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**TROPHIC RELATIONSHIPS BETWEEN BENTHIC FAUNA
AND EARLY SALMONID STAGES ON THE ALLIPEN RIVER
ECOSYSTEM, TOLTEN BASIN, LA ARAUCANÍA REGION,
CHILE.**

**DOCTORAL THESIS IN FULFILLMENT OF
THE REQUERIMENTS FOR THE DEGREE
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SALMONID STAGES ON THE ALLIPEN RIVER, TOLTEN BASIN, LA
ARAUCANÍA REGION, CHILE.**

Esta tesis fue realizada bajo la supervisión del Dr. Jorge Farías Avendaño perteneciente al Departamento de Ingeniería Química, Facultad de Ingeniería y Ciencias de la Universidad de La Frontera.

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“Sea lo que sea en lo que te conviertas, no me decepcionarás; no tengo preconcepciones. No deseo predecir en lo que te convertirás, sólo deseo descubrirte. No me decepcionarás”

[-Mary Haskell-]

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Summary and thesis outline.

Animals often shift their diets in response to changes in resource availability, ontogenetic stage competition, and interactions with predators or parasites. Consequently, food web structure varies in both space and time. In order to assess the conditions of river ecosystems, the use of more than one taxonomic group assures the best predictive results, this is because each assemblage is associated with different environmental variables, a condition given by differences in the species' life history, habitat preferences, and behavior. Fish and macroinvertebrates are good indicators of river ecosystem conditions since they respond differently to environmental conditions. The aquatic biota of the Allipen river is scarcely known; the community of benthic invertebrates has high species richness and low diversity. Moreover, not only benthic invertebrates are little known, but also there is scarce knowledge about fish fauna of the Allipen river. *Oncorhynchus mykiss* (Rainbow trout) and *Salmo trutta* (Brown trout) are introduced fish species, naturalized in river basins from Chile, these are the main ichthyophagous (fish-eating) salmonid species reported, both representing a threat to native species. On the other hand, the current distribution of *Oncorhynchus tshawytscha* (Chinook salmon) in Chile extends from La Araucanía Region to the fiords of Patagonia. Because of its size and meat quality, this species sustains a vast commercial and recreational community of local fishermen in the Allipen river basin. Benthic macroinvertebrates are one of the most important components of freshwater ecosystems. These are mainly immature stages of insects, most of which spend at least one stage of their life cycle in aquatic systems before emerging to the adult state. So that the following hypothesis is proposed: The Allipen river fish community is dominated by juvenile salmonids, both resident and anadromous, which feed preferentially on benthic macroinvertebrates in a selective way and without a wide dietary overlap.

In Chapter I, general introduction, hypotheses, and general and specific objectives are presented. The general objective of this Doctoral Thesis is to determine the trophic interactions between the juveniles of the introduced fish species and the benthic fauna of the Allipen river, established whether there is dietary overlap between fish species in the Allipen river of La Araucanía Region.

Chapter II corresponds to the manuscript entitled “First reports on seasonal abundances on Malacostracans in the Allipen river (38°51's North Patagonia, La Araucanía Region, Chile)”. In this chapter, the spatial pattern of distribution of an *Aegla abtao* and *Hyaella patagonica* population from the Allipen river was described. In this chapter, the need for basic, adequate and accurate taxonomic information on the species distribution throughout Chile was fulfilled.

Chapter III corresponds to the manuscript entitled “First report of the identification of juveniles of *Oncorhynchus tshawytscha* in the Allipen river (La Araucanía Region, Chile) using *microsatellite DNA markers*”. In this chapter, juveniles of *Oncorhynchus tshawytscha* in Allipen river were identified through microsatellites. Twenty microsatellites were used to identify juveniles of *O. tshawytscha*. It was concluded that the identification of juvenile *O. tshawytscha* was possible through these 5 microsatellites that were successfully amplified. Highlighting this research as the first report that allows the identification of this species youth in the Allipen river and validates it through molecular markers.

Chapter IV corresponds to the manuscript entitled “Community structure of benthic invertebrates in the Allipen river basin, North Patagonia, La Araucanía Region (39° S, Chile)”. In this chapter, the community structure of benthic invertebrates in the Allipen river was determined by describing their composition, richness, and abundance of species through richness models and null models based on presence/absence. The

results reveal a high species richness and low diversity. The Arthropoda phylum was the most representative in abundance and richness. The information provided here contributes to the understanding of the ecological patterns of the invertebrate communities in the Allipen river, establishing the basis for more complex ecological studies.

Chapter V corresponds to the manuscript entitled “Trophic ecology of introduced fish species from the Allipen river basin, a sparsely studied ecosystem of the La Araucanía Region.” The objective of this chapter was to determine the trophic level of exotic fish species in the river system, to investigate the prey spectrum consumed by these species, and to identify the degree of overlap (competition for food resources).

Finally, Chapter VI corresponds to general discussion, conclusions, and future directions.

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CHAPTER I

General Introduction

1.1.Introduction

The study of the effects and impacts caused by biological invasions to local ecosystems and the species inhabiting them, allows the evaluation of ecological state of these ecosystems, being a critical first step towards efficiently managing catchments. Ecosystem impacts caused by invasive species can be associated with altering the hydrological fluxes, mass balance and energy of the ecosystem (Ehrenfeld 2010; Simberloff 2011). Thereby, invasive species generate changes in the structure and function of natural systems. For instance, invasive species can change ecosystem processes through their resource acquisition, by altering disturbance regimes, or by altering the trophic structure and/or food webs of the invaded system (Miranda *et al.*, 2018). Predicting invasive species impacts is an essential aspect for their management, providing the basis for prioritizing interventions according to the ecological risk of different species (Guo *et al.*, 2016). According to Fierro *et al.*, (2019), in order to assess the conditions of river ecosystems, the use of more than one taxonomic group assures the best predictive results. This is because each assemblage is associated with different environmental variables, a condition given by differences in the species life history, habitat preferences, and behavior (Ruaro *et al.*, 2016). Fish, macroinvertebrates, and benthic algae are good indicators of river ecosystem conditions since they respond differently to environmental conditions (Hill *et al.*, 2003; Terra *et al.*, 2013; Silva *et al.*, 2017). Ecological indicators based on assemblages are frequently used in bioassessments, the absence or reduced diversity and abundance of these assemblages reflect the environmental status from previous months (Fierro *et al.*, 2019). In addition, the composition of freshwater fish populations has been used to assess long-term stream perturbations. Since fish are mobile organisms, they respond to disruptions such as flow

alteration, impact of exotic species, or habitat fragmentation (Karr 1981; Penaluna *et al.*, 2009).

Recent studies indicate that Chilean fish fauna is being affected by biotic homogenization resulting from the introduction of species from other regions and the extinctions or extirpations of native species (Rojas *et al.*, 2019). In addition, different authors recognize the information gaps that Chilean fish fauna has with respect to its taxonomy and distribution (Habit *et al.*, 2006a; Vila & Habit 2015; Vila & Quezada-Romegialli 2018). Fish species introduction started in the 19th century, associated with commercial, ornamental, sporting, and biological control activities (Iriarte *et al.*, 2005). Furthermore, there is strong evidence suggesting that interaction between introduced and native species would adversely disrupt the latter, negatively affecting the abundance and distribution of native species (Arismendi *et al.*, 2009; Habit *et al.*, 2010; Arismendi *et al.*, 2011; Correa *et al.*, 2012). Juveniles of the introduced fish species in Chile have generalist trophic habits, and prey on (Vila *et al.*, 2006; Habit *et al.*, 2015) or compete for resources with native fish species (Soto *et al.*, 2007; Penaluna *et al.*, 2009; Correa *et al.*, 2012; Elgueta *et al.*, 2013; Habit *et al.*, 2015).

The Allipen river is located in the south of Chile (38°51`S and 71°45`W) at 463 m above sea level, this is part of the watershed of the Toltén river, which has an area of 7,886 km² and is located in La Araucanía Region (Vargas *et al.*, 2010). It rises in the Andes Mountains; annual precipitation can reach 3,000 mm and mean monthly temperatures oscillate between -3 and 18 °C. The watershed receives mostly pluvial precipitation, with slight snow influence in the upper part, presenting a mixed hydrological regime (pluvio-nival) (Medina & Muñoz 2020). The aquatic biota of the Allipen river is scarcely known; the community of benthic invertebrates has a high species richness and low diversity. Arthropoda phylum is the most representative in abundance and richness, dominated by

orders, Trichoptera and Diptera (Solis-Lufi *et al.*, 2021). Benthic macroinvertebrates are one of the most important components of freshwater ecosystems (Fierro *et al.*, 2015). These are mainly immature stages of insects, most of which spend at least one stage of their life cycle in aquatic systems before emerging to the adult state (Hauer & Resh 2007). Moreover, aquatic benthic macroinvertebrates are organisms presenting temporal and spatial variation due to several factors, one of which is the diverse food resources available in rivers (Fierro *et al.*, 2015). Not only benthic invertebrates are little known, but also there is scarce knowledge about fish fauna of the Allipen river (Vargas *et al.*, 2010). Determined preferences and the degree of microhabitat overlap between the juvenile Chinook salmon and native catfish (*Trichomycterus areolatus*) in the Allipen river results in a potentially negative interaction between the two species through interactive habitat segregation, which could represent a threat to the stability of catfish populations. In addition, Gomez-Uchida *et al.*, (2016) reported Chinook salmon reproductive nests in tributary rivers of the Allipen river and the presence of other salmonids co-inhabiting with Chinook, Rainbow trout (*Oncorhynchus mykiss*) and Brown trout (*Salmo trutta*), and the native species puyes (*Galaxias* spp.), pochas (*Cheirodon* spp.), catfish (*Diplomystes* spp.), and lampreys (*Geotria* sp.).

Moreover the Allipen river is of great importance for local economic activities such as tourism and recreational fishing, so that recreational fishing for Chinook salmon is currently restricted in this zone to protect and encourage spawning sites between the months of April and August each year (Gomez-Uchida *et al.*, 2016). The effects generated by Chinook salmon populations in continental aquatic systems are of different origin and magnitude according to the analyzed life stage. On one hand, there are effects associated with return adult and their subsequent deaths in low order rivers, which have been relatively well studied (Naiman *et al.*, 2002; Soto *et al.*, 2006; Soto *et al.*, 2007). However,

on the other hand, the invasion of these salmons also implies the establishment, by one or two years, of new members of the fish communities, corresponding to juveniles, for which background information is lacking. Given the short period of time in which adult Chinook salmon is found in the rivers of Southern Chile (Soto *et al.*, 2007; Correa & Gross 2008), it is reasonable to think that potentially negative interactions with native fish could derive from competitive relationships (food and/or habitat) only with Chinook salmon juveniles, they feed on terrestrial and aquatic insects, amphipods and other crustaceans while they are young, and mainly on other fish when they are older (Healey 1991). At age of 1+, juvenile Chinook enter aquatic systems as piscivore predators, and if they reach body sizes > 8.5 cm LT before the first year, they also behave as piscivores, selecting native fish as prey (Ibarra *et al.*, 2011); therefore, this species establishes new negative interaction with the native fish fauna, both by predation (mediated by size) and competition for benthic species. Furthermore, due to the scarcity of reports on the presence of juveniles of Chinook Salmon in the Allipen river, and since in the initial stages of their life cycle they present morphological characteristics similar to their salmonid congeners (Pollard *et al.*, 1997), more specific studies are required, such as the use of molecular genetic markers that allow evaluating the characteristics of each species and allow the correct identification of the species, as it was reported by Docker & Heath (2002). Molecular methods have been particularly useful to solve taxonomic issues (Weber & Montoya-Burgos 2002; Montoya-Burgos 2003; Cardoso *et al.*, 2016, 2019). The information accessed using genetic markers continuously contributes to a better understanding of the evolutionary and ecological aspects of species biology (Mezzomo *et al.*, 2020).

Chinook salmon (*Oncorhynchus tshawytscha*) was naturalized in many river systems from South America following aggressive introduction efforts (Irgang, 2019). Its current

distribution in Chile extends from La Araucanía Region to the fiords of Patagonia (Correa & Gross, 2008). In recent studies, Gomez-Uchida *et al.*, (2018) confirm Chinook salmon population in the Allipen river is a result of the release of unknown broodstock to Estero El Membrillo (inlet tributary of Allipen river) from failed hatchery operation in 1995, with a current population of around 12,000 returning adults reported during austral spring and summer in 2014-2015. Because of its size and meat quality, this species sustains a vast commercial and recreational community of local fishermen (Myers *et al.*, 1998).

