Universidad de Concepción

Facultad de Ciencias Ambientales
Programa de Doctorado en Ciencias Ambientales mención Sistemas Acuáticos Continentales

Establecimiento de impactos de actividades antropogénicas sobre la integridad biótica en ríos de la ecorregión Mediterránea

Tesis para optar al grado de

Doctor en Ciencias Ambientales con mención en Sistemas Acuáticos Continentales

PABLO IGNACIO FIERRO RETAMAL

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PABLO IGNACIO FIERRO RETAMAL

Profesor Guía: Dr. Claudio Valdovinos Zarges

Departamento de Sistemas Acuáticos, Facultad de Ciencias Ambientales
Universidad de Concepción

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2018

Comisión Evaluadora de Tesis de Grado:

Dr. Claudio Valdovinos Zarges
Director de Tesis
Departamento de Sistemas Acuáticos
Facultad de Ciencias Ambientales
Universidad de Concepción

Dr. Iván Arismendi
Cotutor de Tesis
Department of Fisheries and Wildlife
College of Agricultural Sciences
Oregon State University

Dr. Luis Vargas Chacoff
Instituto de Ciencias Marinas y Limnológicas
Facultad de Ciencias
Universidad Austral de Chile

Esta tesis está dedicada a mi familia que amo,

Patricio, Luz Marina, Carla y Loretto

La inteligencia consiste no solo en el conocimiento,

sino también en la destreza de aplicar los conocimientos en la práctica

Aristóteles

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Resumen

Las diversidad actividades antropogénicas están provocando cambios en las comunidades de los ríos y por lo tanto provocando en casos extremos la perdida de la biodiversidad. En el mediterráneo Chileno las diversas actividades que están afectando al ensamble acuático aún no han sido bien entendidas, por lo que este trabajo busca generar conocimiento de cuáles son las principales actividades que provocan estrés sobre los ecosistemas acuáticos y de que manera están afectando los ríos. En una primera parte de esta tesis nosotros establecimos que macroinvertebrados acuáticos y peces han sido listados como buenos bioindicadores, utilizados principalmente en índices ecológicos. Luego nosotros establecimos que el cambio en el uso de suelo, la introducción de especies exóticas y los contaminantes provenientes de efluentes industriales y domésticos son las principales amenazas para la comunidad acuática en el mediterráneo Chileno. En una tercera parte, demostramos como las algas bentónicas, macroinvertebrados y peces respondieron de diferente manera frente a las mismas perturbaciones. En el cuarto capítulo demostramos que el cambio en el uso de suelo tuvo efectos alterando las tramas tróficas acuáticas, medidas a través de las presas e ingesta de un pez introducido. Finalmente en el quinto capítulo nosotros creamos un índice multimétrico que utiliza cuatro métricas, capaces de diferenciar sitios altamente perturbados de sitios de referencia. Estos resultados demuestran fuertemente el negativo efecto del cambio de uso de suelo producto de las actividades antropogénicas, con cambios principalmente provenientes del bosque nativo a plantaciones forestales exóticas, y un crecimiento de las áreas agrícolas y urbanas. Por otro lado, este cambio en el uso de suelo que es una perturbación a escala de cuenca, también se suma a los efectos que provocan las perturbaciones a escala local, que están ocurriendo dentro o cercanos a los ríos. Si el cambio en el uso de suelo continúa en el

Mediterráneo Chileno, nosotros predecimos que ocurrirá una perdida en la biodiversidad dulceacuática.

Introducción

Las relaciones entre el ambiente y las actividades humanas es un objetivo primario de las Ciencias Ambientales. Las múltiples actividades antropogénicas a las que han estado sometidos los ecosistemas del globo han puesto en evidencia la necesidad de evaluar el estado ecológico de estas, siendo los ecosistemas de agua dulce una prioridad (Dudgeon et al., 2006). Las altas tasas de degradación sobre estos ecosistemas, producto de las contantes amenazas a la cual están siendo sometidos, están teniendo un impacto negativo sobre la biodiversidad acuática (Allan, 2004; Saunders et al., 2002). Dentro de las ecorregiones del globo, la ecoregiones mediterráneas han sido reconocidas como un hotspot de biodiversidad mundial (Myers et al., 2000). La ecorregion mediterránea Chilena se caracteriza por un alto nivel de endemismo, teniendo una elevada riqueza de peces, invertebrados, anfibios y plantas acuáticas, en comparación al resto de las ecorregiones del país (Ramírez and San Martin, 2005; Habit et al., 2006; Valdovinos, 2006; Vidal et al., 2009). A pesar de estas características, las ecorregiones mediterráneas a nivel mundial han estado sometidas a un gran estrés por las actividades antropogénicas que allí se desarrollan, siendo comparativamente la ecoregión mediterránea Chilena una de las menos estudiadas mundialmente (Gasith and Resh, 1999). Los impactos ambientales relacionados a actividades humanas han devastado grandemente esta región en Chile (Romero and Ordenes, 2004), y considerando que las actividades humanas aquí continúan en alza, junto a que este país está catalogado en vías de desarrollo, los efectos de múltiples estresores antropogénicas sobre los ríos necesitan ser bien entendidos.

Para la evaluación de la calidad del agua y el efecto sobre la estructura y funcionamiento de las comunidades acuáticas, el método más utilizado han sido los parámetros físico-químicos del agua. Sin embargo estas evaluaciones son puntuales en el tiempo, por lo que es posible que el efecto de algún contaminante no logre ser medido en el momento (Oberdoff and Hughes, 1992). Es por esto que análisis complementarios son necesarios, como la evaluación mediante la comunidad acuática, la cual estaría integrando a través del tiempo los efectos de estos contaminantes y por lo tanto reflejarían el estado ecológico de la comunidad (Karr, 1987; Hilsenhoff, 1988; Whittier et al., 2007). En este sentido han surgido diferentes métodos, los cuales se han basado primariamente en el estudio de cada ensamble frente a algún estresor en particular. El monitoreo biológico ha sido ampliamente empleado debido a que los organismos pueden integrar características físicas, químicas y biológicas de las condiciones de los ríos.

Peces, macroinvertebrados bentónicos y algas bentónicas han aparecido por ser los principales proxies en la bioevaluación (Kerans and Karr, 1994; Delgado et al., 2012; Jia and Chen et al., 2013) Estos grupos están presentes en la mayoría de los cuerpos acuáticos y pueden responder de manera diferente a las condiciones ambientales. Es así que el conocimiento de cada ensamble a la misma perturbación es primordial, debido a que la tolerancia al estrés de cada grupo taxonómico puede variar frente a un mismo estresor (Hering et al., 2006). Una manera de resumir las respuestas de las comunidades para evaluar la calidad ecológica de los ríos es a través de índices bióticos, siendo los índices multimétricos una buena herramienta para conocer y entender los patrones ecológicos de las comunidades acuáticas (Herman and Nejadhashemi, 2015). A través de estos índices se puede evaluar rápidamente y de manera menos costosa (*i.e.* parámetros físico-químicos) los ríos que están siendo afectados por actividades antropogénicas.

Las grandes cuencas en la región Mediterránea de Chile, donde se localizan los sitios de muestreo de esta tesis, drenan los sistemas fluviales desde las montañas de la cordillera de los Andes, mientras que cuencas de menor tamaño drenan las aguas de la cordillera de la Costa. El clima es caracterizado por una marcada estación húmeda y una estación seca, caracterizándose además las cuencas en las montañas costeras por una influencia oceánica. La precipitación anual varía desde 200 a 700 mm. El paisaje consta de un mosaico de diferentes tipos de cubierta de suelo, mayormente por espinos xerofíticos secos, dominados por arbustos de hoja caduca, junto con una extensiva actividad agrícola, plantaciones forestales y un incesante crecimiento urbano (Armesto et al., 2007). Los ecosistemas mediterráneos han estado grandemente perturbados por actividades antropogénicas, siendo los principales estresores el cambio de uso de suelo, debido a la agricultura, monocultivo de plantaciones forestales y desarrollo industrial y urbano (Pauchard et al., 2006; Fierro et al., 2012; Hernández et al., 2016). Este clima junto con una única geografía e historia geológica, hacen del ecosistema Mediterráneo uno de los más complejos y ricos globalmente en términos de biodiversidad.

Hipótesis y Objetivos específicos

Debido a que diversas perturbaciones antropogénicas, tanto a escala de cuenca como cambios en el uso de suelo, y a escala local, como actividades mineras o alteración de flujo, producen alteraciones en la salud del ecosistema, se espera que en los ecosistemas mediterráneos;

- ✓ Ríos con mayor grado de perturbación presenten menor calidad de hábitat para la fauna acuática, lo que producirá cambios negativos en la comunidad acuática.
- ✓ Diferentes variables ambientales expliquen el ensamble de macroinvertebrados bentónicos, peces, y algas bentónicas frente a un mismo estresor.
- ✓ Cambios en el uso de suelo produzcan cambios en el ensamble de macroinvertebrados acuáticos y estos se vean reflejados en una ingesta diferenciada de presas en peces.
- ✓ Métricas que describan atributos estructurales y funcionales del ensamble de macroinvertebrados sean capaces de determinar la calidad ecológica de los ríos mediterráneos de Chile discriminando ríos impactados de ríos de referencia.

El objetivo general de esta tesis es identificar las actividades antropogénicas que generan mayor estrés sobre los ríos de la zona mediterránea de Chile, evaluando el efecto y magnitud de estas sobre la integridad biótica. Esta será medida a través de la estructura del ensamble de macroinvertebrados bentónicos, peces, perifiton y la dieta de peces introducidos.

Los objetivos de cada capítulo serán:

1.1 Analizar la literatura existente en búsqueda de la evaluación de la calidad del agua mediante indicadores bióticos.

2.1 Seleccionar las principales amenazas de origen antropogénico en las cuencas mediterráneas de Chile a través de una revisión bibliográfica.

2.2 Clasificar las amenazas identificadas en el objetivo anterior y graduarlas a través de una encuesta a científicos en el área dulceacuática.

3.1 Caracterizar la diversidad de macroinvertebrados bentónicos, peces y algas bentónicas en cuencas con uso de suelo de vegetación nativa, plantaciones forestales, agricultura y urbano.

3.2 Evaluar los parámetros ambientales que están afectando al ensamble de macroinvertebrados bentónicos, peces y algas bentónicas.

4.1 Evaluar los impactos ambientales que tiene el uso de suelo de plantación forestal sobre el ensamble de macroinvertebrados bentónicos.

4.2 Evaluar la dieta de la trucha arcoíris como potencial bioindicador en ríos mediterráneos de Chile.

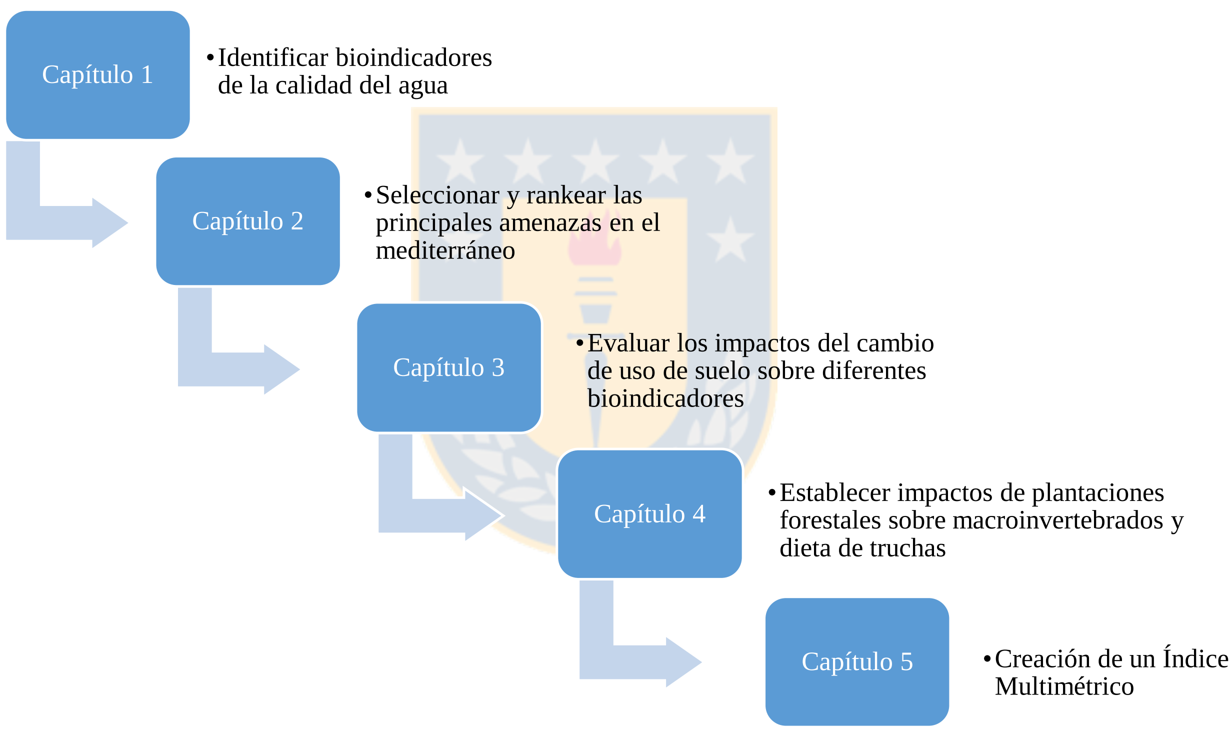
5.1 Determinar un gradiente de perturbación en los ríos mediterráneos de Chile.

5.2 Escoger métricas más adecuadas basadas en macroinvertebrados bentónicos que sean capaces de diferenciar sitios altamente perturbados de sitios poco perturbados.

5.3 Crear un índice multimétrico basado en macroinvertebrados bentónicos

Estructura de la tesis

Para el desarrollo de esta tesis, se muestrearon cinco cuentas andinas y dos cuencas costeras. Las cuencas andinas fueron la cuenca del río Aconcagua, río Maipo, río Cachapoal, río Mataquito, y río Maule. En tanto, las cuencas costeras correspondieron a la del río Moncul y río Queule. En cada cuenca se muestrearon ríos de referencia (*i.e.* con nula o poca intervención antropogénica) y ríos impactados por actividades humanas, muestreándose macroinvertebrados bentónicos acuáticos, peces y algas bentónicas. Entre los peces introducidos capturados nosotros analizamos el contenido estomacal de la trucha arcoíris. Adicionalmente, en cada sitio de muestreo se tomaron muestras de agua para evaluar la calidad físico-química de esta. Para clarificar los pasos de esta investigación, en el siguiente esquema se representa el desarrollo de esta tesis y a continuación se describe detalladamente que aborda cada capítulo.



Esquema 1. Representación esquemática de las etapas de esta tesis.

En el **Capítulo 1** se analiza la literatura existente en búsqueda de la evaluación de la calidad del agua mediante indicadores bióticos, estableciendo ventajas y desventajas de estos mismos. Además se da una introducción a la integridad ecología en los ríos y como esta puede ser medida a través de los índices de integrad biótica. Por último se establece los efectos de la agricultura sobre la integridad en macroinvertebrados y peces, usando como caso de estudio los ríos del centro-sur de Chile.

En el **Capítulo 2** se establece un método estandarizado para colectar los datos bibliográficos en relación a la amenazas antropogénicas a las cuales están sometidos los macroinvertebrados acuáticos, peces, anfibios y plantas de agua dulce. Para esto se hizo una revisión bibliográfica identificando las amenazas antropogénicas en el ecosistema mediterráneo Chileno. Con esta información se realizaron encuestas a la comunidad científica con *expertise* en cada grupo taxonómico, de manera de evaluar estas amenazas e identificar la falta de conocimiento en algún área.

Dado que en el capítulo uno se establecieron los grupos taxonómicos usados en la bioindicación, y en el capítulo dos se identificaron las principales amenazas antropogénicas a los ecosistemas mediterráneos que los afectan. En el **Capítulo 3** se analizaron los impactos que tienen el cambio de uso de suelo, plantaciones forestales, agricultura, y urbano sobre el ensamble de macroinvertebrados acuáticos, peces y algas bentónicas, en ríos ubicados en cuencas andinas. Los patrones de distribución se estudiaron a escala regional y latitudinal durante la época de verano. Se analizaron además las variables ambientales que estarían explicando la composición de cada ensamble biótico.

En el **Capítulo 4** se analizó los impactos que tienen el cambio de uso de suelo desde boque nativo a plantaciones forestales sobre el ensamble de macroinvertebrados bentónicos acuáticos en cuencas costeras. Conjuntamente se analizó el contenido estomacal de la trucha arcoíris y se analiza su uso potencial como bioindicador. Este estudio se realizó estacionalmente durante las cuatro estaciones del año.

Una vez analizadas las respuestas de los diferentes grupos biológicos frente a diferentes usos de suelo, finalmente en el **Capítulo 5** se formuló un índice multimetrico (MMI) basado en macroinvertebrados bentónicos para la evaluación de la calidad ecológica en los ríos mediterráneos. Para esto primariamente se estableció un gradiente de perturbación entre todos los sitios de muestreo, los cuales estuvieron basados en índices de afectación del hábitat a escala de cuenca y a escala local. De esta manera se pudo establecer los sitios de referencia en la ecorregión mediterránea. Luego a través de análisis estadísticos se escogieron las mejores métricas basadas en macroinvertebrados bentónicos que pudieron establecer diferencias entre sitios altamente perturbados y poco perturbados.

Capítulo 1: Macroinvertebrates and fishes as bioindicators of stream water pollution

Este capítulo está basado en:

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Macroinvertebrates and fish as bioindicators of stream water pollution

Pablo Fierro[1,2,*], Claudio Valdovinos[1], Luis Vargas-Chacoff[2,3], Carlos Bertrán[2], Ivan Arismendi[4]

[1]Departamento de Sistemas Acuáticos, Facultad de Ciencias Ambientales y Centro EULA, Universidad de Concepción, Concepción, Chile.

[2]Institute of Marine Science and Limnology, Universidad Austral de Chile, Valdivia, Chile.

[3]Centro Fondap de Investigación de Altas Latitudes (IDEAL). Universidad Austral de Chile, Valdivia, Chile.

[4]Department of Fisheries and Wildlife, Oregon State University, Corvallis, USA.

* Corresponding Author: pablofierro@udec.cl

Introduction

Of all the water on earth, freshwater accounts for just 0.01% and covers only 0.8% of the planet's surface (Dudgeon et al., 2006). Freshwaters are among the most threatened ecosystems of the world and thus, understanding their health statuses is of special relevance. Indeed, the physical, chemical, and biological integrities of water are highly important for successfully implementing conservation and management strategies before ecosystem health or biotic integrity are affected (Butcher et al., 2003; Herman and Nejadhashemi, 2015; Lyons et al., 1995). This chapter provides a review of known biotic integrity indicators, including of benthic macroinvertebrate and fish communities that have been proposed to serve as water quality indicators. In addition, the pros and cons of using aquatic communities as water quality indicators are discussed. Finally, we present a research case study in which benthic macroinvertebrate and fish communities are used as bioindicators, in addition to discussing the effectiveness of using illustrative examples for streams subject to several agriculture uses in a region of Chile dominated by agricultural activities.

Worldwide, a primary threat to freshwater ecosystems is the rapid changes occurring in land uses (*Figure_1*), a situation that has intensified over the last decade (Barletta et al., 2010; Fierro et al., 2016). Most recent land use conversion has been for crop production, which notably impacts proximal ecosystems due to changes over extensive crop areas (Allan, 2004). In particular, the fertilizers and pesticides used in agriculture negatively affect freshwater ecosystems by draining into rivers, where eutrophication and other negative effects, such as high sediment deposits and post-sedimentation, subsequently occur. Furthermore, the extensive land use of farming many times results in landscape deforestation, which often arrives to the riverbank itself. This deforestation can increase the temperature of and quantity of light in river water. When coupled with eutrophication, the trophic changes within the aquatic ecosystem can be disturbed, causing, for example, a decreased quantity of aquatic taxa as compared to rivers with fewer alterations (Fierro et al., 2015; Wang et al., 2007).

Figure 1. Examples of land use in the central-south of Chile. Left: Stream nearby corn crops, Right: Stream borderer by native forest of the Maule Region watershed (Photographs by P. Fierro).

Indicators of aquatic ecosystem health

The definition of a healthy ecosystem has been widely debated in the literature. Nevertheless, the definition proposed by Rapport is one of the most widely accepted (Fu-Liu and Shu, 2000). This definition states that a healthy ecosystem is defined by the "absence of danger signals in the ecosystem, the ability of the ecosystem to quickly and completely recover (resilience), and/or the lack of risks or threats that push the ecosystem composition, structure, and/or function." The purpose of monitoring aquatic ecosystem health is to identify physicochemical and biological changes arising from anthropogenic impacts (Hughes et al., 1992). This information is crucial for managers and policy makers to make informed decisions towards improving the environment and, consequently, human health (Weigel et al., 2002).

Traditional techniques for measuring water quality and to establish aquatic health assess a number of physical and chemical parameters of the water. However, these measurements do not accurately account for the real impacts that physicochemical activities have on freshwater ecosystems (Oberdoff and Hughes, 1992). Indeed, these parameters interact and evidence accumulative effects over time, the impacts of which can finally affect aquatic biota (Roldan, 1999). Due to this, other measurements that consider non-natural disturbing effects on ecological integrity should be used to calculate the quality of aquatic resources (Oliveira and Cortes, 2006). Indices based on aquatic biota have been widely successful in determining the integrity of aquatic ecosystems (Karr, 1987).

The use of indices that evaluate water quality through biological parameters, such as freshwater ecosystem structure and performance, has considerably increased in recent years and has gained recognition as an important measure for calculating the global integrity of freshwater ecosystems (Barbour et al., 1999; Karr and Chu, 2000; Ollis et al., 2006). Biological monitoring is advantageous in that it can integrate and reflect accumulative changes over time, which is in contrast to a number of other methods, such as flow regimen, energetic resources, and biotic interactions (Alba-Tercedor, 1996; Cairns and Pratt, 1993). Another benefit is that the high fauna diversity found in aquatic ecosystems, which include microorganisms, algae, periphyton, phytoplankton, zooplankton, macroinvertebrates, fish, and mammals, can be included in evaluations of river health (Herman and Nejadhashemi, 2015).

Among fauna, fish and macroinvertebrate assemblages have been highlighted as good bioindicators for monitoring ecosystem degradation related to farming and forestry, as well as to urban and industrial effluents (Dos Santos et al., 2011; Fierro et al., 2015).

Diverse proxies are used to measure ecosystem condition, such as species density and the presence/absence of several species in assemblage structures (Hilty and Merenlender et al., 2000). A notable advantage of using these aquatic biota is the relative simplicity of their capture and sampling (Li and Li, 2007; Merrit and Cummins, 2007). In particular, the sampling of fish assemblages can be performed via electrofishing, a highly common tool, while macroinvertebrate sampling is facilitated and simplified by Surber, D-frame dip, and kick nets (*Figure_2*).

Furthermore, recent studies report that the stomach contents of salmonids (i.e., *Oncorhynchus mykiss* and *Salmo trutta*) contain a diversity of invertebrate prey present in the benthos of non-intervened (hereafter termed "native") basins, thereby reflecting anthropogenic impacts to the basin (Vargas-Chacoff et al., 2013). Related to this, Fierro et al. (2016) reported similarities in stomach contents and prey diversity of the benthos in river sections with land use different than in the basin. Likewise, similarities have been found between rivers with more local perturbation, such as through the effects of dams (Rolls et al., 2012; Veloso et al., 2014). Therefore, the *O. mykiss* diet might represent an effective bioindicator for evaluating environmental disturbances within the entire basin (Fierro et al., 2016).

Figure 2. Left: Fish communities sampled using electrofishing. Right: Aquatic macroinvertebrates sampled using a Surber Net (Photograph by P. Fierro).

Among the ecological indices commonly used to evaluate river health, three primary groups exist – biotic indices, multivariate methods, and multimetric indices (Barbour et al, 1999; Karr, 1987; Karr and Chu, 2000; Oliveira and Cortes, 2006; Ollis et al, 2006). Of these, multimetric indices are the most recommended since a large quantity of data can be considered and since these indices may also identify the cause(s) of degradation. This information can then be applied to obtain better understandings of ecosystem status (Herman and Nejadhashemi, 2015). In turn, biotic indices evaluate river health based only on organism tolerance to organic pollution. One of the most well-known biotic indices is the Hilsenhoff Biotic Index (Hilsenhoff, 1988), which has been widely used and adapted around the world (e.g., Fierro et al. 2012; Figueroa et al. 2003, Lenat, 1993). Continuing, multivariate methods require the use of models that relate physicochemical properties of rivers with observed organisms, which are represented under reference (relatively pristine) conditions. These models then compare the observed organisms with those that were “expected.” This comparative method can ultimately detect potentially degraded areas. The most widely used multivariate index is the River Invertebrate Prediction and Classification System (Wright et al., 1988), which was first implemented in the UK and then adapted to other countries, including Australia (Davies, 2000). Finally, multimetric indices capture broad characteristic of community structure and function (metric), thus providing a broader understanding of the events occurring in the river (Reynoldson et al., 1997). Multimetric indices are powerful tools for establishing the consequences of human activities. These effects may include a high amount of specific and blurred disturbances (non-point pollutant discharge), which encompass impacts arising from agriculture, grazing, deforestation, physical alterations of river or bank habitats, damps, sewage discharges, urban areas, and mining (Barbour et al., 1996; Varandas and Vitor, 2010). These indices can be applied in several animal assemblages, plant communities, and ecosystems, including terrestrial, marine, and freshwater environments (Reynoldson et al., 1997). Corresponding indices of integrity are frequently performed and applied in fish [38] and macroinvertebrates (Griffith et al., 2005). A summary that contrasts among the three types of indices is presented in *Table_1*.

	Biotic Indices	Multivariate methods	Multimetric indices
Examples	Hilsenhoff Biotic Index. Fish Species Biotic Index.	River Invertebrate Prediction and Classification System. Australian River Assessment Scheme.	Index of Biotic Integrity. Benthic Index of Biotic Integrity.
Advantages	Simple, measure only one disturbance (e.g. organic pollution tolerance)	Model created to predict the species and number of organisms that would be expected to appear in a stream system.	Include diverse disturbances. Applicable in several animal/plant groups. Incorporates temporal and spatial scale attributes.
Disadvantages	Organisms do not respond to only one disturbance; many more stressors affect distribution in the wild.	Created models can be easily changed, making the results uncertain. These methods were developed to find patterns and not establish impact.	Limited by sampling technique efficiencies. Seasonal migration of biota influence results. Easy confusions with natural perturbations.

Table 1. Summary of the characteristics considered with stream health indices (adapted from Herman and Nejadhashemi, 2015).

Assessing the ecological integrity of streams

Ecological integrity, which is also referred to as river health or ecological status, is a measure of the global condition of an aquatic ecosystem. This measurement integrates physical, chemical, and biological integrity elements (Barbour et al., 1999; Karr, 1987; Oliveira and Cortes, 2006). Importantly, biological integrity is defined as the ability of aquatic ecosystems to support and maintain a balanced and integrated community with adapted organisms and a composition, diversity, and functional organization comparable to natural habitats within the same region (Angermeier and Karr, 1986; Karr, 1991; Karr and Dudley, 1981). Therefore, a loss of integrity indicates any human-induced positive or negative divergence of the system from a natural, model condition (Westra et al., 2000).

The Index of Biotic Integrity (IBI), which was initially developed for western USA rivers by Karr (1981), is the most used index based on fish assemblages. Consequently, the IBI has been adapted for use to numerous rivers on all continents to evaluate stream health [Herman and Nejadhashemi, 2015; Veloso et al., 2014). Indeed, since the creation of the IBI, over 2.374 researchers, as of 2014, have used, modified, or mentioned the importance of the IBI (Google Scholar). Furthermore, the number of citations for the IBI grew exponential until 2005, at which point citations "stagnated" near 140 studies per year (*Figure_3*).

Worth highlighting, of the studies presented in this review, the most important milestone occurred from 1986-1990. During this period, researchers first began adapting and making modifications to indices based on fish, in addition to these indices being applied in reports to the US government. Between 1991 and 1995, integrity indices were developed for several groups, including macroinvertebrates, birds, and zooplankton. Furthermore, this period was witness to index adaptations to marine and estuary environments. Even terrestrial environments were assessed by the IBI to measure the environmental quality of forests. Between 1996 and 2000, the IBI continued to expand to other groups and environments, such as periphyton communities, macrophytes, corals, and wetlands. Corresponding adaptations of the IBI to other continents, including Africa, Europe, and South America (Brazil), also occurred (De Freitas Terra et al., 2013; Tiku Mereta et al., 2013). Since 2001, this index is in use on almost all continents and has been adapted several times to different ecoregions within the same countries.

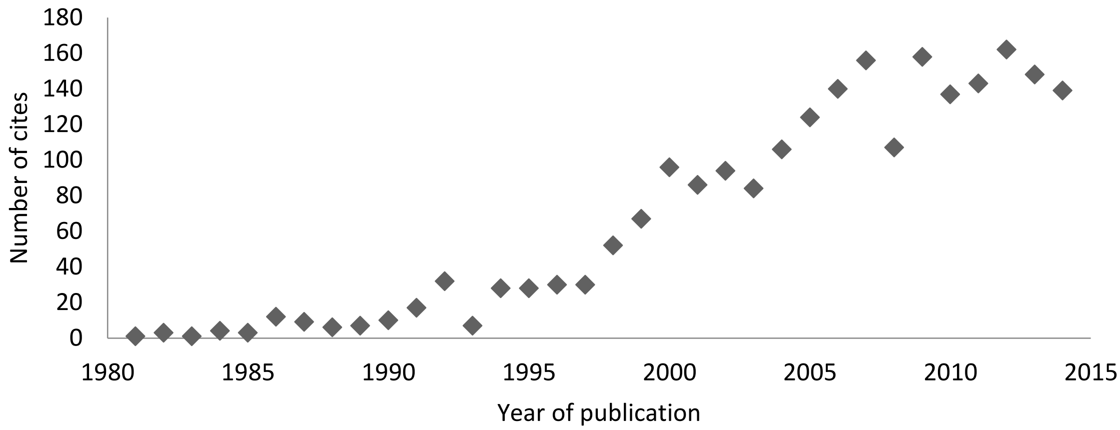

Figure 3. Accumulative number of worldwide publications on the index of biotic integrity around the world, starting with the first related publication by [28] (Source: own elaboration).

The advantage of establishing the biotic integrity of rivers based on fish arises as these organisms are present in all, or almost all, rivers, even those that are polluted. Additionally, extensive life history information is available for many species, and fish assemblages generally represent a variety of trophic levels. Indeed, fish are located within the top of the aquatic food chain and can thus help to provide an integrated view of basin environments. Other benefits of the IBI using fish are that fish populations are relatively stable in the summer, when most monitoring occurs; fish are easily identifiable; and the general public can relate to statements about the conditions of fish assemblages. On the other hand, a noted disadvantage of the IBI is that fish are highly mobile, making sampling difficult. Indeed, large groups of personnel, various tools, and an extended period in the field are needed to record daily and seasonal variations (Figueroa et al., 2003).

Although less used, the Benthic Index of Biotic Integrity (B-IBI) was developed by Kerans and Karr (1994) for rivers of the Tennessee Valley (USA), using the IBI as an initial base (Karr, 1981). The advantages of using macroinvertebrates as bioindicators are a great biodiversity and an extreme sensitivity and fast response of many taxa to pollution. This quick response is likely due to many macroinvertebrates being sessile and having aquatic life cycles, thus any alterations in environmental limits could lead to death (Roldan, 1999). One significant disadvantage of the B-IBI is that a taxonomic specialist is needed to identify the macroinvertebrate species, which takes a long time. To address this limitation, Rolls et al. (2012) used higher levels of taxonomic identification (e.g. genus, family, or both) as a method for adequately describing taxa traits for B-IBI use. Through this technique, a greater cost-benefit might be obtained as less time will be required to taxonomically identify species. Indeed, in countries with few taxonomists and without access to species-level identification keys, application of the B-IBI is very important, as is the case in Chile. Other disadvantages include widespread ignorance about the life histories of many species. Furthermore, it is more difficult for the general public to feel connected to index results based on macroinvertebrates. Finally Karr and Chu (1997) reported that B-IBI requires a large number of samples and multiple metrics to correctly establish the biological condition of a river.

Chile: a case study

Mediterranean-climate ecosystems are priority areas of conservation efforts; however, these ecosystems remain threatened globally due to environment degradation (García and Cuttelod., 2013; Myers et al., 2000). Of the five regions worldwide that present this climate, Chile is the least studied in regards to aquatic ecology (Gasith and Resh, 1999). This is despite reporting high national endemism and being considered among the 34 biodiversity hotspots in the world (Myers, 2003; Myers et al., 2000).

The Mediterranean-climate ecosystem basins of Chile are host to significant industrial activities. This constitutes an increasing problem for aquatic ecosystems due to severe site degradations. Of the various human activities that threaten this region, land use and land cover conversion are highly ranked (Aguayo et al., 2009). Indeed, while many activities directly or indirectly influence aquatic ecosystems, land use is the principal determinant of water quality and of water quantity entering aquatic ecosystems (Cuevas et al., 2014). Furthermore, land cover conversions for crop production or monoculture plantations directly affect freshwater fauna, decreasing, for example, aquatic insect densities and possibly inducing local extinction (Fierro et al., 2015).

In Chile, the use of bioindicators to assess water quality is limited, with applications focused on benthic macroinvertebrate assemblages through a modified Hilsenhoff Biotic Index (e.g., Fierro et al., 2012; Figueroa et al., 2003; Figueroa et al., 2005). Notably, these studies were conducted only as a part of basic scientific research as no regulations or laws in Chile stipulate the use of biological criteria for measuring water quality. In contrast, bioindicators are widely used in other countries for assessing and monitoring water quality, often times to meet governmental regulations. In the United States, for example, the Environmental Protection Agency established the "Use of Biological Assessments and Criteria in the Water Quality Program" (EPA, 1991), while the European Environment Agency has used biomarker-based monitoring in a number of countries (e.g., Austria 1968 and United Kingdom 1970; EEA, 2016).

Effects of agricultural land use on aquatic ecosystems

Agricultural land use can increase the delivery of several compounds, such as phosphorous and nitrogen, to fluvial ecosystems. In turn, this can produce eutrophication and, consequently, limit the presence of some macroinvertebrate and fish species. For example, when 22 streams were sampled across five Mediterranean-climate watersheds in the farming, central-south region of Chile, agricultural land use was found to be an important predictor of both macroinvertebrate and fish assemblages. Specifically, significant differences in the composition of macroinvertebrate (*Figure_4*; ANOSIM $r = 0.203$ $P = 0.01$) and fish (*Figure_5*; ANOSIM $r = 0.563$ $P = 0.01$) assemblages between land use types were found. In addition, taxonomic diversity of macroinvertebrates were higher in native streams than agricultural streams (Average Shannon-Wiener index in Native streams: 1.5, Agricultural streams: 1.1).

