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# A benthic macroinvertebrate multimetric index for Chilean Mediterranean streams

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#### ABSTRACT

Increased anthropogenic disturbances affecting streams worldwide have resulted in declines of freshwater biodiversity. Mediterranean ecoregions are very sensitive to such disturbances because of their high levels of natural hydrological variability and increasing trends in human population growth. The use of aquatic macroinvertebrate assemblages as bioindicators is a commonly applied approach for evaluating water body condition. However, extensive use of macroinvertebrates across similar ecoregions may be limited because of natural local differences in biodiversity and differing anthropogenic disturbances. Here, we present a multi-metric index (MMI) based on macroinvertebrate assemblages from Chilean Mediterranean streams. To evaluate the relative level of disturbance among sampling sites with respect to multiple anthropogenic perturbations, we used an integrated disturbance index based on catchment and local scale disturbances. We sampled 95 sites from streams and rivers during the 2016 austral summer including 26 least-disturbed reference sites, 13 highly disturbed sites, and 56 intermediately disturbed sites. In addition, we re-sampled 14 sites during winter and spring to validate our findings. Using a set of screening criteria, we evaluated 74 candidate macroinvertebrate metrics, representing diversity, composition, trophic structure, and tolerance to pollution. The resulting MMI included Diptera taxa richness, total macroinvertebrate density, number of Ephemeroptera-Plecoptera-Trichoptera individuals, and predator taxa richness. The final MMI scores classified the 95 sampling sites into three categories of biotic condition, including good (n = 46), fair (n = 18), and poor (n = 31). Our approach is transferable to other rivers in the region and is a sufficient tool to evaluate the condition of sites affected by several human perturbations at both local and catchment scales in Chilean Mediterranean streams

#### 1. Introduction

Freshwater ecosystems are among the most threatened systems globally because of human-related influences (Saunders et al., 2002). These environments provide ecosystem services such as water quality and quantity, recreational uses, habitat for species, biodiversity maintenance, and tourism (Malinga et al., 2015). Until the 1980s, the majority of research about the impacts of human activities causing degraded freshwaters was based on the evaluation of water quality stressors, however, this approach considers only conditions at the time and place of sampling (Oberdorff and Hughes, 1992; Fierro et al., 2017a). More recently, research has been focused on the use of biological indicators, such as benthic macroinvertebrates, that could reflect longer term conditions of aquatic ecosystems (Karr, 1987; Hilsenhoff,

1988). In particular, multimetric indices (MMIs) based on benthic macroinvertebrates have been widely used in many ecoregions of the world (Kerans and Karr 1994; Whittier et al., 2007; Mondy et al., 2012; Chen et al., 2014; Lake and Moog, 2015; Silva et al., 2017). Yet, little attention has been paid to systems with high levels of biological isolation and endemism, such as southern South America (Fierro et al., 2015, 2016).

An advantage of using MMIs is their ability to integrate multifaceted biological attributes of benthic macroinvertebrate assemblages (e.g., taxonomic richness, habitat and trophic guild composition, health and abundance) into a score that indicates the biological condition of a site (Hughes et al., 1998). Because multiple perturbations occurring in streams simultaneously will result in different biological responses depending on each particular biome, a universal MMI does not exist and

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thus, a unique index may be needed for each major ecoregion (Stoddard et al., 2008; Pont et al., 2009; Herman and Nejadhashem, 2015). MMI scores are assigned depending on the degree of biological disturbance found at each site, with high scores associated with reference sites and low scores associated with highly altered sites (Sánchez-Montoya et al., 2009). Therefore, MMI scoring to understand anthropogenic impacts on freshwaters in understudied and rapidly changing regions warrants careful selection of least-disturbed reference sites to assess the impact of current human interventions, as well as to better interpret the impacts of future disturbances.

The Chilean Mediterranean Ecoregion has been recognized as a global biodiversity hotspot (Myers et al., 2000). This region is characterized by a high level of biological endemism as a result of geographic isolation by the Atacama Desert in the north, glaciers in the south, the Andean Range in the east, and the Pacific Ocean in the west (Unmack et al., 2009; Vila and Habit, 2014). The region encompasses the greatest species richness of fishes, invertebrates, amphibians and aquatic plants in Chile (Ramírez and San Martín, 2005; Habit et al., 2006; Valdovinos, 2006; Vidal et al., 2009). In recent decades, the Chilean Mediterranean Ecoregion has been severely threatened by changes in land use from native shrublands to agriculture, urbanization, and plantations of exotic pine and eucalyptus trees (Pauchard et al., 2006; Fierro et al., 2012; Hernández et al., 2016). In addition, streams in this region are increasingly being dammed and have received numerous fish introductions including salmonids and cyprinids (Arismendi et al., 2014; Vargas et al., 2015). Whereas most research in Mediterranean ecoregions has focused on terrestrial ecosystems, little is known about their freshwaters (Cooper et al., 2013). Rivers and streams are increasingly affected by multiple physicochemical and biological stressors globally (e.g., Schinegger et al., 2012; Wohl et al., 2017), thus the Chilean Mediterranean ecoregion is of particular interest to better assess its current environmental conditions (Fierro et al., 2017a).