One of the reasons for the successful establishment of Chinook salmon populations in Southern Chile is due to the high plasticity in its life cycle (Ciancio *et al.*, 2005; Soto *et al.*, 2007; Arismendi *et al.*, 2009), a characteristic that makes it an invasive species par excellence (Vargas *et al.*, 2010). Chinook salmon have an anadromous life cycle that favors wide distribution and in turn magnifies the risks to marine and freshwater communities by its ease of generating cascading effects at the food chain level (Pascual & Ciancio, 2007). These species, unlike trout, have an anadromous and semelparous life cycle, which means that they are a migratory species that moves between freshwater (spawn and die) and marine ecosystems (grow and mature) (Flecker *et al.*, 2010). The above, implies an additional risk for the ecosystems where they are introduced since there is a possibility that salmon generate an increase from direct fertilization to the freshwater ecosystem where they spawn by accumulating their carcasses, which can facilitate the production of lower trophic levels such as benthic invertebrates and stream fish (Wipfli *et al.*, 2003; Lessard & Merritt, 2006).

Regarding the effects generated by Rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792) and Brown trout (*Salmo trutta* (Linnaeus, 1758) in the Southern Hemisphere, they have been accountable for the decline of some native fishes (galaxiids), because of direct predation and food competition (Soto *et al.*, 2006; Arismendi *et al.*, 2011). Both are

introduced fish species, naturalized in river basins from Chile, spreading from Atacama to Patagonia and Tierra del Fuego (Habit *et al.*, 2006a; Arismendi *et al.*, 2009, 2011; Habit *et al.*, 2015; Vila & Habit, 2015) They are the main ichthyophagous (fish-eating) salmonid species reported, both representing a threat to native species (Macchi *et al.*, 1999; Milano *et al.*, 2002; Milano *et al.*, 2006; Aigo *et al.*, 2008; Ortiz-Sandoval 2014; Habit *et al.*, 2015). In addition to the effects of predation, native species have been reported competitively disadvantageous compared to salmonids (McIntosh 2000), producing the consequent displacement and diminution of the native fish fauna (Soto *et al.*, 2006; Soto *et al.*, 2007; Habit *et al.*, 2010). Moreover, Penaluna *et al.*, (2009) suggest the existence of trout microhabitat segregation from *O. mykiss* and *S. trutta* to *Trichomycterus areolatus* (Eigenmann, 1846) to native fish.

Furthermore, trout tend to become piscivorous as they grow so it is important to consider ontogenetic diet shifts when evaluating trout predation (McIntosh 2000; Lattuca *et al.*, 2008; Arismendi *et al.*, 2009; 2011). In addition to the effects of predation (McIntosh 2000), displacement, population diminution (Soto *et al.*, 2006; Soto *et al.*, 2007; Habit *et al.*, 2010) and the microhabitat segregation of the native fish fauna (Penaluna *et al.*, 2009) previously mentioned.

The diet is an important characteristic of the organisms that allows to determine the position within the trophic network and to know the role it plays in an ecosystem. It is closely related to food availability, i.e. such food preferences by a fish towards its prey are due to ecological conditions such as food abundance and availability (Baquemenoscal *et al.*, 2012; Fair *et al.*, 2013). Predation has a very important role in the structure and organization of communities and acts by reducing diversity, species abundance, population structure and cohorts (cannibalism), spatial distribution, and habitat occupation (Granados 2000; Peterson *et al.*, 2017). Many evolutionary biologists

believe that predation has played a major role in determining patterns in the history of life on this planet, such as the increase in the maximum complexity of organisms (Vermeij 1994; Levy 1999). The evolution of traits related to predation cannot be understood without some insight of the dynamics of interacting populations that do not exhibit any significant evolutionary change, there are very few species that are not engaged in some form of predator-prey interaction (Abrams 2000). To comprising this interaction requires a description of the potential dynamics of one or more traits in one or both species through time (Van der Laan & Hogeweg 1995). A predator is a taxon that consumes another; therefore, the term “predation” is used to describe an interaction in which individuals of one species kill and can consume a fraction of the biomass of individuals of another species. Prey are those individuals that are detritivorous, herbivorous or fungorous (Jeffries & Lawton 1985; Abrams 2000; Muñoz *et al.*, 2009).

Food web studies, both theoretical and empirical, have increased substantially in recent decades (Layman *et al.*, 2015). Animals often shift their diets in response to changes in resource availability (Buren *et al.*, 2012), ontogenetic stage (Peterson *et al.*, 2017) competition (Kie & Bowyer 1999), and interactions with predators or parasites (Thompson & Townsend 1999; Siepielski *et al.*, 2016). Consequently, food web structure varies in both space and time (Winemiller 1990; McCann 2011; McMeans *et al.*, 2015). The technique of stomach content analysis can be used to characterise the strength of interactions between consumers and their prey by determining trophic connections between species expressed as consumption of individuals (Tavares-Cromar & Williams 1996; Hall *et al.*, 2000; Muñoz *et al.*, 2009). Knowing the feeding habits of a particular species allow a better understanding of its life cycles (Wootton 1990; Saucedo-Lozano *et al.*, 1999; Bocanegra-Castillo *et al.*, 2000; Gámez *et al.*, 2014). According to Silva *et al.*, (2014), there are different methodologies for this type of analysis, each one responding

to particular assumptions and adapting to specific characteristics. The most appropriate would be the one that considers the particularities of the diet of the species under study, since fish are a highly diverse group with a wide range of functions in the systems in which they live, from primary consumers to the most carnivorous. (Helfman 2009; Torres-Rojas 2011; Ramírez-Herrejón *et al.*, 2013). Niche amplitude is a parameter that purports to quantify how specialized a species is within a given environment (Krebs 1989). It is possible to determine this parameter through diversity indices, which in the feeding context are called niche amplitude indices. Determining the diet of the fish allows us to obtain, In addition to the compiled data about this community, information about higher trophic levels, which this group generally occupies. The proportion in abundance or biomass of each of these strategies helps to understand the structure of the community in relation to the available resources (Muñoz *et al.*, 2009).

Consequently, the objective of this study is to determine the trophic interactions between the juveniles of the introduced fish species and the benthic fauna, established whether there is dietary overlap between fish species, in the Allipen river of La Araucanía Region.

1.2 Hypotheses:

The Allipen river fish community is dominated by juvenile salmonids, both resident and anadromous, which feed preferentially on benthic macroinvertebrates in a selective way and without a wide dietary overlap.

1.3 General objective:

Determine the trophic interactions between the juveniles of the introduced fish species and the benthic fauna of the Allipen river, established whether there is dietary overlap between fish species in the Allipen river of La Araucanía Region.

1.4 Specific objectives:

1. To characterize the communities of benthic macroinvertebrates and fish fauna of the Allipen river.
2. To confirm the presence of juvenile Chinook Salmon in the Allipen river.
3. To analyze the diet of juvenile salmonid assemblages in the Allipen river.

CHAPTER II

“First reports on seasonal abundances on Malacostracans Allipen river (38°51's North Patagonia, La Araucanía Region, Chile)”.

Associated to specific objective 1

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NOTES AND NEWS

First reports on seasonal abundances on Malacostracans Allipen river (38°51'S North Patagonia, Araucanía Region, Chile).

by

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Introduction and Methods

The benthic crustaceans in Chilean rivers their low species number and marked endemism (Jara *et al.*, 2006, Jara, 2013; Rudolph, 2013). Ecological studies have described mainly benthic communities in central and southern-central Chilean rivers (33-38°S), the community composition is variable as a function of water quality, and the water quality varied according to the use of the surrounding basin for human activities (Figueroa *et al.*, 2003, 2007). These rivers are characterized by their mixed regime with combined conditions of relatively large quantities of water all year (Vega *et al.*, 2018, in press) and a specific composition in the benthic communities that are adapted to these particular flow conditions (Figueroa, 2000; Colin *et al.*, 2012; Piedra *et al.*, 2012).

The benthic crustaceans reported from central Chilean rivers are mainly amphipods (genus *Hyaletta*) and decapods (families Aeglidae and Parastacidae) (Jara *et al.*, 2006; Jara 2013). The decapods have microendemic species that are reported in specific rivers and their respective basins, are the largest group, made up of shrimps from the following families Palaemonidae (a species) and Parastacidae (five species), more anomuros or “pancoras” from the family Aeglidae (18 species and two subspecies) (Jara *et al.*, 2006; Rudolph, 2013). All species are currently classified in one genus, *Aegla*, with 67 species distributed mainly in Brazil and Chile (Oyanedel *et al.*, 2011).

The Allipen river is located in the south of Chile (38°51'S y 71°45'W) at an altitude of 463 m above sea level (Figure 1). It presents a pluvio-nival hydrologic regime in a mediterranean-like bioclimatic zone with temperated rainy weather (annual average precipitation: 1500 mm) (Vargas *et al.*, 2010). The highest flows occur between May and August, due to the winter rains, between October and December, a slight snow influence, then decrease during the summer months. Is part of hydrological basin the Toltén river and is located in the Region of Araucanía. The aim of the present study was a description of the spatial pattern of distribution of an *Aegla abtao* and *Hyaletta patagonica* population from the Allipen river.

The sampling sites are located on the Allipen river, a tributary of the Toltén river basin in southern Chile (38°51'S and 71°45'W) at an elevation of 463 meters above sea level. The samples were collected in four sampling campaigns (high and low flow) between the months of November (Spring) 2016, January (Summer), May (Autumn) and August (Winter) 2017, at three sites distributed along the Allipen river (Figure 1, Table I).

Benthic crustacean samples were collected using a Surber net of 0.25m² surface. The samples were stored in plastic bags, labeled and fixed in Kahle solution for 36-48 h

(Peña 2006), and then preserved in 95 % alcohol for subsequent separation and identification of organisms to the lowest possible taxonomic level using an Olympus SZ 2-ILST model stereomicroscope and dissection material. The results were expressed in numbers per square metre. The identification of the taxa was performed as described by González (2003) and Fernández & Domínguez (2009).

Results and Discussion

The results revealed low density of *Aegla abtao* among Allipen river, also, all specimens were collected third site specifically in Summer and Autumn, whereas this species was absent in the first and second sites, whereas *H. patagonica* was present in first site at spring, autumn and winter, and in second site was present only in autumn (Table I). The results about seasonality agree with similar results for Chillan river (36°S), where the minimum diversity is in winter whereas the maximum diversity is in summer (Figuroa *et al.*, 2007), similar results were described for close Cautin river (38°S; Figuroa, 2000), coastal rivers in northern Patagonia (38°S; Fierro *et al.*, 2012) and central Patagonian rivers in Aysen region (45° S; Oyanedel *et al.*, 2008; Moya *et al.*, 2009; Valdovinos *et al.*, 2010).

Other important factor that would affect the malacostracan abundances would be the introduced salmonids presence among Allipen river, such as was reported for Vargas *et al.*, (2010), the presence of salmonids have important ecological role because they are opportunistic predators on benthic fauna in Patagonian rivers (Vila *et al.*, 1999; Soto *et al.*, 2006, 2007; Vargas *et al.*, 2010; Encina *et al.*, 2017). The salmonids can be active predators on crustaceans, in according to literature descriptions for Cautin river, that is close to studied site (Vega *et al.*, 2017; Vega *et al.*, in press), and coastal rivers and streams in the same latitude of studied site (Fierro *et al.*, 2012).

In this scenario, it would be necessary do more integrative studies at seasonal scales, that involves the presence of predator fishes, specially salmonids considering their active predation mainly on invertebrates, that would be a complex system (Arismendi *et al.*, 2009; Ings *et al.*, 2009; Woodward *et al.*, 2010; Schmid-Araya *et al.*, 2012; Gray *et al.*, 2015), that would explain the temporal and spatial abundance of crustacean species in Patagonian rivers.