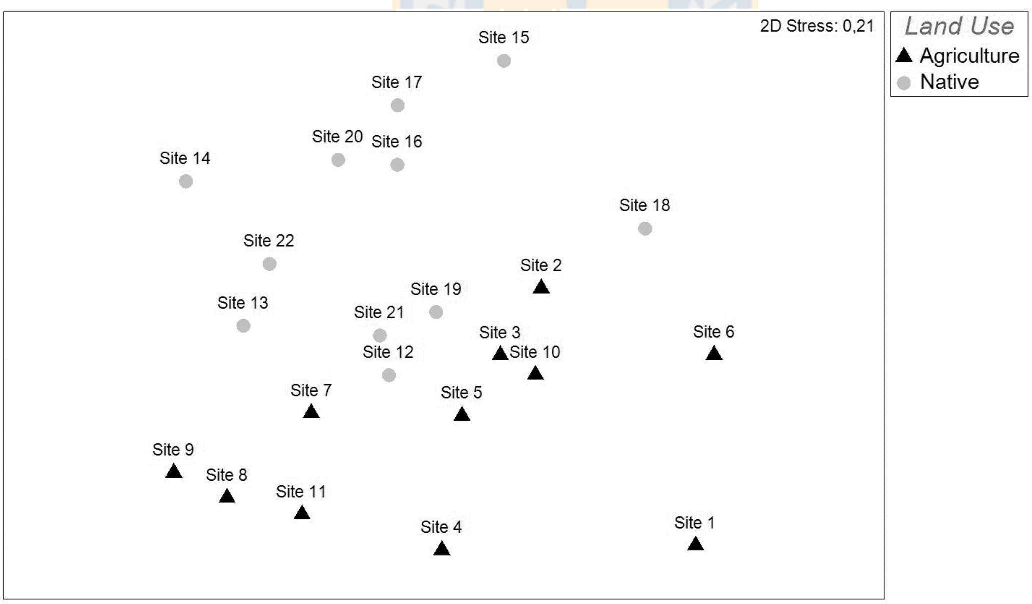

Figure 4. nMDS plot based on the composition of macroinvertebrates in 11 native streams and 11 agriculture streams in Mediterranean-climate ecosystems in the farming, central-south region of

Chile. The data matrix was constructed using the Bray-Curtis Similarity Index with the square-root transformation of data (9999 restarts). Axes are relative scales and therefore appear without legends (personal data P. Fierro).

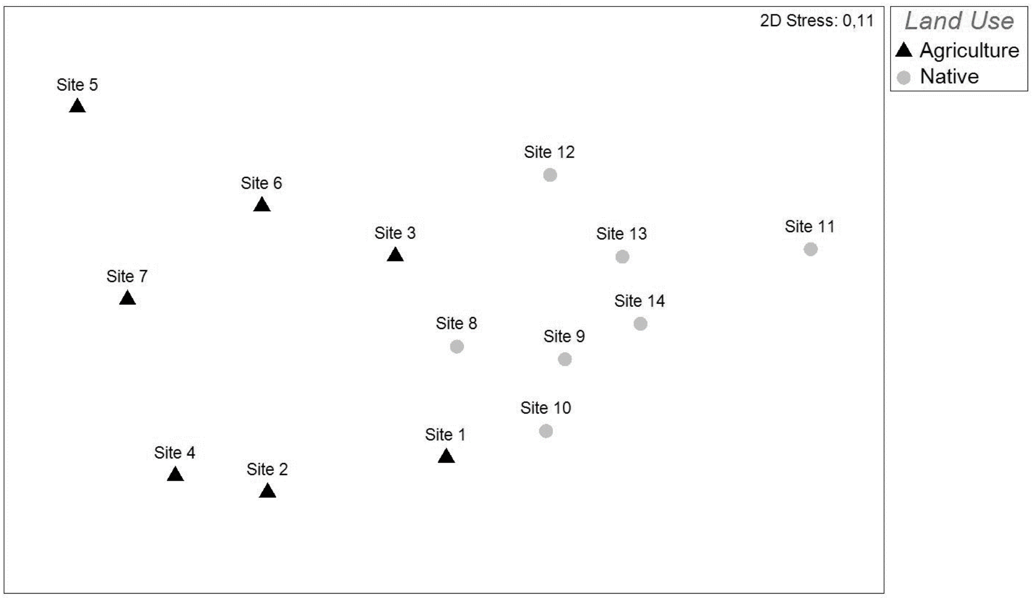

Figure 5. nMDS plot based on the composition of fish in seven native streams and seven agriculture streams in Mediterranean-climate ecosystems in the farming, central-south region of Chile. The data matrix was constructed using the Bray-Curtis Similarity Index with the square-root transformation of data (9999 restarts). Axes are relative scales and therefore appear without legends (personal data P. Fierro).

The principal difference in both assemblages was community heterogeneity, where native streams were constituted by greater abundances of Ephemeroptera larvaes and presented Plecoptera larvaes, while in agriculture streams, Diptera larvaes and gastropods were more abundant (*Figure_6*). Regarding fish assemblages, a higher amount of taxa were recorded in native streams, and included exotic trout (e.g., *O. mykiss* and *S. trutta*; *Table_2*). These species are unique to environments with low temperatures and high oxygen content, indicators of good water quality. In contrast, the catfish *Trichomycterus areolatus* (*Figure_7*) was recorded at all native and agriculture sites, supporting the broad environmental tolerance of catfish species in general (Habit et al., 2005).

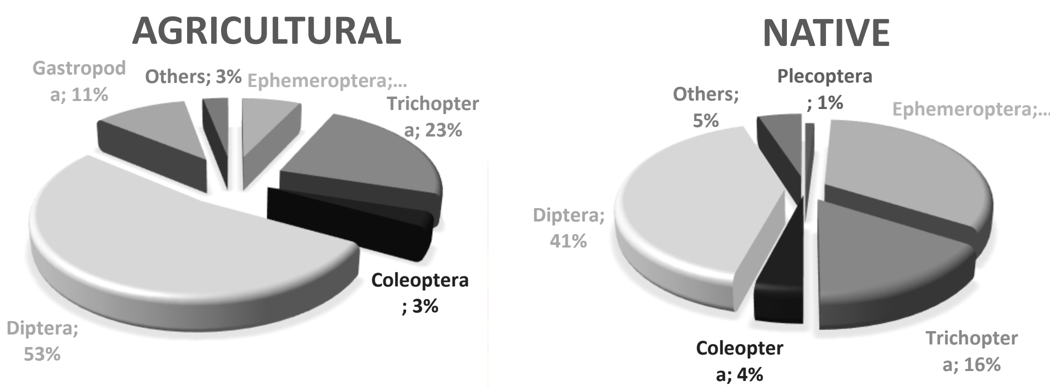

Figure 6. Macroinvertebrate classes found in agricultural dominated and reference streams (N= 22) (Unpublished data P. Fierro).

	Agriculture	Native
Diplomystes nahuelbutensis	0%	4,4%
Trichomycterus areolatus	20,9%	34,1%
Brachygalaxias bullocki	0,2%	0%
Cheirodon galusdae	3,5%	0,6%
Percilia gillisi	20,4%	28,7%
Basilicthys microlepidotus	0%	1,6%
Percichthys trucha	3,2%	0,8%
Gambusia holbrooki *	50,3%	0%
Cnesterodon decemmaculatus *	0,1%	0%
*Oncorhynchus mykiss**	1,8%	26,7%
*Salmo trutta**	0%	3,2%
*Cyprinus carpio**	0,5%	0%

Table 2. Species richness and relative abundances of fish species in agriculture and native streams the farming, central-south region of Chile. * Exotic species (Unpublished data P. Fierro).

Figure 7. Left: Catfish, *Trichomycterus areolatus*, Siluriformes, 9 cm in total length. Center: *Andesiops torrens*, Ephemeroptera, 0.5 cm in total length. Right: *Antarctoperla michaelseni*, Plecoptera, 0.8 cm in total length. All individuals were collected from streams in the farming, central-south region of Chile (Photographs by P. Fierro).

Conclusion

Macroinvertebrates and fish are used to evaluate the health of streams worldwide. The case results presented in this chapter evidence the importance of using one or more taxonomic groups in bioassessments, where both evaluated assemblages efficiently responded to pressures of human agricultural activities. These results suggest that macroinvertebrates and fish can be used as indicators of water pollution in monitoring programs. Using both assemblages as bioindicators presents several methodological advantages as compared to only assessing physicochemical parameters. These include low costs, easily identifiable fish, and, principally, the sensitivity of both assemblages to different stressors. For example, macroinvertebrates responded differently to substrate compositions than fish, which, in turn, responded to variables such as stream morphometry.

Rivers are increasingly affected by multiple physicochemical and biological stressors. Considering the ongoing rise in environmental management programs for aquatic communities, one related future goal is to develop appropriate indices, such as multimetric or biotic integrity indices, to differentiate between taxonomic groups, thereby facilitating assessments of stream health. However, the effectively of these indices will be highly dependent on applicability in different ecoregions.

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Capítulo 2: Anthropogenic threats to the Mediterranean freshwater ecosystem in Chile

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Assessing anthropogenic threats to the freshwater ecosystem: Review of the Chilean Mediterranean

Pablo Fierro[a,b,*], Claudio Valdovinos[a], Ivan Arismendi[c], Gustavo Díaz[a], Margarita Ruiz de Gamboa[d], Loretto Arriagada[a]

[a] *Center of Environmental Sciences EULA-CHILE, Universidad de Concepción, Chile*
[b] *Institute of Marine Science and Limnology, Universidad Austral de Chile, Chile*
[c] *Department of Fisheries and Wildlife, Oregon State University, Corvallis, USA*
[d] *Facultad de Recursos Naturales Renovables, Universidad Arturo Prat, Chile*

*Corresponding *E-mail*: pablofierror@gmail.com

Running head: Threats to freshwater ecosystems in Chile

Introduction

Threats to freshwater biodiversity can be grouped within five categories: natural resource overexploitation; water pollution; flow modification; habitat degradation or destruction; and invasion by exotic species (Dudgeon et al., 2006). This list has more recently expanded to include climate change (Thomas et al., 2004; Bellard, Bertelsmeier, Leadley, Thuiller, Courchamp, 2012). The reach of these threats is intensifying (Klausmeyer & Shaw, 2009) and, when coupled with a lack of local knowledge, determining and comparing impacts to aquatic ecosystems becomes an ever-more-complicated task. Considering the negative consequences of these threats to biodiversity, there is an urgent need to identify high-risk zones and to establish priority conservation areas. Such actions will ensure timely responses to human disturbances, thus improving habitats for species adaptation (Médail & Quézel, 1999). Adequate decision making on natural resource management requires a baseline of knowledge on specific systems. This information is crucial for determining the consequences of natural and anthropogenic forces on biodiversity loss. Ultimately, knowledgeable policy decisions not only aid in environmental preservation, but also in preventing irreversible environmental damage (Mayer-Pinto et al., 2015).

A number of methods exist for evaluating anthropogenic threats. Nevertheless, these methods frequently focus on single group of species or ecosystem (e.g. Whitfield, Ruddock, & Bullman, 2008; Cinner et al., 2013; Marr et al., 2013). This dispersed information can be synthesized by literature reviews, which can ultimately provide detailed and quantified information on threats. Literature reviews also serve to establish the current status of knowledge regarding a certain subject. This is in addition to often highlighting existing knowledge gaps, which can aid in directing future research. However, literature reviews do not necessarily extend to areas of less scientific interest, lending to taxonomic bias towards charismatic species and their systems (Halpern, Selkoe, Micheli, & Kappel, 2007). Additionally, literature reviews do not facilitate ranking which threats are of greater/lesser relevance to distinct ecosystems. At present, a number of methods have been developed and tested to establish the risks to which specific ecosystems are exposed. Among these methods, expert opinions are a good alternative for ranking ecosystem-specific threats and impacts (Kleypas & Eakin, 2007; Smith et al., 2015).

Expert opinions can be used to classify threats based on a combination of factors, including the degree, frequency, and functional impact of disturbances and the resistance and recovery time of ecosystems (Halpern, Selkoe, Micheli, & Kappel, 2007; Selkoe, Halpern, & Toonen, 2008). Indeed, surveys are an effective method for synthesizing expert opinions. The use of expert opinions can also record realities and complexities that monitoring programs many times are unable to establish (Hockings, 2003). Nevertheless, while opinion surveys of experts are a validated approach for evaluating threats, researchers may present inherent response biases that could result in misinterpretations (Halpern, Selkoe, Micheli, & Kappel, 2007). More specifically, responses might be biased towards the research areas or regions of focus respective to each investigator (Kleypas & Eakin, 2007). To mitigate this possible bias, opinions should ideally be collected from different members of the scientific community and from enough individuals to serve as a representative population of those working on the subject.

Another promising alternative to control for survey biases would be to identify the different threats reported in the literature for particular ecosystems (e.g., literature review) and subsequently have experts evaluate these threats (e.g., surveys). Identifying and evaluating threats is especially relevant for vulnerable ecosystems that have been subjected to a history of anthropogenic impacts. One of the few studies that has employed expert opinions to evaluate freshwater ecosystems concluded that 50 stressors affect the large lakes of the Northern Hemisphere, with invasion by mussels and climate change being the stressors with the greatest impact potential (Smith et al., 2015). Such approximations are often used by decision makers when addressing environmental issues. However, this is not the case when dealing with topics of conservation (Donlan, Wingfield, Crowder, & Wilcox, 2010).

The Mediterranean ecosystems of the world are located in central Chile, the Mediterranean basin, southwestern Africa, southern California, and southwestern/southern Australia. This ecosystem is characterized by strong seasonal rains and fluctuations in air temperature. Heavy rainfall and flooding typically occurs in winter, months that are notably humid and cold, whereas the summer is long and dry (Gasith & Resh, 1999; Bolle, 2003). These climatic patterns, together with unique geographies and geological histories, make the

Mediterranean ecosystem one of the most complex and rich globally in terms of biodiversity (Blondel, Aronson, Boudiou, & Boeuf, 2010). Although Mediterranean ecosystems are one of the most well-studied regions worldwide (De Figueroa, López-Rodríguez, Fenoglio, Sánchez-Castillo, & Fochetti, 2013), the Mediterranean Chilean Ecoregion (MCE) is the exception. Aquatic ecosystems in the MCE, in particular, have been comparably overlocked (Gasith & Resh, 1999; Figueroa et al., 2013).

Mediterranean ecosystems in general have been widely threated by human activities. Most of the research conducted on these systems focusses on terrestrial ecosystems. Such is the case in the EMC, where changes in land use include extensive farms and grasslands, as wells as incessant urban growth (Pauchard, Aguayo, Peña, & Urrutia, 2006; Hernández, Miranda, Arellano, & Dobss, 2016). Studies on aquatic ecosystems are more limited (Cooper, Sake, Sabater, Melack, & Sabo, 2013). The biotic integrity of rivers is affected by human activities, thus stressing the need for more data and knowledge towards understanding the organization and functioning of these habitats, as well as of respective key environmental factors (Gasith & Resh, 1999). Various studies have evaluated the effects that different human disturbances have on ecological communities within the MCE. However, this information has never been synthesized and processed. As such, the status of knowledge regarding threats to this region remains unknown.

The aim of this study was to determine the baseline of existing knowledge for threats to the MCE. In addition to a literature review, expert opinions were collected to rank threats and to identify knowledge gaps, information that can be used to direct future investigations. This combined approximation (i.e., literature review + expert surveys) represents a clear, detailed methodology applicable to other zones with a Mediterranean ecosystem, thus allowing for comparative, global analyses of threats, using the MCE as a case study. Such comparative assessments would be useful in directing new research on the causes for biodiversity loss in highly threatened systems, ultimately contributing towards the design and implementation of adequate management strategies for aquatic resources.

Methods

Study area

The MCE is recognized as one of the 34 diversity hotspots on the planet (Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000; Conservation International, 2007). The MCE is known for high endemism levels and low species richness, which has been shaped by natural geographical features – the Atacama Desert to the north, glaciers to the south, the Andes Mountain Range to the east, and the Pacific Ocean to the west (Vila & Habit, 2014). The MCE approximately extends from 25°S to 39°S. This area is known for its terrestrial and aquatic floral/faunal diversity (Cowling, Rundel, Lamont, Arroyo, & Arianoutsou, 1996). Regarding aquatic biodiversity, fish, macroinvertebrates, amphibian, and plant richness is highest near the southern limit of this Mediterranean region (Ramírez & San Martín, 2005; Habit, Dyer, & Vila, 2006; Valdovinos, 2006; Vidal, 2008). The MCE is host to 415 aquatic plant species, of which, 30% are native species (Ramírez & San Martin, 2006). Chile has a recorded 46 native fish species, with 21 existing in the MCE and 17 of these being endemic (Habit, Dyer, & Vila, 2006; Vila & Habit, 2014). For aquatic macroinvertebrates, nearly 1,000 species have been reported in Chile, with many of them endemic or, even, micro-endemic, i.e., restricted to only some streams (Valdovinos, 2006). Finally, of the 63 amphibian species present in Chile, 41 are endemic, and 43 inhabit the MCE (Arroyo et al., 2006; Correa, Donoso, & Ortiz, 2016; Vidal, 2008).

Literature review

To determine possible threats to aquatic Mediterranean ecosystems, a systematic literature review was performed. This review included literature in which aquatic macroinvertebrates, fish, amphibians, and aquatic plants were the primary groups under threat. A systematic search for publications was conducted using the Web of Science, Google Scholar, and Google. We used keywords that includes “threats,” “Chile,” “human disturbance,” and “Mediterranean,” both English and Spanish. Accepted publications included papers, books, book chapters, and guides dealing general subjects of the biota. Neither undergraduate/postgraduate theses nor species indices were included due to difficulties in verifying peer-reviewed publication elsewhere. Likewise, we excluded technical reports, such as local environmental impact studies and baseline reports due to the low reliability of their datasets. We recorded the listed threats and respectively threatened taxonomic groups from each revised publication.

Survey methods

We conducted expert surveys were conducted after completing the literature review and compiling the threat list. The interviewed individuals all had research experience or expertise in some ecological aspect(s) of the freshwater MCE and included academic scientists, governmental scientists, students (PhD or MSc), and other experts (e.g., environmental consultants). These experts were contacted at the 8th Annual Chilean Limnology Congress, at the 7th Chilean Amphibian and Reptile Congress, and through email between October 2016 and January 2017. Participants were informed of the purpose of the survey, the scope thereof, how to fill out the survey, and of attribute definitions (Table 1). Respondents generally completed the survey within one day. Participants recruited from the aforementioned congresses were personally given the survey, and emails containing clear instructions for survey completion were sent to each participant. Emails were also sent to increase the probability of receiving a response, thus increasing the total number of completed surveys. Any doubts that the participants had regarding survey completion were promptly clarified via email.

Participants were asked to evaluate each of the threats detected through the previously completed literature review (Table 1). To establish the relative vulnerability of each taxonomic group to each threat, the following five attributes were established according to Halpern, Selkoe, Micheli, & Kappel (2007) and Selkoe, Halpern, & Toonen (2008): (1) spatial scale on which the threat causes effects; (2) temporal frequency of the threat; (3) functional impact of the threat on groups (i.e., population, ensemble, community); (4) resistance of individuals to the threat, measured as the ability of individuals to return to a normal state following a disturbance; and (5) recovery time, measured as the time required by individuals to return to a natural state following a disturbance. Certainty was also established as an attribute to measure the degree of confidence in responses given by participants based on their experience. Certainty scores serve as an indicator for knowledge gaps, which is useful for determining the areas in which future research should be conducted (Selkoe, Halpern, & Toonen, 2008) (Table 1).

Data analysis

To establish a threat ranking, “scale” and “resistance” values were standardized between 0 and 4 (multiplied by 4/5 and 4/3, respectively). This standardization allowed all attributes to be compared. Vulnerability to each threat was established as the average of the five criteria.

The vulnerability scores of each taxonomic group to each threat were then averaged using the responded surveys (n = 46). To obtain a global vulnerability score of aquatic biodiversity to each threat, the values for each studied taxonomic group were averaged. This methodology resulted in a ranking of each threat by its respective vulnerability score, where higher vulnerability scores reflected greater threats for the Mediterranean ecosystem. The same procedure was applied to establish response certainty. To determine the higher-risk disturbances for the MCE, vulnerability scores were graphed with the degrees of response certainty. All of these methods were conducted following Halpern, Selkoe, Micheli, & Kappel (2007) and Selkoe, Halpern, & Toonen (2008).

Results and Discussion

Published literature

The 79 assessed publications reported on four taxonomic groups studied within the MCE (Appendix I, Literature Review). Specifically, 32 reported on threats to fish, 17 on benthonic macroinvertebrates, 18 on amphibians, and 8 on freshwater plants. Four publications studied two threatened taxonomic groups (i.e., fish-aquatic plants and fish-macroinvertebrates). These reports identified a total of 14 threats, which were categorized into the following five groups: exotic species, habitat loss and degradation, contamination, and climate change (Table 2). The following specific threats were reported: changes in land use (33 reports, 18%), the introduction of exotic species (28 reports, 16%), and contamination from industrial and residential effluents (23, 13%) (Table 2). Threats related to habitat loss and degradation were reported across all of the taxonomic groups.

Survey analysis

A total of 115 surveys were delivered personally or via email. Forty-six were completed, corresponding to a 40% response rate. Among the participants, 57% were academic instructors, 7% were government scientists, 15% were post-graduate students, and 22% were classified as other, most of whom were professionals related to environmental consultancies. The distribution of responses obtained for each taxonomic group was 33% for amphibians, 30% for fish, 24% for macroinvertebrates, and 13% for aquatic plants.

Threat ranking

The three greatest threats recorded in the literature were changes in land use, mining, and urbanization (Fig. 1, Table 3). Fish were the most threatened taxonomic group, followed by amphibians, macroinvertebrates, and aquatic plants. For fish, the greatest threats were hydropower plants, urbanization, and industrial/residential effluents. For aquatic plants, the greatest threats were mining, changes in land use, and the introduction of exotic species. In turn, the greatest threats to amphibians were changes in land use, urbanization, and water extraction. Finally, the greatest threats to macroinvertebrates were industrial and domestic effluents, mining, and drought. Of note, illegal trade was the only item that ranked zero for aquatic plants, indicating a lack of threat for this taxonomic group.

Knowledge gaps

Certainly scores were used to determine the existing baseline of knowledge regarding aquatic Mediterranean ecosystems (Table 4). Illegal trade was the threat with the highest uncertainty, while the introduction of exotic species had the lowest uncertainty score. For the taxonomic groups, aquatic plants obtained the lowest uncertainty score (i.e., 1.2), whereas macroinvertebrates received the highest uncertainty score (i.e., 1.5), indicating a lack of knowledge on respective threats.

In turn, the highest baselines of knowledge existed for aquatic plants and the introduction of exotic species. The lowest baselines of knowledge were found for macroinvertebrates and illegal trade/fires, respectively (Table 4). In line with these findings, threats with the lowest baselines of knowledge matched to threats with fewer literature citations (Table 2). Similarly, the highest baseline of knowledge existed for the introduction of exotic species, one of the three most cited threats in the literature. These observations support a coherency between the conducted literature review and the administered surveys.

Threats to biodiversity in Mediterranean ecosystems

Most of the studied disturbances represent a high risk for the MCE. Changes in land use and the introduction of exotic species were ranked highly by the surveyed experts who, notably, showed a greater confidence in these replies. This resulted in high-risk rankings for the MCE (lower right corner, Fig. 2). In contrast, some disturbances, such as illegal trade and recreational fishing, were ranked as low-risk due to low threat and confidence scores (Fig. 2).

Habitat loss and degradation

Changes in land use were ranked first among threats by survey respondents. This threat was also the most reported in the literature, affecting all four taxonomic groups. Changes in land use are a primary threat to biodiversity globally (Cooper, Sake, Sabater, Melack, & Sabo, 2013), and this factor is a key determinant for the quality and quantity of water flow into freshwater systems (Cuevas et al., 2014).

Riparian vegetation influences aquatic ecosystems in a number of ways, such as controlling biochemical cycles, influencing the water quality and quantity (i.e., organic matter and sediments), regulating river temperatures, and acting as natural buffer in the land-water interface (Fierro et al., 2017; Poff et al., 2011; Romero, Cozano, Gangas, & Naulin, 2014). In addition to physical and chemical modifications, changes in land use directly affect the ecology of aquatic communities. In effect, riverside vegetation of headwaters is the principal source of energy and nutrients in trophic chains, providing food for various invertebrate taxa and shelter for fish. Consequently, a decrease in or the disappearance of this vegetation can alter trophic food webs or, even, trigger the loss of aquatic species (Fierro et al., 2015, 2016).

Studies on the MCE conclude that the greatest change in land use over recent decades has been the transformation of native vegetation into grasslands/scrublands, farms, and exotic tree plantations. Indeed, the land area occupied by native forests has decreased by a third within the last 20 years (Aguayo, Pauchard, Azócar, & Parra, 2009; Nahuelhual, Carmona, Lara, Echeverría, & González, 2012). In effect, the MCE is known worldwide for fruit exports (Retamales et al., 2014; Jara-Rojas, Guerra, Adasme-Berrios, Engler, & Valdés, 2015), and few control or management measures have been taken to protect terrestrial-aquatic biological communities. Research on land-use changes within the MCE have primarily focused on farming activities and exotic tree plantations, demonstrating how these practices have affected aquatic communities. In particular, species of sensitive fish and macroinvertebrates have suffered decline or disappearance (Figueroa, Palma, Ruiz, & Niell, 2007; Fierro, Valdovinos, Vargas-Chacoff, Bertrán, & Arismendi, 2017). Amphibians are similarly affected, with changes in land use possibly accounting for the population decline and near extinction of Darwin's frog (*Rhinoderma rufum*) (Cuevas, 2014).

Other noteworthy disturbances within this category are canal construction and gravel extraction, activities that principally affect the mid to lower parts of MCE basins. These disturbances can alter the geomorphology and hydrology river channels, which translates into low variability in habitat condition. For example, Zawiejska, Wyzga, & Radecki-Pawlik (2015) reported a greater quantity of fine sediment at and downriver from gravel extraction sites, which would be the result of fine particles from the riverbed being exposed to and dragged by the current, thus degrading the riverbed. This, in turn, negatively impacts freshwater fauna (Wyzga, Amirowicz, Radecki-Pawlik, & Zawiejska, 2009). This has serious implications on fish with benthonic habitats, such as the native catfish (*Trichomycterus areolatus*), a high-density species within the MCE. The presence of fish in a river can drastically decrease when spawning sites are impoverished or eliminated by a greater amount of sediment (Kondolf, 1997; Brown, Lyttle, & Brown, 1998; Wyzga, Amirowicz, Radecki-Pawlik, & Zawiejska, 2009). Furthermore, physical modifications to channels can alter invertebrate abundances and biomass by restricting breathing or movement due to high turbidity. River dredging can also impact functional food groups, the downstream food sources of which would be affected by an increase in inorganic material and decrease in organic matter (Brown, Lyttle, & Brown, 1998; Mori, Simčič, Lukančič, & Brancelj, 2011). The only study in the MCE regarding dredging was by Ortiz-Sandoval, Ortiz, Cifuentes, González, & Habit (2009), who recorded low fish diversity post-dredging. However, this metric recovered over time, evidencing the high resilience of fish to this disturbance.

Despite the expanse of Chile, most water extraction projects, such as hydropower plants, dams, and aqueducts, are concentrated within the MCE (Lacy, Meza, & Marquet, 2017). Hydropower plants alter the magnitude and intensity of seasonal flood pulses, while increasing daily pulses and, consequently, stress to aquatic organisms (Brittain & Saltveit, 1989). One of the main consequences of hydropower plants to aquatic ecosystems is a changed flow regime, which affects aquatic biota. In assessing the effects of a damn within the MCE, Moya, Valdovinos, & Olmos (2002) reported lower diversity in downstream versus upstream invertebrate assemblies. Likewise, fish downstream of hydropower plants present decreases in species richness and abundance as a result of channel fluctuations (Habit, Belk, & Parra, 2007; García, Jorde, Habit, Caamaño, & Parra, 2011).

Finally, limited research exists related to recreational activities in the MCE. While results of the present study categorized this disturbance as low-risk, some authors postulate that these activities could be responsible for amphibian population declines, as due to habitat alterations (Soto-Azat et al., 2013a). On a global scale, aquatic recreational activities negatively affect fish. Boating, for example, can lead to high larvae mortalities and changes in swimming behaviour (Wolter & Arlinghaus, 2003).

Exotic species

The introduction of invasive species is the second leading factor contributing to reduced biodiversity worldwide (Vitousek, D'Antonio, Loope, Rejmánek, & Westbrooks, 1997). Chile has a reported 128 invasive species across aquatic and terrestrial environments. Of these, 27 have been prioritized as a threat to biodiversity. The invasive species that directly influence freshwater biodiversity in the MCE are trout (e.g., *Oncorhynchus mykiss*, *Salmo trutta*), Didymo algae (*Didymosphenia geminata*), the red-eared slider (*Trachemys scripta elegans*), and the African clawed frog (*Xenopus laevi*).

Salmonidae are the most widely propagated fish family by humans. Chile currently has ten Salmonidae species, which were introduced to the country for a number of reasons, including recreation, aquaculture, as a biological control, and for ornamental purposes (Marr et al., 2010; Arismendi, Sanzana, & Soto, 2011; Arismendi et al., 2014; Vargas, Arismendi, Gomez-Uchida, 2015). Importantly, salmonids impact food chains through trophic interferences exerted on other native species. For example, amphibian larvae are hunted by salmonids (Veloso & Nuñez, 2003). In turn, galaxiids are native fishes affected by the trophic interference of salmonids, which further affect local fish through habitat overtaking and distribution changes (Habit, González, Ortiz-Sandoval, Elgueta, & Sobenes, 2015; Vargas, Arismendi, Larga, Millar, & Peredo, 2010; Vargas, Arismendi, & Gomez-Uchida, 2015). Research carried out in southern Chile, show that both habitat use (Penaluna, Arismendi, & Soto, 2009) and diets (Elgueta, González, Ruzzante, Walde, & Habit, 2013) of native fishes change when trout is present.

Didymo algae were first reported in the south of Chile during the 1960s. Since then, distribution of this alga has extended northwards to the southern limit of the MCE (Montecino et al., 2016). This algae reproduces in rivers with low nutrient levels (Rivera, Basualto, & Cruces, 2013), meaning that distribution in rivers of the MCE is primarily limited to the headwaters of Andean basins. Although no investigations have assessed the impacts of this species to aquatic communities within the MCE, global reports indicate that it may homogenizes the invertebrate community, increasing the density of chironomid and oligochaete worms (Kilroy, Larned, & Biggs, 2009). Most studies conducted in Chile predict the north expansion towards the MCE (Jaramillo, Osman, Caputo, & Cardenas, 2015; Montecino et al., 2016).

The African clawed frog and red-eared slider are serious threats for aquatic ecosystems. The diet of these species includes invertebrates, fish, and amphibians in various ontogenetic states. This dietary range is a constant cause of concern regarding fauna within the MCE (Lobo & Measey, 2002). Furthermore, this species is a recognized disease vector, including of Chytridiomycosis, a fungus associated with the decline of amphibian worldwide (Pounds et al., 2006). This fungus has already been detected in 18 amphibian species in Chile, and infection is a possible cause for the decline and disappearance of species such as *Rhinoderma rufum* and *R. darwinii* (Correa, Donoso, & Ortiz, 2016; Soto-Azat et al., 2013b).

Contamination

Contamination is a global issue that affects most ecosystems. This threat is particularly linked to developing countries, where expanded land use for residential, industrial, and agricultural ends has tended to increase in recent decades (Pauchard, Aguayo, Peña, & Urrutia, 2006; Azócar et al., 2007). Contamination sources can be either punctual or diffuse. One diffuse pollution source for aquatic systems is agricultural runoff. Contamination through runoff waters and infiltration is a noted issue in the south of Chile, with wastes typically include nutrients and pesticides that can cause the eutrophication of freshwater ecosystems (Alfaro & Salazar, 2005).

Most of the Chilean population, as well as all exported fruit species, are located within the MCE. While the potential contamination risks of farming activities in Chile are not well understood, some authors (Figueroa, Valdovinos, Araya, & Parra, 2003; Fierro et al., 2012), report a strong relationship between nutrient concentrations (i.e., phosphates and nitrates) and rivers located in proximity to farms/ranches. In other Mediterranean ecosystems, contamination resulting from agriculture can increase the biomass of algae and chlorophyll a (Von Schiller, Martí, Riera, Ribot, Marks, & Sabater, 2008).

The majority of urban centres in Chile are located within the MCE. Large and medium cities are impacting biodiversity in both the urban and suburban areas. This has been demonstrated through a decrease in bird species within cities, as well as by the replacement of native plant species with exotic species (Pauchard, Aguayo, Peña, & Urrutia, 2006). Furthermore, urban areas within the MCE contribute towards the overall concentration increase of total solids in rivers, probably as derived from wastewaters and agro-industrial activities (Pizarro, Vergara, Morales, Rodríguez, & Vila, 2014). The chemical contamination of the MCE has altered the gonads of introduced amphibians, serving as the first indicator that these contaminants could alter reproductive processes for native amphibian species (Larenas et al., 2014; Correa, Donoso, Ortiz, 2016).

Mining significantly affects freshwater communities worldwide. Most threats to aquatic ecosystems and biota arise as a result of modified water quality. For example, fish inhabiting rivers proximal to mining activities in France have high hepatic and muscle concentrations of metals (Monna et al., 2011). Macroinvertebrate assemblies are similarly affected, with impacts including a reduction in species diversity and increase in the dominance of diptera (Smolders, Lock, Van der Velde, Medina Hoyos, & Roelofs, 2003). Examining rivers in Bolivia, Moya, Hughes, Domínguez, Gibon, Goitia, & Oberdoff (2011) arrived at the conclusion that mining negatively affects macroinvertebrate assemblies to a greater degree that in rivers impacted by cities and agriculture. In one of the few studies conducted within the EMC, Alvial, Orth, Durán, Álvarez, & Squeo (2013) reported low macroinvertebrate diversity and density in rivers exposed to naturally high metal concentrations.

Overexploitation

Recreational fishing and illegal trade were ranked as low-risk activities. Recreational fishing, defined as fishing for pleasure, exists worldwide as an economical and, for some countries, culturally important activity (Hughes, 2015). Recreational fishing in Chile is regulated by laws that simultaneously encourage this activity while conserving hydrobiological species and protecting the ecosystem. Although these laws restrict the capture of most native fish species as a means of protection, the capture of introduced species is not well-regulated. In the "*Exotic species*" subsection, discussion has been given on the dangers of introduced species. However, the eradication of these species in Chile is still a long way off, particularly when considering that some species, such as the rainbow trout (*O. mykiss*) exist across the entire MCE. The only regulation on salmonid fishing is a three-fish daily limit per individual. Furthermore, capture is not allowed during the reproductive months of these species. Recreational fishing within the MCE is focussed on salmonids, which, while typically captured using lures, are sometimes baited using live organisms, such as crabs and native fish. Some native fish, such as silverside (Atherinidae) and puyes (Galaxiidae) are also consumed by humans, particularly in rural areas. Measures to protect native species within the MCE have been taken by the Chilean government, including fines for recreational fishermen. Nevertheless, it remains unknown if the fishing of native species continues to affect freshwater communities.