The main objective of this study was to assess the ecological integrity of Chilean Mediterranean streams under multiple human disturbance pressures by using a MMI based on freshwater benthic macroinvertebrates. Specifically, we defined a gradient of disturbance distributed along multiple sites, including sites with low and high anthropogenic influences. Then, we identified potential metrics based on the composition of benthic macroinvertebrates and selected those metrics that best distinguished most- from least-disturbed sites. Lastly, we developed and validated a MMI that can be transferable across similar Mediterranean streams. To our knowledge, this study is the first that develops a MMI to monitor and evaluate the ecological condition of streams in this region of the world.

#### 2. Materials and methods

#### 2.1. Study area

We conducted this study in five large river basins of the Mediterranean Ecoregion of Chile: Aconcagua ( $7340 \text{ km}^2$ ), Maipo ( $15,304 \text{ km}^2$ ), Rapel ( $13,695 \text{ km}^2$ ), Mataquito ( $6190 \text{ km}^2$ ), and Maule ( $20,295 \text{ km}^2$ ) (Fig. 1). The climate is characterized by a dry season (November–May) and a wet season (June–October). Annual precipitation varies from 200 to 700 mm. The landscape consists of a mosaic of different natural land cover types, mostly dry xerophytic thorn, dominated by deciduous shrubs and succulents (Armesto et al., 2007). Extensive agriculture and tree plantations have been accompanied by rapid and uncontrolled urban growth (Pauchard et al., 2006; Hernández et al., 2016).

#### 2.2. Site selection and data collection

We sampled 95 stream sites, including 23 from the Aconcagua Basin, 17 from the Maipo Basin, 20 from the Rapel Basin, 13 from the Mataquito Basin, and 23 from the Maule Basin (Fig. 1). Stream sites ranged from first- to sixth-order (i.e., 1-81 m wetted channel width; 12-2106 m.a.s.l.). Samples were collected during the Austral summer (December 2015-March 2016). At each site, we measured in situ conditions of temperature (°C), pH, conductivity (µs·cm<sup>-1</sup>), total dissolved solids  $(mgL^{-1})$ , and dissolved oxygen  $(mgL^{-1})$  using a Hanna Multiparameter Model HI 9828 Meter. Within each site, three equidistant transects were placed perpendicular to the channel. We evaluated stream channel conditions including average depth, mean active channel width, and mean wetted width using a tape measure. Average depth was estimated from three measurements along each transect across the site. We visually estimated the in-stream percent areal coverage of macrophytes, leaves, large wood, and substrate particle size (silt-clay: < 0.03 mm, sand: 0.03-1 mm, gravel and pebble: 2-64 mm, cobble: 64–256 mm, and boulder: > 256 mm) using a 1-m<sup>2</sup> grid. Three substrate observations in each site were conducted at the stations selected for sampling.

#### 2.3. Macroinvertebrate sampling

Six separate samples were taken from riffle habitats by using a Surber net (500  $\mu$ m mesh size; 0.09 m<sup>2</sup> area). The samples were fixed *in situ* with 90% ethanol and then transported to the laboratory where they were separated and preserved in 70% ethanol. All individuals from each taxon were identified and counted under a stereomicroscope (Zeiss, model Stemi Dv4). Organisms were identified to the lowest possible taxonomic resolution, using the taxonomic key developed by Domínguez and Fernandez (2009). All aquatic invertebrates were identified by the first author to maintain consistency among sample sets.

#### 2.4. Determination of disturbance gradient

We determined least-disturbed sites along a quantified disturbance gradient. To determine the disturbance gradient, we used an integrated disturbance index (IDI) following Terra et al. (2013), Ligeiro et al. (2013), and Macedo et al. (2016). They proposed combining a catchment disturbance index (CDI) and a local disturbance index (LDI) into an integrated disturbance index (IDI). The CDI was calculated based on weighted land use types in the catchment (Rawer-Jost et al., 2004; Ligeiro et al., 2013). The catchment percentages of each land use were estimated for each site by screening digitized satellite images. We used 1:12.000 scale photos that were freely available from Sistema de Información Satelital, Ministerio de Agricultura, Chile (http://sit.conaf. cl/). Land use types were determined using ArcGis 10 (ESRI, 2007) and classified as urban, agricultural, or tree plantation. The urban, pasture and agricultural land uses were weighted following Ligeiro et al. (2013) and Terra et al. (2013). The tree plantation weight was adapted from Miserendino et al. (2011) and Fierro et al. (2015). These authors showed that tree plantations are often associated with less disturbance of aquatic systems than pastures. The CDI was calculated as the sum of land use types, each one weighted differently as:

Catchment disturbance index (CDI) =  $4 \times$ %urban +  $2 \times$ %agricultural +  $1 \times$ %pasture +  $0.5 \times$ %tree plantation

For quantifying the LDI, we followed Kaufmann et al. (1999) who developed the W1\_HALL metric. This metric is calculated from the sum of eleven types of anthropogenic disturbances observed in the channel and in a  $10 \times 10$  m riparian zone plot (i.e., buildings, agriculture, trash, logging, mining, parks and lawns, effluent, pasture, pavement, road, channel revetment). We adapted some disturbances to reflect the activities present in our study area. Specifically, we replaced logging, park and lawns, gravel extraction, and water extraction. We weighted observed local disturbances according to Kaufmann et al. (1999) where the proximity to the stream channel is the main factor. We weighted

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