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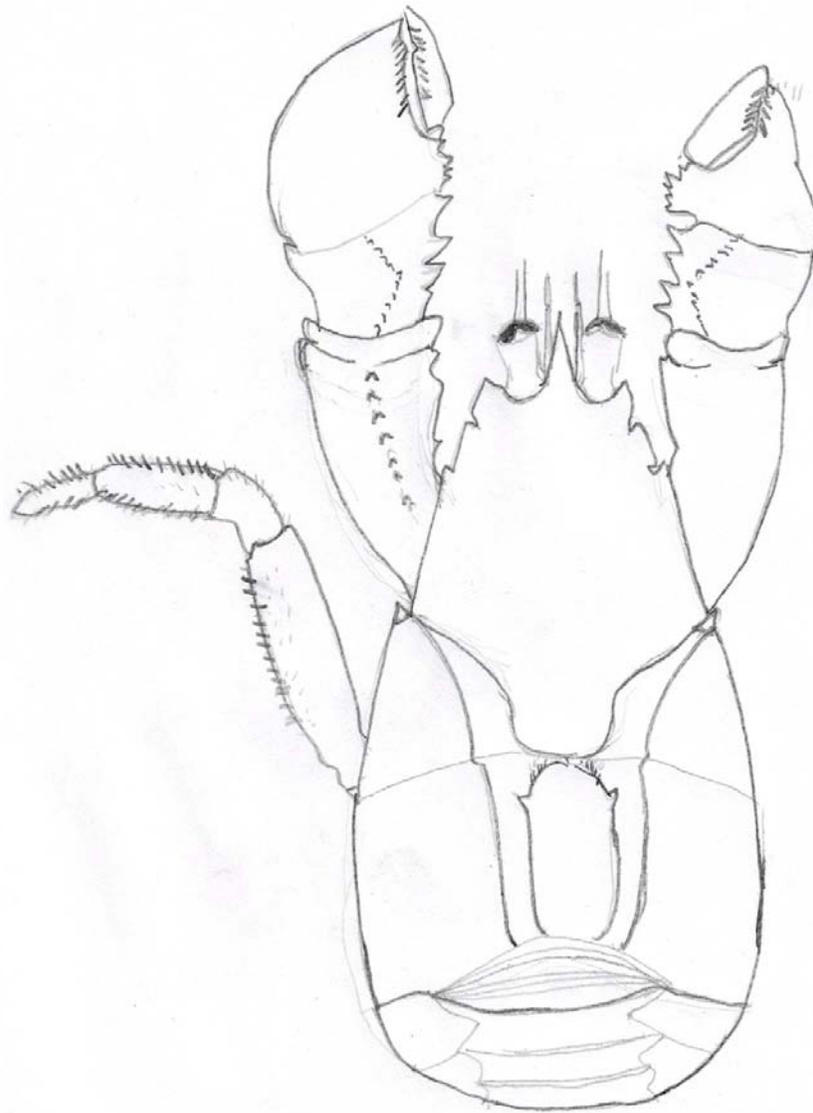
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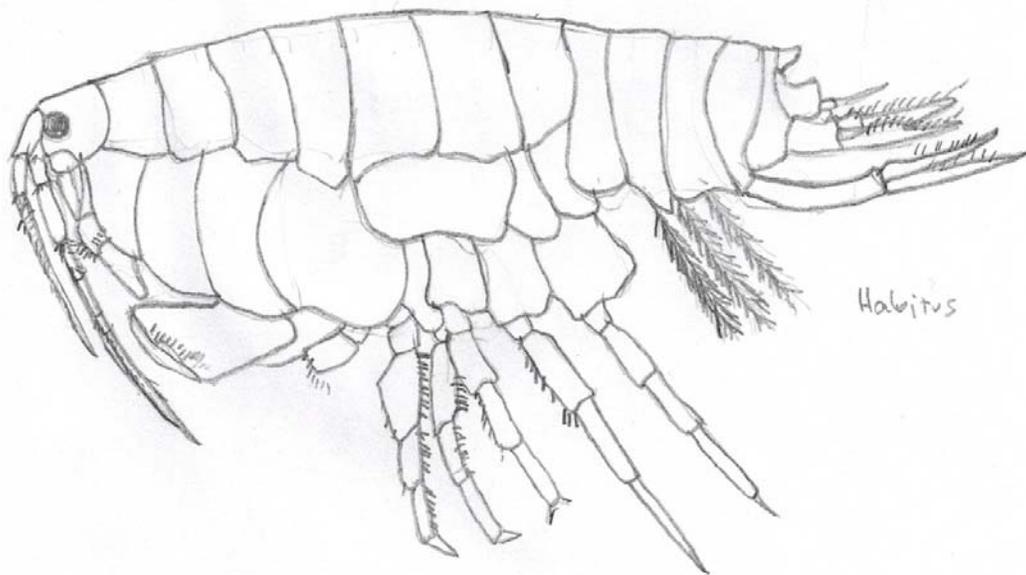
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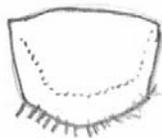
Aegla abtao



Habitus



Male first gnathopod.



Telson

Hyalella patagónica

CHAPTER III

***“First report of the identification of juveniles of
Oncorhynchus tshawytscha in the Allipen river
(La Araucanía Region, Chile) using
microsatellite DNA markers”.***

Associated to specific objective 2

Paper major revision in *Environmental Biology of Fishes*.

The format was adapted for this Doctoral Thesis.

First report of the identification of juveniles of *Oncorhynchus tshawytscha* in the Allipen river (La Araucanía Region, Chile) using microsatellite DNA markers

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Abstract

The detection of fish populations of commercial interest that have been introduced into aquatic ecosystems other than their origin, allows studies of relationships within communities that guarantee the implementation of measures for the preservation of all species present in the area, both the local species such as exotic ones. *Oncorhynchus tshawytscha* (Walbaum, 1792) is a salmonid species introduced into Allipen river (La Araucanía, Chile), which has generated a negative impact on the ecosystem by affecting various communities, an increasing problem day by day due to the juveniles presented a morphology similar to others salmonid species that inhabit the area. An effective way to identify these populations at early ages when they present morphological similarity with other species is through molecular markers, therefore, the objective of this study was to identify juveniles of *Oncorhynchus tshawytscha* in Allipen river through microsatellites. Twenty microsatellites were used to identify 4 juveniles from the autumn run and 22 from the spring run and compared to two adults *Oncorhynchus tshawytscha* and two adults of *Salmo trutta*. Five of the 20 microsatellite loci analyzed were successfully amplified (Ogo 2, Ogo 4 and Oke 4, Ots G249 and Ots G78b), identifying the 26 juveniles as *Oncorhynchus tshawytscha*. There was no evidence of genetic difference between the different run (autumn and spring). It was concluded that the identification of juvenile *Oncorhynchus tshawytscha* was possible through these five microsatellites, highlighting this research as the first report that allows the identification of this species in Allipen river and validates it through molecular markers.

Keywords

Oncorhynchus tshawytscha, Chinook Salmon; Allipen river; loci of microsatellites; genetic variation.

Introduction

Chinook salmon (*Oncorhynchus tshawytscha*) is an anadromous and semelparous salmonid species (NOAA 2016) with sexual dimorphism in the adult stage (Gómez-Uchida *et al.*, 2016). They feed on terrestrial and aquatic insects, amphipods and other crustaceans when they are young, and mainly on other fish when they are older, which is possible because they are the largest salmonid species, some specimens reaching 45 kg and more than 1m in length (Healey 1991). In relation to its geographical distribution, as a native species it can be found on the Pacific coast of North America (Alaska, Canada, and northern North America), as well as in northern Asia Pacific (Russia, northern Japan). However, according to Welcomme (1988), *Oncorhynchus tshawytscha* was the first Pacific Salmon introduced into environments that are not its native environment, which has generated that it is currently widely distributed throughout the world.

The introduction of this species in areas that are beyond its natural distribution has been a process generated by anthropogenic causes for economic purposes to develop aquaculture and increase tourism through recreational fishing and artisanal fishing (Pascual *et al.*, 2001, 2002; Soto *et al.*, 2001, 2006). In Chile, the introduction of Chinook salmon began more than 30 years ago in the Chiloé and Magallanes area through the technique of "farming on ocean farms" (releasing juveniles in freshwater) (Di Prinzio & Pascual 2008; Correa & Gross 2008), distributing from the La Araucanía region to the fjords of Patagonia (Correa & Gross 2008). Although there is information on the behavior and biology of *Oncorhynchus tshawytscha* in the Los Lagos and Aysén regions, their ecology, abundance, and life stories on the Toltén river are poorly understood. This distribution limit was determined by Correa & Gross (2008), confirming the presence of spawning adults. However, the records do not include juvenile stages despite the fishing effort through direct observations using the snorkeling technique.

Allipen river, which is part of the hydrological basin of the Toltén river, is characterized by the greater availability of *Oncorhynchus tshawytscha*, generating a value chain related to economic activities, such as tourism, recreational fishing and others, which constitute an opportunity for the economic development of the riparian communities (Gómez-Uchida *et al.*, 2016). This situation contrasts completely with the biological importance of this species, where it should be noted that unlike the natural balance established in the ecosystems of the northern hemisphere where salmon have natural predators such as brown and gray bears and coyotes (*Ursus arctos* Linnaeus 1758, *Ursus americanus* Pallas 1780 and *Canis latrans* Say 1823), in the southern hemisphere these large mammals are not present and native fish species are under constant pressure from these exotic species due to predation and competition for habitat and food, which generates a negative impact on ecosystems and aquatic biodiversity (Mack *et al.*, 2000; Soto *et al.*, 2006; Correa & Gross 2008; Habit *et al.*, 2015). This is how the introduction of salmon in Chile has caused great ecological impacts on aquatic ecosystems (Arismendi *et al.*, 2014), specifically *Oncorhynchus tshawytscha* that negatively affects native fauna (Vargas *et al.*, 2010; Hamann & Kennedy 2012; Habit *et al.*, 2012, 2015), a situation that is strongly observed in the Allipen river. For this reason, biological invasions are of scientific interest because they are considered one of the most attractive natural experiments in biogeography and ecology, and for their effect on ecosystems and biodiversity (Callaway & Maron 2006).

The first records of sightings of Chinook salmon in the Allipen river date back to 1998 & 1999, by local fishermen whose attention was attracted by the large sizes of the species. It was not until the years 2007 & 2008 that the first report of the presence of juveniles of this species in said river was obtained (Vargas *et al.*, 2010), which is due, in part, to the fact that juveniles begin their migration to the twelve to eighteen months after hatching (Gómez-Uchida *et al.*, 2016), since in the initial stages of their life cycle they present

morphological characteristics similar to their salmonid congeners (Pollard *et al.*, 1997), therefore, as it was reported by Docker & Heath (2002), more specific studies are required, such as the use of molecular genetic markers that allow evaluating the characteristics of each species and allow the correct identification of the species. Thus, due to the scarcity of reports on the presence of juveniles of Chinook Salmon in the Allipen river, and so far none of these studies have used molecular markers to identify the specimens, which the objective of this research was to identify juveniles of *Oncorhynchus tshawytscha* in the Allipen river through genetic markers corresponding to microsatellites.

Materials and methods

Animals and sample collection

Tissue samples from the caudal fin of the specimens were obtained from the Allipen river, located in south of Chile (38°51`S and 71°45`W) at an altitude of 463 m above sea level (Figure 1). Thirty specimens of salmonids were used in the present study, of which 26 correspond to fall- and spring-run juvenile of the *Oncorhynchus tshawytscha*, 2 correspond to adult of the *Oncorhynchus tshawytscha* used as positive control and 2 to adult of the *Salmo trutta* used as negative control. The captures were made in fall of 2016 and spring of 2017.

Genetic Analysis

First, a polymerase chain reaction (PCR)-based method was used to identify Chinook DNA sequences that are specific to primer selected, hence PCR used to exponentially amplify specific fragments of DNA of juvenile and compare these to a known marker. A set of 20 primers distributed among the 68 (2N) chromosomes were initially screened for evaluation and 5 exhibiting higher levels of polymorphism were selected (Ots G249, Ots G78b, Ogo 2, Ogo 4, Oke 4; Table 1). These primer sequences were obtained from

previous studies of Williamson *et al.*, (2002), Greig *et al.*, (2003) & Garza *et al.*, (2008). Reaction conditions were performed in 25 μ L total volume made up of 0.1 μ M each primer, 1 unit Taq DNA polymerase, 0.2 μ M each dNTP, 10 mM Tris-HCl pH 7.2, 50 mM KCl, 1.5 mM MgCl₂, DMSO (50%), and 120 μ g DNA. The reaction was amplified in DNA Eppendorf® 5331 MasterCycler Gradient Thermal programmed for one cycle at 95 °C for 5 min followed by 35 cycles of 95 °C for 1 min, 55-65 °C (in accordance with the primer) for 2 min, and an extension period at 72 °C for 7 min. A 1.5 μ L aliquot of PCR products was loaded onto 6% denaturing polyacrylamide gels and run in 0.5 \times TBE buffer at 1800 V for about 2 h. Gels were stained with silver nitrate to visualize DNA fragments according to Promega's protocol (Madison, Wisconsin, USA). Allele sizes were estimated based on Perfect DNA 50 bp (EMD Chemicals, Madison, Wisconsin, USA) and PCR marker 50-2000bp (EMD Chemicals) ladders.

Statistical analysis

For the analysis of similarity between the samples, exploratory analysis of the band patterns of the samples obtained in the analysis with agarose gel were performed, they were visualized through a conglomerate analysis (cluster) based on the Jaccard test (presence/ absence) using the PAST 3.2 program. Additionally, a multidimensional scaling analysis without metrics (nMDS) was performed.

Results and Discussion

The development of genetic markers has allowed great advances in the quantitative analysis of traits of interest in different species (Gutierrez *et al.*, 2012), where DNA markers are particularly useful because samples can be collected without killing the organism and many markers (for example, microsatellites) show a high level of polymorphism (Docker & Heath 2002). In addition, DNA markers are currently recognized as attractive tools in salmon management, due to their less stringent storage

requirements and potentially higher resolution (Rasmussen *et al.*, 2003). In relation to *microsatellite DNA markers*, these are characterized by being highly polymorphic, providing greater statistical power to detect kinship than alloenzymes previously provided. (Banks *et al.*, 2000); in addition, they have a high power of discrimination to solve the complex genetic structure of salmon populations within a hydrographic basin (Banks *et al.*, 2000). Another characteristic that highlights the importance of their use is the technique by which they are analyzed, which corresponds to PCR amplification, which stands out for being a fast method that requires only small amounts of tissue that can be obtained in a non-lethal way, which is favorable for the work with juvenile fish (Rasmussen *et al.*, 2003). Nowadays, many ecological and evolutionary studies in Pacific salmon, as well as management and conservation efforts, require molecular genetic markers that describe population structure and variation (Docker & Heath 2002).