Illegal trade within the MCE has historically centred on terrestrial vertebrates (Iriarte, Feinsinger, & Jaksic, 1997). However, modern-day illegal trade is drastically reduced as a result of governmental regulations. A freshwater species affected by illegal trade was Darwin's frog, which was extracted for illegal sale in the United States and Europe (Soto-

Azat et al., 2013a). The Chilean frog (*Calyptocephalella gayi*) continues to suffer illegal extraction for human consumption (Veloso & Nuñez, 2013).

Climate change

According to climate change models, the MCE will undergo widespread, extensive periods of drought in the summer. This will mean insufficient water flow, while rainfall will be concentrated within a few months (Garreaud, 2011). The greatest negative climate impacts will be felt by the northern zone of Chile and in the MCE (Henriquez, Aspee, & Quense, 2016). Research on climate change within the MCE has focused primarily on amphibians, due to the contributions that droughts and fires have already had in the extinction of local populations.

Fire is one of the greatest consumers of vegetation worldwide, making this force of nature a key factor in landscape formation. The MCE has suffered a sustained increase in forest fires within recent decades, with 99% of these fires caused by humans (González, Lara, Urrutia, & Bosnich, 2011). In 2014, for example, close to 1,240 forest fires were reported within the MCE. These fires primarily occur on the outer periphery of cities, at the urban-rural interface where a high concentration of vegetation exists (Atienza, Muños, & Balladares, 2012). The effects of drought and fire not only impact terrestrial landscapes, but can also negatively modify aquatic ecosystems. The absence of a tree canopy, for example, can mean wider thermal ranges, less relative humidity, and increased solar exposure. These consequences of tree loss can directly impact freshwater ecosystems by, for example, increasing water temperatures (Blackhall, Raffaele, & Veblen, 2015; Pedreros, Guevara-Mora, Urrutia, & Stehr, 2016). In reviewing the effects of fire on amphibians in North America, Pilliod, Bury, Hyde, Pearl, & Corn (2003) highlighted that this disturbance can affect the different life stages of amphibians, whether in the aquatic, riverside, or terrestrial environment. Climate change, expressed as an increase in droughts or fires, is a strong desiccating force for aquatic environments of the MCE, resulting in the death of sensitive aquatic species (Acuña-O, Vélez-R, Mizobe, Bustos-López, & Contreras-López, 2014; Vidal, Novoa-Muñoz, Werner, Torres, & Nova, 2017).

Conclusions

Mediterranean ecosystems worldwide are under notable threat due to high rates of destruction and degradation caused by anthropogenic activities (Garcia & Cuttelod, 2013). This report provides the first synthesis of published information regarding anthropogenic threats to the Mediterranean Chile Ecosystem. This information was complemented by a systematic ranking of threats by experts. The literature review identified 14 threats to the MCE, and these were then assessed through 46 surveys administered to area experts from distinct public/private institutions. Survey results were used to rank the risk presented by each threat to the MCE. Both approaches coincided on the primary threats to the MCE, with changes in land use ranked as the greatest threat. This disturbance obtained the highest confidence scores among the surveyed experts and was also the threat most mentioned in the literature. Other primary threats to ecosystem structure and functioning were mining, urbanization, droughts, industrial/residential effluents, and hydropower plants. All of the threats cited in the literature for the MCE have been reported in other Mediterranean ecosystems as threats to freshwater biodiversity (Cooper, Sake, Sabater, Melack, & Sabo, 2013; Cuttelod, Garcia, Malak, Temple, & Katariya, 2008; Underwood, Viers, Klausmeyer, Cox, & Shaw, 2009). Threat risks were generally comparable among the studied taxonomic groups, and, as such, future research should similarly review threats in other aquatic and terrestrial groups.

Compared to other aquatic ecosystems worldwide, fauna within the EMC is species-poor. However, these species are highly valuable in the contexts of biogeography and conservation. The threats to which these species are exposed currently mean that most freshwater flora and fauna have a "vulnerable" conservation status, while other species even rank as "endangered" or "critically endangered". Future public policies should focus on mitigating the high-risk threats catalogued by this study. Additionally, as more information is collected, adequate policies for the continued management of aquatic resources can be designed and implemented.

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Tables and Figures

Table 1. System used to rank each evaluated threat. Adapted from Halpern, Selkoe, Micheli, & Kappel (2007) and Selkoe, Halpern, & Toonen (2008).

Value	Scale	Frequency	Functional impact	Resistance	Recovery time	Confidence
0	No impact	No impact	No impact	No impact	No impact	Very high
1	< 100 m²	Rare	Species	High	< 1 year	High
2	100 m² - 1 km²	Occasional	Single trophic group	Moderate	1-10 years	Medium
3	1 km² - 10 km²	Regular	Multiple trophic groups	Weak	10-100 years	Low
4	10 km² - 1000 km²	Constant	Entire community, even habitat		> 100 years	
5	> 1000 km²					

Table 2. Threats reported in the literature for fish, macroinvertebrates, amphibians, and aquatic plants within the Mediterranean Chile Ecosystem. Shown are the number of publications (N) and relative abundances (%) for each respective threat.

Category	Threat	N	%
Exotic species	Introduction of exotic species	28	16
Habitat loss and degradation	Land use change	33	18
	Water extraction	16	9
	Dredged and canalized streams	18	10
	Hydropower plant	12	7
	Urbanization	14	8
	Ecotourism and recreation	4	2
Contamination	Industrial and domestic effluents	23	13
	Nutrients by agricultural activities	8	4
	Mining	4	2
Overexploitation	Sport fishing	3	2
	Illegal trade	4	2
Climate change	Fire	2	1
	Drought	10	6

Table 3. Threat scores by taxonomic group. Changes in land use included farming, deforestation, forest plantations, and pastures. The mean values for each threat are bolded, and the top three threats within each taxonomic group are shaded in grey.

	Fish	Aquatic Plants	Amphibians	Macroinvertebrates	***Mean***
Land use change	3.01	2.92	3.53	2.84	**3.08**
Mining	3.02	3.04	2.91	3.19	**3.04**
Urbanization	3.13	2.51	3.22	3.05	**2.98**
Drought	2.94	2.66	3.04	3.18	**2.95**
Industrial and domestic effluents	3.15	2.48	2.83	3.27	**2.93**
Hydropower plant	3.17	2.20	3.00	3.16	**2.88**
Water extraction	3.01	2.49	3.16	2.69	**2.84**
Introduction of exotic species	3.09	2.85	2.51	2.76	**2.80**
Nutrients by agricultural activities	2.99	2.43	2.70	2.94	**2.76**
Dredged and canalized streams	2.82	2.61	2.72	2.83	**2.75**
Fire	2.44	1.58	2.83	2.31	**2.29**
Ecotourism and recreation	2.40	2.46	1.77	1.98	**2.15**
Sport fishing	2.10	1.23	1.20	1.59	**1.53**
Illegal trade	1.85	0.00	1.95	1.07	**1.22**
Mean	**2.79**	**2.25**	**2.67**	**2.63**	

Table 4. Confidence scores by taxonomic group.

	Fish	Aquatic Plants	Amphibians	Macroinvertebrates	***Mean***
Land-use change	1.3	0.7	1.0	1.0	**1.0**
Urbanization	1.4	1.5	1.4	1.4	**1.4**
Water extraction	1.3	1.0	1.5	1.5	**1.3**
Mining	1.6	1.4	1.4	1.5	**1.5**
Industrial/residential effluents	1.2	1.0	1.5	1.5	**1.3**
Drought	1.6	1.2	1.2	1.2	**1.3**
Hydroelectric plant	1.1	1.0	1.3	1.3	**1.2**
Agricultural runoff	1.5	1.0	1.6	1.4	**1.4**
Dredged and channelized streams	1.3	1.0	1.3	1.7	**1.3**
Illegal trade	1.8	1.7	1.6	2.4	**1.9**
Introduction of exotic species	0.9	0.0	1.2	1.2	**0.8**
Fire	1.8	1.7	1.3	2.3	**1.8**
Recreational fishing	1.6	1.7	1.9	1.4	**1.6**
Ecotourism and recreation	1.8	1.7	1.5	1.1	**1.5**
Mean	**1.4**	**1.2**	**1.4**	**1.5**	

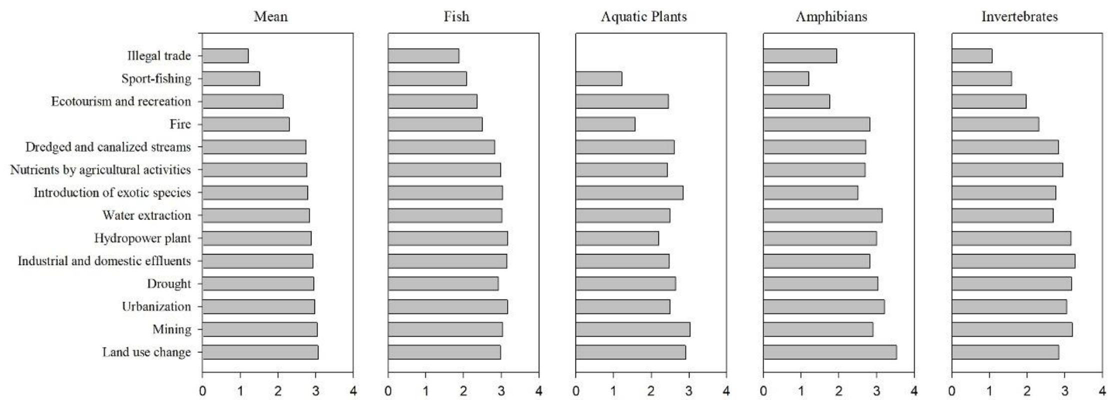

Figure 1. Ranking of threats to each taxonomic group. Provided is a visual synthesis of the information provided in Table 3.

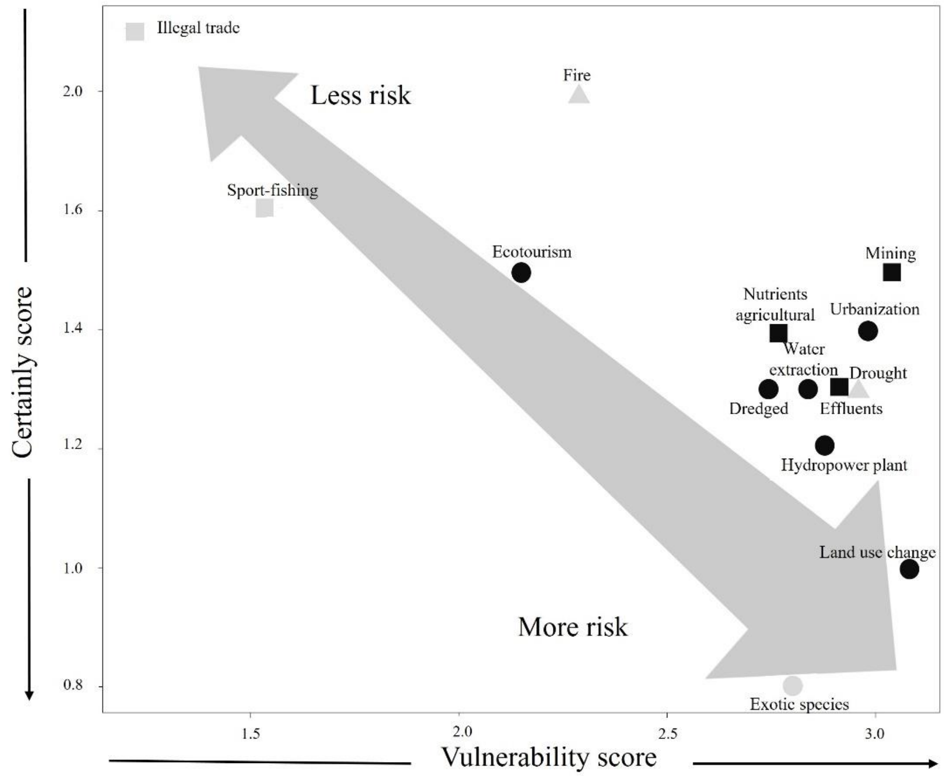

Figure 2. Risk assessment of threats to aquatic ecosystem within the Mediterranen Chile Ecosystem. From left to right, the doublé-arrow indicates threats rankings from less risk to more risk. Black circles = habitat loss and degradation; Black squares = contamination; Grey squares = overexplotation; Grey triangles = climatic change; Grey circles = introduction of exotic species.

Appendice

Appendix I. Literature review of 79 scientific articles related to the Mediterranean Chile Ecosystem. Abbreviations: ES, Introduction of exotic species; LC, Land-use change; WE, Water extraction and dams; DC, Dredged and channelized streams; HP, Hydropower plant; U, Urbanization; ER, Ecotourism and recreation; IDE, Industrial and domestic effluents; NA, Nutrients by agricultural activities; M: Mining; SF: Sport-fishing; IT: Illegal trade; F: Fire; D: Drought.

Acuña, P., Vila, I., Pardo, R., & Comte, S. (2005). Caracterización espacio-temporal del nicho trófico de la fauna ictica andina del río Maule. Chile. *Gayana*, 69, 175-179.
Assemblage: Fish; Threats: ES.

Acuña-O, P.L., Vélez-R, C.M., Mizobe, C.E., Bustos-López, C., & Contreras-López, M. (2014). Mortalidad de la población de rana grande chilena, *Calyptocephalella gayi* (Calyptocephalellidae), en la laguna Matanzas, del humedal El Yali, en Chile central. *Anales del Museo de Historia Natural de Valparaíso*, 27, 35-50.
Assemblage: Amphibians; Threats: DC, D.

Arancibia, J., & Araya, M.P. (2014). Diversidad, abundancia y distribución de la flora vascular del estero de Viña del mar, región de Valparaiso, Chile. *Anales Museo de Historia Natural de* Valparaiso, 27, 15-27.
Assemblage: Aquatic plants; Threats: ES, DC, IDE, NA.

Aranda, J., Muñoz, J.V., & Olivares, H.G. (2014). Valoración del ecosistema estero Limache, Región de Valparaíso (Chile central), mediante la aplicación del índice de funcionalidad fluvial. *Anales Museo de Historia Natural de* Valparaíso, 27, 7-14.
Assemblage: Macroinvertebrates; Threats: LC, WE, IDE.

Arenas, J. (1995). Composición y distribución del macrozoobentos del curso principal del río Biobío, Chile. *Medio Ambiente*, 12, 39-50.
Assemblage: Macroinvertebrates; Threats: LC.

Arratia, G. (1978). Comentario sobre la introducción de peces exóticos en aguas continentales de Chile. *Ciencias Forestales*, 1, 21-30.
Assemblage: Fish; Threats: ES.

Campos, H. (1970). Introducción de especies exóticas y su relación con los peces de agua dulce de Chile. *Museo Nacional de Historia Natural, Noticiario Mensual*, 162, 3-9.

Assemblage: Fish; Threats: ES.

Campos, H., Ruiz, V.H., Gavilán, J.F., & Alay, F. (1993). Peces del Río Bío-Bío. Serie Publicaciones de Divulgación EULA, Universidad de Concepción.
Assemblage: Fish; Threats: ES, HP.

Charrier, A., Correa-Quezada, C.L., Castro, C., & Mendes-Torres, M.A. (2015). A new species of (Anura: Alsodidae) from Altos de Cantillana, central Chile. *Zootaxa*, 3915, 540-550.
Assemblage: Amphibians; Threats: LC, M, F.

Chiang, G., Munkittrick, K., Saavedra, F., Tucca, F., McMaster, M., Urrutia, R., … Barra, R. (2011). Seasonal changes in reproductive endpoints in *Trichomycterus areolatus* (Siluriformes: Trichomycteridae) and *Percilia gillissi* (Perciformes, Perciliidae), and the consequences for environmental monitoring. *Studies on Neotropical Fauna and Environment*, 46, 185-196.
Assemblage: Fish; Threats: IDE.

Chiang, G., McMaster, M., Urrutia, R., Saavedra, F., Gavilán, J.F., Tucca, F., … Munkittrick, K.R. (2011). Health status of native fish (*Percilia gillissi* and *Trichomycterus areolatus*) downstream of the discharge of effluent from a tertiary-treated elemental chorine-free pulp mill in Chile. *Environmental Toxicology Chemistry*, 30, 1793-1809.
Assemblage: Fish; Threats: IDE.

Chiang, G., Munkittrick, K., McMaster, M., Barra, R., & Servos, M. (2014). Regional Cumulative Effects Monitoring Framework: Gaps and Challenges for the Biobío River Basin in South Central Chile. *Gayana*, 78, 109-119.
Assemblage: Fish, Aquatic plants; Threats: WE, HP, IDE.

Copaja, S.V., Muñoz, G.S., Nuñez, V.R., Pérez, C., Vila, I., & Véliz, D. (2016). Effects of a dam reservoir on the distribution of heavy metals in two Chilean native freshwater fish species. *Bulletin of Environmental Contamination and* Toxicology, 97, 24-30.
Assemblage: Fish; Threats: WE, M.

Córdova, S., Gaete, H., Aránguiz, F., & Figueroa, R. (2009). Evaluación de la calidad de las aguas del estero Limache (Chile central), mediante bioindicadores y bioensayos. *Latin American Journal of Aquatic* Research, 37, 199-209.
Assemblage: Macroinvertebrates; Threats: LC, WE, IDE.

Correa, C., Donoso, J.P., & Ortiz, J.C. (2016). Estado de conocimiento y conservación de los anfibios de Chile: una síntesis de los últimos 10 años de investigación. *Gayana*, 80, 103-124.
Assemblage: Amphibians; Threats: LC, D.

Cuevas, C.C. (2014). Native forest loss impact's on anuran diversity: with focus on *Rhinoderma rufum* (Philippi 1902) (Rhinodermatidae) in coast range, south-central Chile. *Gestión Ambiental*, 27, 1-18.

Assemblage: Amphibians; Threats: LC.

Dyer, B. (2000). Systematic review and biogeography of the freshwater fishes of Chile. *Estudios Oceanológicos*, 19, 77-98.
Assemblage: Fish; Threats: ES.

Fierro, P., Valdovinos, C., Vargas-Chacoff, L., Bertrán, C., & Arismendi, I. (2017). Macroinvertebrates and Fishes as Bioindicators of Stream Water Pollution. In *Water Pollution*, Tutu, H. (ed). Intechopen, 23-38.
Assemblage: Fish, Macroinvertebrates; Threats: LC.

Figueroa, R., Palma, A., Ruiz, V., & Niell, X. (2007). Análisis comparativo de índices bióticos utilizados en la evaluación de la calidad de las aguas en un río mediterráneo de Chile: río Chillán, VIII Región. *Revista Chilena de Historia Natural*, 80, 225-242.
Assemblage: Macroinvertebrates; Threats: LC, IDE, NA.

Figueroa, R., Suarez, M.L., Andreu, A., Ruiz, V.H., & Vidal-Abarca, M.R. (2009). Caracterización ecológica de humedales de la zona semiárida en Chile Central. *Gayana*, 73, 76-94.
Assemblage: Macroinvertebrates; Threats: LC, DC, U, IDE, IT.

Figueroa, R., Ruiz, V.H., Berrios, P., Palma, A., Villegas, P., & Andreu-Soler, A. (2010). Trophic ecology of native and introduced fish species from the Chillán River, South-Central Chile. *Journal of Applied Ichthyology*, 26, 78-83.
Assemblage: Fish; Threats: ES.

Figueroa, R., Bonada, N., Guevara, M., Pedreros, P., Correa-Araneda, F., Díaz, M.E., & Ruiz, V.H. (2013). Freshwater biodiversity and conservation in mediterranean climate streams of Chile. *Hydrobiologia*, 719, 269-289.
Assemblage: Macroinvertebrates; Threats: LC, HP, IDE.

García, A., Jorde, K., Habit, E., Caamaño, D., & Parra, O. (2011). Downstream environmental effects of dam operations: changes in habitat quality for native fish species. *River Research and Applications*, 27, 312-327.
Assemblage: Fish; Threats: HP.

Garin, C., & Lobos, G. (2008). Generalidades sobre anfibios y reptiles. In *Herpetología de Chile*, Vidal M, & Labra A. (eds). Science Verlag, 51-75.
Assemblage: Amphibians; Threats: ES, LC.

Garin, C.F., & Hussein, Y. (2013). Guía de reconocimiento de anfibios y reptiles de la region de Valparaiso. Espinoza, A., & Benavides, D. (eds). Servicio Agricola y Ganaero (SAG).
Assemblage: Amphibians; Threats: ES, U, IDE, IT, D.

Goodwin, P., Jorde, K., Meier, C., & Parra, O. (2006). Minimizing environmental impacts of hydropower development: transferring lessons from past projects to a proposed strategy for Chile. *Journal of Hydroinformatics*, 8, 253-270.

Assemblage: Fish; Threats: HP.

Habit, E., Bertrán, C., Arévalo, S., & Victoriano, P. (1998). Benthonic fauna of the Itata river and irrigation canals (Chile). *Irrigation Science*, 18, 91-99.
Assemblage: Macroinvertebrates; Threats: WE.

Habit, E., & Parra, O. (2001). Impacto ambiental de los canales de riego sobre la fauna de peces. *Ambiente y Desarrollo*, 17, 50-58.
Assemblage: Fish; Threats: DC.

Habit, E., Gonzalez, S., & Victoriano, P. (2002). Alcances sobre el uso sustentable de la ictiofauna de sistemas fluviales. *Theoria*, 11, 15-20.
Assemblage: Fish; Threats: ER.

Habit, E., Victoriano, P., & Rodríguez-Ruiz, A. (2003). Variaciones espacio-temporales del ensamble de peces de un sistema fluvial de bajo orden del centro-sur de Chile. *Revista Chilena de Historia Natural*, 76, 3-14.
Assemblage: Fish; Threats: LC, U.

Habit, E., Parra, O., & Valdovinos, C. (2005). Ictiofauna de un sistema fluvial receptor de aguas servidas: respuestas a una nueva planta de tratamiento (río Quilque, Chile Central). *Gayana*, 69, 94-103.
Assemblage: Fish; Threats: IDE.

Habit, E., Belk, M.C., Cary Tuckfield, R., & Parra, O. (2006). Response of the fish community to human-induced changes in the Biobío River in Chile. *Freshwater Biology*, 51, 1-11.
Assemblage: Fish; Threats: HP, IDE.

Habit, E., Belk, M.C., & Parra, O. (2007). Response of the riverine fish community to the construction and operation of a diversion hydropower plant in central Chile. *Aquatic conservation: Marine and Freshwater Ecosystems*, 17, 37-49.
Assemblage: Fish; Threats: HP.

Habit, E., Piedra, P., Ruzzante, D.E., Walde, S.J., Belk, M.C, Cussac, V.E., … Colin, N. (2010). Changes in the distribution of native fishes in response to introduced species and other anthropogenic effects. *Global Ecology and Biogeography*, 19, 697-710.
Assemblage: Fish; Threats: ES.

Hauenstein, E., Muñoz-Pedreros, A., Yánez, J., Sánchez, P., Möller, P., Guiñez, B., & Gil, C. (2009). Flora y vegetación de la Reserva Nacional Lago Peñuelas, Reserva de la Biósfera, Región de Valparaíso, Chile. *Bosque*, 30, 159-179.
Assemblage: Aquatic plants; Threats: LC.

Ibarra-Vidal, I.L., Ortiz, J.C., & Torres-Pérez, F. (2004). *Eupsophus septentrionalis* n. sp., nueva especie de Leptodactylidae (Amphibia) de Chile central. *Boletin de la Sociedad de Biología de Concepcion*, 75, 91-102.
Assemblage: Amphibians; Threats: LC.

Iriarte, J.A., Lobos, G.A., & Jaksic, F.M. (2005). Invasive vertebrate species in Chile and their control and monitoring by governmental agencies. *Revista Chilena de Historia Natural*, 78, 143-154.
Assemblage: Fish; Threats: ES.

Jara, C.G., Rudolph, E.H., & González, E.R. (2006). Estado de conocimiento de los malacostráceos dulceacuícolas de Chile. *Gayana*, 70, 40-49.
Assemblage: Macroinvertebrates; Threats: LC, WE, DC, U, SF.

Jofré, C., & Méndez, M.A. (2011). The preservation of evolutionary value of Chilean amphibians in protected areas. In: Biodiversity Conservation in the Americas: Lessons and Policy Recommendations, Figueroa, E. (ed). Editorial FEN-Universidad de Chile, 81-112.
Assemblage: Amphibians; Threats: ES, LC, IDE, D.

Lacy, S.N., Meza, F.J, & Marquet, P.A. (2017). Can environmental impact assessments alone conserve freshwater fish biota? Review of the Chilean experience. *Environmental Impact Assessment Review*, 63, 87-94.
Assemblage: Fish; Threats: WE, DC, HP.

Lobos, G., Vidal, M., Correa, C., Labra, A., Díaz-Páez, H., Charrier, A., … Tala, C. (2013a). Anfibios de Chile, un desafío para la conservación. Ministerio del Medio Ambiente, Fundación Facultad de Ciencias Veterinarias y Pecuarias de la Universidad de Chile y Red Chilena de Herpetología, Santiago. 104 pp.
Assemblage: Amphibians; Threats: LC.

Lobos, G., Cattan, P., Estades, C., & Jaksic, F.M. (2013b). Invasive African clawed frog Xenopus laevis in southern South America: key factors and predictions. *Studies on Neotropical Fauna and Environment*, 48, 1-12.
Assemblage: Amphibians; Threats: ES.

Mancilla, G., Valdovinos, C., Azócar, M., Henriquez, M., & Figueroa, R. (2009). Aproximación multimétrica a la evaluación de la calidad del agua en cuencas con diferentes niveles de intervención antrópica. *Interciencia*, 34, 857-864.
Assemblage: Macroinvertebrates; Threats: LC, IDE.

Mao, L., Ugalde, F., Iroume, A., & Lacy, S.N. (2016). The effects of replacing forest on the quantity and impacts of in-channel pieces of large wood in Chilean streams. *River research and applications*, 33, 73-88.
Assemblage: Fish; Threats:LC.

Marr, S., Marchetti, M., Olden, J.D., Garcia-Berthou, E., Morgan, D., Arismendi, I., … Skelton, P. (2010). Freshwater fish introductions in mediterranean-climate regions: are there commonalities in the conservation problem? *Diversity and Distributions*, 16, 606-619.
Assemblage: Fish; Threats: ES, ER.

Mera, C.R., & Lobos, G.A. (2008). Anfibios y reptiles en el imaginario cultural de Chile. In *Herpetología de Chile*, Vidal, M.A., & Labra, A. (eds). Science Verlag, 55-76.
Assemblage: Amphibians; Threats: SP.

Mizobe, C.E., Contreras-López, M., Acuña-O, P.L., Vélez-R, C.M., & Bustos-López, C. (2014). Mortalidad masiva reciente de la rana grande chilena (*Calyptocephalella gayi*) en la Reserva Nacional El Yali. *Biodiversidata*, 2, 30-34.
Assemblage: Amphibians; Threats: DC, D.

Moya, C., Valdovinos, C., & Olmos, V. (2002). Efecto de un embalse sobre la deriva de macroinvertebrados en el río Bio-Bío (Chile central). *Boletín de la Sociedad de Biología de Concepción*, 73, 7-15.
Assemblage: Macroinvertebrates; Threats: WE.

Muñoz, E., Mendoza, G., & Valdovinos, C. (2001). Evaluación rápida de la biodiversidad en cinco sistemas lénticos de Chile central: macroinvertebrados bentónicos. *Gayana*, 65, 173-180.
Assemblage: Macroinvertebrates; Threats: LC.

Muñoz-Ramírez, C.P., Victoriano, P.F., & Habit, E. (2015). Inter-basin dispersal through irrigation canals explains low genetic structure in *Diplomystes cf. chilensis*, an endangered freshwater catfish from Central Chile. *Limnologica-Ecology and Management of Inland Waters*, 53, 10-16.
Assemblage: Fish; Threats: DC.

Ortiz, J.C., & Díaz-Páez, H. (2006). Estado de conocimiento de los anfibios de Chile. *Gayana*, 70, 114-121.
Assemblage: Amphibians; Threats: LC.

Ortiz-Sandoval, J.J., Ortiz, N., Cifuentes, R., González, J., & Habit, E. (2009). Respuesta de la comunidad de peces al dragado de ríos costeros de la región del BioBío (Chile). *Gayana*, 73, 64-75.
Assemblage: Fish; Threats: DC.

Orrego, R., Marshall Adams, S., Barra, R., Chiang, G., & Gavilan, J.F. (2009). Patterns of fish community composition along a river affected by agricultural and urban disturbance in south-central Chile. *Hydrobiologia*, 620, 35-46.
Assemblage: Fish; Threats: LC, U, IDE.

Palma, B., San Martín, C., Rosales, M., Zuñiga, L., & Ramírez, C. (1987). Distribución espacial de la flora y vegetación acuática y palustre del estero Marga - Marga en Chile Central. *Anales del Instituto de Ciencias del Mar y Limnología*, 14, 125- 132.
Assemblage: Aquatic plants; Threats: DC, IDE.

Palma, A., Figueroa, R., & Ruiz, V.H. (2009). Evaluación de ribera y hábitat fluvial a través de los índices QBR e IHF. *Gayana*, 73, 57-63.
Assemblage: Macroinvertebrates; Threats: LC, U.

Parada, E., & Peredo, S. (2005). La relocalización como una herramienta de conservación y manejo de la biodiversidad: Lecciones aprendidas con *Diplodon chilensis* (Gray 1828) (Bivalvia, Hyriidae). *Gayana*, 69, 41-47.
Assemblage: Macroinvertebrates; Threats: ES, WE, DC, U, NA.

Parada, E., & Peredo, S. (2006). Estado de conocimiento de los bivalvos dulceacuícolas de Chile. *Gayana*, 70, 82-87.
Assemblage: Macroinvertebrates; Threats: LC, U, NA.

Parra, O. (1989). La eutroficación de la Laguna Grande de San Pedro, Concepción, Chile: un caso de estudio. *Ambiente y Desarrollo*, 1, 117-136.
Assemblage: Aquatic plants; Threats: ES, U, IDE.

Parra, O., Valdovinos, C., Urrutia, R., Cisternas, M., Habit, E., & Mardones, M. (2003). Caracterización y tendencias tróficas de cinco lagos costeros de Chile central. *Limnetica*, 22, 51-83.
Assemblage: Fish, Macroinvertebrates; Threats: ES, LC, U, ER, IDE.

Ramírez, C., & San Martin, C. (2006). Flora Acuática. In *Biodiversidad de Chile: patrimonies y desafíos*, CONAMA (ed). CONAMA, 364-369.
Assemblage: Aquatic plants; Threats: ES.

Ramírez, C., Fariña, J.M., Contreras, D., Camaño, A., San Martín, C., Molina M, … Pérez Y. (2014). La diversidad florística del humedal" Ciénagas del Name" (región del Maule) comparada con otros humedales costeros de Chile. *Gayana Botánica*, 71, 108-119.
Assemblage: Aquatic plants; Threats: LC, WE, NA, D.

Ramírez, D. (2015). Nueva localidad para *Alsodes tumultuosus* Veloso, Iturra & Galleguillos, 1979 (Amphibia, Alsodidae) en la Cordillera de los Andes, Region de O'Higgins, Chile. *Biodiversity & Natural History*, 1, 1-5.
Assemblage: Amphibians; Threats: HP.

Rossel, V., & De la Fuente, A. (2015). Assessing the link between environmental flow, hydropeaking operation and water quality of reservoirs. *Ecological Engineering*, 85, 26-38.
Assemblage: Aquatic plants; Threats: WE.

Ruiz, V.H. (1993). Ictiofauna del río Andalien. *Gayana*, 57, 109-278.
Assemblage: Fish; Threats: ES, DC, U, IDE, NA, D.

Ruiz, V.H. (1996). Ictiofauna del río Lja (VIII Region, Chile: Una evaluación preliminar. *Boletín de la Sociedad de Biología de Concepción*, 67, 15-21.
Assemblage: Fish, Macroinvertebrates; Threats: WE.

Ruiz, V.H., Moyano, H., & Marchant, M. (1992). Aspectos biológicos del pez exótico *Cichlasoma facetum* (Jenys, 1842) (Pisces, Cichlidae) en aguas dulces de Concepción. *Boletin de la Sociedad de Biología de Concepción*, 63, 193-201.

Assemblage: Fish; Threats: ES.

Ruiz, V.H., & Marchant, M. (2004). Ictiofauna de Aguas Continentales Chilenas. Universidad de Concepción.
Assemblage: Fish; Threats: ES, DC, HP, IDE.

San Martín, C., Ramírez, C., San Martín, J., & Villaseñor, R. (2001). Flora y vegetación del estero Reñaca (V Región, Chile). *Gayana Botánica*, 58, 31-46.
Assemblage: Aquatic plants; Threats: DC, IDE.

Soto-Azat, C., Valenzuela-Sánchez, A., Ortiz, J.C., Díaz-Páez, H., Castro, C., Charrier, A., … Angulo, A. (2015). ASG Chile Leads Update of the Extinction Risk of Chilean Amphibians for the IUCN Red List of Threatened Species. *FrogLog*, 23, 6-7.
Assemblage: Amphibians; Threats: ES, LC, WE, U, M, F.

Soto-Azat, C., Valenzuela-Sánchez, A., Collen, B., Rowcliffe, J.M., Veloso, A., & Cunningham, A.A. (2013a). The population decline and extinction of Darwin's frogs. *PLoS ONE*, 8, e669567.
Assemblage: Amphibians; Threats: LC, U, ER, IT.

Uribe-Rivera, D.E., Soto-Azat, C., Valenzuela-Sánchez, A., Bizama, G., Simonetti, J.A., & Pliscoff, P. (2017). Dispersal and extrapolation on the accuracy of temporal predictions from distribution models for the Darwin's frog. *Ecological Applications*, 5, 1633-1645.
Assemblage: Amphibians; Threats: D.

Valdovinos, C. (2001). Riparian leaf litter processing by benthic macroinvertebrates in a woodland stream of central Chile. *Revista Chilena de Historia Natural*, 74, 445-453.
Assemblage: Macroinvertebrates; Threats: LC.

Valdovinos, C. (2006). Invertebrados dulceacuícolas, Diversidad de especies. In *Biodiversidad de Chile: Patrimonios y desafíos*, CONAMA (ed). CONAMA, 202-223.
Assemblage: Macroinvertebrates; Threats: ES, LC, WE, DC, HP, U, IDE, NA, M, IT, D.

Valdovinos, C., Moya, C., Olmos, V., Parra, O., Karrasch, B., & Buettner, O. (2007). The importance of water-level fluctuation for the conservation of shallow water benthic macroinvertebrates: an example in the Andean zone of Chile. *Biodiversity Conservation*, 16, 3095-3109.
Assemblage: Macroinvertebrates; Threats: WE.

Vidal, M.A., Novoa-Muñoz, F., Werner, E., Torres, C., & Nova, R. (2017). Modeling warming predicts a physiological threshold for the extinction of the living fossil frog *Calyptocephalella gayi*. *Journal of Thermal Biology*, 69, 110-117.
Assemblage: Amphibians; Threats: D.

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Capítulo 3: Impacts of anthropogenic disturbed-streams on macroinvertebrate, fish and periphyton assemblages

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Impacts of anthropogenic disturbed streams on macroinvertebrate, fish and periphyton assemblages

Pablo Fierro[1,2,*], Claudio Valdovinos[1], Ivan Arismendi[3], Gustavo Díaz[1], Alfonso Jara-Flores[4], Evelyn Habit[1] & Luis Vargas-Chacoff[2,*]

[1] Department of Aquatic Systems of Environmental Science and Center of Environmental Sciences EULA-CHILE, Universidad de Concepción, Chile.

