | 1     |
| 2.5 SP| Ecuador | San Pedro | 3 300        | 110             | 85  | 85  | 7.34          | 88.33                | 7.29| 0.04           | 8.41            | 1     |
| 2.6 SP| Ecuador | San Pedro | 3 300        | 110             | 85  | 80  | 6.73          | 80.5                 | 7.61| 0.04           | 9.62            | 1     |
| 3.1 SP| Ecuador | San Pedro | 2 787        | 120             | 90  | 90  | 5.54          | 243                  | 7.28| 0.06           | 10.78           | 1     |
| 3.2 SP| Ecuador | San Pedro | 2 750        | 118             | 90  | 80  | 8.99          | 210.67               | 7.38| 0.06           | 12.52           | 2     |
| 3.3 SP| Ecuador | San Pedro | 2 800        | 110             | 85  | 80  | 7.46          | 148                  | 7.13| 0.3            | 15.2            | 2     |
| 3.4 SP| Ecuador | San Pedro | 2 953        | 110             | 85  | 65  | 8.09          | 127.73               | 6.76| 0.07           | 12.62           | 2     |
| TAMB-1| Ecuador | San Pedro | 2 812        | 102             | 78  | 65  | 8.01          | 178.6                | 6.74| 0.91           | 13.3            | 2     |
| TAMB-2| Ecuador | San Pedro | 2 812        | 68              | 37  | 0   | 7.7           | 181.6                | 7.63| 0.74           | 13.5            | 2     |
| JAM-1 | Ecuador | San Pedro | 3 002        | 102             | 75  | 70  | 7.7           | 267                  | 8.2  | 2.14           | 11.7            | 3     |
| PH1   | Ecuador | Pita   | 2 600         | 84              | 66  | 20  | 8.32          | 242.65               | 7.45| 0.54           | 15.8            | 3     |
| Site     | Country | Basin | Altitude m asl | Reference Score | IHF | QBR | Oxygen (mg/l) | Conductivity (μS/cm) | pH   | Nitrates (mg/l) | Temperature (°C) | Order |
|----------|---------|-------|----------------|----------------|-----|-----|---------------|----------------------|------|-----------------|------------------|-------|
| PI2(3.1 PI) | Ecuador | Pita  | 2 804          | 110            | 89  | 65  | 8.07          | 174.3                | 8.1  | 0.12            | 13.6             | 2     |
| PI3(3.2 PI) | Ecuador | Pita  | 2 805          | 110            | 89  | 60  | 7.87          | 210.1                | 7.82 | 0.17            | 13.4             | 2     |
| PI4(3.4 PI) | Ecuador | Pita  | 2 843          | 102            | 87  | 85  | 8.39          | 154.5                | 7.79 | 0.18            | 12.1             | 4     |
| 1.1 PI   | Ecuador | Pita  | 3 824          | 118            | 76  | 25  | 7.17          | 463.56               | 7.89 | 0.24            | 11.87            | 2     |
| 1.2 PI   | Ecuador | Pita  | 3 743          | 118            | 66  | 25  | 6.43          | 132.23               | 8.16 | 0.03            | 10.61            | 3     |
| 1.3 PI   | Ecuador | Pita  | 3 754          | 118            | 70  | 25  | 6.38          | 85.11                | 8.55 | 0.03            | 18.4             | 1     |
| 1.4 PI   | Ecuador | Pita  | 3 694          | 116            | 66  | 25  | 6.15          | 417.19               | 8.43 | 0.03            | 15.13            | 1     |
| 2.1 PI   | Ecuador | Pita  | 3 300          | 110            | 89  | 80  | 6.3           | 59.42                | 7.29 | 0.07            | 9.5              | 1     |
| 2.2 PI   | Ecuador | Pita  | 3 180          | 106            | 84  | 65  | 5.71          | 29.81                | 7.44 | 0.04            | 10.23            | 2     |
| 2.3 PI   | Ecuador | Pita  | 3 295          | 110            | 77  | 70  | 6.11          | 92                   | 7.64 | 0.13            | 8.7              | 1     |
| 2.4 PI   | Ecuador | Pita  | 3 290          | 110            | 87  | 60  | 7.34          | 162.41               | 8.1  | 0.19            | 12.13            | 2     |
| 3.3 PI   | Ecuador | Pita  | 2 900          | 106            | 83  | 70  | 7.25          | 49.78                | 7.59 | 0.96            | 12.45            | 2     |
| STA CLARA-1 | Ecuador | Pita  | 2 557          | 82             | 70  | 30  | 8.32          | 254.6                | 7.18 | 0.51            | 14.8             | 1     |
| STA CLARA-2 | Ecuador | Pita  | 2 509          | 76             | 62  | 15  | 7.95          | 286.1                | 8.19 | 0.75            | 14.8             | 1     |