Chinook salmon, in Allipen river, was one of the salmonid species that was introduced in Chile many years ago, so it was expected that it would present genetic differences with the rest of the populations of *Oncorhynchus tshawytscha* in the North Pacific Ocean, moreover considering the exposed by Beacham *et al.*, (2006a), in which they indicated that there is an evident genetic differentiation between populations of Chinook Salmon in the different geographical regions, this because the differentiation of the populations is the result of two forces that interact and configure the genetic variation: 1) time from radiation of the population, and 2) the magnitude of the subsequent gene flow between different populations, both forces produce genetic signatures (Garza *et al.*, 2008). For this reason, *microsatellite DNA markers* were used to identify juvenile *Oncorhynchus tshawytscha*, initially, a set of 20 primers was examined for evaluation through Agarose Gels, of the 20 markers used in this study (Table 1), only the 5 microsatellite loci that presented the highest levels of polymorphism were taken into account, which it did not

represent a problem for obtaining reliable results, since according to Beacham *et al.*, (2003), when more than eight loci are used to estimate stock compositions, the increase in precision is marginal and the least effective loci provide only a modest increase in accuracy. Furthermore, not all loci can be amplified in all species under the given set of PCR conditions, and the degree of polymorphism at each locus differs between species (Williamson *et al.*, 2002). Thus, for *O. tshawytscha*, loci with more than 20 alleles are probably more effective for identifying individual or stock than loci with fewer alleles (Beacham *et al.*, 2003). Taking this information into account, in this study the Ots G249 microsatellite loci was not considered a valid marker for the identification of juveniles *Oncorhynchus tshawytscha* in Allipen river, since despite having been used successfully in juveniles of this species, has less than 20 alleles (Evans *et al.*, 2010). It has also been used to identify Chinook Salmon from Steelhead, Atlantic, Brook, Coho and Cutthroat (Williamson *et al.*, 2002), so it is not convenient to use it exclusively for the identification of salmon species. On the other hand, the Ots G78b microsatellite loci has been shown to only amplify in Chinook and Coho, so, although not exclusive to Chinook, it is more selective (Williamson *et al.*, 2002). In addition, within the selected microsatellite loci there are three, Ogo 2, Ogo 4 and Oke 4, which have been widely used in other studies, demonstrating a high identification of species because they are present in many alleles, which increases the probability of appearance during PCR amplification (Beacham *et al.*, 2006a, Beacham *et al.*, 2006b, Withler *et al.*, 2007, Garza *et al.*, 2008). Of the five microsatellite loci analyzed, none were amplified for the negative control used (samples from *Salmo trutta*).

Genetic variation can be used as a very effective tool to assess the structure of the salmonid population, it is a key component in the elucidation of management units or conservation units in a species, and it can be applied to manage the fisheries that specific

salmon populations are exploited (Beacham *et al.*, 2006a). In order to determine the genetic relationship between the samples evaluated, a Cluster analysis was carried out, in which the similarity between the StS10616B and StS10616A samples grouped with 80% was evidenced, and in turn it was observed that they presented a great difference with respect to the rest of the samples (Figure 2). Similarly, samples StS10616B and StS10616A were determined to correspond to the two samples of *S. trutta* used as a negative control within the analysis. The multidimensional scaling analysis confirmed what was observed in the dendrogram where the two specimens are distant from the rest and close to each other, confirming that it is a different species from the rest of the sample. In turn, the other 28 specimens are close in the scale, confirming that they are the same species. The positive control (OtS10917D1 and OtS10917C4) used in the study was effective since there were no differences between the adult specimens of *O. tshawytscha* and the rest of the juveniles of the same species. In turn, adults used as a positive control could be considered to be genetically related to juveniles from autumn and spring runs. Although juveniles were caught in the fall (OtS20516, OtS20516B, OtS20516D, OtS20516E) and in spring (the rest of the sample) they were not located differentially within the dendrogram. This may indicate that the markers used were not sufficient to establish both runs as different populations and/or that there are no great differences between the genome of the juveniles captured in autumn and spring, which contrasts with that observed by Garza *et al.*, (2008) in the Feather river Hatchery, where spring and fall fish were not closely related and found that the vast majority (> 98%) of genetic variation was between individuals within populations, which is the expected pattern for generalized species with substantial gene flow between populations. The segregation in clades presented by the sample is also evident and that these clades are made up of very few specimens (in some cases only three), which gives indications of the genetic variability

that exists between the individuals of both populations. For the Fraser river chinook salmon, the genetic variation attributable to population differentiation was approximately eight times the variation attributable to annual variation within populations (Beacham *et al.*, 2003), whereas in this study, from the results obtained in the dendrogram it can be inferred that the introduction of Chinook Salmon in the Allipen river could have been at two different times, which allowed these salmon to genetically diversify into two variants, since only one of the autumn samples was it is more genetically distant from the rest of the population and the other three samples are more closely related to specimens of the spring run, it being evident that all these samples have a common ancestor or, on the other hand, that when the species was introduced into this river, there was selective pressure that caused a part of the population to undergo genetic variations - for example due to mutations - that were established in the following generations, generating these two variants. In any case, these populations do not meet the necessary premises for the Hardy-Weinberg equilibrium to occur, so it is expected that genetic diversity will be obtained within the population. The results obtained agree with a study carried out on Chinook Salmon from the Central Valley by Garza *et al.*, (2008) where said population did not meet the Hardy-Weinberg equilibrium. Bottlenecks and genetic drift, which can affect highly polymorphic microsatellite markers in particular (Hedrick 1999), these may be some of the reasons that have caused the genetic divergence observed for the Ots20516 specimen with respect to the rest of the sample.

The results of this work, although preliminary, indicate that these DNA markers can be highly effective for the identification of populations of juveniles of *O. tshawytscha* in Allipen river, since they allow them to be identified with greater certainty than is currently achieved due to morphological characteristics. The potential for discrimination that is achieved with microsatellite loci to identify juvenile populations of Chinook Salmon is

of obvious importance for the management and handling of this species introduced into Allipen river. Due to the effect that Chinook Salmon has on the native species of Allipen river, it is necessary to have precise information on their presence and abundance so that the relevant organizations make regulatory decisions for the control of these populations and the management of native species in a context of conservation of aquatic ecosystems. In countries like United States, this species is threatened in California Valley due to massive habitat destruction, exploitation, and artificial propagation (Banks *et al.*, 2000), therefore it is also important to have valid reports of its presence elsewhere of the world. These microsatellite loci are expected to be useful in a variety of studies, given their efficacy in identifying juveniles of the species, the low cost of PCR assays, and their high performance relative to other DNA-based methods.

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Tables

Table 1

Microsatellite locus and PCR primer summary. Forward and reverse primers used for PCR are listed. The first five microsatellite loci were the most polymorphic and were taken into account for the study results.

Locus	Primer	Primer Sequence (5' to 3')	Citation
<i>Ogo 2</i>	F R	ACA TCG CAC ACC ATA AGC AT GTT TCT TCG ACT GTT TCC TCT GTG TTG AG	Garza <i>et al.</i> , 2008
<i>Ogo 4</i>	F R	GTC GTC ACT GGC ATC AGC TA GAG TGG AGA TGC AGC CAA AG	Garza <i>et al.</i> , 2008
<i>Oke 4</i>	F R	AGG CCC AAA GTC TGT AGT GAA GG GAT GAA TCG AGA GAA TAG GGA CTG AAT	Garza <i>et al.</i> , 2008
<i>Ots G78b</i>	F R	GTC CCT TGA ATT GAA TTG ATT AGA CAG CCT ACT GCA GTT CAA TAG ACT	Williamson <i>et al.</i> , 2002
<i>Ots G249</i>	F R	TTC TCA GAG GGT AAA ATC TCA GTA AG GTA CAA CCC CTC TCA CCT ACC C	Williamson <i>et al.</i> , 2002
<i>Omm 1032</i>	F R	GCG AGG AAG AGA AAG TAG TAG CCC ATC TTC TCT CTG ATT ATG	Garza <i>et al.</i> , 2008
<i>Omm 1080</i>	F R	GAG ACT GAC ACG GGT ATT GA GTT ATG TTG TCA TGC CTA GGG	Garza <i>et al.</i> , 2008
<i>Omy 77</i>	F R	CGT TCT CTA CTG AGT CAT GTC TTT AAG GCT TCA CTG CA	Garza <i>et al.</i> , 2008
<i>Omy 325</i>	F R	TGTGAGACTGTCAGATTTTGC CGGAGTCCGTATCCTTCCC	Garza <i>et al.</i> , 2008
<i>Ots M2</i>	F R	ACA CCT CAC ACT TAG A CAG TGT GAA GGA TAT TAA A	Garza <i>et al.</i> , 2008
<i>Ots 4</i>	F R	GAA CCC AGA GCA CAG CAC AA GGA GGA CAC ATT TCA GCA G	Garza <i>et al.</i> , 2008
<i>Ots M9</i>	F R	ATC AGG GAA AGC TTT GGA GA CCC TCT GTT CAC AGC TAG CA	Garza <i>et al.</i> , 2008
<i>Ots 201b</i>	F R	CAG GGC GTG ACA ATT ATG C TGG ACA TCT GTG CGT TGC	Garza <i>et al.</i> , 2008
<i>Ots 211</i>	F	TAG GTT ACT GCT TCC GTC AAT G	Greig <i>et al.</i> , 2003

	R	GAG AGG TGG TAG GAT TTG CAG	
<i>Ots 213</i>	F R	CCC TAC TCA TGT CTC TAT TTG GTG AGC CAA GGC ATT TCT AAG TGAC	Greig <i>et al.</i> , 2003
<i>Ots G83b</i>	F R	TAG CCC TGC ACT AAA ATA CAG TTC CAT TAA TCT AGG CTT GTC AGC AGT	Williamson <i>et al.</i> , 2002
<i>Ots G311</i>	F R	TGC GGT GCT CAA AGT GAT CTC AGT CA TCC ATC CCT CCC CCA TCC ATT GT	Williamson <i>et al.</i> , 2002
<i>Ots G432</i>	F R	TGA AAA GTA GGG GAA ACA CAT ACG TAA AGC CCA TTG AAT TGA ATA GAA	Williamson <i>et al.</i> , 2002
<i>Ssa 85</i>	F R	AGG TGG GTC CTC CAA GCT AC ACC CGC TCC TCA CTT AAT C	Garza <i>et al.</i> , 2008
<i>Ssa 408</i>	F R	AATGGATTACGGGTACGTTAGACA CTCTTGTGCAGGTTCTTCATCTGT	Garza <i>et al.</i> , 2008

Figure Captions

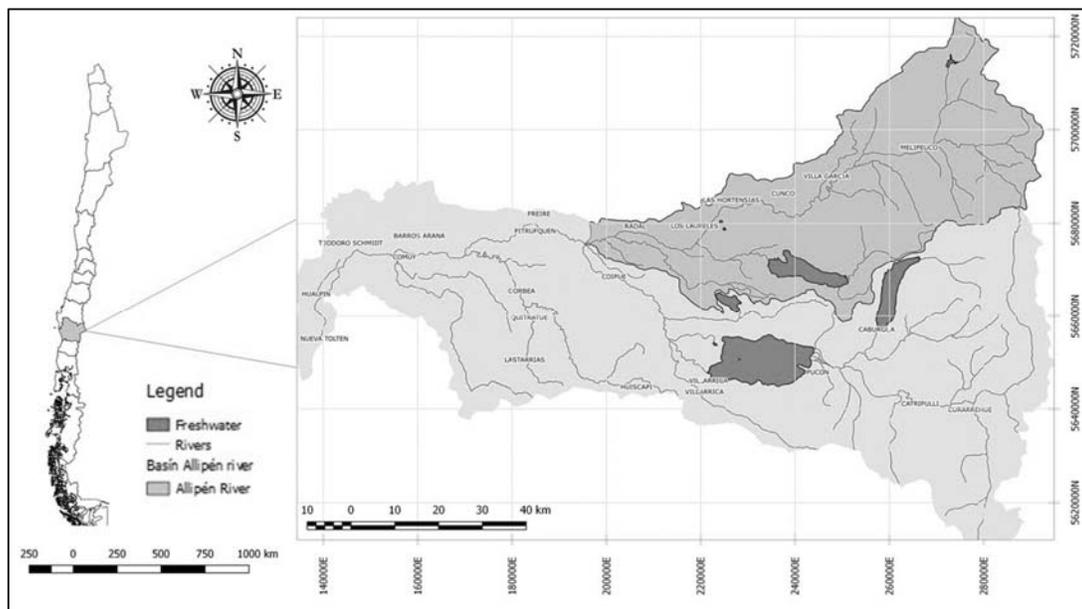


Figure 1. Map of Allipén river, Tolten river basin, Chile.