[2] Institute of Marine Science and Limnology, Universidad Austral de Chile, Chile.

[3] Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR, 97330 USA.

[4] Department de Zoology, Universidad de Concepción, Chile.

[*] Corresponding author E-mail: pablofierror@gmail.com

Introduction

Streams are the ecosystems most threated around the world (Allan, 2004). The anthropogenic disturbances are affecting this ecosystems can be summarized in both local and landscape scale perturbations, which are strongly associated with changes in stream community (Habit et al., 2006; Fierro et al., 2017a). Local disturbances are affected directly stream channel, including trash and wastewater, meanwhile landscapes disturbances can be affected by the catchment scale, being land-use changes one of the main disturbance. Overall, anthropogenic disturbances in streams are the principal degradation sources of freshwater and therefore to the global freshwater biodiversity changes (Dudgeon et al., 2006).

Assessing ecological condition of streams is a critical step previous to conduct efficient management of catchments, being biological monitoring one of the principal tools to asses this condition (Hughes et al., 1998). Biological monitoring have been widely employed since organisms can integrate physical, chemical and biological features of river conditions (Barbour et al., 1996). In this regard, fish, macroinvertebrates, and benthic algal assemblage have been proposed as good biological indicators, since there are present in almost all freshwater and because they respond at different environmental conditions (Terra et al., 2013; Hill et al., 2003; Silva et al., 2017). Knowing the response of each stream assemblage to the same anthropogenic perturbation is important, because stress tolerance of each one could be vary significantly to the same stressor (Hering et al., 2006). As example, agricultural and urban streams, the absence of riparian vegetation are increasing water temperature and conductivity, which in addition to nutrients input, can result in largely biomass benthic algae (Miserendino et al., 2008). Meanwhile macroinvertebrates and fish had shown a relatively lower richness and diversity, and tolerant species increased their densities (Gerth et al., 2017; Von Schiller et al., 2008).

Freshwater macroinvertebrates assemblages have been largely used to assess multiple perturbations in streams. These organisms asses the river health, since they respond to several perturbations, both natural and anthropogenic origin, integrating impacts of chemical pollution, physical perturbations and biological stress, besides there are able to respond to land-use changes (Fierro et al., 2012, 2016, 2017; Luo et al., 2017; Miserendino et al., 2016). Compared to others biological assemblages, the macroinvertebrates are relatively easy to sample and identification keys are available, being an advantage. As characteristics, these organisms have low mobility, their absence or reduced abundances can be reflecting the environment status from preceding months (Zamora-Muñoz et al., 1995).

Among freshwater vertebrates, fish composition is determinate both a local process and catchment-scale processes (Terra et al., 2016). Therefore, they have been used principally for assessing stream perturbations, included changes in substrate type, depth, land-use change, flow modification and biological stressors as exotic species (de Carvalho et al., 2017; Karr, 1981; Penaluna et al., 2009). Since that fish can respond to anthropogenic changes at the individual, population or assemblage level, stream fish bioassessment methods can include indices of biological integrity, community-based and biomarkers, among others (Colin et al., 2015; Jia and Chen et al., 2013, Pont et al., 2009).

The use of benthic algae as bioindicators in streams has increased during the last years (Delgado et al., 2012). The response of benthic algae often is to small-scale or site-specific factors, as nutrients concentrations, light intensity, flow velocity and substrate (Dodds et al., 2002; Taylor et al., 2004). Benthic algae are often used to assessing nutrient enrichment in streams (Sonneman et al., 2001), which usually originate from effluent direct discharges, as wastewater or diffuse sources, being agriculture a key source of diffuse pollution.

In Mediterranean ecoregions the use of this organisms often have been widely used in biotic indices (e.g. Mondy et al., 2012; Navarro-Llácer et al., 2010). Exception is Chile, where only recent studies on macroinvertebrates have been considered (Figueroa et al., 2003; Fierro et al., 2012), while the use of fish and benthic algae biomonitoring is incipient (Fierro et al., 2017b). Mediterranean ecosystems has been largely perturbed by anthropogenic activities, being the principally stressor loss habitat due to agricultural, monocultures and urban/industries (Pauchard et al., 2006; Aparicio, 2008). Chilean Mediterranean ecoregions is considered a hotspot of biodiversity in the world (Myers et al., 2000), however studies on aquatic ecosystems are limited, compared to terrestrial ecosystems (Cooper et al., 2013). The Chilean Mediterranean ecoregions is characterized by heavy rainfall and flooding typically occurs in winter, months that are notably humid and cold, whereas the summer is long and dry (Gasith and Resh, 1999; Bolle, 2003). These climatic patterns, together with unique geographies and geological histories, make the Mediterranean ecosystem one of the most complex and rich globally in terms of biodiversity (Blondel et al., 2010).

Comparative studies using macroinvertebrates, fish and benthic algae have been carried out poorly in Mediterranean regions to measure the response to the same stressor. Therefore, our objective was to determine the response of macroinvertebrate, fish and benthic algae assemblages, the last one measure as biomass, at different land-uses in Chilean Mediterranean streams. Our sampling design was performed in streams affected by exotic forest plantations, agriculture and urbanization, and native vegetation streams as references. Because three groups differ in longevity and mobility, we tested two hypothesis: (1)

community streams will be respond differently to perturbations both at the watershed scale as local habitat variables; (2) the references streams will have higher macroinvertebrates diversity and fish species sensitive to pollution, while chlorophyll-*a* concentrations will be lower compared to perturbed streams.

Methods

2.1 Study area

The study area belong to Mediterranean bioclimatic zone (Amigo and Ramírez, 1998) and is located in Central Chile (33°55'S, 70°31'W; 36°14'S, 71°26'W) (Fig. 1). The climate is characterized by dry season (November-May) and wet season (June-October). Average yearly precipitation is between 200 to 700 mm, and average annual temperature is 14 °C. Landscapes present are similar in orography, gradient and geology (Donoso, 1982). The Chilean Mediterranean presents a highly heterogeneous vegetation mosaic, and the vegetation types are dry xerophytic thorn scrub and succulents and mesic communities dominated by evergreen sclerophyllous trees in the coastal and Andean foothills (Armesto et al., 2007). Extensive agriculture and exotic forest plantation areas have been accompanied by incessant urban growth (Pauchard et al., 2006; Hernández et al., 2016).

2.2 Land-use categories and sampling sites

Our research focused in for land-use categories, native covert, exotic forest plantation, agriculture and urban land. Native covert was principally composed of evergreen vegetation (spinal *Acacia caven*, Chilean wine palm *Jubaea chilensis*, Litre *Lithraea caustica*, conifers

Ausrocedtrus chilensis) and deciduous vegetation (*Nothofagus obliqua*, *N. glauca*, *N. alessandri*, *N. alpina*) (Donoso, 1982). The exotic forest plantation included eucalyptus *Eucaliptus globulus*, started roughly 5-10 from the stream edge, leaving a riparian vegetation compromised of mix native and exotic species. Agricultural land were primarily dedicated to fruit crops and vineyards. Urban stream stations were sampled downstream of Curicó city (aprox. 100,000 inhabitants), Longavi city (aprox. 29,000 inhabitants), San Fernando city (aprox. 49,000 inhabitants) and San Vicente de Tagua Tagua city (aprox. 40,000 inhabitants). Riparian plant coverage on both sides of stream in agricultural and urban streams presented exotic species, including willows (*Salix* spp.) and poplars (*Populus nigra*).

All catchments areas were in similar gradient, geology, slope and Strahler stream order. The assessed streams were free-flowing and perennial. Four basin were defined (Maipo, Rapel, Mataquito and Maule basins), with a total of 20 sampling sites in 10 streams were established. In each one land-use were assigned five sampling sites (Fig.1, Table 1). 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 and cover were determined using ArcGis 10 (ESRI, 2007) and classified as urban, agricultural, forest plantation, native cover and others (water and land without vegetation).

2.3 Environmental collection

Field samples were collected during the Austral summer (December 2015 to March 2016). This season was selected for sampling due to river-flow stabilities. We sampled water quality and habitat, benthic macroinvertebrates, fish and benthic algae at the same time in each one sampling site.

At each site, we measured *in situ* conditions of temperature (°C), pH, conductivity ($\mu s \cdot cm^{-1}$), total dissolved solids ($mg \cdot l^{-1}$), and dissolved oxygen ($mg \cdot l^{-1}$) using a Hanna Multiparameter Model HI 9828. We evaluated stream channel conditions that included average depth, mean active channel width using a tape measure. We visually estimated the in-stream percent areal coverage of macrophytes 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^2 grid.

2.4 Biological sampling

Macroinvertebrates were sampled from riffle habitats, the most common habitat. Six separate samples were taken by using a Surber net (250 µm mesh size; 0.09 m^2 area). The samples were fixed *in situ* with 70% ethanol and then transported to the laboratory where they were separated and preserved in 90% 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, usually genus or species, exception lower Diptera and Trichoptera families, 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.

Fish were sampled using backpack electrofisher in different microhabitats, including patches with and without submerged and emerged vegetation, different substrate types, water current velocity and depth. Electrofishing pass was conducted by one operator managing the anode and two others collected fish using 1-mm mesh dip nets. All retrieved fish were identified in situ, quantified and returned alive to their habitat.

Biomass benthic algae were sampled with BenthoTorch® on 9 cobbles underwater sampled randomly at maximum 0.3 m. BenthoTorch® development by BBE Moldaenke GmbH (Schewntinental, German) is an instrument that allows quick and easy measurement of algal biomass (Kahlert and McKie, 2014). The BenthoTorch measures the resulting fluorescence of Chl-a emitted at 680 nm. Data produced by the BenthoTorch are given for three photosynthetic groups: cyanobacteria, diatoms, and green algae. The calculation of the respective biomasses of the photosynthetic groups is via an algorithm based on the fluorescence responses to all different excitation wavelengths. Biomasses are expressed as a Chl-a equivalent per unit of surface (μg cm^{-2}). The pre-programmed factory settings of the BenthoTorch were used for all measurements and total Chl-a concentrations were deduced by summing biomasses values for the three photosynthetic groups (Echenique-Subiabre et al., 2016; Harris and Graham, 2015; Kahlert and McKie, 2014).

2.5 Data analysis

We calculated a set of macroinvertebrate assemblage descriptors for each sampling site. These included richness measures: taxa richness, EPT richness (Ephemeroptera, Plecoptera and Trichoptera), diversity measures: Shannon-Weaver diversity index (H'), and abundance measures: Diptera abundance and % non-insect individuals. A biotic index previously adapted to Chile, FBI was calculated (Fierro et al., 2012).

Dissimilarities in the physic-chemical parameters, taxonomic composition of macroinvertebrate, fish and benthic algae assemblages, and macroinvertebrate assemblage descriptors among land-uses were established using a one-way Permutational Multivariate

Analysis of Variance (PERMANOVA; 9999 permutations), with land-use as a fixed factor. Prior to analysis physic-chemical and biological data were log x+1 transformed and then was constructed three Euclidean and Bray-Curtis distance matrices respectively.

A preliminary detrended correspondence analysis (DCA) was primary conducted on assemblage data, and revealed a short gradient length (< 3) suggesting a linear response. Redundancy analysis (RDA) ordination technique were thus conducted to explore site distribution based on physic-chemical variables and macroinvertebrates, fish and benthic algae assemblage's metrics. All variables included in Table 1 and 2 were used as environmental data, which prior to RDA analysis were transformed to log x+1 (except pH), to put them all at the same scale so as to avoid influence on the analysis. Abundance and biomass assemblages were Hellinger transformed. All ordination techniques were performed with the R statistical package (R Development Core Team, 2016).

Results

3.1 Characteristics of environmental variables

Environmental variables were different among land-use. Elevation of sampling sites ranged among 141 and 914 m.a.s.l., Strahler order stream, ranged among 2 and 4, and slope among 0.01 and 0.09 $m.m^{-1}$, had agricultural and urban stream the lowest slopes. The substrate particle size ranged among boulder and sand, having agricultural stream significantly higher proportion of soft sediments (PERMANOVA, $p < 0.05$, Table 1). Percentage aquatic plant coverage was higher in agricultural and urban stream, ranged among 0 and 60% cover stream, however not significantly different was recorded.

Channel width ranged was significantly different among land use, ranged among 2.7 and 45 m (PERMANOVA, $p < 0.05$, Table 1), while depth were similar in all land-uses (Table 1).

Mean conductivity and total dissolved sites were significantly higher in native covert streams than the other land-use streams (PERMANOVA, $p < 0.05$, Table 1), values ranged among 44 and 352 µS cm^{-1} and 22 and 304 mg l^{-1} respectively. Dissolved oxygen contents was lower in urban streams and water temperature was higher in agricultural and urban stream, however both were not significantly differences. Finally pH showed high mean values at all land-uses (values > 7).

3.2 Response of benthic algae

The benthic algae composition was different among four land-uses (Table 2). Chlorophyll-*a* biomass was significantly different among land-uses (Table 2, Fig. 4), having native vegetation a mean value of 11.97 mg-m^{-1}, followed by forest plantation with 26.46 mg-m^{-1}, agricultural streams 31.39 mg-m^{-1} and urban streams had the higher biomass 36.47 mg-m^{-1} (Fig. 2). Cyanobacteria and diatoms had significantly higher biomass in agricultural and urban streams (Table 2, Fig. 4). In agricultural streams the green algae presented lower biomass compared to all study area (Fig. 2), however significantly differences was not recorded among land-uses (Table 2).

First two axes of RDA analysis accounted for 90.9% of the total variance in the benthic algae assemblage data and was significant ($p < 0.005$) (Fig. 5a). The 1st axis represented an environmental gradient defined by local habitat variable, while 2st axis represented a land-use coverage associated to native covert and urban coverage (Fig. 5a). According to these, cyanobacteria were associated to urban sites, while diatoms were associated mostly to agricultural and urban sites.

3.3 Response of benthic macroinvertebrates

A total of 58 taxa were collected and identified from all sites. The orders more diverse were Diptera (17%), Ephemeroptera (16%), Trichoptera (14%), Mollusca (12%) and Coleoptera (10%). Benthic macroinvertebrate assemblage was different among land-uses (Table 2). Number of benthic macroinvertebrate taxa differed among land-uses (Table 2), ranged from 17 to 23 taxa in native cover streams, 16 to 25 taxa in forest plantation streams, 13 to 19 taxa in agriculture streams, and 8 to 18 taxa in urban streams (Table 3, Fig. 3). Respect to density, 706 to 2468 ind.m^{-2} were recorded in native cover streams, 612 to 2948 ind.m^{-2} in forest plantation streams, 640 to 2808 ind.m^{-2} in agriculture streams, and 438 to 16957 ind.m^{-2} in urban streams (Table 3). EPT richness showed a similar pattern, having significantly higher richness native vegetation and forest plantation than the rest of land-uses (Table 2, Fig. 3). Shannon-Weaver diversity was significantly different among land-uses, having urban streams the lower diversity (Table 2, Fig. 2). Diptera density was higher in urban streams (Fig. 2), however not significantly differences were recorded (Table 2). Proportion of individual non-insect was significantly higher in agricultural and urban streams (Table 2, Fig. 3). Analysis of FBI showed four water quality class, having urban streams significantly the worst water quality (fairly poor water quality) (Table 2), whereas agriculture streams had poor water quality, native vegetation and forest plantation streams ranged among good and poor water quality, and one sampled site of native vegetation had a very good water quality (Fig. 3).

First two axes of RDA analysis accounted for 47.3% of the total variance in the macroinvertebrate assemblage data and were significant ($p < 0.005$) (Fig. 5b). The 1st axis represented an environmental gradient defined by land-use coverage, while 2st axis represented a local variable associated to temperature (Fig. 5b). Higher water temperature were associated to sites with urban covert. Gastropods *Chilina* sp and *Physa chilensis* were associated with urban and agricultural stream in the lower left and right quadrants.

3.4 Response of fish

12 fish species were recorded in all study area, being eight native species, and the remaining exotic (*Gambusia hoolbroki*, *Cnesterodon decenmaculatus*, *Oncorhynchus mykiss* and *Salmo trutta*) (Table 3). Fish composition was significantly different among land-uses (Table 2). Three native species were present in all land-uses streams: *Trychomycterus areolatus* (catfish), *Cheirodon galusdae* (endemic characid) and *Percilia gillissi* (endemic perch) (Fig. 4). Native vegetation did not have any exclusive species. The endangered endemic catfish *Diplomystes nahuelbutaensis* was a rare species in the study area, found only in two forest plantation streams. Native species *Brachygalaxias bullocki* and *Geotria australis* were only collected in agricultural streams, meanwhile the introduce *Cnesterodon decenmaculatus* was only found in urban streams (Tabla 3). The most common fish assemblage in all stream was composed by two native fish (*T. aerolatus* and *P. gillissi*). They varied among different land-uses streams, including other species: *O. mykiss* in native covert and forest plantation streams, *Ch. galusdae* and *Basilichthys microlepidotus* in agricultural streams, and *Gambusia hoolbroki* in urban streams (Fig. 4). First axis of RDA analysis accounted for 33.2% of the total variance in the fish assemblage data and was significant ($p < 0.005$), being only

agricultural coverage significant (Fig. 5c). Two non-native species best explain the resulted arrangement, with the introduce rainbow trout more related with native vegetation, and forest plantation streams, and *Gambusia* and *Cheirodon* strongly related to agricultural and urban areas.

Discussion

The effects of land-use change are complex, local variables are highly influenced by catchment land use, and therefore both scales are influenced aquatic biota. Aquatic fauna of Mediterranean streams can respond to perturbations both at the watershed scale (e.g. land use) as local habitat variables. Our results suggest that local habitat variables and land user had principally contribution to the observed variation in macroinvertebrates and benthic algae assemblages, while land cover was the unique variable contributed to observed variation in fish. These results are consistent with previously studies, in particular with Lammert and Allan (1999) and Macedo et al. (2014), suggest benthos and fish assemblages have differing sensitivities to environmental variables, being benthos more sensible to perturbations than fish, which can move in response to perturbations.

Native vegetation and forest plantation streams showed better water quality than agricultural and urban streams, based on physic-chemical variables and biotic index. In addition, they shelter macroinvertebrate communities with higher richness and diversity. Instead, fish assemblages in this streams were characterized by lower species richness, with presence of exotic trout, adapted cold-water and sensitive to environmental perturbation. Agricultural and urban streams had lower macroinvertebrates diversity, higher Diptera density and non-insect taxa. Moreover, fish assemblages were represented by species with higher tolerance to pollution, mainly the introduced *G. holbrooki*. Regarding benthic algae, we detected significant increase in biomass cyanobacteria, diatoms and chlorophyll-*a* from native vegetation, to forest plantation, agriculture and urban streams.

Agricultural and urban cover were factors landscapes influencing macroinvertebrate composition, whereas temperature was a local factor significantly associated to macroinvertebrates distribution. Land use surrounding can be influencing the temperature of the streams, overall absence or riparian habitat destruction contributed to increasing water temperature (Baillie et al., 2005; Fierro et al., 2017a). In our study area, temperature water was positively related to agricultural and urban land use, concordantly this sites present a reduction of the riparian canopy. The temperature has been largely described by induce in macroinvertebrates distribution (Lessard and Hayes, 2002; Miserendino et al., 2016). Gastropods as *Physa chilensis* and *Chilina* sp. were positively related to agriculture, urban land uses and water temperature. These species, catalogued as scrapers feeding are favored in this streams types, due to high growth of benthic algae biomass. Although this taxa were recorded in other land-uses, the density was major in urban and agriculture streams. Our results, contrary to what is expected in agricultural streams, exhibit high macroinvertebrate diversity (H´) together with native vegetation and forest plantation streams. This assumption would explain by taxa exchange, decreasing intolerant richness (e.g. EPT taxa) and increasing others, mostly non-insect taxa. This observations are in agreement with Gerth et al. (2017) in Mediterranean USA and with Walsh et al. (2001) in Mediterranean Australia, who reported negative correlation between EPT taxon richness in urban streams and positive correlation between non-insect taxa and agricultural.

Fish assemblages were different among land uses, being agriculture cover the unique factor influencing fish composition. The negative effect of this land use on water quality and biota has been largely described (Dala-Corte et al., 2016; Gerth et al., 2017; Tanaka et al., 2016). Agricultural land can cause loss habitat to aquatic fauna increasing fine sediments and nutrient load in streams (Ribbe et al., 2008), being taxon richness and sensitive organisms factors strongly influenced. This is consistent with other studies in Mediterranean streams, researchers reported that agricultural streams affect negatively the fish fauna (Brown, 2000; Colin et al., 2015). In turn, land-use change have been described by explain Chilean fish assemblages (Habit et al., 2006; Habit & Victoriano, 2005), well reflected in our study by two contrasting life-histories species: *O. mykiss* and *G. holbrooki*. In this study a group of cold-water fish species in native and exotic forest plantation was dominant, while warm-water species were more abundant in agriculture and urban streams. Among the native species an increase of *Ch. galusdae* was recorded in agricultural streams. In these streams water current velocity and higher temperature were more common, which represent the typical habitat of this species (Habit & Victoriano, 2005; García et al., 2012). Notwithstanding this specie was reported in high densities in agricultural streams, these was recorded in all land uses, together with *T. areolatus* and *P. gillissi*, consistently with the broad environmental tolerance of this species (Habit et al., 2005; Fierro et al., 2017b). *D. nahuelbutaensis* an specie endemic from central-south Chile, classified as “danger of extinction”, appear to be related to natural low densities in least human-impacted streams (Habit, 2005). In our study was only recorded in two streams in exotic forest plantation, while in the other land-uses it was not captured, suggest that land-use change is a serious threat to native fish of Chile.

The occurrence of biomass algal depends of a varied number of chemical variables (such as light, substrate, nutrients) (Urrea-Clos et al., 2014) and catchment-scale factors (such as urban and agriculture land) (Taylor et al., 2004). In the current investigation, native vegetation, urban areas and pH were factors significantly associated with benthic algae biomass. Lower biomass density of benthic algae was reported in native vegetation, probably due to lower light input and nutrients compared to others land use. The effects of urbanization related to high nutrients concentration level on diatoms and macroinvertebrates has been widely reported in streams (Sonneman et al., 2001; Walsh et al., 2001). In areas of high population density and intensive agriculture high values of chlorophyll-*a* are reported, specifically higher than 70 $mg.m^2$ are considered excesive, indicators of high pollution level (Dodds et al., 2002; Urrea-Clos et al. 2014). Our Mediterranean streams presented variable values chlorophyll-*a* concentration (3-108 $mg.m^2$), present a clearly tendency to increase from native vegetation, to exotic forest plantation, agriculture and finally urban streams, had higher values of chlorophyll-*a*. The pH was the unique local variable associated with benthic algae biomass, tends to alkalinity (i.e. values over 7). Concordantly, our urban areas present wastewater treatments plants and therefore the input of nutrients could be high, present excessive algal growth. This high growth induce to photosynthesis activities, increase oxygen dissolved and pH in column water (Wallace et al., 2016).

It has been well documented that human perturbations, including land-use change to forest plantation, agriculture and urbanization, have negative impacts on aquatic biodiversity (Allan, 2004; Miserendino et al., 2011; Fierro et al., 2015). During decades, Mediterranean-climate regions has been submitted to a long history of human occupation, therefore watersheds have been seriously modified. Chilean Mediterranean ecoregions have a clearly tendency to increase both agricultural and forest plantation land (Armesto et al., 2007; Schulz et al., 2010). Indeed, the high rates production of some species fruit crops (e.g. berries and avocado), has resulted Chile have one of the largest area planted in the world, turning among the leading producers and exporters of these species worldwide (Jara-Rojas et al., 2015; Retamales et al., 2014).

Conclusions

Our results highlights the negative effect of land-use change on macroinvertebrates, fish and benthic algae assemblages. Taxa richness, diversity, EPT richness and score water quality biotic index decrease with exotic forest plantation, agriculture and urbanization gradient. Cyanobacteria, diatoms and total chlorophyll-*a* increasing in the same gradient perturbation. Variables at local and catchment scale were predictive to each one assemblage. Specifically, macroinvertebrate were explained by urban and agricultural coverage areas and temperature, fish were explained by agricultural coverage, and benthic algae assemblage were explained by native vegetation, urban coverage areas and pH. At general in the Mediterranean ecosystems, exotic forest plantations and agriculture are the first transformation from native vegetation, followed by urban areas (Pauchard et al. 2006). According to the same gradient, we found that urbanization resulted in the most dramatic changes in water quality and aquatic fauna, followed by agricultural areas, whereas forest plantation was the land use more similar to native vegetation. Change in land-use from native covert to agriculture, forest plantation, pasture and urban-industrial uses, continue to be a challenge for global conservation efforts (Manuschevich & Beier 2016). We supposed that if land-use trend continue in Chilean Mediterranean, the aquatic biodiversity loss will continue to increase.

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Tables and Figures

Table 1. Characteristics physicals of 20 sampled sites in Chilean Mediterranean. Land-use coded: NF: native vegetation; FP: forest plantation; Ag: Agriculture; Ur: Urban. Bo: boulder, Co: cobble, Gra: gravel, Sa: sand. *PERMANOVA significant difference among land-uses ($p < 0.05$).

.Site Code	Elevation (m.a.s.l.)	Catchment size (ha)	Stream order	Slope $m.m^{-1}$	Substrate type	% Sand *	% Aquatic plant coverage	Channel wet width (m) *	Depth (m)	Temperature (°C)	Dissolved oxygen (mg.L)	Conductivity µS/cm *	pH	Total dissolved solids mg l^{-1} *	% Native vegetation	% Forest plantation	% Agriculture	% Urban areas	% Water body and others
NV 1	654	13925.8	3	0.04	Bo/Co	10	0	16	0.3	19.1	8.5	80	7.8	32	99.5	0	0.5	0	0
NV 2	914	13867.0	4	0.09	Bo/Co	10	5	9.5	0.5	13.7	9.4	44	7.0	22	77.1	0.0	0.0	0.0	22.9
NV 3	635	10990.0	3	0.03	Bo/Co	5	30	3	0.2	25.2	7.4	122	9.2	78	92.2	0.0	0.0	0.0	7.8
NV 4	643	27051.6	3	0.07	Bo/Co	10	0	11	0.3	12	9.5	98	7.5	66	77.8	0.4	0.0	0.0	21.8
NV 5	566	14952.5	3	0.03	Co/Gra	15	30	2.7	0.1	26	6.8	96	9.2	63	91.1	0.2	0.9	0.0	7.8
EP 1	471	4115.6	2	0.03	Co/Gra	5	30	3.9	0.2	13	9.9	150	7.7	50	56.8	39.0	4.2	0.0	0.0
EP 2	489	53486.0	4	0.04	Co/Gra	0	0	47	0.5	13.2	8.9	168	8.2	112	72.3	4.3	0.5	0.0	22.9
EP 3	390	21243.5	3	0.04	Bo/Co	10	0	8.8	0.3	14.2	8.2	66	7.7	44	93.4	5.6	0.9	0.0	0.1
EP 4	501	4793.2	3	0.04	Bo/Co	5	40	8	0.2	20.5	7.5	406	7.8	272	94.7	3.2	2.1	0.0	0.0
EP 5	196	97666.6	4	0.04	Co/Gra	5	45	30.3	0.4	25	9	166.8	8.5	83.4	58.9	23.4	17.2	0.0	0.5
AG 1	456	199945.9	3	0.01	Gra/Co	10	60	5.5	0.2	23.1	7.3	234	7.6	155	86.9	0.2	12.6	0	0.3
AG 2	190	6041.0	2	0.02	Gra/Sa	30	0	2	0.5	18.1	9.3	87.9	7.4	44	56.6	20.6	22.8	0	0
AG 3	179	8201.0	2	0.02	Gra/Sa	20	50	5	0.2	18.1	9.3	90.6	7.1	45.3	41.7	26.2	32.1	0.0	0.0
AG 4	376	24034.9	3	0.01	Gra/Sa	30	50	3	0.1	28.2	7.6	301	9.0	202	84.8	0.6	13.9	0.1	0.6
AG 5	273	8779.9	2	0.01	Gra/Sa	30	50	9	0.15	22	6.7	177	7.7	120	55.5	0.9	43.6	0.0	0.0
UR 1	141	37068.0	3	0.01	Gra/Sa	10	60	8.6	0.2	23.7	9.27	126.6	8.4	63.3	11.2	17.3	70.7	0.7	0.1
UR 2	332	25674.6	3	0.02	Co/Gra	10	30	15.4	0.3	24.2	5.4	286	7.6	192	79.4	0.5	15.5	4.0	0.6
UR 3	301	27640.6	3	0.02	Co/Gra	0	50	19.3	0.2	26.7	7.5	456	8.8	304	76.2	0.5	18.4	4.3	0.6
UR 4	201	79485.8	4	0.01	Co/Gra	20	60	45	0.4	20	7.6	352	7.7	234	58.3	0.7	27.6	1.4	12.0
UR 5	200	10929.3	4	0.02	Co/Gra	10	50	25	0.15	24.2	7.1	304	8.4	202	49.3	8.1	38.8	3.8	0.0

Table 2. Results from multivariate PERMANOVA analyses for differences in macroinvertebrate, fish and benthic algae assemblage, and mean values (±SE) for several metrics of macroinvertebrates and biomass benthic algae in each land use (native vegetation, forest plantation, agriculture, urban).

					PERMANOVA		
Source	Native Vegetation	Forest Plantation	Agriculture	Urban	Pseudo-F	*P* (perm)	Perms
Macroinvertebrates assemblage					2.03	**0,0008**	9880
Fish assemblage					3.64	**0,0002**	9928
Benthic algae assemblage					3.08	**0,0055**	9927
Macroinvertebrates richness	18.6 (2.6)	18.8 (3.7)	15.8 (2.3)	13.4 (3.8)	3.23	**0,0282**	9824
Macroinvertebrates Diversity (H)	1.72 (0.24)	1.66 (0.36)	1.72 (0.15)	1.07 (0.58)	2.51	**0,0098**	9907
EPT Richness	8 (2.3)	8.8 (3.7)	3.6 (0.9)	3.8 (2.6)	4.94	**0,0045**	9869
Diptera Density	468.7 (302.6)	603.2 (275.8)	243.1 (214.2)	4398.7 (6690.2)	2.11	0,0679	9942
%Non-Insect Individuals	5.1 (7.3)	6.8 (6.1)	33.6 (14.5)	29.8 (34)	2.06	**0,0450**	9925
IBF	5.5 (0.7)	5.5 (0.5)	5.9 (0.2)	6.9 (0.5)	6.27	**0,0031**	9962
Cyanobacteria	2.87 (2.55)	5.81 (2.68)	7.25 (4.48)	11.56 (10.48)	2.40	**0,0431**	9952
Green Algae	1.17 (1.55)	2.33 (3.19)	0.20 (0.24)	2.60 (4.92)	1.15	0,2287	9144
Diatoms	7.92 (4.73)	18.31 (4.37)	23.91 (5.40)	22.31 (31.86)	3.95	**0,0080**	9943
Total Chlorophyll-*a*	11.97 (6.58)	26.46 (2.79)	31.39 (5.19)	36.47 (40.66)	3.54	**0,0039**	9940

Note: Values with bold letters were significantly different with PERMANOVA ($p < 0.05$).

Table 3. Mean relative abundance (percentages) of macroinvertebrates and fish, and biomass benthic algae (mg.m^2) in 20 sampling sites of Mediterranean Chilean ecoregions.

	NF 1	NF 2	NF 3	NF 4	NF 5	EP 1	EP 2	EP 3	EP 4	EP 5	AG 1	AG 2	AG 3	AG 4	AG 5	UR 1	UR 2	UR 3	UR 4	UR 5
Macroinvertebrates																				
Plecoptera																				
Antarctoperla michaelseni (Am)	0.0	0.0	0.0	6.5	0.0	0.6	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Notoperlopsis femina (Nf)	0.2	0.0	0.0	0.0	0.0	0.9	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pelurgoperla personata (Pp)	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ceratoperla schwabei (Cs)	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neonemura sp. (Neo)	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ephemeroptera																				
Andesiops peruvianus (Ap)	0.2	0.3	4.7	3.6	3.9	6.0	1.1	0.0	0.5	0.5	3.8	0.9	0.1	6.1	2.6	0.0	0.0	2.7	2.1	0.0
Andesiops torrens (At)	0.0	3.9	0.3	28.1	0.0	2.1	7.8	0.0	1.9	0.1	0.0	0.0	0.0	1.1	4.0	0.0	0.2	0.0	0.0	0.0
Camelobaetidius sp. (Cam)	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.8	0.0
Caenis chilensis (Cc)	17.8	0.0	48.7	1.8	28.3	0.3	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chiloporter eatoni (Ce)	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Meridialaris diguillina (Md)	0.0	4.7	0.3	36.1	0.0	2.1	20.0	0.2	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nousia maculata (Nma)	0.0	0.0	0.0	0.4	0.1	1.2	1.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nousia minor (Nmi)	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Penaphlebia chilensis (Pc)	0.0	1.2	4.7	0.1	1.0	6.9	0.2	2.0	0.1	29.0	0.0	2.6	0.0	0.0	1.6	0.0	0.2	0.0	6.8	0.0
Trichoptera																				
Austrotinodes sp. (Aus)	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hydrobiosidae (H)	0.0	0.0	0.0	0.0	0.0	0.6	2.9	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.2
Metrichia sp. (Met)	5.1	1.5	0.9	0.2	0.7	0.6	1.3	0.7	2.3	0.0	2.6	0.0	9.4	6.9	1.4	4.1	2.8	1.5	1.3	0.0
Oxyethira sp. (Oxy)	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Smicridea sp. (Smi)	0.6	13.0	0.3	9.4	0.3	42.0	18.5	0.2	54.9	24.1	41.6	35.5	22.0	15.7	56.5	6.9	19.5	2.7	1.3	0.0
Mastigoptila sp. (Mas)	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Brachysetodes sp. (Bra)	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 3 continued

	NF 1	NF 2	NF 3	NF 4	NF 5	EP 1	EP 2	EP 3	EP 4	EP 5	AG 1	AG 2	AG 3	AG 4	AG 5	UR 1	UR 2	UR 3	UR 4	UR 5
Oecetis sp. (Oec)	0.4	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Coleoptera																				
Staphylinidae (Sta)	0.0	0.2	0.0	0.1	0.0	0.3	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hydrophilidae (Hy)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Elmidae (Elm)	12.5	4.7	6.0	4.6	7.7	1.8	2.6	14.4	0.1	1.3	11.6	0.0	0.0	10.5	0.0	0.0	9.8	3.6	0.0	3.9
Gyrinidae (Gyr)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hydraenidae (Hyd)	2.6	0.0	0.3	0.2	0.0	0.0	0.0	0.2	0.5	0.0	1.7	0.0	2.2	3.3	0.2	0.0	0.0	0.1	3.0	0.1
Tychepsephenus felix (Tfe)	0.0	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hemiptera																				
Corixidae (Cor)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	0.0	0.0	0.4	0.0
Megaloptera																				
Protochauliodes sp. (Pro)	0.6	0.0	0.6	0.0	0.1	3.0	0.1	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
Odonata																				
Aeshna sp. (Aes)	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lestes sp. (Les)	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Diptera																				
Athericidae (Ath)	6.5	1.8	5.0	3.8	0.0	3.3	3.1	4.4	0.0	0.3	0.3	0.0	3.8	0.0	0.0	0.4	0.0	0.0	0.0	0.0
Ceratopogonidae (Cer)	1.4	0.0	0.3	1.3	35.8	0.6	0.1	0.0	0.0	0.0	1.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Empididae (Emp)	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Simuliidae (Sim)	0.0	1.5	0.0	0.0	0.0	3.6	0.2	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.2
Psychodidae (Psy)	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tipulidae (Tip)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hexatoma sp. (Hex)	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Limonia sp. (Lim)	0.4	2.4	0.0	0.0	0.1	1.2	0.0	0.5	0.0	4.5	0.0	0.4	2.6	0.0	0.0	0.8	0.0	0.0	1.3	0.0
Blephariceridae (Ble)	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chironomidae (Chi)	33.5	62.9	21.7	2.4	19.9	17.2	36.0	64.5	23.5	33.7	13.9	7.9	14.5	27.5	14.6	8.5	64.9	73.9	28.3	94.9
Collembola (Coll)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Amphipoda																				
Hyalella sp. (Hay)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.2	0.4	0.0

Table 3 continued

	NF 1	NF 2	NF 3	NF 4	NF 5	EP 1	EP 2	EP 3	EP 4	EP 5	AG 1	AG 2	AG 3	AG 4	AG 5	UR 1	UR 2	UR 3	UR 4	UR 5
Decapoda																				
Aegla sp. (Aeg)	0.2	0.0	0.0	0.3	0.0	1.2	0.0	3.4	0.0	4.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	7.2	0.0
Acari																				
Hydracarina (Hydr)	0.0	0.0	0.0	0.0	0.4	0.0	0.2	0.2	0.0	0.0	0.6	0.7	0.1	0.2	0.0	0.1	0.3	0.0	0.0	0.0
Mollusca																				
Physa chilensis (Pch)	6.9	0.0	0.0	0.1	0.6	0.0	0.0	0.0	0.0	0.0	19.4	0.7	0.7	24.5	0.2	72.0	0.6	0.7	0.0	0.0
Littoridina sp. (Lit)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0	2.0	0.1	0.0	3.0	0.0	0.1	0.0	0.0	0.0
Lymnaea sp. (Lym)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chilina sp. (Chil)	1.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	1.9	0.3	0.0	0.2	4.2	0.0	13.0	0.1	0.0	0.0	34.2	0.0
Uncancylus sp. (Unc)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	13.7	0.0	0.3	37.7	38.3	0.2	0.2	0.4	0.0	0.0	1.3	0.0
Gundlachia sp. (Gun)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pisidium sp. (Pis)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Annelida																				
Tubifex sp. (Tub)	9.7	0.2	5.0	0.0	0.3	0.6	1.2	2.7	0.1	0.3	2.3	5.7	1.9	3.6	0.4	3.2	0.6	11.3	11.0	0.2
Lumbriculidae (Lum)	0.0	0.2	0.0	0.0	0.4	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.2	1.2	0.4	0.1	0.6	0.0	0.6
Glossiphoniidae (Glo)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
Platyhelminthes																				
Dugessidae (Dug)	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.2	0.2	0.0	0.4	0.1	0.0	2.7	0.0	0.0
Nematoda (Nem)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
Total richness	19	17	17	23	17	25	19	18	16	16	15	19	15	13	17	13	18	12	16	8
Mean abundance (ind.m^2)	934	1221	706	2469	1277	612	2060	757	2949	2140	640	840	2808	1161	914	1341	4449	3678	438	16957
Fish																				
Siluriformes																				
Diplomystes nahuelbutaensis (Dn)	0.0	0.0	0.0	0.0	0.0	0.0	11.1	31.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Trichomycterus areolatus (Ta)	52.9	88.7	46.8	7.7	62.0	6.0	7.8	18.4	44.7	21.4	31.5	21.1	62.5	81.9	57.1	8.3	66.3	24.8	5.1	98.8
Osmeriformes																				
Brachygalaxias bullocki (Bb)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Characiformes																				

Table 3 continued

	NF 1	NF 2	NF 3	NF 4	NF 5	EP 1	EP 2	EP 3	EP 4	EP 5	AG 1	AG 2	AG 3	AG 4	AG 5	UR 1	UR 2	UR 3	UR 4	UR 5
Cheirodon galusdae (Cg)	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	14.3	11.7	36.8	18.8	0.8	21.4	27.4	10.9	1.4	1.3	0.0
Perciformes																				
Percilia gillisi (Pg)	33.5	0.0	14.3	0.0	16.3	62.0	51.1	34.2	27.6	46.4	13.5	0.0	12.5	5.3	17.9	3.6	12.0	6.4	44.3	1.2
Percichthys trucha (Pt)	8.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.3	14.3	0.0	5.3	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Arheriniformes																				
Basilichthys microlepidotus (Bm)	0.0	0.0	0.0	0.0	6.2	0.0	0.0	0.0	0.0	0.0	40.5	0.0	0.0	11.6	0.0	0.0	0.0	0.5	1.3	0.0
Petromyzontiformes																				
Geotria australis (Ga)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.6	0.0	0.0	0.0	0.0	0.0
Cyprinodontiformes																				
Gambusia hoolbroki (Gh)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	36.8	0.0	0.0	0.0	60.7	10.9	35.3	48.1	0.0
Cnesterodon decemmaculatus (Cd)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	31.7	0.0	0.0
Salmoniformes																				
Oncorhynkus mykiss (Om)	2.6	3.2	33.8	66.7	15.5	28.0	30.0	15.8	18.4	3.6	2.7	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Salmo trutta (St)	0.0	8.1	5.2	25.6	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total richness	5	3	4	3	4	4	4	4	5	5	5	4	4	6	4	4	4	6	5	2
Benthic algae																				
Cyanobacteries	3.4	0.7	1.3	7.1	1.9	5.1	3.0	8.6	8.7	3.7	4.2	5.6	9.2	3.1	14.1	4.4	7.3	29.8	10.8	5.5
Green Algae	0.6	0.0	3.7	0.0	1.5	8.0	0.0	1.2	1.3	1.2	0.2	0.2	0.0	0.6	0.0	11.3	0.0	0.0	0.0	1.7
Diatoms	13.1	2.4	5.2	12.6	6.3	12.9	25.0	18.4	18.4	16.9	28.7	19.0	29.5	24.6	17.7	5.8	6.9	79.0	13.8	6.1
Total Chlorophyll-*a*	17.2	3.2	10.1	19.7	9.7	25.9	28.0	28.3	28.3	21.8	33.1	24.9	38.7	28.4	31.9	21.6	14.2	108.7	24.6	13.3

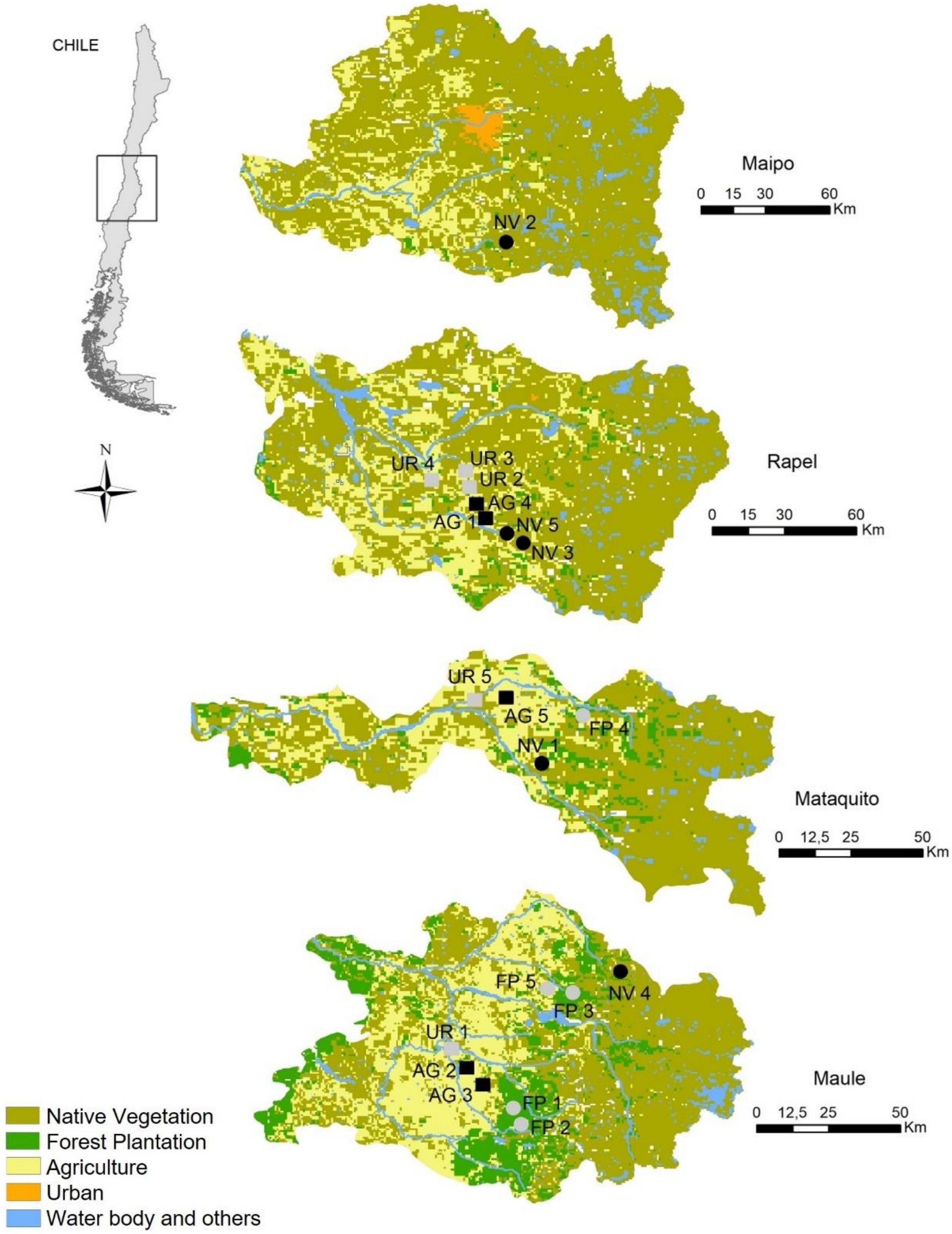

Fig. 1. Map of the study area, showing location of 20 sampling sites in Chilean Mediterranean across four land-uses. Sites native vegetation (●), forest plantation (●), agriculture (■) and urban (■).

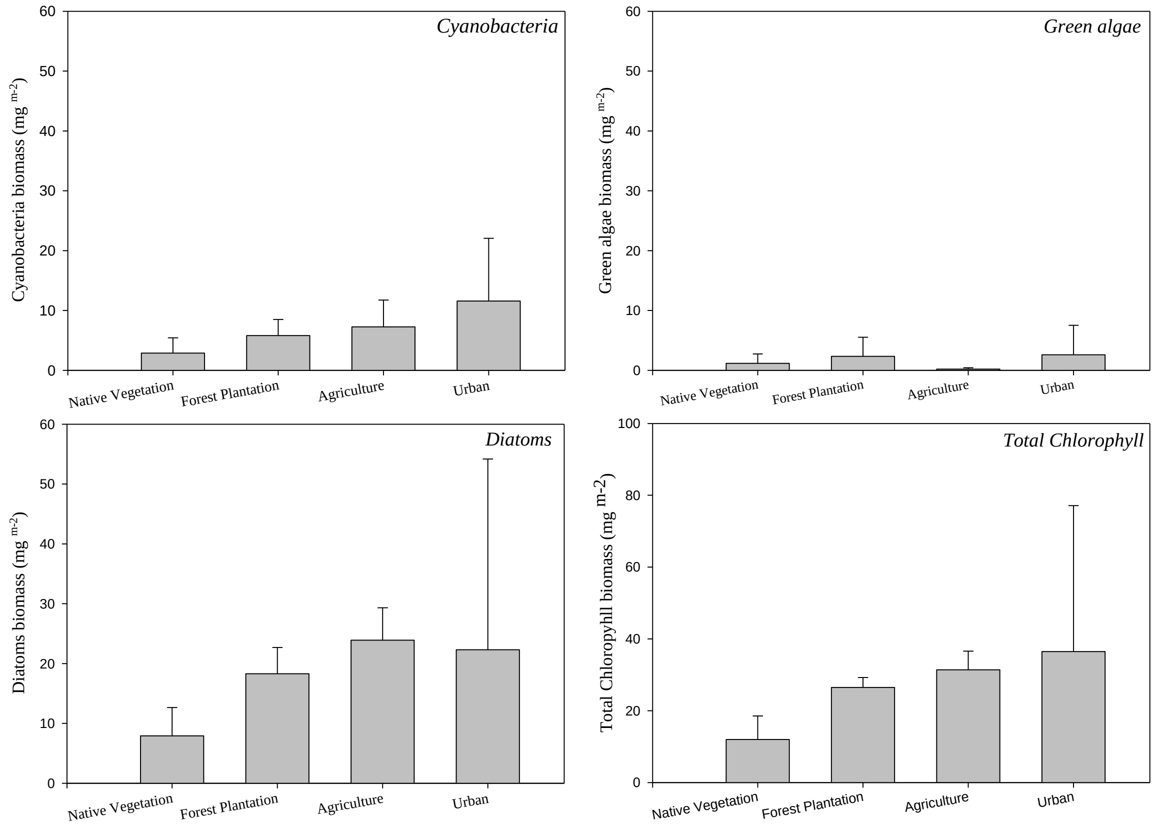

Fig. 2. Biomass (mg m^{-2}) of assemblage benthic algae and total chlorophyll-*a* in 20 sampling sites across four land uses in Chilean Mediterranean ecoregions. Bars represent the mean and standard deviation.

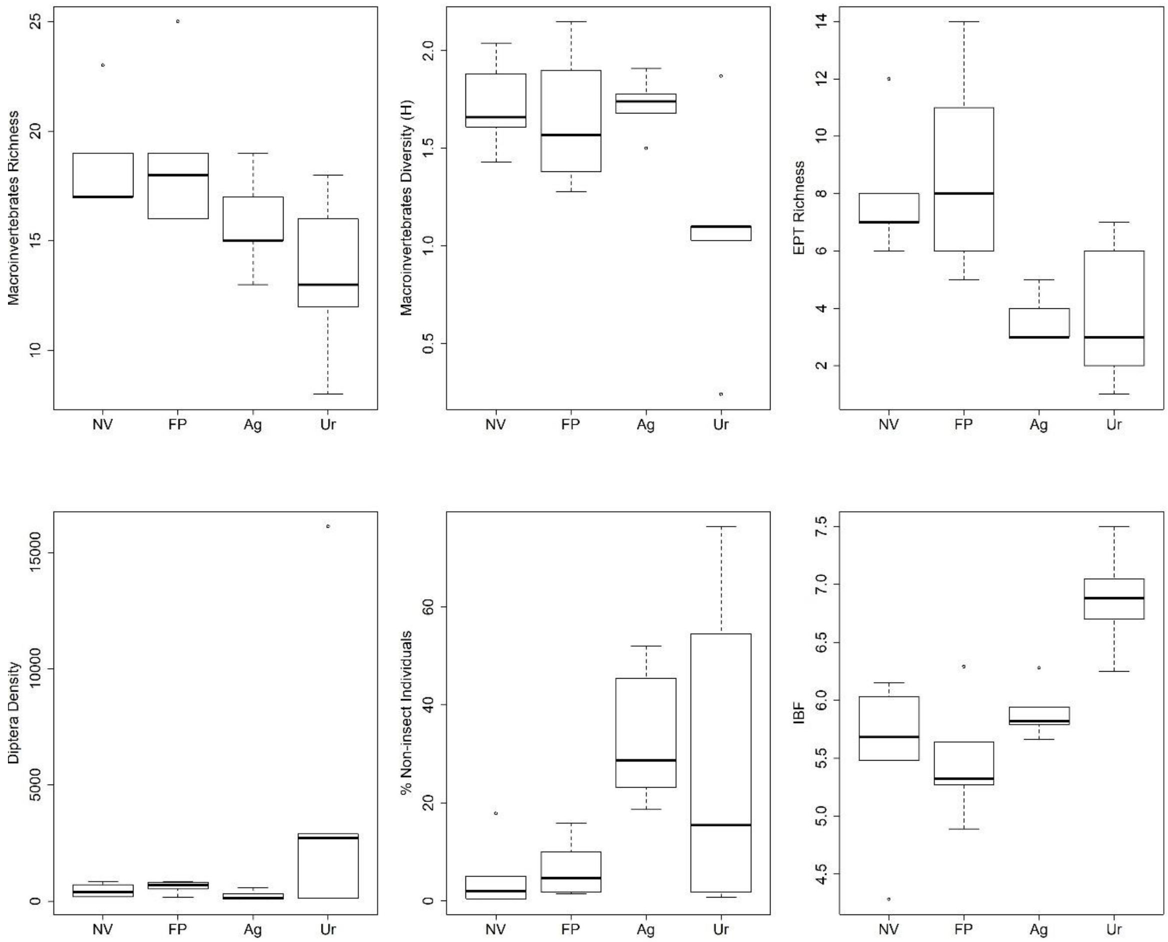

Fig. 3. Macroinvertebrate metrics for taxa richness, diversity (Shannon-Weaver), EPT richness, Diptera density, % non-insect individuals and FBI quality index. Range bars show maxima and minima, boxes are interquartile ranges (25–75%), Dark lines are medians. Land-uses: NV: Native Vegetation, FP: Forest Plantation, Ag: Agriculture, Ur: Urban.

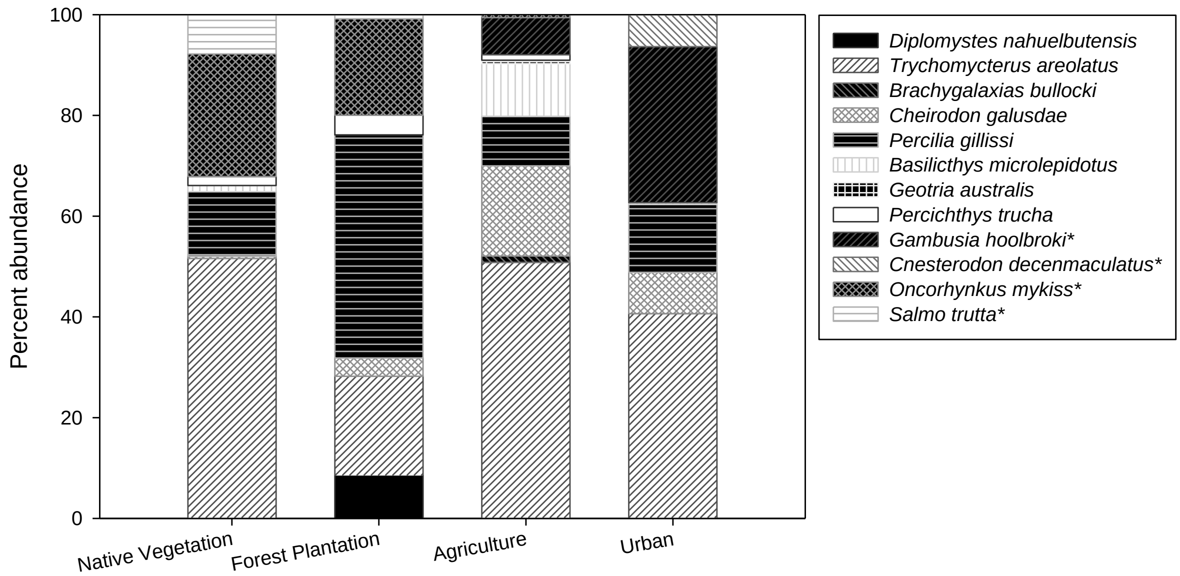

Fig. 4. Relative fish abundance in Chilean Mediterranean streams with different land use. *Exotic species

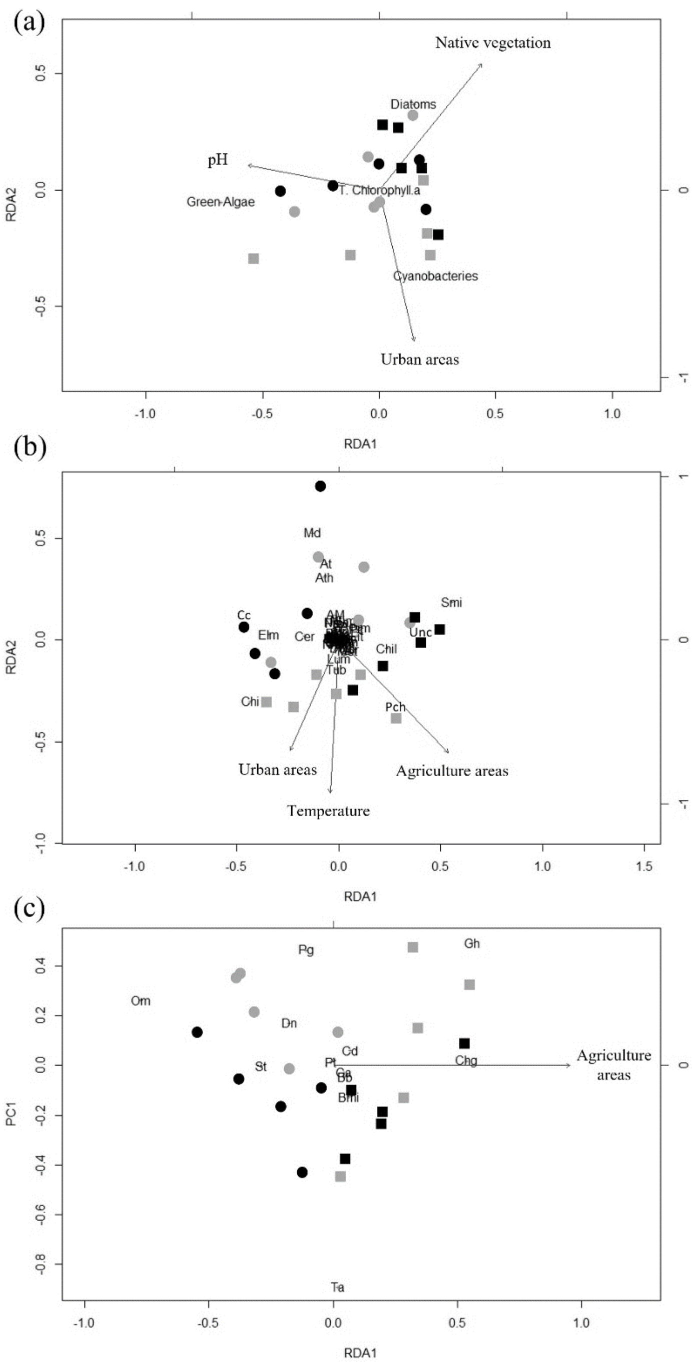

Fig. 5. Ordination triplot of RDA on sampling sites, environmental variables and (a) macroinvertebrate taxa, (b) fish species and (c) perifiton biomass, in Chilean Mediterranean ecoregions. Environmental variables are represented by arrows. Codes of taxa in Table 2. Sites native vegetation (●), forest plantation (●), agriculture (■) and urban (■).

Capítulo 4: Rainbow Trout diets and macroinvertebrates assemblages responses from watersheds dominated by native and exotic plantations

Este capítulo está basado en:

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Rainbow Trout diets and macroinvertebrates assemblages responses from watersheds dominated by native and exotic plantations

Pablo Fierro[1,2], Loreto Quilodrán[1], Carlos Bertrán[1], Ivan Arismendi[3], Jaime Tapia[4], Fernando Peña-Cortés[5], Enrique Hauenstein[5], Raúl Arriagada[1], Eduardo Fernández[5] & Luis Vargas-Chacoff[1*]

[1] Institute of Marine Science and Limnology, Universidad Austral de Chile, Chile

[2] Center of Environmental Sciences EULA-CHILE, Universidad de Concepción, Chile

[3] Department of Fisheries and Wildlife, Oregon State University, USA

[4] Institute of Chemistry and Natural Resources, Universidad de Talca, Chile

[5] Territorial Planning Laboratory, Universidad Católica de Temuco, Chile

[*] Corresponding author: Tel.: +56 63 221648. Fax: +56 63 221315

E-mail: luis.vargas@uach.cl

Introduction

Freshwater ecosystems are among the most seriously threatened in the world (Saunders et al., 2002; Barletta et al., 2010). During the recent decades, the loss of freshwater biodiversity has been accentuated mainly due to changes in land use from human-related activities (e.g., forestry and livestock or arable farming) that have resulted in habitat destruction, fragmentation and eutrophication (e.g., Encalada et al., 2010; Miserendino et al., 2011; Lunde and Resh, 2012). In particular, because of the economic benefit from the cellulose industry (Valdovinos, 2006), the replacement of native forest by plantations of exotic species (i.e., monocultures of conifers and eucalyptus) has been a widespread forestry practice all over the world (Hartley, 2002).

In headwaters of forested watersheds, riparian vegetation is a major source of energy and nutrients for stream food webs through the introduction of dead leaves and large woody debris (Vannote et al., 1980). In these environments, the relatively high velocity of water and extensive shade from the canopy limit the autochthonous production (Vannote et al., 1980; Wallace et al., 1997). Therefore, modifications of riparian vegetation can modify the quality of leaf-litter inputs and alter processes in aquatic ecosystems such as the trophic structure and composition of aquatic communities (Abelho and Graça, 1996; Martínez et al., 2013).

Since the beginning of the 19th century, varying degrees of anthropogenic disturbance along coast of southern Chile (southern South America) have occurred (Peña-Cortés et al., 2011a). This includes an over-exploitation of the soil and the replacement of the native forest by agriculture, urbanisation, and plantations of exotic tree species (Sala et al., 2000; Peña-Cortés et al., 2006; Aguayo et al., 2009). The consequences of these activities upon aquatic food webs are still not well understood. Recently, it has been reported that among the most threatened communities by such changes in land use are the benthic aquatic macroinvertebrates (Fierro et al., 2015). A few studies conducted in headwaters (e.g., Larrañaga et al., 2009; Miserendino and Masi, 2010) have shown higher shredder richness in streams dominated by native forest compared to streams dominated by exotic plantations. More recently, Fierro et al. (2015) showed higher invertebrate densities and richness in streams dominated by native forest. Because macroinvertebrates assemblages represent intermediate trophic links between primary and tertiary consumers (Jensen et al., 2012; Bertrán et al., 2013; Cornejo-Acevedo et al., 2014; Fierro et al., 2014) as fish food sources, their availability can affect fish carrying capacity of these low-to-medium order streams populations. If prey availability is limiting, prey fish would be affected (Pequeño et al., 2010). Therefore, any change in the assemblage of macroinvertebrates, would result in changes in the functioning of aquatic ecosystems and restructuring of food chains (Richards et al., 1996; Vargas-Chacoff et al., 2013; Tiziano et al., 2014).

Further, non-native fish introductions represent one of the greatest threats to freshwater ecosystems in southern Chile (Arismendi et al., 2014). In this region, salmonids have been introduced into freshwaters, mainly for recreational fisheries and aquaculture purposes (Arismendi et al., 2014). Rainbow Trout (*Oncorhynchus mykiss*, Walbaum) is one of the most successful introduced species, and currently it is widely distributed in southern South America, reaching higher abundances than native fishes (Arenas, 1978; Soto et al., 2006; Arismendi et al., 2012, 2014). Like other salmonids in the region, Rainbow Trout is known as generalist and largely opportunistic feeder (e.g., Arenas, 1978; Campos et al., 1984; Ruiz et al., 1993; Berrios et al., 2002; Palma et al., 2012; Arismendi et al., 2012; Vargas-Chacoff et al., 2013). Most of these studies have related the diet of Rainbow Trout with the availability of macroinvertebrates in the environment in summer, but few of them have investigated this across seasons (Buria et al., 2009; Di Prinzio et al., 2013).

The first goal of this study is to characterize macroinvertebrate assemblages and functional feeding groups from two land use types (native forest and exotic plantations). The second goal is to examine whether diets of Rainbow Trout can be used as predictors of macroinvertebrate assemblage composition from these two land use types. Collectively, our study provides an assessment of the influences of eucalypt plantations on macroinvertebrate functional feeding groups and fish diets. This could help to clarify how land use change may impact aquatic food webs, contribute to the development of management practices on freshwater ecosystems, and serve as a baseline for future investigations of ecological processes in streams under human-related disturbances.

Materials and methods

Study area

Field sampling was conducted seasonally during 2010, in summer (10-13 January), autumn (10-13 May), winter (10-13 August) and spring (10-13 November) at the coastal zone of the Araucanía Region (Fig. 1). We sampled water quality, macroinvertebrates and stomach contents from streams between 2rd and 4th order (n = 12; Table 1, Fig.1). The climate in this area is maritime with a mediterranean influence; the average annual precipitation is between 1200 mm and 1600 mm (Di Castri and Hajek, 1976). The landscape geomorphology varies from mountain systems to marine abrasion platforms, with elevations ranging between 870 masl and -2 masl (Peña-Cortés et al., 2009; Peña-Cortés et al., 2011b). Our sites encompassed two watersheds with varying land uses: the Moncul River located in the northern part of the region is dominated by forest practices on exotic species – mainly *Eucalyptus globulus* (Labill); the Queule River, located in the southern part of the region is dominated by forest practices on native forest, the dominant species being *Nothofagus dombeyi* (Oersted), *Nothofagus obliqua* (Oersted) and *Drimys winteri* (Forster & Forster). The study sites within each watershed were selected according to the proportion covered by riparian vegetation type, including up to 60% of exotic vegetation in the Moncul watershed, dominated by *Eucalpytus* spp., and up to 60% of native forest in the Queule watershed, dominated by *Nothofaguss* spp. (Vargas-Chacoff et al., 2013). The eucalyptus plantations have mostly been planted during the last 20 to 25 years, while the native forest sections have been present for over 50 years.

Sampling

Environmental characteristics

The water samples were collected in duplicate in the morning (8-11 AM) from the centre of the active channel, deposited in bottles and taken to the Analytical Chemistry Laboratory of the Institute of Chemistry and Natural Resources, Universidad de Talca, for the following parameters to be determined: bio-chemical oxygen demand, suspended solids, dissolved oxygen, chlorides, sulphates, dissolved solids, apparent colour, nitrates and phosphates. All the analyses were carried out following standard methods for water and waste water (APHA, 2005). The temperature, pH and conductivity were measured *in situ* with a pH meter (WTW pH model 330i/SET), and a conductivity meter (WTW cond. Model 330i/SET).

Availability of prey

Together with the water samples in each sampling station, three separates samples were taken in a zone of riffles (the most common habitat type) using a Surber net with 500 µm mesh (0.09 m^2 area). The samples were fixed *in situ* with 90% ethanol and then taken to the Benthos Laboratory of the Institute of Marine and Limnological Sciences, Universidad Austral de Chile, where they were separated, identified and counted under stereo microscope (Olympus, model SZ 51, 40x) and optical microscope (Olympus, model CX 31, 100x) at lowest possible taxonomic resolution following Domínguez and Fernández (2009). The taxa identified were assigned to seven functional feeding groups (FFG): shredders, collector-gatherers, collector-filterers, grazers, predators, detritivores and parasites, following the criteria of Merrit and Cummins (1996) and Fierro et al. (2015).

Fish sampling

Individuals of Rainbow Trout were captured using an electrofishing equipment (EFKO, model FEG 1000, 1 KW, 150-600 V) at the same sampling sites where the invertebrates were collected. The electrofishing method was carried out on a 100 m stretch of stream for 15 minutes. The fish captured were fixed and preserved in ethanol 90% and then transported to the Benthos Laboratory of the Institute of Marine and Limnological Sciences, Universidad Austral de Chile, where the individuals were measured (standard length, 0.1 mm) and weighed (0.001 g accuracy).

Diet of Rainbow Trout

The stomach contents extracted from each fish were emptied into a Petri dish. The prey organisms were removed and identified to the same taxonomic level as the benthic organisms. The contribution of each prey type was assessed using two methods following

Hyslop (1980) including the frequency of appearance (*%F*), corresponding to the number of stomach samples containing each taxon, expressed as a percentage of the total stomach samples, and abundance of occurrence (*%N*), corresponding to the total number of individuals of each taxon expressed as a percentage of the total stomach samples.

To assess the particular contribution of each prey, the Index of Relative Importance (IRI) established by Pinkas et al. (1971), and used by other studies in the region (e.g., Bertrán et al., 2013; Cornejo-Acevedo et al., 2014; Fierro et al., 2014), was applied. The relative importance of each food item was calculated as follows:

$$IRI = \%F \times \%N \times 100^{-1}$$

Statistical analyses

Associations between the physical, chemical and biological data for each site within the two watersheds were examined using multivariate statistical procedures within the software package PRIMER V.6.1.2 software (Clarke and Gorley, 2006) and PERMANOVA v.1 software (Anderson et al., 2008). The physical-chemical data were first transformed (square root) and normalised, and a matrix of Euclidian distance was constructed. These transformed and normalised data were subjected to principal component analysis (PCA) to order the sampling sites along the environmental gradient. To assess the degree of similarity between the sampling sites of the two watersheds by season, the biological data (abundance of macroinvertebrates, abundance of functional feeding groups and index of relative importance) were transformed (square root) in order to construct three Bray-Curtis similarity matrixes. To test significant differences ($P < 0.05$) between the two watersheds by season, two-way fixed factors were used: watershed and season, which were tested using a

Permutational multivariate analysis of variance (PERMANOVA; 9,999 permutations). This nonparametric method is similar to the analysis of variance, using the permutations method to test the difference between groups (Anderson et al., 2008). A RELATE analysis (Clarke and Gorley, 2006) was used to determine the significance of the correlation between the Bray-Curtis similarity matrices of the benthic data and the index of relative importance. A Spearman correlation ranking was used to determine the coefficient level between the two matrices (benthic data and index of relative importance).

Results

Environmental characteristics

Chemical and physical data provided a clear distinction between native watershed sites and exotic watershed sites (Table 2, Fig. 2). Significant statistical differences between the watersheds was found (PERMANOVA: $F = 1.010$, $P = 0.001$). Of all the variables measured, in the PCA analysis, it was established that the strongest relationships with the sites in watersheds dominated by exotic vegetation were with total dissolved solids, suspended solids, nitrates, chlorides and sulphates (Fig. 2). These variables showed higher mean values at exotic watershed sites than at native watershed sites.

However, when the physical and chemical data were compared among seasons, we found non-significant statistical differences (PERMANOVA: $F = 21.779$, $P = 0.416$). A synthesis of physical and chemical data of the sites is presented in Table 2.

Availability of prey invertebrates

A total of 103 taxa of macroinvertebrates were identified during the study time period (Appendix I), with the most represented orders being Diptera (26%), Ephemeroptera (16%), Plecoptera (16%) and Trichoptera (16%). There was a significant statistical difference in the composition of macroinvertebrate communities between the two watersheds (Fig. 3) (PERMANOVA: F = 2.545, P = 0.002) and among seasons (PERMANOVA: F = 3.075, P < 0.001). In general, taxa richness and total density were lowest in the exotic vegetation watershed streams (richness: 42-59, density: 796 – 2.079 individuals/m^2, respectively) and highest in the native vegetation watershed streams (richness: 56-70, density: 722-2.660 individuals/m^2) (Fig. 3 and Appendix I). The lowest abundance of macroinvertebrates occurred in winter, while the highest abundances were recorded in summer and autumn at both watersheds.