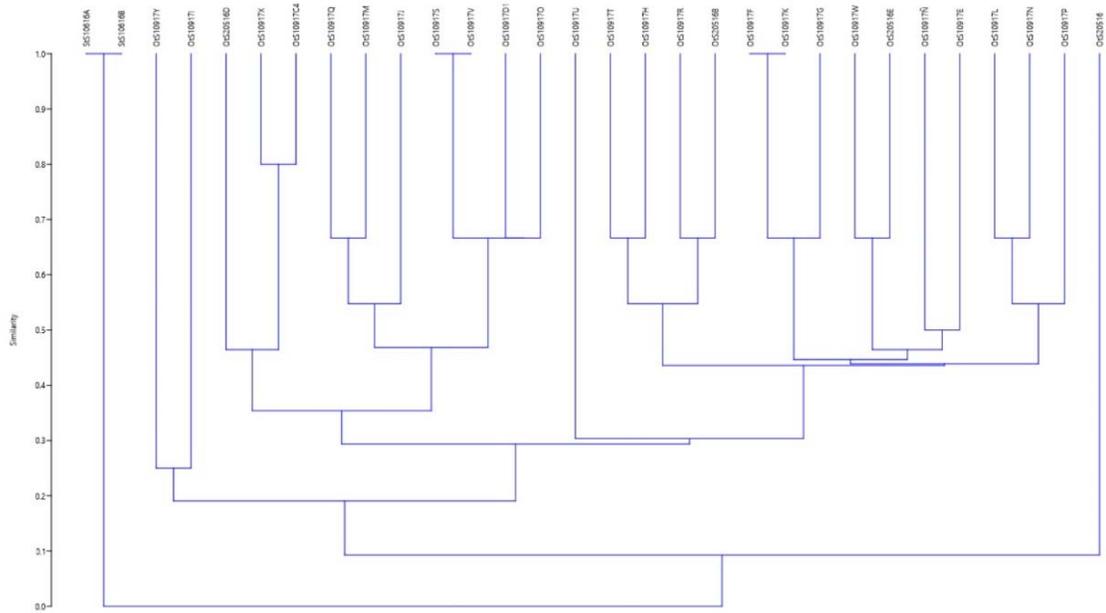


Figure. 2 Cluster analysis performed through a dendrogram using data from five microsatellite loci (Ots G249, Ots G78b, Ogo 2, Ogo 4, Oke 4) for 28 samples of *Oncorhynchus tshawytscha* and two samples of *Salmo trutta* from Allipen river, Region of La Araucanía, Chile. The scale indicates genetic distance

CHAPTER IV

***“Community structure of benthic invertebrates in
Allipen river basin, La Araucanía Region”.***

Associated to specific objective 1

Paper accepted in *Brazilian Journal of Biology*

The format was adapted for this Doctoral Thesis

Community structure of benthic invertebrates in the Allipen river basin, North Patagonia, Araucanía region (39° S, Chile).

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(With 4 figures)

Running title: Benthic invertebrates of the Allipen river

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Abstract

One of the biological indicators most used to determine the health of a fluvial ecosystem are the benthic macroinvertebrates. The presence of recurrent species in a wide gradient of latitudes, dominates the biogeographic pattern of the benthic macroinvertebrates in Chilean fresh waters, nevertheless the knowledge on the communitarian ecology of these in the Chilean rivers continues to be scarce. Null models became a powerful statistical tool for describing the ecological mechanisms that drive the structure of an ecological community and the underlying patterns of diversity. The objective of this study was to determine the community structure of benthic invertebrates in the Allipen river by describing their composition, richness and abundance of families through richness models and null models based on presence/absence. The results reveal a high species richness and low diversity, three phyla, five classes, 11 orders and 28 families were identified in the study area during the four seasons of the year. The Arthropoda phylum was the most representative in abundance and richness. Regarding to richness, Trichoptera (7 families) and Diptera (6 families) followed by Ephemeroptera (3 families) were the orders that showed the greatest diversity of families, however, a low diversity with a $H' \leq 1.5$ nit was registered in the study area. We demonstrated through the null models, the randomization in the species associations corresponding to the three analyzed sites. The information provided here contributes to the understanding of the ecological patterns of the invertebrate communities in the Allipen river, establishing the basis for more complex ecological studies.

Key words: Macroinvertebrates, null models, fluvial habitat, Toltén river basin.

**Estrutura comunitária de invertebrados bentônicos na bacia do rio Allipen,
Patagônia Norte, Região da Araucanía (38° S, Chile).**

Resumo

Um dos indicadores biológicos mais utilizados para determinar a saúde de um ecossistema fluvial são os macroinvertebrados bentônicos. A presença de espécies recorrentes em um amplo gradiente de latitudes domina o padrão biogeográfico dos macroinvertebrados bentônicos nas águas doces do Chile; no entanto, o conhecimento sobre a ecologia comunitária destes nos rios chilenos continua escasso. Os modelos nulos se tornaram uma poderosa ferramenta estatística para descrever os mecanismos ecológicos que orientam a estrutura de uma comunidade ecológica e os padrões subjacentes da diversidade. O objetivo deste estudo foi determinar a estrutura da comunidade de invertebrados bentônicos no rio Allipen, descrevendo sua composição, riqueza e abundância de espécies através de modelos de riqueza e modelos nulos baseados na presença / ausência. Os resultados revelam alta riqueza de espécies e baixa diversidade, sendo identificados três filos, cinco classes, 11 ordens e 28 famílias na área de estudo durante as quatro estações do ano. O filo de Arthropoda foi o mais representativo em abundância e riqueza. Em relação à riqueza, Trichoptera (7) e Diptera (6) seguidos por Ephemeroptera (3) foram as ordens que mostraram a maior diversidade de famílias, no entanto, uma baixa diversidade com $H' \leq 1,5$ nit foi registrada na área de estudo. Demonstramos através dos modelos nulos, a randomização nas associações de espécies correspondentes aos três locais analisados. As informações aqui fornecidas contribuem para a compreensão dos padrões ecológicos das comunidades de invertebrados no rio Allipen, estabelecendo a base para estudos ecológicos mais complexos.

Palavras-chave: Macroinvertebrados, modelos nulos, habitat fluvial, bacia do rio Toltén.

1. Introduction

Freshwater courses represent an essential component in the protection of the natural and cultural community heritage; biologically speaking, they support one of the richest and most diverse habitats, but also include one of the most threatened ecosystems worldwide (Dudgeon *et al.*, 2006; Gioria *et al.*, 2010). An adequate description of the biological community can give indications about the general state of the system, since the properties of the biota depend on environmental processes that can determine distribution and abundance patterns in the river ecosystem (Vannote *et al.*, 1980; Yoder, 1995; Resh *et al.*, 1996). The evaluation by bioassays and bioindicators complemented with the physical and chemical components, leads to a comprehensive water quality biomonitoring, which contributes to provide information to determine environmental risk (Gerhardt *et al.*, 2004). Anthropic activities are the modulating forces of ecosystem changes, which in most cases cause a decrease in water quality. In order to quickly know if the environmental pressures generate changes in the system, the use of biological indicators is recommended, which behave according to the physical-chemical changes, facilitating an integral analysis of the water resource (Springer *et al.*, 2010).

Benthic macroinvertebrates are one of the biological indicators mostly used to determine the fluvial ecosystem health, which reflect the environmental conditions in a specific time and space because the different benthic species have specific tolerance to environmental conditions, that would imply the presence of specific groups under different environmental conditions (Figueroa *et al.*, 2003, 2007). The response to these changes is observed in an increase or decrease of species within the community matrix (Machado *et al.*, 1988). Therefore, the community composition serves as a predictor of the habitat quality and the biotic integrity of the water system (Rosenberg & Resh, 1993; Allan & Castillo, 2007).

The benthic communities of central-southern Chile rivers (located between 33°S and 39°S) have a specific composition that has adapted to the particular flow conditions that move mostly through mountainous regions, showing a turbulence effect on its route to the ocean, in addition to the relatively large amount of water during much of the year, as a product of the rains in the area in winter and snow melting in summer (Niemeyer & Cereceda, 1984; Arenas, 1995; Vega *et al.*, 2017). These river ecosystems harbor multiple invertebrates' species with high conservation value which, at the same time, are influenced by human intervention (Figuroa *et al.*, 2013). Although there are several studies on benthic macroinvertebrates in various watersheds from Chile, the information gaps are evident, being this, one of the main obstacles for water resources management and conservation (Ramírez & Gutierrez-Fonseca, 2014). Therefore, information of the invertebrate fauna community ecology in Chilean rivers and their specific environmental drivers, remains scarce (Figuroa *et al.*, 2003, 2007, 2010; De los Ríos-Escalante *et al.*, 2015a, 2015b).

In this sense, null models are a powerful statistical tool to describe the ecological mechanisms that drive the structure of an ecological community and the underlying diversity patterns (De los Ríos *et al.*, 2019). Null models for the co-occurrence of species assume a random pattern of associations (Tiho & Josens, 2007), and the absence of overlapping niches, that is, the absence of interspecific competition (Gotelli & Ellison, 2013; Carvajal-Quintero *et al.*, 2015).

The objective of this study was to determine the community structure of benthic macroinvertebrates in the Allipen river describing their composition, the richness and abundance, through models of wealth and null models based on the presence / absence of species.

2. Materials and Methods

The Allipen river rises in the Andes Mountains, in the southern part of Chile, specifically in the La Araucanía region (Medina & Muñoz, 2020; Vargas *et al.*, 2010), afterwards receives the waters of Trifultruful river and Zahuelhue river, it has a 108 km length and a 2,325 km² basin area. The river drains to the northern part of the Toltén basin and ends its route 15 km east of Pitrufrquén, leading to the Toltén river (38°51'S and 71°45'W) (DGA, 2004). Sampling collection were carried out the months the times of drought or low flow (November 2016 and January 2017) and months the times of flood or high flow (May and August 2017). Three collection points were established along the ecological gradient of the river, from forests with little intervention to grassland areas and forest plantations.

The quantitative samples were carried out using a Surber network with a sampling surface area of 0.09 m² and 250 µm mesh opening, considering three replicates per site, standardizing the sampling sites with pebble bottoms (between 6-10 cm), with moderate velocities (0.1-0.2 m s⁻¹) and at a depth between 0.20 and 0.25 m (Figueroa *et al.*, 2007), and were stored in plastic bags, labeled and fixed in Kahle solution for 36-48 h (Peña, 2006), and then preserved in 95% alcohol. The identification was made up to family taxonomic level with an Olympus SZ 2-ILST stereomicroscope model and dissection material. Taxa identification was performed as described by McLellan *et al.*, (2005), González (2003) and Domínguez & Fernández (2009). The results were expressed in an abundance matrix for each taxon per sampling station (Ind. * m⁻²), where the community variables were analyzed, such as specific richness, Shannon Log₂ (H') and Simpson (D) diversity indexes (Table 5). Statistical analysis was performed through multiple regression using the R software (R Development Core Team, 2009) and the HSAUR R package (Everitt & Hothorn, 2016). Two models were run: the first used the number of

species and the second used the total abundance as a dependent variable (Tondoh, 2006; Tiho & Josens, 2007). In addition, a matrix of species' presence / absence was constructed. The "C-score" was calculated as a quantitative occurrence index that measures the degree to which the species coexist, due to causality, less frequently than expected (Gotelli, 2000). The community is structured by competition when the C score is significantly higher than the expected by causality (Gotelli, 2000; Tondoh, 2006; Tiho & Josens, 2007). Consequently, we compared the co-occurrence patterns with the null expectations through simulation using Null Fixed-Fixed statistical models (Gotelli & Ellison, 2013). In this model, the row and column sums in the matrix are preserved and each random community contains the same number of families (fixed column), and each family occurs with the same frequency as the original community (fixed row).

3. Results

The fauna richness found in the study area is given in Table 1. A total of 3 phyla, 5 classes, 11 orders and 28 families were identified in the study area during the four seasons of the year, most of these are immature insect stages which account for 95% of the total. Specifically, 13 families were reported in autumn, 8 in winter, 12 families in spring and 13 families in summer (Tables 1; 2 and 3). Due to the impossibility of identifying all the taxa found at the species level, they are treated indistinctly (family, genus, species) as individual units. The Arthropoda phylum was the most representative in abundance and richness than the others phyla.

The orders that showed the greatest diversity of families were Trichoptera (7 families) and Diptera (6 families) followed by Ephemeroptera (3 families) were however, a low diversity with a $H' \leq 1.5$ nit was registered in the total study area (Table 4). The results for the null model analysis revealed that the associations for the registered

families, are random, this mean that there are not structured pattern in species associations observed in studied site (Table 5).