Species diversity of benthic macroinvertebrates were different between watersheds, some species were recorded on native vegetation watershed but not on exotic vegetation watershed. Moreover, 91 taxa were recorded in native vegetation watershed, while only 81 taxa were recorded in exotic vegetation watershed. The macroinvertebrates only present in sites in the native vegetation watershed were principally immature stages of Trichoptera and Diptera.

From total of 103 taxa, being 38 collector-gatherers, 27 predators, 15 shredders, 10 scrapers, 6 collector-filterers, 5 detritivores and 2 parasites (Appendix I). Non-significant statistical differences between the two watersheds were observed (PERMANOVA: F = 2.028, P = 0.258). Collector-gatherers were the most abundant group in both cases (44-77% relative abundance respectively), followed by shredders (13–35%) and predators (2–20%). Other functional feeding groups were poorly represented in both watersheds. However, the relative abundance of each functional feeding group showed seasonal changes

(PERMANOVA: F = 4.647, P = 0.037). Although the collector-gatherers had the highest proportion at both watersheds year round, the shredders increased in winter while the predators increased in spring.

Diet of Rainbow Trout

We analysed a total of 244 stomachs from Rainbow Trout that ranged between 3.3 to 19.8 cm SL, and between 0.19 and 252.87 g in mass (Table 3). The diet consisted of 79 taxa of animal origin, from 12 orders. Benthic macroinvertebrates, especially immature insects (Ephemeroptera, Plecoptera, Trichoptera, Diptera and Coleoptera) were the most common diet items (Appendix II, Fig. 4a). However, the diet consumed by Rainbow Trout differed between the two watersheds (PERMANOVA: F = 1.870, P = 0.013). The number of taxa consumed in the watershed dominated by native vegetation was higher (76 taxa) than the exotic plantation watershed (56 taxa) (Appendix II, Appendix III). Likewise, diets changed across seasons (PERMANOVA: F = 2.327, P = 0.001) (Fig. 5, Appendix II).

The taxa registered in the stomach contents of Rainbow Trout were often found in great abundance in the benthos in both watersheds during the year (Appendix I and II). Nevertheless some taxa present in the benthos were absent in the stomach contents (Appendix III). Using the RELATE analysis showed strong correlation between the matrices of Bray-Curtis similarities of the benthic data and the index of relative importance (RELATE R = 0.577, P = 0.001). Similar to the taxonomic analysis, there was a strong association between the benthos and diets at the functional feeding groups level (RELATE R = 0.486, P = 0.007). In general, the collector-gatherers were the best represented functional group in the diets at both watersheds across seasons. In winter and spring, shredders, predators and grazers were consumed in a greater proportion. The other functional groups (collectors-filterers and detritivores) were poorly represented (Fig. 4b).

Discussion

This study examined the influence of changes in land use on aquatic food webs in native forest watershed and exotic plantation watershed streams, and explores how these relationships change with seasons. Benthic invertebrate abundance, richness and aquatic prey ingested by Rainbow Trout were higher on native forest sites. This suggests that trophic structure is different between land uses, and provide evidence that diets are representative of taxa from the benthos.

The influence of riparian vegetation on freshwater ecosystems has been widely discussed elsewhere (e.g., Miserendino et al., 2011; Da Silva et al., 2012; Fierro et al., 2015). Most of the studies have concluded than allocthonous organic matter is a key component that sustains food webs in the aquatic systems of mountain streams. Therefore, any alteration in its quality or quantity can affect the aquatic biota (Abelho and Graca, 1996).

We show that exotic vegetation influenced chemical and physical variables (see also Harding and Winterbourn, 1995; Miserendino and Masi, 2010). Streams dominated by exotic vegetation led to higher concentrations of nutrients, minerals and solids. This finding is in agreement with other studies in streams from coastal watershed in southern Chile (Fierro et al., 2012; Fierro et al., 2015). Direct effects from forest practices including road-building, fertiliser application and erosion due to forest management increasing find sediment entering streams (Kansagaki et al., 2008; Peña-Cortés et al., 2011a).

As in other studies in southern South America, the diet of Rainbow Trout consists mainly of benthic aquatic macroinvertebrates and a few terrestrial taxa, confirming the generalist-opportunist diet of this species in southern Chile (Arismendi et al., 2012; Di Prinzio et al., 2013; Vargas-Chacoff et al., 2013). The response of Rainbow Trout to food availability in this study, suggest that the diet is influenced by the type of riparian vegetation. Aquatic invertebrates are less available in the watershed with eucalyptus plantations. Indeed, some taxa that are not found in this watershed may be excluded from these streams, and thus a lower availability of prey to trout (Duffy et al., 2010).

Vegetation effects on prey availability in this study are concordant with previous studies (Romero et al., 2005; Mancilla et al., 2009) that suggests conversion of native forest to monoculture plantations may influence aquatic macroinvertebrate composition. The higher richness and abundance of macroinvertebrates in the native vegetation watershed may be explained by the preference of certain taxa for the organic matter derived from native forest. Abelho and Graça (1996) reports that aquatic macroinvertebrates use fewer leaves in streams dominated by eucalyptus than in streams dominated by native forest. A consequence of exotic plantations is the contribution of particulate organic matter and/or the food quality of the detritus to freshwaters ecosystems (Larrañaga et al., 2009). This low preference of macroinvertebrates, may be related to the characteristics of the eucalyptus leaves, for example the quantity of nutrients, and presence of secondary compounds (e.g. tannins) (Peralta-Maraver et al., 2011). It is difficult to conclude which of these factors accounts for the changes observed in our study, so future work should focus on resolving which are the principal consequences produced by eucalyptus species affecting benthic aquatic macroinvertebrates.

Although prey availability in this study was different between watersheds, we found no evidence for differences in FFG. This supports the hypothesis of Pozo et al. (1998) and Peralta-Maraver et al. (2011), who indicate that the organic matter contributed by eucalyptus (e.g., leaves and branches) is also colonised by collector-gatherers, shredders and predators. These groups wait for a time during which the leaves would be pre-conditioned by fungal and bacterial activity to then can consume. We suggest than the diet of rainbow trout based on FFG was not affected by watershed characteristics because the diet was comprise primarily on most abundant functional feeding groups recorded in the study area year round. Other FFG, like grazers or detritivores, had low presence at the sampling sites (prey availability and stomach content), would be limited by the scarce presence of periphyton or macrophytes. Both FFGs feeding on these elements, which have lower abundance and biomass in mountain headwaters, therefore may restrict the presence of this feedings groups. Temporal patterns of macroinvertebrate availability in these streams were similar to seasonal tendencies observed in other streams of South America (Hollmann and Miserendino, 2008; Epele et al., 2011; Fierro et al., 2015). The dependence of Rainbow Trout on this temporal pattern availability may be caused than their diet varied during the year, presenting a significant relation with the presence of prey items. The more numerous terrestrial species prey in the Rainbow Trout stomachs was in spring, compared to other seasons. This difference between seasons in the abundance of terrestrial prey ingested could be explained by higher reproduction of prey during this time, making them more abundant in riparian habitats and thus more available for consumption (Romero et al., 2005). This situation, together with the emergence of aquatic insects and their reduced presence in the benthos, would influence the diet of fish at this time of year (Buria et al., 2009; Da Silva et al., 2012).

Nevertheless, it is surprising to find that of the total taxa recorded in the benthos during the year, slightly over half were consumed. In fact, some taxa which were present in very low abundance in the benthos were well represented in the Rainbow Trout stomachs (e.g., some species of crustaceans). This difference could be explained since salmonids are mainly visual predators (Eggers, 1978), and prey which are large in size but few in number in the benthos are more exposed to predation (Buria et al., 2007). In addition other taxa recorded in the benthos were not recorded in the stomachs, may be smaller prey species can seek protection in safe refuges among the rocks, where they are at less risk of predation because they are less visible (McCutchen, 2002). It should be noted however, that the individuals examined in the stomach often are much degraded, therefore many times was not possible identify to level genera o specie, as if was possible in benthos sample. Anyway, these results show that the prey selectiveness of the *O. mykiss* is conditioned by the seasonal availability of the prey, and by their size and accessibility.

Conclusion

This study shows that exotic vegetation may produce an impact on environmental variables and benthic macroinvertebrates communities, leading to changes in stream food webs. Our findings show that land use changes in southern Chile, mainly due to monoculture plantations of forestry species replacing native forest, seem to affect the dissolved solids, suspended solids, nitrates, chlorides and sulphates on streams. Furthermore, these land use changes appear to affect the composition of aquatic macroinvertebrate assemblages. However, these effects are not seen at the functional feeding group level due to the fact that collectors-gatherers are still the most abundant group under both land use types. Lastly, the diet of

Rainbow Trout is based mainly on the most abundant taxa and FFG in the benthos in both watersheds all year round. Therefore, the use of diets of Rainbow Trout may serve as a good tool for stream ecosystem assessment. Lastly, if the deforestation of native forest in the watersheds of southern Chile continues to increase, accompanied by an increase in plantations of exotic species, we may expect the disappearance of certain species of benthic macroinvertebrates, especially in the most vulnerable systems.

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Tables and Figures

Table 1. Summary of watershed characteristics at the study sites (n = 12) in southern Chile.

Land use (%)	Site code	Basin/ Sub-basin	Watershed size (km^2)	Stream order	Altitude (masl)	Active channel width (m)	Water Velocity ($m\ s^{-1}$)	Depth (m)	Substrate type[1]
		Moncul							
> 60 % Exotic forestry species	E1	Danquil	19.23	2	19	3.50	0.49	0.80	Peb/Gra
	E2	Cabrero	26.15	3	36	5.00	1.55	0.60	Peb/Cob
	E3	El Peral	67.93	4	30	25.00	0.66	0.50	Peb/Cob
	E4	Puyanhue	108.53	4	125	15.00	0.55	0.90	Peb/Cob
		Queule							
> 60% Native forest	N1	Boldo River	308.31	4	99	20.00	0.90	0.50	Bou/Peb/Cob
	N2	Boldo River	308.31	3	101	5.00	1.70	0.30	Bou/Peb/Cob
	N3	Lovera stream	24.28	3	25	18.00	1.14	0.45	Peb/Cob
	N4	Ramírez stream	21.89	3	27	8.00	0.80	0.50	Peb/Cob
	N5	Boroa River	82.93	3	66	10.00	2.51	0.60	Bou/Peb/Cob
	N6	Lovera stream	24.28	3	25	6.00	1.78	0.70	Peb/Cob
	N7	Piren stream	48.15	3	26	8.00	1.50	0.90	Peb/Cob
	N8	Piren stream	48.15	3	25	18.00	1.08	0.50	Peb/Cob/Gra

[1]Bou: boulders, Peb: pebbles, Cob: cobbles, Gra: gravel.

Table 2. Physical and chemical characteristics of streams across seasons. Values represent average ± SD.

Basin	Sites	Temperature (°C)	Electrical conductivity (µS cm^{-1})	Total dissolved solids (mg L^{-1})	pH	Suspended solids (mg.L^{-1})	Dissolved oxygen (mg. L^{-1})	DBO5 (mg .L^{-1})	Phosphates (µg L^{-1})	Nitrates (mg L^{-1})	Apparent colour (Pt.Co^{-1})	Chlorides (mg L^{-1})	Sulphates (mg L^{-1})
Exotic	E1	10.35 ± 3.1	57.80 ± 1	49.38 ± 17.6	6.79 ± 0.3	7.50 ± 3.9	10.86 ± 1.1	1.80 ± 0.8	43.10 ± 22.3	1.73 ± 0.3	27.75 ± 15	16.55 ± 2.9	2.36 ± 2.7
	E2	10.80 ± 3.2	40.20 ± 2.6	35.00 ± 12.9	6.9125 ± 0.3	5.80 ± 1.2	10.98 ± 1.2	2.25 ± 0.6	47.60 ± 52.1	1.25 ± 0.6	32.00 ± 8.9	13.53 ± 1.6	0.83 ± 0.2
	E3	10.98 ± 3.4	44.23 ± 9.3	27.13 ± 13.5	6.82 ± 0.3	6.99 ± 8.6	11.10 ± 1.2	2.38 ± 0.4	41.98 ± 31.9	1.45 ± 0.3	36.97 ± 11.7	11.98 ± 2.1	1.13 ± 0.2
	E4	10.30 ± 3.0	40.88 ± 0.3	27.13 ± 13.5	6.65 ± 0.3	8.57 ± 5.4	10.65 ± 1.1	2.10 ± 0.7	58.03 ± 30.9	1.38 ± 0.4	42.43 ± 7.8	14.30 ± 1.6	1.42 ± 0.7
Native	N1	10.40 ± 4.0	24.70 ± 0.8	29.38 ± 14.9	6.95 ± 0.3	1.83 ± 0.8	11.15 ± 1.1	2.55 ± 1	59.88 ± 26.7	0.75 ± 0.2	39.10 ± 14.3	9.65 ± 0.6	1.87 ± 1.4
	N2	10.48 ± 3.6	22.78 ± 1.3	29.38 ± 14.9	6.74 ± 0.3	3.02 ± 1.3	10.93 ± 1.3	2.25 ± 0.7	76.48 ± 15.1	1.10 ± 0.2	37.95 ± 13.2	11.28 ± 1.7	1.27 ± 0.3
	N3	12.25 ± 3.2	34.08 ± 2.1	37.88 ± 19.5	6.70 ± 0.1	5.55 ± 4.9	8.20 ± 5	1.78 ± 0.5	72.93 ± 27.8	0.90 ± 0.4	44.35 ± 19.4	12.58 ± 0.9	1.77 ± 0.9
	N4	12.35 ± 3.8	33.35 ± 1.3	18.00 ± 9.3	6.80 ± 0.2	4.25 ± 1.6	8.33 ± 5.1	1.78 ± 0.1	71.70 ± 31.6	0.90 ± 0.2	40.58 ± 16.9	11.18 ± 2.3	1.02 ± 0.3
	N5	11.28 ± 2.7	33.85 ± 0.9	16.13 ± 8.3	6.76 ± 0.1	2.45 ± 1.9	10.88 ± 1	2.35 ± 0.6	61.93 ± 44.4	0.70 ± 0.1	39.28 ± 15.7	10.83 ± 1.1	1.06 ± 0.3
	N6	10.95 ± 2.7	37.28 ± 1.9	24.13 ± 12.4	6.83 ± 0.1	6.29 ± 3.3	10.60 ± 0.8	2.05 ± 0.4	54.88 ± 22.5	1.08 ± 0.2	45.38 ± 19.2	10.95 ± 1.6	1.37 ± 0.3
	N7	10.38 ± 2.6	40.50 ± 1.6	28.88 ± 14.9	6.71 ± 0.2	6.82 ± 1.6	10.85 ± 1	2.60 ± 0.8	71.30 ± 53.6	0.98 ± 0.2	35.60 ± 11.7	12.53 ± 2.4	1.06 ± 0.1
	N8	10.38 ± 2.2	36.43 ± 0.7	25.63 ± 12.3	6.65 ± 0.2	1.44 ± 1.2	11.25 ± 1.2	2.93 ± 1.1	62.10 ± 35.8	1.05 ± 0.3	39.80 ± 13.9	9.98 ± 1.4	1.12 ± 0.3

Table 3. Frequency, standard length and weight of *Oncorhynchus mykiss* in the Araucanía Region (Chile) during the study period.

	N		**Lenght (mm)**			**Weight (g)**		
	Native	Exotic	Min	Max	Average	Min	Max	Average
Summer	29	23	3.3	18.7	7.83	0.19	55.5	7.32
Autumn	69	35	5.4	19.2	9.12	1.82	70.07	10.99
Winter	38	7	5.6	16.5	9.95	1.51	30.12	9.71
Spring	35	8	4.6	19.8	12.57	1.68	252.87	29.76

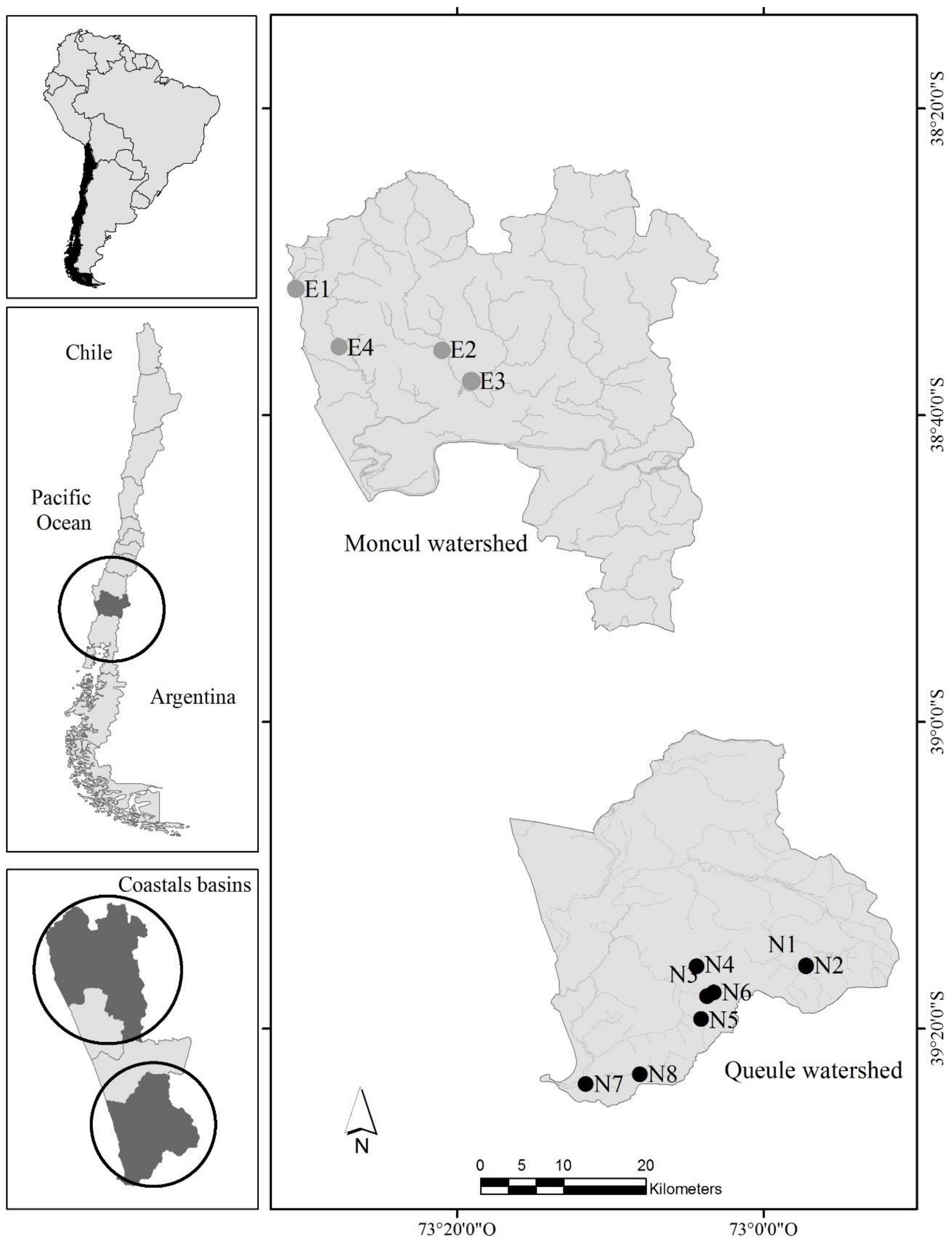


Fig. 1. Map of the study area and study sites (n = 12) from two watersheds (Moncul watershed – exotic vegetation; Queule watershed – native vegetation) of the Araucanía Region in southern Chile.

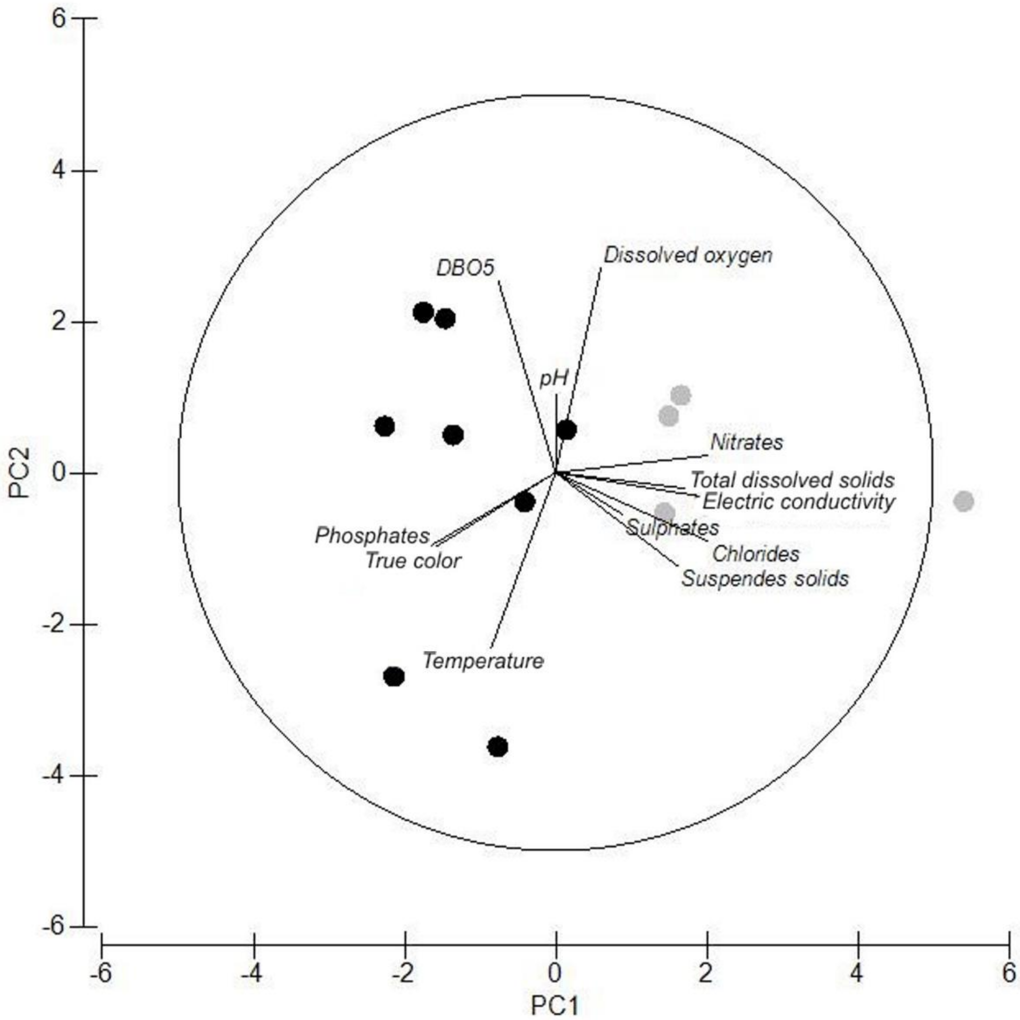


Figure 2. Principal component analysis (PCA) of environmental variables at sites dominated by exotic vegetation (grey circles) and at sites dominated by native vegetation (black circles).

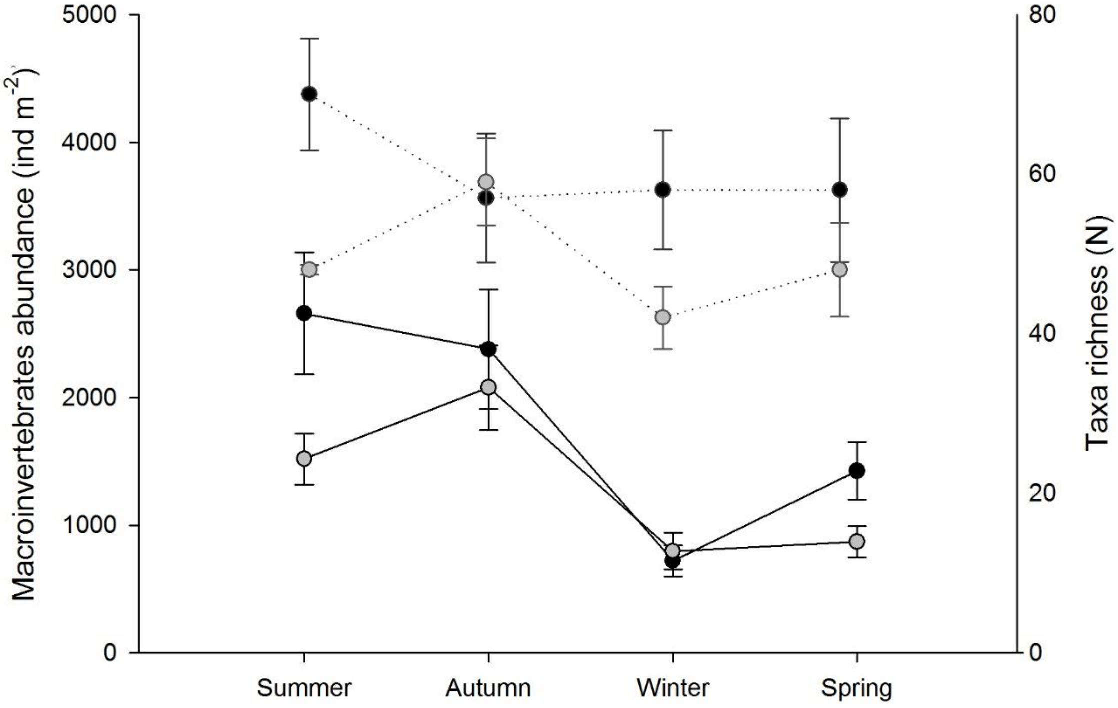
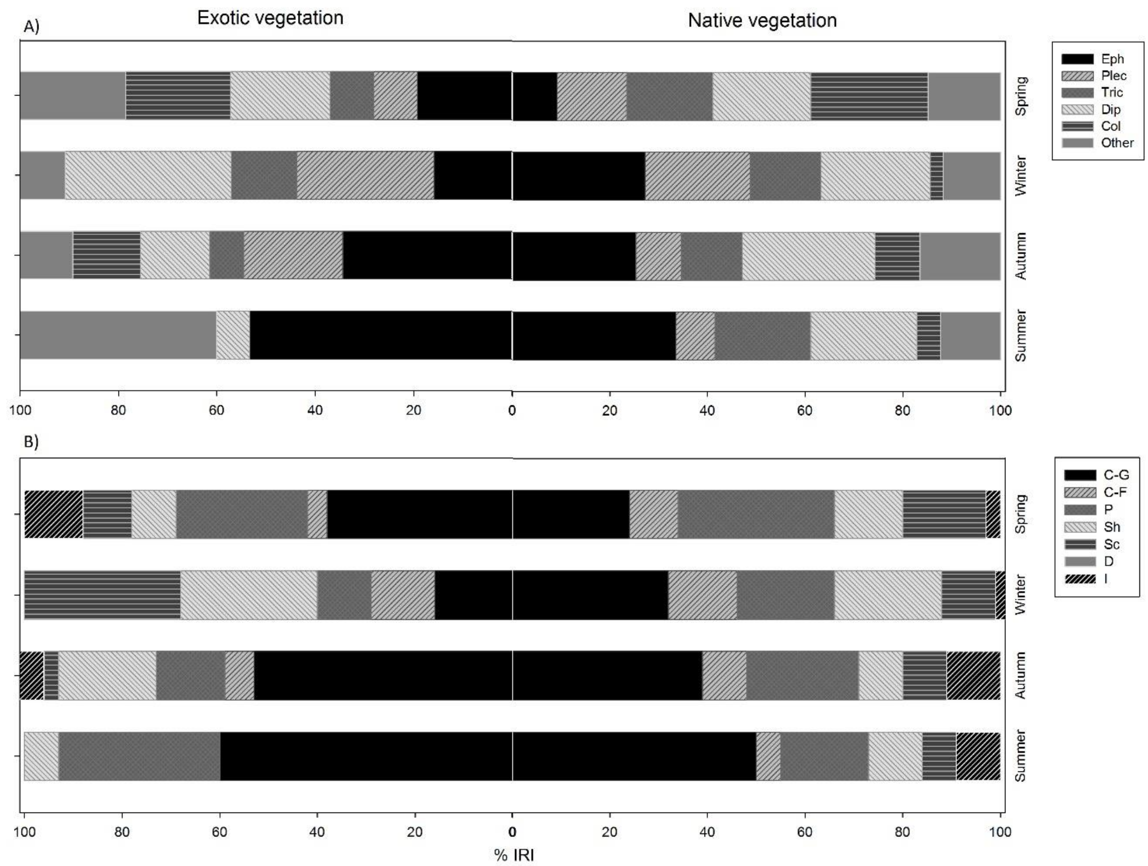


Fig. 3. Average ± SD of macroinvertebrate abundances (solid lines - ind m^{-2}) and number of taxa (dotted lines) across seasons at the watershed dominated by native vegetation (black circles) and the watershed dominated by exotic vegetation (grey circles).



.**Fig. 4.** Index of Relative Importance (IRI) of prey items by taxa (A) (Eph: Ephemeroptera; Plec: Plecoptera; Tri: Trichoptera, Dip: Diptera; Col: Coleoptera; Other: Other taxa) and functional feeding groups (B) (C-G: Collector-gatherer; C-F: Collector-filterer; P: Predator; Sh: Shredder; Sc: Scraper; D: Detritivore; I: Indeterminate) across seasons from stomach contents of trout (*O. mykiss*) at the watershed dominated by native vegetation (right panel) and the watershed dominated by exotic vegetation (left panel).

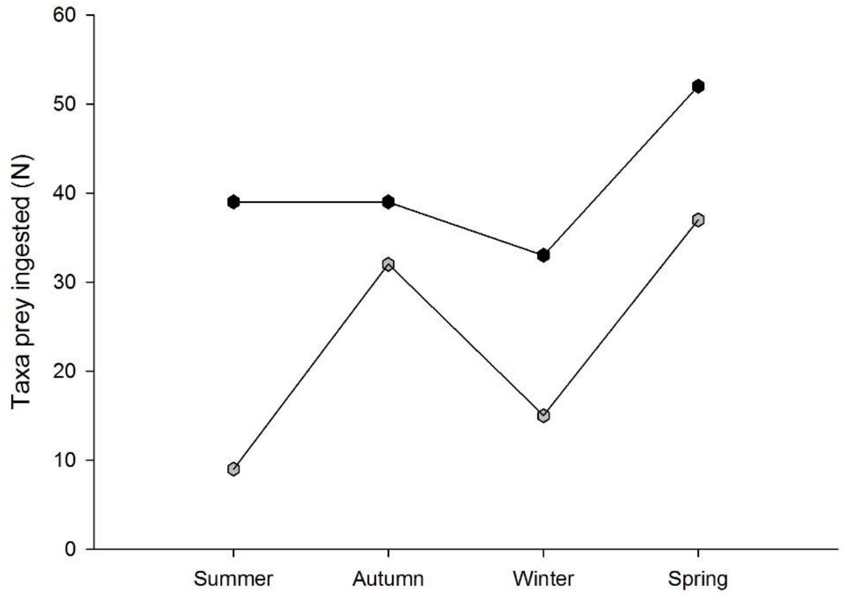


Fig. 5. Seasonal changes in the mean number of taxa ingested in trout (*O. mykiss*) at the watershed dominated by native vegetation (black circles) and the watershed dominated by exotic vegetation (grey circles).

Appendice

Appendix I. Summary of abundances (ind m-2) of benthic macroinvertebrates by taxa sampled from two watersheds in southern Chile. The Functional Feeding Group (FFG) for each taxon are also indicated.

		Summer		Autumn		Winter		Spring	
	FFG	Native	Exotic	Native	Exotic	Native	Exotic	Native	Exotic
Andesiops torrens	CG	59.43	5.89	36.40	9.39	5.38	10.78	21.29	9.73
Andesiops peruvianus	CG	17.76	29.78	15.70	15.78	4.04	5.22	12.13	7.13
Chiloporter eatoni	P	0.83	0.00	0.27	0.00	0.33	0.00	1.04	0.13
Chaquihua bullocki	P	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00
Caenis chilensis	CG	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Siphonella guttata	CG	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Murphyella needhami	CF	0.24	0.11	0.23	0.17	0.33	0.11	1.38	0.00
Nousia maculata	CG	0.00	7.56	0.67	7.83	0.29	0.11	0.50	0.53
Nousia delicata	CG	0.67	0.00	2.77	0.22	0.17	0.00	0.08	0.00
Nousia sp.	CG	0.00	0.11	0.00	0.11	0.00	0.00	0.00	0.00
Meridialaris diguillina	CG	53.24	12.33	46.07	20.17	13.58	17.22	18.96	14.07
Meridialaris chilooense	CG	6.14	0.00	0.20	0.00	0.00	0.00	0.04	0.00
Hapsiphlebia anastomosis	CG	5.74	0.11	1.03	0.44	0.13	0.11	0.29	6.07
Massarttellopsis irrarazavali	CG	0.05	0.00	5.47	0.00	0.33	3.56	0.00	0.00
Penaphlebia chilensis	CG	1.40	1.67	9.67	8.39	1.13	2.11	0.63	1.87
Penaphlebia vinosa	CG	0.00	0.00	0.00	0.00	0.00	1.11	0.00	0.13
Penaphlebia sp.	CG	0.00	0.33	0.00	0.00	0.13	0.44	0.00	0.00
Diamphipnopsis samali	S	6.67	2.56	0.80	0.56	0.63	0.00	9.21	0.13
Diamphipnoa helgae	S	1.14	0.00	0.17	0.17	0.83	0.33	0.21	0.20
Diamphipnoidae	S	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00
Kempnyella genualis	P	0.05	0.33	0.33	0.11	0.08	0.00	0.29	0.00
Inconeuria porteri	P	0.36	0.22	0.23	0.56	0.00	0.00	0.13	0.20
Pictoperla gayi	P	0.00	0.00	0.00	0.06	0.04	0.00	0.00	0.00
Perlidae	P	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00
Neuroperlopsis patris	S	0.19	0.44	0.27	0.83	0.00	0.56	0.25	0.33
Penturoperla barbata	S	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00
Klapopteryx armillata	S	10.24	1.22	3.80	0.83	0.58	0.00	11.54	0.73
Udamocercia sp.	SC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.27
Astronemoura chilena	S	0.69	0.78	0.13	1.61	0.00	0.00	0.04	0.27
Pelurgoperla personata	S	0.43	6.11	0.00	0.94	0.08	0.00	0.17	1.53
Limnoperla jaffueli	S	15.45	8.78	41.43	33.00	22.54	19.67	17.54	9.67
Notoperlopsis femina	S	8.57	1.67	0.53	0.50	4.21	13.89	0.00	0.00
Antarctoperla michaelseni	S	0.05	0.00	0.37	12.11	0.04	0.00	0.00	0.00

Ceratoperla schwabei	S	0.00	0.44	0.00	0.11	0.00	0.00	0.04	0.00
Ecnomidae	CG	0.79	0.33	1.13	1.56	0.04	0.00	0.08	0.20
Hydrobiosidae	P	0.76	1.44	0.00	5.89	0.58	1.00	0.58	0.60
Leptoceridae	SC	0.05	0.11	0.00	0.06	0.08	0.11	0.04	0.00
Hydroptilidae	SC	0.19	0.00	0.00	0.06	0.00	0.00	0.04	0.00
Hydroptila sp.	CG	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Smicridea annulicornis	CF	0.57	2.56	6.47	17.61	0.00	0.00	0.00	0.00
Smicridea sp.	CF	1.21	21.11	0.70	2.56	1.38	0.67	2.46	1.20
Triplectides sp.	S	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00
Metrichia sp.	CG	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.20
Neotrichia sp.	CG	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00
Neotrichia chilensis	CG	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Austrotinodes sp.	CG	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00
Dolophilodes sp.	S	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00
Parasericostoma sp.	S	0.10	0.00	0.20	0.11	0.04	0.00	0.00	0.00
Polycentropus sp.	P	0.00	0.00	0.07	0.06	0.00	0.11	0.08	0.20
Brachysetodes sp.	SC	1.81	0.00	0.00	0.00	0.08	0.00	0.13	0.07
Rheocorema sp.	P	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00
Psychodidae	CG	0.00	0.00	0.13	0.00	0.04	0.00	0.04	0.00
Ephydridae	P	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Empididae	P	0.14	0.00	0.00	0.22	0.00	0.00	0.00	0.00
Hemerodromia sp	P	0.36	0.11	0.00	0.06	0.04	0.11	1.13	0.20
Simulium sp.	CF	1.52	5.78	5.63	20.33	2.25	1.22	0.67	0.47
Arauchnephioides sp.	CF	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07
Gigantodax sp.	CF	0.52	0.56	0.13	0.06	0.21	0.11	0.04	0.00
Blephabericeridae	SC	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00
Tipula sp.	P	1.29	0.00	0.00	0.00	0.00	0.11	1.42	0.13
Atherix sp.	P	1.48	0.11	3.37	2.83	0.50	0.11	0.83	0.40
Hexatoma sp.	P	2.12	0.11	9.37	7.61	8.88	12.89	18.83	9.27
Limonia sp.	P	2.24	1.22	0.33	1.78	0.25	0.56	1.42	6.20
Tipulidae	P	0.57	0.00	0.00	0.17	0.00	0.00	0.00	0.00
Stilobezzia sp.	P	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00
Alluaudomyia sp.	P	1.14	0.22	0.40	0.17	0.33	0.11	0.46	0.27
Corynoneura sp.	CG	5.52	1.89	7.30	0.56	0.92	0.89	1.25	0.40
Eukiefierella sp.	CG	8.55	24.44	1.90	24.44	0.17	0.11	10.50	3.40
Dicrotendipes sp.	CG	0.24	0.00	0.07	0.44	0.08	0.00	0.00	0.00
Coelotanypus mendax	CG	0.10	0.11	0.07	0.00	0.00	0.00	0.08	0.13
Lopescladius sp.	CG	1.38	1.89	1.80	0.56	0.42	1.00	3.00	5.07
Orthocladius sp.	CG	28.55	12.11	3.33	11.56	4.96	1.11	6.54	6.00
Paratrichocladius sp.	CG	0.00	0.11	0.00	0.11	0.04	0.00	0.00	0.00
Pentaneura sp.	CG	9.02	1.78	0.20	0.17	0.04	0.11	1.00	0.40
Rheotanytarsus sp.	CG	0.00	0.00	0.00	0.00	0.08	0.00	2.25	0.00
Tanytarsus sp.	CG	0.14	0.33	0.00	1.28	0.00	0.00	0.88	0.00

Thienemaniella sp.	CG	5.05	0.11	12.70	2.06	3.08	2.67	1.67	0.33
Symbiocladius wygodzinskyi	PA	0.43	0.00	0.13	0.06	0.00	0.00	0.25	0.00
Austrolimnius sp.	CG	22.55	9.00	8.37	7.89	1.58	0.67	3.75	5.93
Austrelmis sp.	CG	0.05	0.00	0.00	0.00	0.00	0.00	0.04	0.40
Luchoelmis sp.	CG	0.05	0.00	0.20	0.28	0.00	0.00	0.13	0.00
Tychepsephenus felix	SC	0.69	0.22	1.17	0.67	0.58	0.00	1.13	0.27
Haliplidae	SC	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Haliplus sp.	SC	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00
Hydrophilidae	P	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Coleoptera	P	0.00	0.00	0.20	0.11	0.00	0.11	0.04	0.00
Protochauliodes sp.	P	0.10	0.00	0.17	0.00	0.00	0.00	0.00	0.13
Neogomphus sp.	P	0.05	0.00	0.43	0.00	0.00	0.00	0.13	0.00
Hydracarina	P	0.43	0.11	0.07	0.11	0.08	0.00	0.00	0.07
Littoridina cumingi	SC	0.19	0.11	0.00	0.00	0.00	0.00	0.08	0.00
Aegla araucaniensis	P	1.40	0.11	0.93	0.50	0.46	0.11	1.46	1.00
Aegla abtao	P	0.14	0.00	0.00	0.00	0.04	0.00	0.04	0.00
Aegla sp.	P	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.07
Chilina dombeyana	SC	0.14	0.00	0.20	0.00	0.04	0.00	0.00	0.07
Dugesia sp.	D	0.00	0.11	0.00	0.00	0.04	0.00	0.00	0.00
Hyalella costera	CG	0.05	0.00	0.00	0.00	0.04	0.00	0.04	0.00
Hyalella sp.	CG	0.29	0.00	0.00	0.17	0.00	0.00	0.00	0.00
Tubifex sp.	D	3.45	1.22	3.83	4.50	1.38	0.33	0.04	0.13
Chaetogaster sp.	D	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00
Lumbriculidae	D	0.00	0.00	0.07	0.17	0.13	0.00	0.00	0.00
Naididae	D	0.10	0.22	0.00	0.00	0.04	0.00	0.00	0.00
Heterias exul	CG	0.10	0.00	0.00	0.00	0.00	0.00	0.08	0.40
Temnocephala chilensis	PA	0.00	0.00	1.87	0.33	0.00	0.00	0.00	0.00

Appendix II. Index of Relative Importance (IRI) of the prey items in the stomach of O. mykiss in the two watersheds in southern Chile during the study period.

	Summer		Autumn		Winter		Spring	
	Native	Exotic	Native	Exotic	Native	Exotic	Native	Exotic
Andesiops peruvianus	1.45	2.57	1.61	1.53	0.89	1.00	0.46	0.77
Andesiops torrens	0.64	1.14	0.86	0.94	0.59	0.27	0.15	0.32
Baetidae	0.00	0.00	0.22	0.13	0.00	0.00	0.23	0.89
Meridialaris sp.	0.19	0.29	0.11	0.28	0.74	0.45	0.13	0.00
Meridialaris diguillina	0.60	0.29	0.00	0.00	0.00	0.00	0.00	0.00
Nousia sp.	0.40	0.00	0.00	0.16	0.55	0.00	0.12	0.00
Leptophlebiidae	0.24	0.00	0.27	0.31	0.18	0.00	0.18	0.44
Hapsiphlebia anastomosis	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Penaphlebia sp.	0.32	0.00	0.00	0.00	0.37	0.00	0.00	0.00
Ephemeroptera	1.72	0.29	0.57	2.00	0.40	0.00	0.24	0.68
Notoperlopsis femina	0.00	0.00	0.13	0.63	0.27	0.45	0.58	0.55
Limnoperla jaffuelli	0.40	0.00	0.11	0.31	0.33	1.64	0.63	0.66
Antactoperla michaelseni	0.00	0.00	0.11	0.31	1.64	0.55	0.00	0.00
Gripopterygidae sp1.	0.40	0.00	0.45	0.19	0.00	0.00	0.00	0.00
Gripopterygidae sp2.	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pelurgoperla personata	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00
Diamphipnopsis samali	0.00	0.00	0.00	0.00	0.15	0.00	0.12	0.00
Diamphipnoa helgae	0.00	0.00	0.00	0.00	0.15	0.00	0.76	0.00
Neuroperlopsis patris	0.00	0.00	0.00	0.00	0.00	0.27	0.13	0.00
Plecoptera	0.16	0.00	0.53	1.67	0.27	0.14	0.13	0.22
Smicridea sp.	0.56	0.00	0.92	0.51	0.59	1.00	0.23	0.33
Anomalopsychidae	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.00
Leptoceridae	0.40	0.00	0.00	0.31	0.00	0.00	0.76	0.00
Limnephilidae	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00
Helicopsychidae	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Helicophidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33
Hydrobiosidae	0.40	0.00	0.00	0.00	0.37	0.45	0.25	0.00
Polycentropus sp.	0.00	0.00	0.00	0.13	0.00	0.00	0.58	0.00
Hydroptilidae	0.00	0.00	0.11	0.00	0.74	0.00	0.13	0.00
Glossosomatidae	0.40	0.00	0.00	0.00	0.00	0.00	0.13	0.55
Ecnomidae	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Trichoptera	0.79	0.00	0.75	0.13	0.30	0.00	0.11	0.22
Psychodidae	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ptychopteridae	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.38
Tipula sp.	0.00	0.00	0.00	0.00	0.37	0.00	0.00	0.55
Limonia sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.38
Tipulidae	0.36	0.00	0.18	0.00	0.00	0.32	0.76	0.33
Simulium sp.	0.40	0.00	0.45	0.45	0.57	0.00	0.13	0.00
Simuliidae sp1.	0.00	0.00	0.00	0.00	0.44	0.00	1.30	0.39
Simuliidae sp2.	0.00	0.00	0.00	0.00	0.26	0.45	0.00	0.00
Alluaudomya sp.	0.36	0.00	0.11	0.00	0.00	0.00	0.13	0.00
Ceratopogonidae	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00
Rheotanytarsus sp.	0.40	0.00	0.00	0.00	0.00	0.00	0.13	0.00
Tanytarsus sp.	0.00	0.29	0.00	0.31	0.00	0.00	0.13	0.44
Paratrichocladius sp.	0.40	0.00	0.00	0.31	0.00	0.00	0.00	0.00
Orthocladius sp.	0.67	0.00	0.95	0.00	0.22	0.00	0.00	0.00
Coryneura sp.	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00

Pentaneura sp.	0.00	0.00	0.45	0.00	0.37	0.00	0.00	0.00
Chironomidae	0.60	0.29	0.19	0.85	0.00	0.00	0.14	0.22
Blephariceridae	0.00	0.00	0.11	0.00	0.37	2.45	0.00	0.00
Diptera	0.24	0.00	1.22	0.26	0.46	0.45	0.46	0.55
Tychepsephenus felix	0.00	0.00	0.18	0.00	0.00	0.00	0.13	0.19
Luchoelmis sp.	0.16	0.00	0.00	0.31	0.00	0.00	0.76	0.22
Austrolimnius sp.	0.16	0.00	0.35	0.88	0.00	0.00	0.13	0.00
Austrelmis sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.00
Phanocerus sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.55
Macrelmis sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.16
Elmidae	0.00	0.00	0.11	0.31	0.00	0.00	0.11	0.22
Dryopidae	0.00	0.00	0.00	0.00	0.00	0.00	0.35	0.55
Dystiscidae	0.40	0.00	0.45	0.31	0.00	0.00	0.36	0.55
Haliplidae	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.00
Hydrophilidae	0.00	0.00	0.11	0.31	0.00	0.00	0.13	0.00
Staphylinidae	0.00	0.00	0.11	0.00	0.00	0.00	0.13	0.22
Coleoptera	0.12	0.00	0.00	0.00	0.37	0.00	0.46	0.77
Belostomatidae	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.55
Corixidae	0.48	0.00	0.63	0.26	0.00	0.00	0.69	0.00
Mesoveliidae	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00
Hemiptera	0.00	0.00	0.18	0.31	0.37	0.00	0.76	0.33
Lepidoptera	0.14	0.57	0.00	0.00	0.00	0.00	0.00	0.00
Formicidae	0.79	0.00	0.00	0.00	0.00	0.00	0.26	0.70
Littoridina cumingi	0.00	0.00	0.45	0.00	0.37	0.00	0.58	0.00
Chilina sp.	0.40	0.00	0.45	0.13	0.00	0.00	0.00	0.00
Chilina dombeyana	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.55
Aegla sp.	0.36	0.00	0.18	0.28	0.37	0.00	0.36	0.00
Crustacea	0.40	2.86	0.13	0.31	0.22	0.00	0.13	0.33
Aranae	0.00	0.00	0.00	0.00	0.15	0.00	0.35	0.22
Heterias exul	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.77
Oligochaeta	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00
Unknown	0.00	0.00	0.87	0.63	0.00	0.00	0.00	0.55

Appendix III. Taxa macroinvertebrates found in the analysed samples from benthos and stomach Oncorhynchus mykiss from the watershed dominated by native vegetation (black circles) and the watershed dominated by exotic vegetation (grey circles).

	Macroinvertebrates		Diet *O. mykiss*	
Land use / Taxa	Native	Exotic	Native	Exotic
Ephemeroptera				
Andesiops torrens	●	●	●	●
Andesiops peruvianus	●	●	●	●
Baetidae			●	●
Chiloporter eatoni	●	●		
Chaquihua bullocki	●			
Caenis chilensis				
Siphonella guttata	●			
Murphyella needhami	●	●		
Nousia maculata	●	●		
Nousia delicata	●	●		
Nousia sp.		●	●	●
Meridialaris diguillina	●	●	●	●
Meridialaris chilooense	●			
Meridialaris sp.			●	●
Hapsiphlebia anastomosis	●	●	●	
Massarttellopsis irrarazavali	●	●		
Penaphlebia chilensis	●	●		
Penaphlebia vinosa		●		
Penaphlebia sp.	●	●	●	
Leptophlebiidae			●	●
Ephemeroptera n/i			●	●
Plecoptera				
Diamphipnopsis samali	●	●	●	
Diamphipnoa helgae	●	●	●	
Diamphipnoidae		●		
Kempnyella genualis	●	●		
Inconeuria porteri	●	●		
Pictoperla gayi	●	●		
Perlidae	●			
Neuroperlopsis patris	●	●	●	●
Penturoperla barbata		●		
Klapopteryx armillata	●	●		
Udamocercia sp.		●		
Astronemoura chilena	●	●		
Pelurgoperla personata	●	●	●	

Limnoperla jaffueli	●	●	●	●
Notoperlopsis femina	●	●	●	●
Antarctoperla michaelseni	●	●	●	●
Ceratoperla schwabei	●	●		
Gripopterygidae sp1			●	●
Gripopterygidae sp2			●	
Plecoptera n/i			●	●
Trichoptera				
Anomalopsychidae			●	
Ecnomidae	●	●	●	
Glossosomatidae			●	●
Helicopsychidae			●	
Helicophidae				●
Hydrobiosidae	●	●	●	●
Leptoceridae	●	●	●	●
Limnephilidae			●	
Hydroptilidae	●	●	●	
Hydroptila sp.	●			
Smicridea annulicornis	●	●		
Smicridea sp.	●	●	●	●
Triplectides sp.		●		
Metrichia sp.	●	●		
Neotrichia sp.	●			
Neotrichia chilensis	●			
Austrotinodes sp.		●		
Dolophilodes sp.	●			
Parasericostoma sp.	●	●		
Polycentropus sp.	●	●	●	●
Brachysetodes sp.	●	●		
Rheocorema sp.	●			
Trichoptera n/i			●	●
Diptera				
Psychodidae	●		●	
Ptychopteridae			●	●
Ephydridae				
Empididae	●	●		
Hemerodromia sp	●	●		
Simulium sp.	●	●	●	●
Arauchnephioides sp.		●		
Gigantodax sp.	●	●		
Simuliidae sp1			●	●
Simuliidae sp2			●	●
Blephabericeridae	●		●	●
Tipula sp.	●	●	●	●

Atherix sp.	●	●		
Hexatoma sp.	●	●		
Limonia sp.	●	●		●
Tipulidae	●	●	●	●
Stilobezzia sp.	●			
Alluaudomyia sp.	●	●	●	
Ceratopogonidae			●	
Corynoneura sp.	●	●	●	
Eukiefierella sp.	●	●		
Dicrotendipes sp.	●	●		
Coelotanypus mendax	●	●		
Lopescladius sp.	●	●		
Orthocladius sp.	●	●	●	
Paratrichocladius sp.	●	●	●	●
Pentaneura sp.	●	●	●	
Rheotanytarsus sp.	●		●	
Tanytarsus sp.	●	●	●	●
Thienemaniella sp.	●	●		
Symbiocladius wygodzinskyi	●	●		
Chironomidae			●	●
Diptera n/i			●	●
Coleoptera				
Austrolimnius sp.	●	●	●	●
Austrelmis sp.	●	●	●	
Luchoelmis sp.	●	●	●	●
Elmidae			●	●
Phanocerus sp.			●	●
Macrelmis sp.			●	●
Tychepsephenus felix	●	●	●	●
Dryopidae			●	●
Dystiscidae			●	●
Haliplidae	●		●	
Haliplus sp.	●			
Hydrophilidae	●		●	●
Staphylinidae			●	●
Coleoptera n/i	●	●	●	●
Hemiptera				
Belostomatidae			●	●
Corixidae			●	●
Mesoveliidae			●	
Hemiptera n/i			●	●
Megaloptera				
Protochauliodes sp.	●	●		

Odonata				
Neogomphus sp.	•			
Lepidoptera			•	•
Hymenoptera				
Formicidae			•	•
Non Insecta				
Hydracarina	•	•		
Araneae			•	•
Littoridina cumingi	•	•	•	
Aegla araucaniensis	•	•		
Aegla abtao	•			
Aegla sp.		•	•	•
Crustacea			•	•
Chilina dombeyana	•	•		•
Chilina sp.			•	•
Dugesia sp.	•	•		
Hyalella costera	•			
Hyalella sp.	•	•		
Tubifex sp.	•	•		
Chaetogaster sp.		•		
Lumbriculidae	•	•		
Naididae	•	•		
Oligochaeta			•	
Heterias exul	•	•	•	•
Temnocephala chilensis	•			
Unknown			•	•
N taxa	91	81	76	56

Capítulo 5: A benthic macroinvertebrate multimetric index for Chilean Mediterranean streams

Este capítulo está basado en:

Fierro P, Arismendi I, Hughes RM, Valdovinos C, Jara-Flores A. A benthic macroinvertebrate multimetric index for Chilean Mediterranean streams. *Submitted to Ecological Indicators*

A benthic macroinvertebrate multimetric index for Chilean Mediterranean streams

Pablo Fierro[a,b,*], Ivan Arismendi[c], Robert M. Hughes[c,d], Claudio Valdovinos[a], Alfonso Jara-Flores[a]

[a] Center of Environmental Sciences EULA-CHILE, Universidad de Concepción, Chile

[b] Institute of Marine Science and Limnology, Universidad Austral de Chile, Chile

[c] Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR, 97330 USA

[d] Amnis Opes Institute, Corvallis, OR 97333

*Corresponding author e-mail: pablofierror@gmail.com.

Introduction

Freshwater ecosystems are among the most threatened systems around the world 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 chemical and physical parameters, however, this approach considers only conditions at the time and place of sampling (Oberdorff and Hughes, 1992; Fierro et al., 2017). More recently, research has been focused on the use of biological indicators, such as benthic macroinvertebrates, that could reflect longer term conditions of aquatic communities (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 et al., 2015; Silva et al., In Press). Yet, little attention has been paid to systems with high levels of isolation and endemism, such as southern South America (Fierro et al., 2015; 2016).

An advantage of using MMIs is their ability to synthesize multifaceted biological attributes of benthic macroinvertebrate assemblages (e.g., taxonomic richness, habitat and trophic guild composition, health and abundance) into a score that represents 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 thus, a unique index may be needed for each major ecoregion (Pont et al., 2009; Herman and Nejadhashem, 2015). Scores of MMIs 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). The adoption of MMIs to understand the impacts of human related influences on freshwaters at understudied and low population density regions of the world warrants a proper selection of least-disturbed locations as reference sites to assess the impact of human interventions as well as 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 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). During recent decades, this Chilean Mediterranean Ecoregion has been under severe threats, principally by changes in land use from native forest to agriculture, urbanization, monoculture plantations of exotic trees (Pauchard et al., 2006; Fierro et al., 2012; Hernández et al., 2016), and nonnative aquatic invasive species (Arismendi et al., 2014; Vargas et al., 2015). Whereas most of the research related to Mediterranean ecosystems has been focused on terrestrial ecosystems, little is known about freshwaters (Cooper et al., 2013). Rivers and streams are increasingly affected by multiple physicochemical and biological stressors and thus, this region is of particular interest to better assess its environmental conditions (Fierro et al., 2017).

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 human-related 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.

Materials and methods

2.1 Study area

We conducted this study in five large river basins of the Mediterranean Ecoregion of Chile: Aconcagua (7,340 km^2), Maipo (15,304 km^2), Rapel (13,695 km^2), Mataquito (6,190 km^2), and Maule (20,295 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 forest plantation areas have been accompanied by incessant urban growth (Pauchard et al., 2006; Hernández et al., 2016).

2.2 Site selection and data collection

We sampled a total of 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). The stream sites ranged from first- to sixth-order (i.e., 1 - 81 m wetted channel width; 12 - 2,106 m.a.s.l.). Field samples were collected during the Austral summer (December 2015 to March 2016). At each site, we measured *in situ* conditions of temperature (°C), pH, conductivity ($\mu s \cdot cm^{-1}$), total dissolved solids ($mg \cdot l^{-1}$), and dissolved oxygen ($mg \cdot l^{-1}$) using a Hanna Multiparameter Model HI 9828. We evaluated stream channel conditions that included average depth, mean active channel width, and mean wetted width using a tape measure. 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^2 grid.

2.3 Macroinvertebrate sampling

Six separate samples were taken from riffle habitats by using a Surber net (500 µm mesh size; 0.09 m^2 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, and forest plantation. The urban and agricultural land uses were weighted following Ligeiro et al. (2013) and Terra et al. (2013). The forest plantation weight was adapted from Fierro et al. (2015). The CDI was calculated as the sum of land use types, each one weighted differently as:

Catchment disturbance index (CDI) = 4 x %urban + 2 x %agricultural + 0.5 x %forest 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 riparian zone (i.e., buildings, agriculture, trash, logging, mining, parks and lawns, effluent, pasture, pavement, roads, channel revetment). We made adaptations to some disturbances to reflect the activities present in our study area. Specifically, we replaced logging, park and lawns, pavement, and channel revetment with erosion, small-head dams, 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 proximity as in-channel or along the river bank (x 1.5), 1-10 m from the river bank (x 1.0), and >10 m from the river bank (x 0.667).

The values of the CDI ranged between 0 (no anthropogenic land use in the watershed) and 400 (all catchment urban). Whereas the value of the LDI can range between 0 (no evidence of anthropogenic perturbation) and 16.5 (evidence of all perturbations), the maximum theoretical value of the LDI is around 7 (Kauffman et al. 1999). Because the CDI and LDI indices do not have the same numerical scale, we standardized them following Ligeiro et al. (2013). We divided the maximum value of each index by its 75th percentile (i.e., CDI values were divided by 300 and LDI values were divided by 5). Then, we calculated the IDI following Ligeiro et al. (2013) as:

Integrated disturbance index (IDI): $[(LDI/5)^2 + (CDI/300)^2]^{1/2}$

We ranked sites from low to high disturbance based on the IDI gradient (Macedo et al. 2016). Mean and standard deviation were calculated from the IDI observations and sites were classified as least-disturbed (below the mean minus one SD) and most-disturbed (above the mean plus one SD). Moderately disturbed sites were those in between the two categories defined above. To validate the IDI we followed Terra et al. (2013) and used physical-chemical variables and habitat metrics calculated in the field (i.e., temperature, dissolved oxygen, conductivity, pH, total dissolved solids, mean depth, mean width, mean wetted width, %clay, %sand, %cobble, %boulder, mean substrate diameter, %macrophyte coverage, %leaves, and %large wood). We conducted a principal component analysis (PCA) using all environmental variables (Table 1) and the IDI classes as factors. After that, we used a nonmetric multidimensional scaling (nMDS) ordination technique to determine the position of samples along the main environmental gradients. The nMDS was based on PCA axis 1 against the IDI scores. We performed these analyses in PRIMER v6.1 software (Clarke and Gorley, 2006).

2.5 Candidate biological metrics

We considered 74 candidate metrics commonly used in previous studies of macroinvertebrate responses to anthropogenic pressures in Chile and elsewhere (Villamarin et al., 2013; Fierro et al., 2015; Macedo et al., 2016, see Appendix A). These metrics represented a range of structural and functional macroinvertebrate assemblage characteristics such as diversity (17.3% of the metrics), species composition (53.3%), trophic structure (17.3%), and tolerance to pollution (12%). Trophic structure metrics were selected based on functional feeding groups (FFGs) following the criteria of Merrit and Cummins (1996) and Fierro et al. (2016). Tolerance metrics were based on taxa organic pollution tolerance scores and Hilsenhoff Biotic Index scores, following Fierro et al. (2012) and Mandaville (2002).

2.6 Metric selection and scoring

We screened a pool of candidate metrics through use of four stepwise criteria. First, we used a range test to eliminate metrics with narrow range or similar scores. Specifically, we eliminated metrics if more than a third of the sites had values of 0 and metrics with a range below three (Stoddard et al., 2008). Second, we determined the cross-correlation of metrics at reference sites to evaluate natural gradients (i.e., catchment area and altitude) unrelated to human-related disturbances. We calibrated those metrics that showed potential influence of natural gradients ($R \geq 0.75$, $p < 0.05$) by subtracting the regression-predicted metric values from each raw value (Klemm et al., 2003; Stoddard et al., 2008; Macedo et al., 2016). We replaced the original values of those metrics with the resulting residuals. Third, we evaluated metric responsiveness using a one-way analysis of variance using permutations PERMANOVA ($p < 0.05$). This method evaluated the ability of metrics to distinguish between least-disturbed and most-disturbed sites using IDI as one fixed-factor and least-disturbed versus most-disturbed sites as two-factors (Terra et al., 2013). Metrics with significantly different values ($p < 0.05$) were further screened through visual examination of boxplots. Fourth, we evaluated redundancy of metrics using the Spearman correlation coefficient ($R \geq 0.70$) and *p-value* < 0.05 (Stoddard et al., 2008; Mereta et al., 2013). We compared correlated metrics and retained those with the greatest PERMANOVA-score.

To reduce the impacts of possible outliers and variability of the MMI, we used the 5th percentiles of raw values of all sites and the 95th percentile of the least-disturbed sites to exclude the effects of extreme values that may impair metric interpretation. Each selected metric was scored continuously from 0 to 10 (Hughes et al., 1998). For metrics responding negatively to disturbance, we set the ceiling at the 95th percentile of the reference value (least-disturbed sites) and the floor at the 5th percentile of all sample value (all sites). For metrics responding positively to disturbance, we set the ceiling at the 95th percentile of all site values and the floor at the 5th percentile of the reference values (Stoddard et al., 2008; Terra et al., 2013; Macedo et al., 2016; Silva et al., In Press). For all statistical analyses we used R statistical software (R Development Core Team 2016) except for the case of PERMANOVA where we used PRIMER + PERMANOVA v6.1 software (Anderson et al., 2008).

2.7 Index construction and validation

We scored site MMI scores as the sum of the individual metric values divided by the total number of metrics, therefore final MMI scores ranged between 0 and 10 (Klemm et al., 2003). The final MMI scores were assigned to three different quality classes. We assigned sites as poor when the MMI value was less than the 5th percentile of the least-disturbed sites, intermediate or fair when MMI scores were between the 5th and 25th percentiles of least-disturbed sites, and good when MMI scores were greater than the 25th percentile of least-disturbed sites (Paulsen et al., 2008; Silva et al; In Press).

We validated the MMI with a Pearson correlation to relate site positions on PCA Axis 1 to the MMI sites scores (Mereta et al., 2013). MMI performance was also correlated with IDI, LDI, and CDI scores. Finally to assess seasonal influence, we tested the MMI with data not used for the index construction (14 sites that were resampled during the Austral winter, August 2016, in locations classified as least-, moderately, and most-disturbed by IDI scores).

Results

3.1 Disturbance gradient

From the total 95 sites, 26 were classified as least-disturbed (IDI < 0.27), 56 as moderately disturbed (IDI 0.30-0.97), and 13 as most-disturbed (IDI > 1.00). PCA axis-1 explained 24.1% of the variability in the data whereas PCA axis-2 explained 18.7% (Fig. 2). Least-disturbed sites were associated with greater mean substrate diameter (boulder, cobble), and higher concentrations of dissolved oxygen. Most-disturbed sites were associated with higher conductivity, TDS, and lower mean substrate diameter (gravel and pebble, sand and finer sediment size; Table 1). The IDI was significantly correlated with PCA Axis-1 (R=0.60, $p < 0.0001$). The nMDS ordination plot showed a clear separation among disturbance categories (Fig. 3).

3.2 Metric selection

Out of 74 candidate metrics we finally selected four. Forty six metrics passed the range test. Five metrics were strongly correlated with catchment area and elevation, and therefore they were adjusted for MMI development. Among the 46 metrics, only 20 were able to distinguish least- and most-disturbed sites through visual examination of boxplots and PERMANOVA. Finally 16 metrics were highly correlated with each other, resulting in four metrics in the final MMI (Fig. 4, Table 2).

3.3 MMI scoring and validation

The MMI scores ranged between 0 and 10 with the three categories good (>4.0), fair (3.9-2.5), and poor (<2.4) resulting in 29 sites as good, 21 as fair, and 45 as poor (Fig. 5). MMI scores distinguished least-disturbed sites from intermediate disturbed and most-disturbed sites, but intermediate disturbed and most-disturbed sites did not differ substantially (Fig. 6). MMI scores negatively correlated with PCA Axis-1 scores (R = -0.20, $p < 0.05$) and IDI scores (R = -0.46, $p < 0.0001$). MMI scores were also negatively correlated with LDI scores (R = -0.47; $p < 0.0001$), but not with CDI scores ($p > 0.05$). For the 14 sites sampled in the winter season, the MMI scores were negatively correlated with IDI scores (winter R = -0.76, $p < 0.001$) and the MMI was able to distinguish poor, fair, and good sites.

Discussion

To our knowledge, this is the first multimetric index (MMI) developed for Chilean Mediterranean rivers. Using 74 candidate metrics hypothesized to be useful in discriminate between reference and impaired sites we selected four metrics that were the most sensitive including Diptera taxa richness, total macroinvertebrate density, number of Ephemeroptera-Plecoptera-Trichoptera (EPT) individuals and predator taxa richness. We focused on aquatic macroinvertebrates because they often respond to most types of stressors (Herlihy et al., 2005; Pace et al., 2012). However, not all aquatic fauna have equal capacities for evaluating the biotic condition of streams (Iliopulou-Georgudaki et al., 2003; Hughes et al., 2009; Marzin et al., 2012). The MMI we propose includes information related to composition, diversity and trophic structure of aquatic macroinvertebrates. Interestingly, our selected metrics are remarkably similar to those proposed in other Mediterranean ecosystems of the world (Fore et al., 1996; Ode et al., 2005; Sánchez-Montoya et al., 2010; Odume et al., 2012; Ntislidou et al., 2013). The high similarities of metrics used to develop MMIs across Mediterranean ecosystems are likely related to similarities in their hydroclimates (Gasith and Resh, 1999). Besides a similar hydroclimate, there are also similar types of human disturbances across Mediterranean streams of the world. Habitat alteration, water pollution, high levels of water extraction, land use change (agricultural, urban), water regulation, and introduction of non-native species likely would result in similar aquatic and terrestrial flora and fauna (Parsons and Moldenke, 1975; Sánchez-Montoya et al., 2009; Marr et al., 2013). Those similarities among Mediterranean ecoregions could explain the structure and functioning of aquatic communities and thus, the use of similar metrics to build our proposed MMI.

Our MMI was effective in capturing different degrees of environmental degradation and illustrates the importance of using several metrics to establish biotic condition of streams along a gradient of human perturbations. It has been shown that including multiple metrics in an MMI such as macroinvertebrate density and EPT individuals often varied by the type of perturbation (Cline et al., 1982). For example, the disturbance-sensitive EPT insect orders have been used to assess the impacts of agricultural and urban land uses (Roy et al., 2003; Gerth et al., 2017). In addition, diversity metrics such as total number of taxa, can also be negatively affected by the type of land use (Lenat and Crawford, 1994; Allan, 2004). Our results are in agreement with Miserendino et al. (2011) who reported that insect richness was higher in streams with native forest than in streams modified by urban, pasture and managed native forest land-use. In fact, urban and agricultural activities have been identified as one of the principal drivers of macroinvertebrate taxonomic richness in Mediterranean streams (Fierro et al., 2017; Gerth et al., 2017). Lastly, metrics related to trophic structure, such as functional feeding groups (i.e., predator richness) can be influenced by the riparian vegetation and location within the stream network given their influence on the availability and distribution of allochthonous material (Vannotte et al., 1980; Fierro et al., 2015; Serrano Balderas et al., 2016). Indeed, higher predator richness has been reported in streams with high proportions of native forest (Miserendino and Masi, 2010); although this was not the case for Amazonian streams, where Odonata richness was lower in preserved sites than in altered or degraded sites (Oliveira-Junior et al. 2015).

Mediterranean ecosystems are among the most devastated ecosystems in Chile because of human-related environmental impacts (Romero and Ordenes 2004). Our MMI scores indicate that the lowest biotic condition in Chilean Mediterranean streams occurs in urban streams followed by agricultural streams during both the wet and dry seasons. This is also supported by other studies conducted in the same ecoregion (Fierro et al., 2017; Figueroa et al., 2013). Future increases in human population and economic growth and their potential consequences on freshwater ecosystems indicate an urgent need to identify reference sites as well as areas needing rehabilitation (Guida-Johnson and Zuleta, 2017). Narrative and numeric biological indexes can communicate to the public the biological condition of streams in a compressed and understandable form (Fore et al., 1996; Paulsen et al., 2008). Our proposed MMI can be used by government agencies and decision makers for such purposes. In addition, the MMI can be used to assess the baseline conditions for future monitoring plans for Chilean Mediterranean streams. Further, an advantage of using MMIs is that the collection of samples has a relatively low cost and taxonomic identification is relatively fast. The use of MMIs requires, however, qualified personnel to identify taxa. Although Moya et al. (2011) developed a nationally applicable for all of Bolivia (ranging from Amazonia to the Altiplano), both region-specific (Stoddard et al., 2008) and human-disturbance type (Carvalho et al. 2017) MMIs have been developed. For example, our MMI is regionally specific, but it is not applicable to natural perturbations (e.g., streams influenced by volcanic eruption or fires).

Conclusions

The MMI proposed here is the first index to assess ecological quality of Chilean Mediterranean streams. The index provides a quick evaluation of stream biotic conditions that is consistent across seasons. This index is based on four macroinvertebrate metrics that effectively discriminate reference from impaired sites, and is a complement to classical physical and chemical assessments to evaluate habitat structure and water quality. Our MMI can be applied to streams experiencing high levels of anthropogenic pressures, including catchment-scale perturbations, such as urbanization, agriculture and conversion of native forest to exotic three plantations, as well as local perturbations, such as small dams, riparian vegetation removal, and gravel extraction.