The largest number of benthic macroinvertebrates taxa occurred during the dry season, including four orders and eight families. The most abundant groups were Elmidae (27%), Hydropsychidae (25%) and Chironomidae (18%), throughout the study area. Also important were the groups Gripopterygiidae, Leptophlebiidae, Baetidae and Blephariceridae. The Aeglidae showed the lowest relative abundance being present only in autumn and the Chilinidae (gastropod) nevertheless, it was found in all the sites studied (Tables 1; 2 and 3).

The Coleoptera order, with the Elmidae, showed the highest individual's relative abundance in the site 1 "Melipeuco", during the dry season (46.4 %) (Table 1; Figure 2), followed by the Trichoptera order in which the Hydropsychidae showed a 40.3% in autumn. The Chironomidae showed a greater contribution in the site 2 "Huereré" (Table 2) with 34.4% for the site and with its maximum abundance (87.8%) during winter time, followed by the Plecoptera order, with the Gripopterygidae which showed an 8.6% abundance on the site and its maximum of 9.8% in autumn, and the Baetidae with 5.7% representativeness, with a maximum of 7.8% in the same season; it should be noted that the three mentioned families were reported in the four seasons of the year (Figure 3). In the Hortensies site 3 (Table 3), eight families were registered and three of them were reported during the four seasons of the year: the Chironomidae, with 43.7%, Elmidae with 18.1%, and Baetidae with 7.4% abundance (Figure 4).

4. Discussion

The Allipen river waters in its downstream route are derived for various anthropogenic activities, receiving wastewater discharges from a range of industrial

activities. In some sections of the river system present an interruption of the natural flow, with the presence of agricultural and forestry activities in the marginal strip of the river, in addition to human settlement during the fishing season (Barile *et al.*, in press). The loss of this natural physical barrier disrupts the dynamics between the river and riverine vegetation favoring the direct entry of an excesses of agrochemicals and fertilizers into the river system, which affects the chemical characteristics and composition of the river biota.

In the upper zone of the basin Allipen river, the number of taxa of the Ephemeroptera, Plecoptera and Trichoptera orders remained relatively constant during the four seasons of the year, being replaced by the Diptera order taxa, in the downstream sites. This would suggest that the waters of the Allipen river in this area are well oxygenated water and in appropriate conditions for the development of these organisms due do ephemeropters prefer to live in good oxygenation places, in stone and sand substrates (Baptista *et al.*, 2006; Romero *et al.*, 2006; Moya *et al.*, 2009; Oyanedel *et al.*, 2008) and individuals of the Trichoptera order, in their larval phase are very well adapted to rapid water courses and stony substrates in high-speed rivers, and are distributed across all types of habitats, being very well represented in all river flows, except in highly polluted areas (Basaguren, 1990).

The Chironomidae, which prevailed in all Allipen river during the four seasons of the year, included individuals with a high capacity to tolerate high organic load concentrations, and long periods with low oxygen concentrations, facilitating high reproduction rates in these environments (Kay *et al.*, 2001; Newall & Tiller, 2002; rivera *et al.*, 2004). This higher organic load concentrations could be associated with both urban centers and fish farms discharges, near the study area (FIP, 2017). According to studies by Figueroa *et al.*, (2003, 2007, 2010), for river ecosystems in central southern Chile,

only in the lower part, these taxa reach high abundance and biomass, being favored by their tolerance and the high organic detritus availability, which is part of their diet. The development of these activities without environmental criteria is leading to an excessive load of organic pollutants discharged, which minimize the waste elimination capacity of these aquatic ecosystems (Alonso & Camargo, 2005), risking its sustainability for food supply and biodiversity.

Some studies (Yam & Dudgeon, 2005) have confirmed the dependence of aquatic invertebrates on allochthonous material that reaches rivers, such as leaves and pieces of wood (detritus) that are consumed as food, and their influence on physical and biological processes, especially in habitat diversification (Nakano *et al.*, 1999; Thompson & Townsend, 2004; Lyon & Gross, 2005; Rios & Bailey, 2006). This aspect becomes vitally important when observe the a partial elimination of riverine vegetation, was detected downstream of the Allipen river (Huereré and Las Hortensias sites) producing an instability in the thermal characteristics of the water column, and an increase of the sediments frequency and aquatic macrophytes, facilitating the contaminants entry into water bodies (Scalley & Aide, 2003). Among the most important consequences of this environmental pressures, are the water quality deterioration and the biodiversity reduction (Corbacho *et al.*, 2003; Bonada *et al.*, 2006). Over the past decade, studies have shown that the changes in land use have been one of the main drivers of biodiversity loss (Diggelen *et al.*, 2005).

The results observed from co-occurrence null model analysis, revealed the absence of structuring pattern in species associations, it is due probably the presence of many species repeated in analyzed sites (Tondoh, 2006; Tiho & Johens, 2007), that is a similar situation observed for Chilean inland waters (De los Ríos *et al.*, 2019; De los Ríos-Escalante *et al.*, 2015b).

Finally, the benthonic macroinvertebrate community in the Allipen river is composed of three phyla; Arthropoda, Mollusca and Annelida five classes; Insecta, Arachnoidea, Malacostraca, Gastropoda and Oligochaeta; eleven orders and twenty-eight families. This background is extremely important due to there is little knowledge about the aquatic biota of this area, moreover knowledge of the diversity of benthic macroinvertebrates in Chile is fragmentary, despite the efforts of many national and foreign researchers, especially since the 19th century (Valdovinos, 2018). From a river conservation perspective, there are no development policies in Chile (Figuroa *et al.*, 2013). In consequence the results presented in this work have a high conservation value and provide unprecedented background information on the bentonite macroinvertebrates of the Allipen river in the Toltén Basin.

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Figures and Tables

Figure 1. Map of Allipen river, Tolten river basin, La Araucanía Region Chile. Where S1 “Melipeuco” (265639 S, 5694829 W), S2: “Huerere” (758774 S- 5681448 W) and S3: “Las Hortensias” (746454 S; 5684086 W) represent the specific sampling stations, respectively.

Figure 2. Mean abundance (individuals/m²) the families more representative of benthic macroinvertebrates at site 1 Melipeuco in the Allipen river.

Figure 3. Mean abundance (individuals/m²) the families more representative of benthic macroinvertebrates at site 2 Huereré in the Allipen river.

Figure 4. Mean abundance (individuals/m²) the families more representative of benthic macroinvertebrates at site 3 Las Hortensias in the Allipen river.

Table 1. Benthic invertebrate abundances (ind/m²) observed for first site (Melipeuco) during sampling period.

Table 2. Benthic invertebrate abundances (ind/m²) observed for second site (Huereré) during sampling period.

Table 3. Benthic invertebrate abundances (ind/m²) observed for third site (Las Hortensias) during sampling period.

Table 4. Results of Shannon Log₂ (H') and Simpson (D) diversity indexes for community of benthic invertebrates the Allipen river.

Table 5. Results of null model analysis for studied sites.

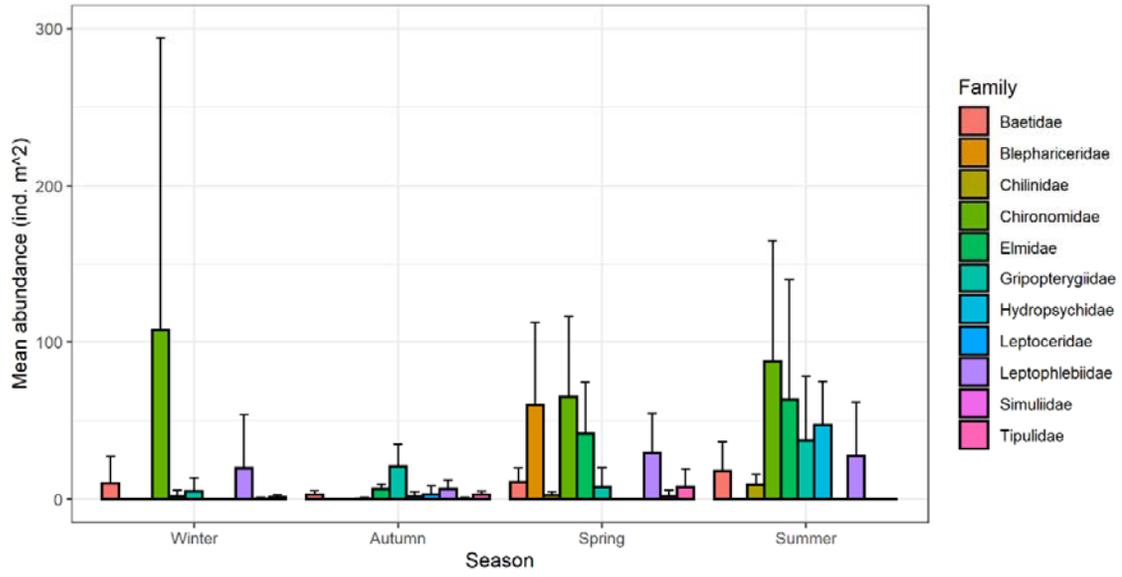


Figure 3.

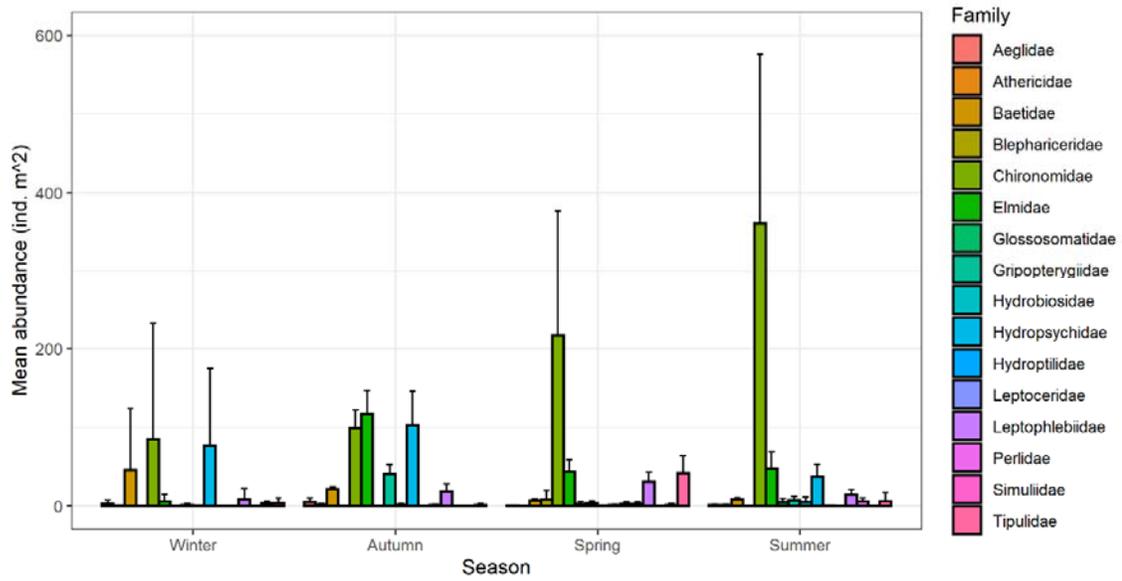


Figure 4.

Table 1.

Phylum	Class	Order	Family	Summer	Spring	Autumn	Winter
Annelida	Oligochaeta	Haplotaxida	Tubificidae	1	0	0	0
Arthropoda	Arachnoidea	Acari	Unidentified	6	0	0	0
	Insecta	Coleoptera	Elmidae	1790	206	488	60
			Psephenidae	0	0	0	0
		Diptera	Athericidae	9	76	13	4
			Blephariceridae	0	1	0	0
			Chironomidae	79	7	169	266
			Empididae	1	0	5	2
			Simuliidae	0	6	35	0
			Tipulidae	1	7	0	50
		Ephemeroptera	Ameltopsidae	0	0	1	0
			Baetidae	78	52	102	42
			Leptophlebiidae	145	191	8	113
		Plecoptera	Austroperlidae	0	0	6	0
			Diamphipnoide	0	1	0	0
			Gripopterygiidae	558	36	300	17
			Notonemouridae	0	0	0	0
			Perlidae	15	3	0	0
		Trichoptera	Glossosomatidae	0	0	0	0
			Hydropsychidae	1167	8	642	138
			Hydroptilidae	0	0	0	0
			Hydrobiosidae	0	4	5	0
			Leptoceridae	0	28	2	1
			Limnephilidae	0	0	0	0
			Polycentropodidae	0	1	2	0
	Malacostraca	Amphipoda	Hyalellidae	0	1	3	4
		Decapoda	Aegliidae	0	0	0	0
Mollusca	Gastropoda	Basommatophora	Chilinidae	0	1	0	0

Table 2.