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Tables and Figures

Table 1. Variables from 95 Chilean Mediterranean sites, classified by the Integrated Disturbance Index: Least-, moderately, and most-disturbed. Mean and SD (standard deviation) are presented.

	Code	Least disturbed		Intermediate		Most disturbed	
		Mean	SD	Mean	SD	Mean	SD
Temperature (°C)	T°	16.6	2.8	19.8	4.6	15.4	3.3
Dissolved oxygen (mg·l^{-1})	DO	8.6	1.2	8.1	1.3	8.1	1.0
Conductivity (µs·cm^{-1})	Con	174.5	141.0	346.8	207.8	788.7	266.8
pH	pH	7.9	0.6	8.1	0.6	8.1	1.0
Total dissolved solids (mg·l^{-1})	TDS	150.7	133.3	210.4	138.0	490.2	173.2
Mean depth (m)	Prof	0.4	0.2	0.4	0.3	1.1	0.7
Mean dry width (m)	Dw	22.1	20.1	30.2	32.8	55.3	48.7
Mean wetted width (m)	Ww	15.0	14.4	15.9	16.6	26.9	19.7
% Fines (< 3.9 µm)	%Fine	1.4	2.2	5.6	8.9	12.5	14.6
% Sand (<1 mm)	%Sand	6.2	5.9	15.5	14.1	27.7	14.2
% Gravel and pebble (2-64 mm)	%Gra	15.7	11.0	37.8	21.2	30.0	16.8
% Cobble (64-256 mm)	Co	29.2	19.6	25.5	14.9	19.2	14.4
% Boulder (> 256 mm)	Bo	47.6	27.4	16.2	21.1	11.5	18.2
Average substrate diameter (cm)	Sub	34.6	10.9	21.1	10.4	20.4	9.9
% Macrophyte coverage	%Macr	1.3	2.9	23.6	28.7	0.0	0.0
% Leaves coverage	%Leav	0.8	2.3	1.6	5.9	0.0	0.0
% Large wood coverage	%Lwd	0.9	2.3	0.1	0.3	0.0	0.0

Table 2. MMI scoring. Metric scores were scored 0-10 by interpolating between floor and ceiling values. We set the ceiling at the 95th percentile of the reference values and the floor at the 5th percentile of all sample values. Final MMI scores were the mean of the selected metric scores and also ranged from 0-10.

Metric score	Number Diptera Taxa	Macroinvertebrate density	Number EPT individuals	Number predator taxa
10	≥ 7	> 429	> 136	≥ 9
9		382-428	121-135	
8	6	335-381	106-120	8
7		288-334	91-105	7
6	5	241-287	76-90	6
5	4	193-240	61-75	5
4	3	146-192	46-60	4
3		99-145	31-45	3
2	2	52-98	16-30	2
1		5-51	1-15	
0	≤ 1	≤ 4	0	≤ 1

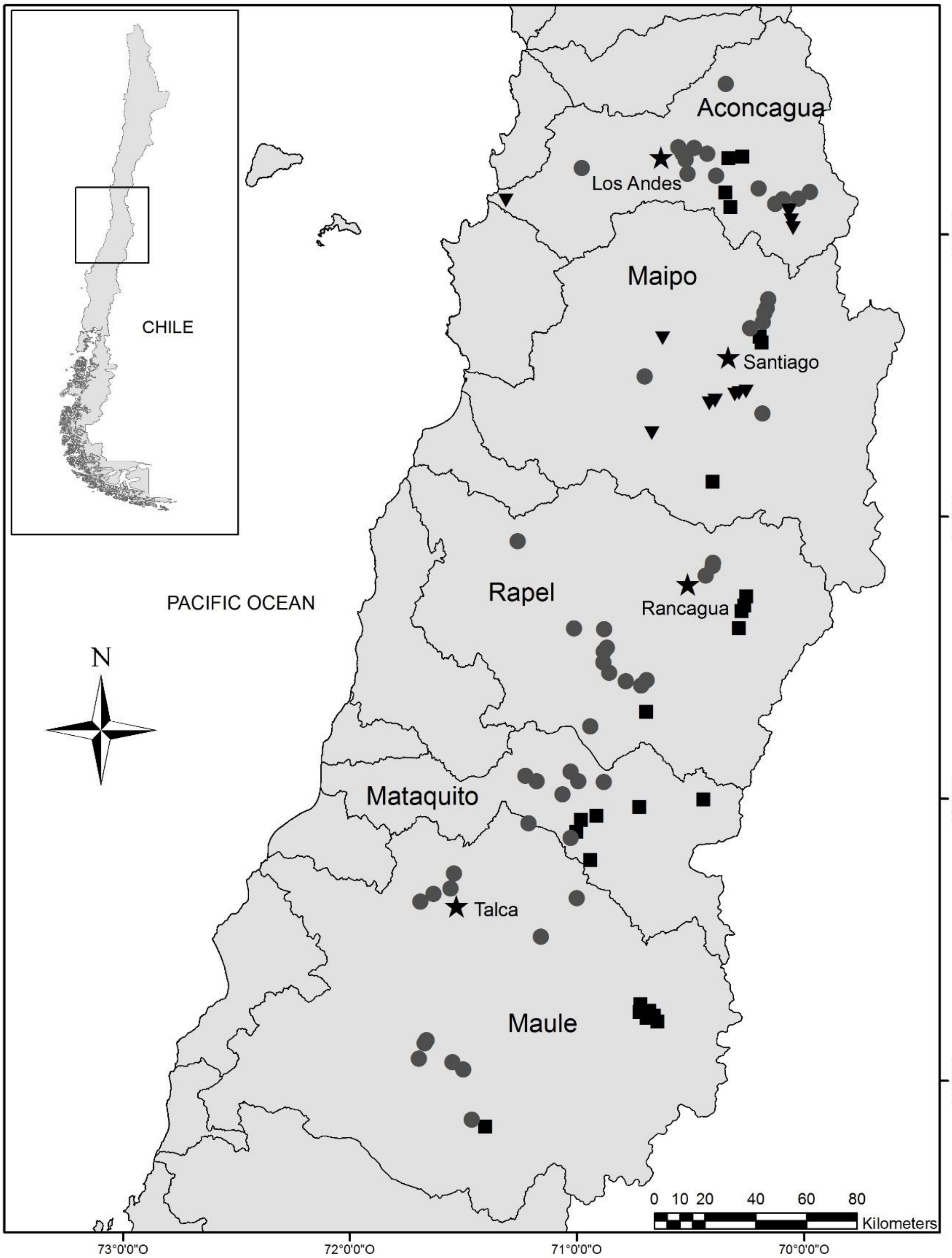

Fig. 1. Locations of the 95 sampling sites in five basins in the Chilean Mediterranean Region. Stars represent the location of major cities in the region. Sites are classified by integrated disturbance index class (squares = least-disturbed, grey circles = moderately disturbed, inverted triangles = most-disturbed).

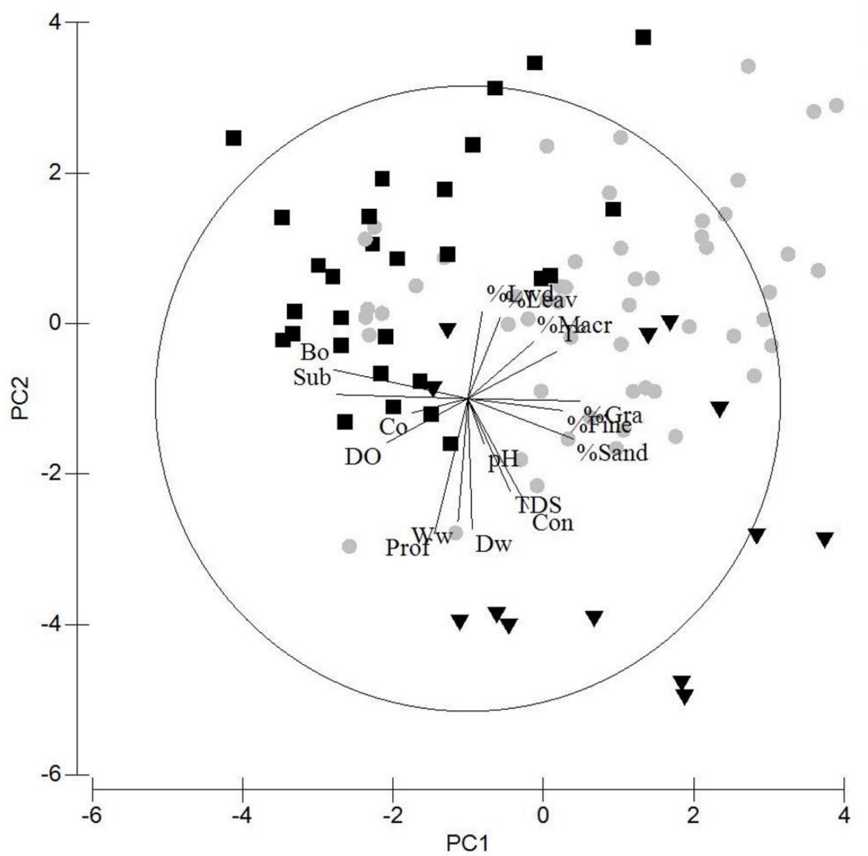

Fig. 2. Principal Component Analysis of environmental variables from 95 Chilean Mediterranean sites plotted by integrated disturbance index class. Codes for environmental variables are described in Table 1 (squares = least-disturbed, grey circles = moderately disturbed, inverted triangles = most-disturbed).

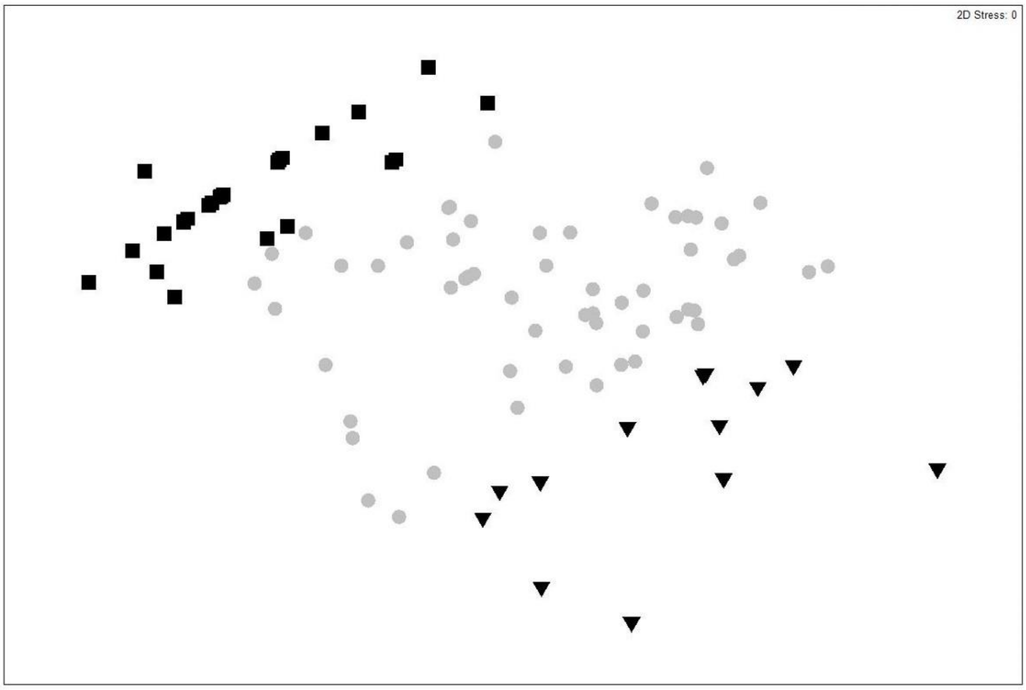

Fig. 3. MDS on PCA Axis-1 scores along a physicochemical gradient. Sites are classified by integrated disturbance index class (squares = least-disturbed, grey circles = moderately disturbed, inverted triangles = most-disturbed).

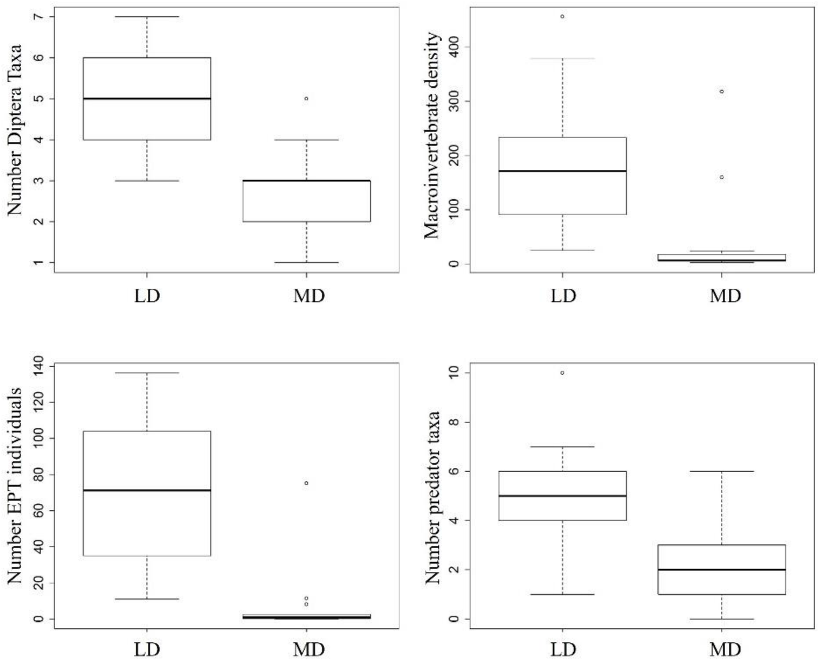

Fig. 4. MMI metrics discriminating least- and most-disturbed sites. Bold horizontal lines are medians, boxes are interquartile ranges (25-75th percentiles), bars are 5th and 95th percentiles, and circles are extreme values.

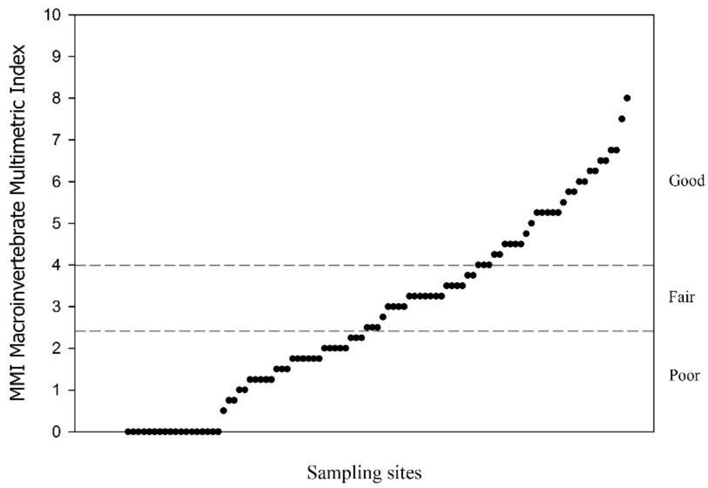

Fig. 5. Classification of final MMI scores. The upper fair boundary is when MMI scores were greater than the 25th percentile of least-disturbed sites, and the lower fair boundary is when the MMI value was less than the 5th percentile of the least-disturbed sites.

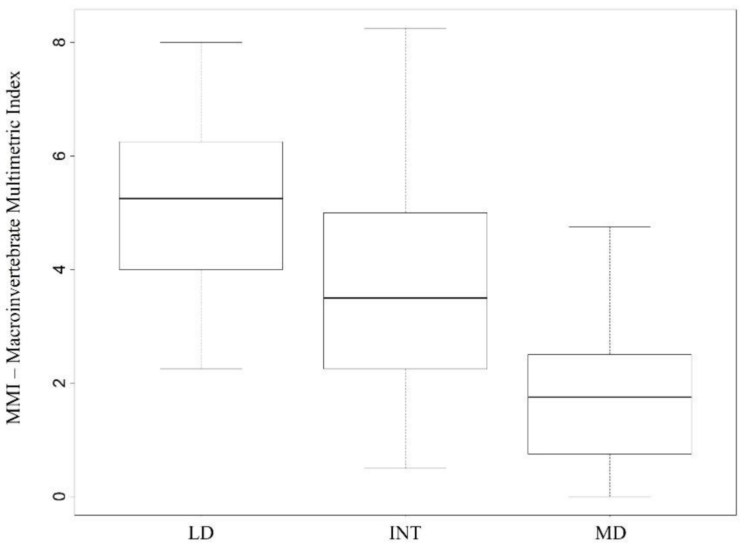

Fig. 6. Relationship of MMI scores to IDI class. LD = least disturbed, INT=intermediate, MD=most disturbed.

Appendice

Appendix A. Seventy four candidate metrics screened in the MMI. Percentage (%) metrics are calculated based on the total number of individuals collected. Class I: diversity metrics, II: composition metrics, III: trophic structure metrics, IV: pollution tolerance metrics.

N	**Metric**	**Class**	**Expected response to disturbance**
1	Total number Taxa / Richness	I	Decrease
2	Total number Taxa Plecoptera	I	Decrease
3	Total number Taxa Ephemeroptera	I	Decrease
4	Total number Taxa Trichoptera	I	Decrease
5	Total number Taxa Coleoptera	I	Decrease
6	Total number Taxa Odonata	I	Decrease
7	Total number Taxa Diptera	I	Decrease
8	Total number Taxa Insecta	I	Decrease
9	Total number Taxa No-insecta	I	Decrease
10	Ephemeroptera and Plecoptera Taxa richness	I	Decrease
11	EPT richness	I	Decrease
12	Taxa richness "legles"" organisms	I	Increase
13	% Taxa Crustacea and Mollusca	II	Variable
14	% Crustacea and Mollusca	II	Variable
15	Sum five most dominant Taxa	II	Increase
16	% (five most dominant Taxa)	II	Increase
17	Macroinvertebrate density	II	Variable
18	% EPT richness	II	Decrease
19	EPT individuals	II	Decrease
20	% EPT individuals	II	Decrease
21	EPT / Chironomidae Individuals	II	Decrease
22	% Insecta	II	Decrease
23	% No Insecta	II	Increase
24	Shannon diversity	II	Decrease
25	Evenness (J)	II	Decrease
26	Simpsons diversity	II	Decrease
27	% Oligochaeta	II	Increase
28	% Odonata	II	Decrease
29	% Ephemeroptera	II	Decrease
30	% Baetidae	II	Decrease
31	% Leptohplebiidae	II	Decrease
32	% Trichoptera	II	Decrease
33	% Hydropsychidae	II	Decrease
34	% Hydrobiosidae	II	Decrease
35	% Plecoptera	II	Decrease
36	% Gripopterygidae	II	Decrease
37	% Coleoptera	II	Decrease

38	% Elmidae	II	Variable
39	% Diptera	II	Decrease
40	% Non-chironomid Diptera	II	Increase
41	% Chironomids	II	Increase
42	% Gastropoda	II	Variable
43	% Amphipoda	II	Variable
44	% Isopoda	II	Variable
45	% Acari	II	Variable
46	% Mollusca and Diptera	II	Increase
47	Chironomidae (abundance)	II	Increase
48	Diptera (abundance)	II	Increase
49	Chironomidae/Diptera individuals	II	Increase
50	Total abundance - Chironomidae Abundance	II	Increase
51	% Planaria+Amphipods	II	Increase
52	% "Legless" organisms	II	Increase
53	Shredders	III	Decrease
54	Scrapers	III	Decrease
55	Gatherers	III	Decrease
56	Filterers	III	Variable
57	Predators	III	Decrease
58	Detritivores	III	Increase
59	% Shredders	III	Decrease
60	% Scrapers	III	Decrease
61	% Gatherers	III	Decrease
62	% Filterers	III	Variable
63	% Predators	III	Decrease
64	% Detritivores	III	Increase
65	Scrapers:Filterers	III	Variable
66	Hilsenhoff´s biotic index	IV	Increase
67	% Super-Tolerant individuals (% Taxa values 9-10)	IV	Increase
68	Super-Tolerant Taxa richness (richness Taxa value 9-10)	IV	Increase
69	% Tolerant individuals (7-8-9-10)	IV	Increase
70	Super-Tolerant Taxa richness (richness Taxa value 7-8-9-10)	IV	Increase
71	% Sensitive individuals (0-1-2-3)	IV	Decrease
72	Sensitive Taxa richness (richness Taxa value 0-1-2-3)	IV	Decrease
73	% Super sensitive individuals (0-1)	IV	Decrease
74	Super Sensitive Taxa richness (richness Taxa value 0-1)	IV	Decrease

Discusión general

Mundialmente los ecosistemas dulceacuáticos han estado sometidos a una degradación producto de un incremento en las presiones humanas desde décadas pasadas. Entre las actividades humanas de mayor impacto sobre estos ecosistemas se encuentra el cambio en el uso de suelo, siendo uno de los mayores conductores en la degradación de los ríos alrededor del mundo, y contribuyendo a la perdida de hábitat, perdida en la biodiversidad y extinción de especies (Allan, 2004). En las regiones mediterráneas, la conversión de cubierta nativa a plantaciones forestales, agricultura y áreas urbanas ha incrementado durante los últimos años. Específicamente, las ecoregiones mediterráneas son muy sensibles a tales perturbaciones, debido a la variabilidad hidrológica natural y al incremento en la tendencia del desarrollo de la población humana (Aparicio, 2008; Cooper et al., 2013).

Para evaluar los impactos de las actividades humanas sobre la calidad del agua, la evaluación tradicional considera el uso de parámetros físico-químicos. Sin embargo, esta aproximación podría ser insuficiente para establecer los impactos de las actividades humanas sobre los ecosistemas dulceacuáticos (Fierro et al., 2012). Por lo tanto, surge una necesidad por evaluar con herramientas alternativas, como los índices de integridad biótica que pueden proveer un complemento a las aproximaciones tradicionales. En la literatura existen diversos ejemplos de indicadores bióticos que han mostrado prominentes resultados en evaluar la calidad del agua, incluyéndose macroinvertebrados, algas bentónicas y peces (Lammert and Allan, 1999, Taylor et al., 2004; Macedo et al., 2014, Gerth et al., 2017). Como un nuevo método de bioevaluación, la dieta de peces aparecer ser una buena herramienta para indicar los potenciales cambios en la composición de macroinvertebrados de ríos en diferentes tipos de uso de suelo. De esta forma el uso de la biota acuática como bioindicador es una aproximación aplicada comúnmente para la evaluación de la condición del cuerpo de agua. Sin embargo el uso extensivo de esta biota, como macroinvertebrados a través de similares ecoregiones, podría verse limitada debido a las diferencias locales naturales en la biodiversidad y también porque las perturbaciones antropogénicas son diferentes (Stoddard et al., 2008). Adicionalmente, en la literatura las amenazas antropogénicas y los impactos sobre los ecosistemas acuáticos han sido tratadas en revisiones de literatura, sin embargo durante la última década, las opiniones de expertos se han vuelto una alternativa popular, dado el complemento en la información a las revisiones bibliográficas (Hockings, 2003; Halpern et al., 2007; Kleypas and Eakin, 2007; Selkoe et al., 2008).

El propósito de esta tesis Doctoral fue evaluar los efectos y magnitud de múltiples actividades antropogénicas derivadas del cambio de uso de suelo en ríos de cuencas mediterráneas sobre la integridad biótica. El ensamble de macroinvertebrados dulceacuícolas, peces y algas bentónicas, dieta de peces introducidos, junto con imágenes satelitales y métodos estadísticos fueron aplicados e integrados en un establecimiento ecológico. El uso de diferentes *proxies* biológicos conduce a un incremento en el entendimiento de los efectos de actividades antropogénicas sobre los ríos de esta región.

En el primer capítulo, esta tesis proveyó una revisión de los indicadores de integridad biótica. Se identificaron los pro y contra del uso de diferentes índices (índices bióticos, métodos multivariados e índices multimétricos) usando la comunidad acuática como indicadores de calidad de agua. Luego definimos y describimos la historia de los índices de integridad biótica usados mundialmente. Finalmente usamos como ejemplo ríos del mediterráneo Chileno y aplicamos datos del ensamble de peces y macroinvertebrados como bioindicadores, los cuales fueron capaces de diferenciar ríos drenando cuencas con vegetación nativa y ríos agrícolas.

En el segundo capítulo nosotros empleamos un método estandarizado para la colecta de datos bibliográficos. Nosotros revisamos 79 artículos científicos que incluyeron amenazas a los peces, macroinvertebrados, anfibios y plantas dulceacuícolas en la ecoregión mediterránea Chilena. Se identificaron 14 amenazas, siendo los cambios en el uso de suelo, introducción de especies, y efluentes industriales y domésticos los más comunes. Estos resultados de la revisión de la literatura coincidieron con los de la opinión de expertos. Las amenazas más comunes detectadas usando la opinión de expertos incluyeron cambios en el uso de suelo, minerías, urbanización, sequias, efluentes industriales y domésticos y plantas hidroeléctricas. Por grupo taxonómico, los expertos coincidieron en que los peces están siendo altamente amenazados por plantas hidroeléctricas, las plantas dulceacuícolas por minería, los anfibios por el cambio en uso de suelo, y los macroinvertebrados por efluentes industriales y domésticos. Las principales amenazas identificadas aquí coinciden con las reportadas para otros ecosistemas mediterráneos, y en Chile estas amenazan continúan en desarrollo.

En el tercer capítulo nosotros muestreamos la comunidad acuática en cuatro cuencas con uso de suelo de cubierta nativa, plantaciones forestales, agricultura y ríos urbanos de la ecoregión mediterránea Chilena. Los tres ensambles bióticos fueron diferentes entre los usos de suelo. Respecto a las algas bentónicas, se registró un incremento en la biomasa de la clorofila-*a* desde vegetación nativa a plantaciones forestales, agricultura y ríos urbanos. Macroinvertebrados mostraron el mismo patrón, la riqueza de taxones, diversidad, ordenes de insectos sensibles Ephemeroptera-Plecoptera-Trichoptera (EPT) y la calidad del agua fueron también negativamente afectados por el cambio en uso de suelo, siendo más evidente en ríos agrícolas y urbanos. En el otro lado, sitios drenando vegetación nativa y plantaciones forestales tuvieron menor densidad de dípteros y % de individuos no insectos. Especies de peces de agua fría (nativas e introducidas) fueron asociadas a ríos de cubierta nativa y plantación forestal, mientras que especies de aguas calidad (mayormente introducidas) fueron asociadas a ríos agrícolas y urbanos. Acordando a análisis multivariados, variables a escala local y de cuenca tuvieron la mayor explicación para cada uno de los ensambles. Nuestros resultados sugieren que algas bentónicas, macroinvertebrados y peces fueron buenos indicadores del impacto del cambio de uso de suelo, teniendo áreas agrícolas y urbanas los mayores efectos negativos sobre la biota acuática.

En el cuarto capítulo, el ensamble de macroinvertebrados y la dieta de la especie introducida “trucha arcoíris” fue estudiada en ríos que drenan cuencas con uso de suelo de bosque nativo y plantación forestal, en dos cuencas costeras. Nosotros registramos mayor riqueza y abundancia de macroinvertebrados en sitios de bosque nativo que en sitios de plantación forestal. Colectores-recolectores fue el grupo funcional alimenticio (GFA) más abundante, sin embargo no hubo diferencia significativa en la composición de los GFA entre las dos cuencas. Diferencias en la disponibilidad de macroinvertebrados en el rio fue mayormente correlacionada con cambios en la dieta de la trucha arcoíris. Específicamente, los taxa consumidos desde la cuenca dominada por bosque nativo fue mayor que en la cuenca con vegetación exótica. Adicionalmente, variables ambientales mostraron diferencias significativas entre las cuencas. Los sitios de vegetación exótica tuvieron las mayores concentraciones de solidos disueltos, solidos suspendidos, nitratos, cloruros y sulfatos. Estos resultados muestran que la estructura del ensamble de macroinvertebrados y la dieta de truchas pueden ser alteradas por el cambio en la vegetación ribereña. La ausencia de taxa específicos de macroinvertebrados en ríos con vegetación exótica fue capturada por la composición de la dieta de las truchas. Esto sugiere que la dieta de la trucha arcoíris puede ser usada como un buen indicador biológico de las prácticas de uso de suelo, y así la dieta puede ser usada como una rápida y efectiva herramienta para evaluar la calidad ambiental.

Finalmente en el quinto capítulo, nosotros creamos un índice multimétrico basado en macroinvertebrados para establecer la integridad ecológica en ríos del mediterráneo Chileno, bajo múltiples actividades antropogénicas. Aquí nosotros evaluamos 76 métricas que representaron diversidad, composición, estructura trófica y tolerancia a la contaminación de taxa de macroinvertebrados. El índice multimétrico resultante incluyo las métricas de riqueza de taxa de Diptera, densidad total de macroinvertebrados, número de individuos de Ephemeroptera-Plecoptera-Trichoptera, y riqueza de taxa depredadores. Los puntajes del MMI final clasificaron los 95 sitios de muestreo dentro de tres categorías de condición biótica, incluyendo buena, regular y pobre. Nosotros postulamos que nuestra aproximación es transferible a otros ríos en la región, y una herramienta suficiente para evaluar la condición de los sitios afectados por diversas perturbaciones humanas a una escala local como de cuenca en ríos del mediterráneo Chileno.

Los datos generados en la presente tesis serán importante no solo para la comunidad científica, sino que también podrán ser usados para enseñar a la población en general. Entendiendo los efectos a nivel de paisaje, como cambio de uso de suelo, y efectos locales, como una contaminación puntual, ambos afectando las condiciones ambientales de los ríos, y como la fauna dulce acuática responde frente a estos, en los casos más extremos, como pérdida de la biodiversidad.

Conclusiones generales

De acuerdo a las hipótesis y objetivos planteados al principio de esta tesis (ver sección de introducción), los cinco capítulos presentados aquí permitieron responder las preguntas y las actividades planteadas. A continuación se describen las conclusiones por cada capítulo detalladamente.

En el Capítulo 1 se concluyó:

- Diferentes grupos taxonómicos pueden ser usados como bioindicadores de la calidad del agua.
- Se propone al ensamble de macroinvertebrados acuáticos y peces como bioindicadores en la zona mediterránea Chilena.
- El uso de índices de índices multimétricos es una de las mejores herramientas en la evaluación de la integridad ecológica.

En el Capítulo 2 se concluyó:

- De los 79 publicaciones revisadas, se identificaron 14 amenazas antropogénicas al ecosistema mediterráneo dulceacuático Chileno, incluyéndose dentro de las categorías de especies exóticas, perdida de hábitat y degradación, contaminación, sobreexplotación y cambio climático.
- Cambios en el uso de suelo, introducción de especies y efluentes industriales y domésticos fueron las amenazas más estudiadas.
- De acuerdo a la encuesta de opinión, la mayor amenaza para los peces son las plantas hidroeléctricas, para las plantas lo fue la minería, para los anfibios el

cambio en el uso de suelo, y para las macroinvertebrados los efluentes industriales y domésticos.

En el Capítulo 3 se concluyó:

- El ensamble de macroinvertebrados, peces y algas bentónicas fue diferente entre usos de suelo con vegetación nativa, plantaciones forestales, agricultura y urbano.
- Incremento en la clorofila-*a* fue registrado desde la cubierta nativa a plantaciones forestales, mientras que ríos agrícolas y urbanos mostraron las mayores concentraciones.
- Ríos agrícolas y urbanos soportaron las menores riquezas de macroinvertebrados, diversidad, insectos sensibles, y las peores calidades del agua.
- Especies de peces de aguas frías fueron asociadas a ríos nativos y de plantaciones forestales, mientras que especies de aguas calidad estuvieron asociadas a ríos agrícolas y urbanos.
- Vegetación nativa, áreas urbanas y pH explicaron la variación del ensamble de algas bentónicas.
- Áreas urbanas, áreas agrícolas y temperatura explicaron la variación del ensamble de macroinvertebrados.
- Áreas agrícolas explicaron la variación del ensamble de peces.

En el Capítulo 4 se concluyó:

- La riqueza y la abundancia de macroinvertebrados fue mayor en bosque nativo que en plantaciones forestales.
- En bosque nativo una mayor cantidad de taxa fue registrado en los estómagos de trucha arcoíris en comparación a plantación forestal.

- Taxa que no se registraron en el bentos en ríos de plantación forestales si fueron registrados en los estómagos de la trucha arcoíris.
- La dieta de la trucha arcoíris puede ser un buen indicador de las prácticas de uso de suelo, pudiendo ser usado como una rápida y efectiva herramienta de biomonitoreo.

En el Capítulo 5 se concluyó:

- De acuerdo al índice integrado de disturbio, de los 95 sitios muestreados en la ecoregión mediterránea Chilena, 26 fueron clasificados como los menos perturbados, 13 como altamente perturbados, y 56 como sitios intermedios.
- De las 76 métricas evaluadas para macroinvertebrados bentónicos, el índice multimetrico (MMI) estuvo finalmente compuesto de riqueza de taxa de Dipteros, densidad total de macroinvertebrados, número de individuos Ephemeropteros-Plecopteros-Trichopteros, y riqueza de taxa depredadores.
- De los 95 sitios muestreados, 29 fueron clasificados según el MMI como de buena calidad, 21 sitios con calidad regular, y 45 con calidad pobre.
- El MMI creado puede ser transferible a otros ríos en la región, siendo una buena herramienta para evaluar la condición de los ríos afectados por perturbaciones humanas a escala de cuenca como locales.

Limitaciones y futuras investigaciones

Esta tesis no estableció la alta variabilidad estacional de la biota acuática. Si bien en el capítulo 4 nosotros registramos la alta variabilidad de los macroinvertebrados a través del año en diferentes usos de suelo, nosotros no muestreamos peces y algas bentónicas estacionalmente, por lo tanto desconocemos las respuesta de estos dos grupos taxonómicos. Es por esto que se recomienda estudios a largo plazo, para entender como las estacionalidad y fenómenos a grandes escalas (*e.g.* El niño, cambio climático) tienen efectos sobre la comunidad acuática.

Dado que a lo largo de todo Chile existen diferentes ecoregiones y diferentes amenazas a los ecosistemas dulceacuáticos, nuestro índice multimétrico solo tiene que ser usado en ríos de la ecoregión mediterránea Chilena. Por lo tanto es recomendable muestrear y crear nuevos índices multimétricos para las demás ecoregiones Chilenas. De esta manera resultados confiables en la integridad biótica podrán ser comparados con los resultados de esta tesis. En conjunto, aumentar el conocimiento sobre la diversidad de fauna acuática es necesario, especialmente sobre macroinvertebrados bentónicos, en los cuales se necesita aumentar la taxonomía de los estados larvales.

Basado en la revisión bibliográfica, las amenazas de alto riesgo catalogadas en este estudio: cambio en uso de suelo, minería, urbanización, sequia, efluentes industriales y domésticos y plantas hidroeléctricas, deberían recibir mayor atención en estudios futuros. Se recomienda que futuras políticas públicas deberían centrarse sobre la mitigación de estas amenazas. Adicionalmente, cuanta más información es colectada, políticas adecuadas para el continuo manejo de recursos acuáticos pueden ser diseñadas e implementadas. Nuestros resultados proveen ideas para el diseño de programas de monitoreo de agua dulce para la detección de impactos antropogénicos en ríos altamente amenazados, como los de la ecoregión mediterránea Chilena.

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