Phylum	Class	Order	Family	Summer	Spring	Autumn	Winter
Annelida	Oligochaeta	Haplotaxida	Tubificidae	0	0	0	0
Arthropoda	Arachnoidea	Acari	Unidentified	0	0	0	0
	Insecta	Coleoptera	Elmidae	45	125	19	6
			Psephenidae	0	0	0	0
		Diptera	Athericidae	1	0	0	0
			Blephariceridae	0	179	0	0
			Chironomidae	11	195	1	323
			Empididae	3	0	0	0
			Simuliidae	0	6	1	1
			Tipulidae	0	23	8	3
		Ephemeroptera	Ameltopsidae	0	1	0	0
			Baetidae	24	32	8	30
			Leptophlebiidae	85	88	19	59
		Plecoptera	Austroperlidae	0	0	0	0
			Diamphipnoide	0	0	0	0
			Gripopterygiidae	308	23	62	15
			Notonemouridae	0	0	0	0
			Perlidae	3	0	0	0
		Trichoptera	Glossosomatidae	0	0	0	0
			Hydropsychidae	661	6	0	0
			Hydroptilidae	0	0	0	0
			Hydrobiosidae	0	0	0	0
			Leptoceridae	0	9	0	0
			Limnephilidae	0	2	0	1
			Polycentropodidae	0	0	0	0
	Malacostraca	Amphipoda	Hyalellidae	0	0	2	0
		Decapoda	Aeglididae	0	0	0	0
Mollusca	Gastropoda	Basommatophora	Chiliniidae	0	7	0	0

Table 3.

Phylum	Class	Order	Family	Summer	Spring	Autumn	Winter
Annelida	Oligochaeta	Haplotaxida	Tubificidae	0	0	0	0
Arthropoda	Arachnoidea	Acari	Unidentified	0	0	0	0
	Insecta	Coleoptera	Elmidae	352	130	350	16
			Psephenidae	0	0	1	0
		Diptera	Athericidae	15	0	4	1
			Blephariceridae	0	23	0	0
			Chironomidae	289	587	296	188
			Empididae	1	0	1	0
			Simuliidae	0	123	0	0
			Tipulidae	24	0	3	0
		Ephemeroptera	Ameltopsidae	0	0	0	0
			Baetidae	0	19	63	9
			Leptophlebiidae	97	92	54	4
		Plecoptera	Austroperlidae	0	0	0	0
			Diamphipnoide	0	0	0	0
			Gripopterygiidae	121	10	121	3
			Perlidae	20	0	0	0
			Notonemouridae	0	0	2	0
		Trichoptera	Glossosomatidae	0	7	0	0
			Hydropsychidae	321	3	308	106
			Hydroptilidae	0	6	0	0
			Hydrobiosidae	0	2	4	0
			Leptoceridae	0	6	3	0
			Limnephilidae	0	0	0	0
			Polycentropodidae	0	0	2	0
	Malacostraca	Amphipoda	Hyaellidae	0	0	1	0
		Decapoda	Aeglididae	15	0	15	0
Mollusca	Gastropoda	Basommatophora	Chilinidae	0	0	10	1

Table 4.

	S1	S2	S3	Average	Standard deviation
Simpson_1-D	0,729	0,608	0,616	0,65	0,068
Shannon_H	1,566	1,314	1,354	1,41	0,135

Table 5.

Species co-occurrence

	Observed index	Mean index	Standard effect size	Variance	P
Melipeuco	0.367	0.359	0.726	< 0.001	0.284
Huerere	0.382	0.366	0.671	< 0.001	0.256
Las Hortensias	0.484	0.499	-0.794	< 0.001	0.804

CHAPTER V

"First description of the fish fauna from the Allipen river with emphasis on trophic ecology of exotic fish."

Associated to specific objective 1 and 3

Paper in preparation

"First description of the fish fauna from the Allipen river with emphasis on trophic ecology of exotic fish."

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Introduction

The Allipen river is located at the southern part of Chile (38°51`S and 71°45`W) at 463 m above sea level; it is part of the watershed of the Toltén river, which has an area of 7,886 km² and is located in the Region of La Araucanía (Vargas *et al.*, 2010). It rises in the Andes Mountains, with an annual precipitation that reaches 3000 mm and monthly temperatures which oscillate between -3 and 18 °C. The watershed receives mostly pluvial precipitation and has a slight snow influence in the upper part, presenting a mixed hydrological regime (pluvio-nival) (Medina & Muñoz, 2020). The aquatic biota of the Allipen river is scarcely known, the community of benthic invertebrates has a high species richness but low diversity. The Arthropoda phylum is the most representative in abundance and richness, dominated by order such as Trichoptera and Diptera (Solis-Lufi *et al.*, 2021). Benthic macroinvertebrates are one of the most important components of freshwater ecosystems (Fierro *et al.*, 2015). These are mainly immature stages of insects, most of which spend at least one stage of their life cycle in aquatic systems before emerging to the adult stage (Hauer & Resh, 2007). There are few previous studies from the Allipen river basin, and the current data (Gomez-Huchida *et al.*, 2016) indicates the presence of seven species of fishes, three introduced or exotic species *Oncorhynchus mykiss*, *Oncorhynchus tshawytscha* and *Salmo trutta*, and four native species with low abundances identified only at the genus level: *Galaxias* sp., *Cheirodon* sp., *Diplomystes* sp. and *Geotria australis*.

The Allipen river is of great importance for local economic activities such as tourism and recreational fishing, due to the annual addition of approximately 12,000 adult Chinook Salmon; moreover with the purpose of protecting this population, recreational fishing for Chinook Salmon is currently restricted in this zone in order to protect and encourage spawning sites between the months of April and August each year (Gomez-

Uchida *et al.*, 2016). The effects generated by Chinook salmon populations in continental aquatic systems are of different origin and magnitude, according to the analyzed life stage. On one hand, there are effects associated to adults returned to the river, and their subsequent deaths in low order rivers, which have been relatively well studied (Naiman *et al.*, 2002, Soto *et al.*, 2006, Soto *et al.*, 2007). However, the invasion of these salmons also implies the establishment, by one or two years, of new members of the fish communities, corresponding to juveniles, which lack background information, due to the short period of time in which adult Chinook salmons are found in the rivers of southern Chile (Soto *et al.*, 2007, Correa & Gross 2008).

The diet is an important characteristic of the organisms that allows to determine the position within the trophic network and know its role in the ecosystem, closely related to food availability, i.e. certain food preferences by a fish towards its prey are due to ecological conditions such as food abundance and availability (Baque-Menoscal *et al.*, 2012, Fair *et al.*, 2013). Predation has a very important role in the structure and organization of communities and acts by reducing diversity, species abundance, population structure and cohorts (cannibalism), spatial distribution, and habitat occupation (Granados 2000, Peterson *et al.*, 2017). A predator is a taxon that consumes another, therefore, the term “predation” is used to describe an interaction in which individuals of one species kill and can consume a fraction of the biomass of individuals of another species; while prey are those individuals that are detritivorous, herbivorous or fungorous (Jeffries & Lawton 1985, Abrams 2000, Muñoz *et al.*, 2009).

Food web studies, both theoretical and empirical, have increased substantially in recent decades (Layman *et al.*, 2015). Animals often shift their diets in response to changes in resource availability (Buren *et al.*, 2012), ontogenetic stage (Peterson *et al.*, 2017) competition (Kie & Bowyer, 1999), and interactions with predators or parasites

(Thompson & Townsend 1999, Siepielski *et al.*, 2016). Consequently, food web structure varies in both space and time (Winemiller 1990, McCann 2011). The stomach content analysis technique can be used to characterize the strength of interactions between consumers and their prey by determining trophic connections between species expressed as consumption of individuals (Tavares-Cromar & Williams 1996, Hall *et al.*, 2000, Muñoz *et al.*, 2009). Knowing the feeding habits of a particular species allow a better understanding of its life cycles (Wootton 1990, Saucedo-Lozano *et al.*, 1999, Bocanegra-Castillo *et al.*, 2000, Gámez *et al.*, 2014). According to Silva *et al.*, (2014), there are different methodologies for this type of analysis, each one responding to particular assumptions and adapting to specific characteristics. Niche amplitude is a parameter that purports to quantify how specialized a species is within a given environment (Krebs, 1989) It is possible to determine this parameter through diversity indices, which in the feeding context are called niche amplitude indices; determining the fish diet allows us to obtain, in addition to our own data about this community, information about higher trophic levels, which this group generally occupies, and the proportion in abundance or biomass of each of these strategies helps to understand the structure of the community in relation to the available resources (Muñoz *et al.*, 2009). Clear and detailed information regarding the aquatic biota in the Allipen river is unavailable, even less concerning the feeding habits, hence the scientific objectives of the study were: (i) identify the fish community of the Allipen river; (ii) determine the prey spectrum consumed of exotic fish species ; and (iii) identify the overlap degree (competition for food resources).

Materials and Methods

Sampling collection were carried out the months the times of drought or low flow (November 2016 and January 2017) and months the times of flood or high flow (May and August 2017). Three collection points were established along the ecological gradient of the Allipen river.

Fish was sampled were obtained from the Allipen river, located in south of Chile (38°51' S and 71°45' W) at an altitude of 463 m above sea level. Using a backpack electroshocker (electric fishing), in shallow littoral zones. We measured multiple traits immediately after capturing individuals. Stomachs were removed and preserved in 95% alcohol for diet analysis. We measured standard length (SL), total length (TL; to the nearest mm), and mass (to the nearest 0.01g). Furthermore, a specific sampling was conducted to capture adult salmonid species using the angling technique, and the help of local fishermen and fishing guides from the study area. Specimens are kept in aquariums adapted for continuous oxygenation and low water temperature. Each caught fish was sedated with anesthetic BZ -20, measured with manual 1 mm precision measuring board, and weighed on a digital scale model Ohaus Scout Pro 400 g. and 0.01 g precision. Finally, native fish was returned to the river at the capture site (site and habitat) after recording their body measurements.

Fish were preserved to perform stomach contents analysis and were fixed in the field by injecting ethanol into their abdominal cavity in order to stop the digestion of their stomach contents. Fish were then frozen in the laboratory for optimal preservation until analysis. In laboratory, stomachs were removed by dissecting the digestive tract from the esophagus to the pyloric blind, and discarding the intestine which, due to the advanced stage of digestion of its contents, makes taxonomic identification difficult. From the stomach of each fish, prey was identified and counted using an "Olympus SZ 2-ILST"

stereomicroscope at a maximum magnification of 45x. The separation of the different food groups found helped the identification up to the family taxonomic level, using specialized bibliography (Bland & Jaques 1978, Fernández & Domínguez 2001, Merritt & Cummins 1984) and subsequently weighted with a 0.0001 g precision “Precisa XB120A” balance. In addition, they were classified as "autochthonous" when they came from the interior of the river and "allochthonous" when the prey origin was terrestrial. All non-animal stomach contents, such as sand, plant tissue or detritus, were grouped into another group called "others".

For the diet analysis, we studied the stomach contents of 163 individuals, and we constructed a gravimetric matrix weighting each prey item by stomach (sample). We estimated the occurrence frequency index (%OF) (Amundsen *et al.*, 1996, Hyslop 1980), numerical abundance index (%N) (García de Jalón *et al.*, 1993) and volumetric abundance index (%V) (Hyslop 1980) to characterize each species diet. Furthermore, the index of importance (IIR) was used this index combines the numerical, gravimetric and frequency of occurrence measurements into one value, which determines the importance of a trophic group within the diet, combining the three previous indexes (Pinkas *et al.*, 1971) as follows: $IIR: (\%F * \%V)/100$, where %F corresponds to the frequency percentage and %V to the volumetric percentage. Finally, we determined the Ivlev selectivity index, to detect species preferences for a particular food item as well as rejection or indifference to others (Lechowicz 1982). This index is expressed as: $E_i = (r_i - p_i) / (r_i + p_i)$, Where r_i is the percentage with which the species uses the resource i and p_i the percentage in which the resource i is available. The values of E_i can fluctuate between -1 and +1, indicating the values between 0 and +1 preference and between 0 and -1 rejection by the dam.

Data analysis

Exploratory analyses were used to find preliminary differences between the species according to their diet. Subsequently Kolmogorov Smirnov's and Levene's tests were used to check the assumptions of normality and homogeneity of variances, respectively. Because variables did not meet the assumptions, we used a Kruskal-Wallis non-parametric median (H) and Dunn test for potential differences between the food "prey" of *Oncorhynchus mykiss*, *Salmo trutta* and *Oncorhynchus tshawytscha*. Statistical analysis was performed with the R software (R Development Core Team, 2009). Furthermore, the Shannon-Wiener H' index (Colwell & Futuyma, 1971) was used to describe the dietary diversity or niche trophic amplitude of the species, while the Pielou index J' (Pielou, 1966) was used to measure if the different prey were uniformly distributed. Likewise, in the analysis of trophic similarity, the simplified Morisite index was applied (Krebs, 2014), the variable used was the %RII of prey consumed by different species; whose values vary between 0 and 1, for the value 1 there is total overlap and consequently interspecific competition, while a value of 0 indicates a total absence of trophic overlap, using the PAST 3.2 program.

Results and Discussion

The study of the food chain and its interaction with other components of the ecosystem with their environment through the description and quantification of the aquatic organisms diet, is the basis for understanding how energy cycles occur (Silva *et al.*, 2014, Trujillo *et al.*, 2016). We determined the fish community present in the Allipen river and analyzed the stomach contents of salmon and trout (exotic fish).

In the four sampling campaigns high abundance of fish was observed throughout the study area. A total of 1125 individuals were captured, 32% of which correspond to

winter season, followed by summer with 26%, spring with 25% and 17% in autumn. We registered a total of 9 species of fish, from those, 6 correspond to native fish species *Percilia gillissi* (Girard, 1855), *Diplomystes camposensis* (Arratia, 1987), *Galaxias maculatus* (Jenyns, 1842), *Geotria australis* (Gray, 1851), *Basilichthys microlepidotus* (Jenyns, 1841), and *Trichomycterus areolatus* (Valenciennes, 1840); and three to introduced or exotic species *Oncorhynchus mykiss*, *Oncorhynchus tshawytscha*, and *Salmo trutta* (Figure 1). These results are consistent with what has been described by there are few previous studies of the Allipen river basin, Gomez-Huchida et al., (2016) indicates the presence of seven species of fishes; three introduced or exotic species *Oncorhynchus mykiss*, *Oncorhynchus tshawytscha*, and *Salmo trutta*; and four native species with low abundances, identified only at the genus level, *Galaxias* sp., *Cheirodon* sp., *Diplomystes* sp., and *Geotria australis*. The greatest abundance was represented by *Trichomycterus areolatus* species throughout the study area. In addition when analyzing abundances for sampling sites, we can observe that an overlap of habitat between salmonid species and native species is not being generated. Because the highest abundances of salmonids occurred in sites 1 and 2, while the highest abundances of native species were found on site 3. This strongly determines the predator/prey relationship that could potentially be generated between the different species. However, there is was observed of an characteristic assemblage of salmonids with two native species, *Trichomycterus areolatus* and *Percilia gillissi*.

In relation to the benthic macroinvertebrate community finding a total of 12 food groups: insects (Diptera, Hemiptera, Coleoptera, Ephemeroptera, Plecoptera and Trichoptera), Crustaceans (Decapoda), fish, plant material, nematodes, detritus and stones. Nematode and stones that are part of the stomach contents are considered casual or occasional, as they would be consumed at the time of ingesting other prey, or being

contained within them (Trias *et al.*, 2005, Bashirullah et al 2005). The three species under study, the diet of a total of 163 stomachs was analyzed, of which 73% had stomach content, a 24 dietary items distributed in 10 Orders and 5 classes were identified, being the most abundant Gripterygidae and Chironomidae (Table 1). Insecta was the most represented prey group, which appeared most frequently in the diets of the studied species. Within the insects, the most important groups, in terms of abundance, were Gripterygidae and Chironomidae families. These results agree with those reported by Figueroa *et al.*, (2010), showing a marked preference for the prey Chironomidae in all fish species from the Chillán river, that has similar characteristics to those of the Allipen river. In addition, a total of 714 prey were counted, of which 93,4% were autochthonous items (zooplankton, insect larvae and nematomorphs), 6,3% of allochthonous origin (adult insects, crustaceans, arachnids and gastropods) and 0,3% fish items. These results are consistent with those described for salmonids by diverse authors. Coche & Muir (1998) noted that natural fish feeds are very diverse and usually consist of a complex mixture of plants and animals, depending on the size of the fish, they can be microscopic in size such as phytoplankton, zooplankton to a relatively large size aquatic insects and land insect larvae. Thus, the size of the fish species is directly related to their feeding habits. This is reflected in the results obtained in the present study, since the size of the fish community in the Allipen river does not exceed 10 cm in length for the species *Oncorhynchus mykiss* and *Salmo trutta* and for *O. tshawytscha* the catches made did not exceed 4.6 cm in total length (see Table 1). For all three species, habits based on allochthonous-type insects are described in the body sizes found in the Allipen river (L'Abée-Lund *et al.*, 1992, Mittelbach & Persson 1998, Ibarra *et al.*, 2011), in consequence the piscivorous behavior, measured as the number of fish per stomach and

prey biomass, was considerably low in the present study, which could be due to the size range of the species found in the Allipen river.

Salmonids are known for their voracious habits and their effect on native fauna, because of direct predation and food competition (Soto *et al.*, 2006, Arismendi *et al.*, 2011), moreover allochthonous prey is also important in the diet of the introduced species *Oncorhynchus mykiss* compared to native species, demonstrating the visual efficiency in which catches its prey directly from the water column (Elliot, 1967; Figueroa *et al.*, 2000, 2010). This results match with the results obtained in this study, where the species with the greatest dietary breadth corresponded to *O. mykiss*. Forty five stomachs were analyzed, finding 18 food items of native origin as allochthonous; only 11% corresponded to empty stomach content. In general, the most frequent food items corresponded to the Plecoptera, Gripopterygidae (167%OF) and Tricoptera of Hydropsichidae (80%OF) (Figure 3), however, the highest volumes corresponded to Nematomorpha and Baetidae with 25,2%V and 17,4%V respectively (Table 1). At the same time, the diet of this species included the item "fish" with 4,4% FO, however, due to the advanced degradation degree of these preys, it was not possible to identify the specific species. When analyzing these results, based on the food supply present in the Allipen river (Solis-Lufi, *et al.*, 2021), we can see that the greater items frequency indicated in the diet agrees with their high availability in the environment since the community of macroinvertebrates of the Allipen river is represented by high abundances of the taxa Elmidae, Hydropsychidae, Gripopterygidae and Chironomidae, mainly.

On the other hand, if we examine their diet with respect to the index of importance in the diet (IIA) we can see their selectivity by prey such as Ceratopogonidae and Scarabaeidae (Ivlev=1 for both), Austroperlidae, Hydroptilidae and Nematomorpha

(Ivlev=0,8 for the three), while Elmidae (Ivlev= -0,1) although it is very abundant in the food supply, it is not in the diet (Table 1).

The analyzed specimens of the *Salmo trutta* species presented an average length of 7,3 cm total length ($\pm 2,4$ DS) where a total of 33 stomachs were analyzed, finding stomach contents in all specimens. The diet of this species is constituted by 8 food items; in general the most frequent food items corresponded to the Plecoptera order, with the item Gripopterygidae (54,5%OF) and to the Ephemeroptera order with the item Leptophlebiidae (24,2%OF), being these the items with greater volume (Figure 4). If we compare these results with the food supply described for the Allipen river (Solis-Lufi *et al.*, 2021), we can determine that the item Leptophlebiidae, even though it is not one of the most abundant, has a high number of individuals with respect to the total of individuals. With respect to the preference of this species for some of the items present in the food supply of the Allipen river, we can appreciate the low selectivity for the items with the highest frequency of occurrence Gripopterygidae (Ivlev= -0,7) and the item Leptophlebiidae (Ivlev = -0,9) presenting a greater preference for the item Perlidae (Ivlev=0,5) which in turn presents a low availability in the environment (Table 1).

The ichthyophagous fish (fish-eating), were considerably low in the present study, which could be due to the range size of the species occurring in the the Allipen river, which are smaller than the sizes reported as potentially piscivorous for both species, in which individuals <13 cm LT (Total length) do not possess this feeding habit (L'Abée-Lund *et al.*, 1992, Mittelbach & Persson 1998).

For the analyzed specimens of the *Oncorhynchus tshawytscha* species, 86 specimens were obtained in total, corresponding to two age ranges, one with an average size of 33 cm total length ($\pm 9,6$ DS) with 98% of stomachs without content and the

remaining 2% with mostly vegetable content, stones and some crustaceans of the genus *Aegla* sp., and the other age group presented specimens with an average size of 4,6 cm total length ($\pm 1,3$ DS) finding content in all stomachs. The diet of this species is constituted by 12 food items of autochthonous origin as allochthonous, being the most frequent food items the order Diptera with the item Chironomidae (41,2%OF), the order Ephemeroptera with the item Leptophlebiidae (17,6%OF) and Plecoptera with the item Gripopterygidae (15,3%OF), these coinciding with the greater volumes present in the diet (Figure 2) which is consistent with previous studies indicating that Chinook juveniles present a diet dominated mainly by benthic macroinvertebrates (Jonsson *et al.*, 1999), autochthonous organisms such as Diptera larvae, larvae and pupae of chironomids and copepods (Limm & Marchetti 2009). On the basis of the obtained results, we can observe that this species incorporates in its diet food groups that are found in high abundances in the environment, however, according to the selectivity analysis carried out, food preferences would be rather associated with the orders Tricoptera of the item Hydrobiosidae (Ivlev= 0,6), Nematomorpha (Ivlev= 0,6) and the order Diptera with the item Empididae (Ivlev= 0,4) (Table 1).

The % RII values show the three trophic groups with the greatest contribution to the diet of the three species studied, corresponding to Gripopterygiidae (22 RII), Leptophlebiidae (11 RII) and Baetidae (10 RII) (Table 1 and Figure2). Our results are in line with those reported by Palma *et al.*, (2002) for *O. mykiss* which indicated that the prey items Leptophlebiidae and Baetidae (Ephemeroptera) are more important in ecosystems with similar characteristics.

Finally, the level of food resources utilization that may be common to the species studied which coexist in the same habitat, was quantitatively evaluated considering the trophic interaction degree between the species, and through the superposition of their

diets, using the modified Morisite index (Krebs, 2014). The overlap of the trophic niches, according to the Morisita Index, is elevated for *Salmo trutta* and *Oncorhynchus tshawytscha*, but not for *Oncorhynchus mykiss*, which has a greater variety of food items, showing that there is a strong association which suggests interspecific competition between the *S.trutta* and *O.tshawytscha*, but not with *O.mykiss* (Figure6). In this case, the trophic overlap acts as an indirect evaluation of resources competition, as already indicated by some authors (Ferriz, 1988; Bocanegra-Castillo *et al.*, 2000, Figueroa *et al.*, 2010). In addition, diet diversity or trophic niche width $H' = 1.17$ *O. mykiss*, 0.80 *S. trutta* and 0.95 *O. tshawytscha*. indicate low diversity. A high diversity is obtained with $H' > 3$. To measure if different preys are uniformly distributed, we used the Pielou index J' where 0.93 was obtained for *O. mykiss*, 0.89 for *S. trutta* and 0.88 for *O. tshawytscha*. A greater equality is obtained with values close to zero. Therefore, the preys are not uniformly distributed. Significant differences were determined ($H=102.57$, $p < 0.05$ /Dunn test) between the food "items" of *O. mykiss* compared to *S. trutta* and *O. tshawytscha*. This is in line with the feeding habits described for these species, where several authors show that *O. mykiss* does not have a preference for a specific stage, and feeds on all kind of insects at any stage of their life cycle (Medina & Paggi, 2004, Castro *et al.*, 2004, Soto *et al.*, 2007, Villalba, 2009, Arismendi *et al.*, 2014).

In conclusion, this research gives a first biological approach of the Allipen river fish fauna and, based on this study, , a posterior research study could be planned in river tributaries, to establish ecosystem interactions that may be occurring with these studied components.

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Tables and Figures

Figure 1. Relative abundance of fish communities found in the Allipen river, where S1: Site 1; S2: Site 2 and S3: Site 3.

Figure 2. Diet composition (expressed as per cent index of relative importance, % RII) of *Oncorhynchus mykiss* (Om), *Salmo trutta* (St) and *Oncorhynchus tshawytscha* (Ot).

Figure 3. Diet composition (expressed as Occurrence frequency index) of *Oncorhynchus mykiss*, where (▨) corresponds to Plecoptera order, (▩) Ephemeroptera order, (▧) Diptera order, (▦) Decapoda order, (▥) Coleoptera order, (▤) Hemiptera order, (▣) Trichoptera order, (▢) Nemathomorpha order and (□) Fish.

Figure 4. Diet composition (expressed as Occurrence frequency index) of *Salmo trutta*, where (▨) corresponds to Plecoptera order, (▩) Ephemeroptera order, (▧) Diptera order, (▦) Decapoda order, (▥) Coleoptera order, (▤) Hemiptera order, (▣) Trichoptera order, (▢) Nemathomorpha order and (□) Fish.

Figure 5. Diet composition (expressed as Occurrence frequency index) of *Oncorhynchus tshawytscha*, where (▨) corresponds to Plecoptera order, (▩) Ephemeroptera order, (▧) Diptera order, (▦) Decapoda order, (▥) Coleoptera order, (▤) Hemiptera order, (▣) Trichoptera order, (▢) Nemathomorpha order and (□) Fish.

Figure 6. Morisite similarity dendrogram between the different fish species studied based on the relative importance index (%RII) of the consumed prey.

Table 1. Dietary composition of the three species under study, where %N: numerical abundance index, %OF: occurrence frequency index, %V: volumetric abundance index, RII: Relative importance index and Ivlev selectivity index, total length is expressed in cm. LT/DS: Average total length in centimeters and standard deviation.

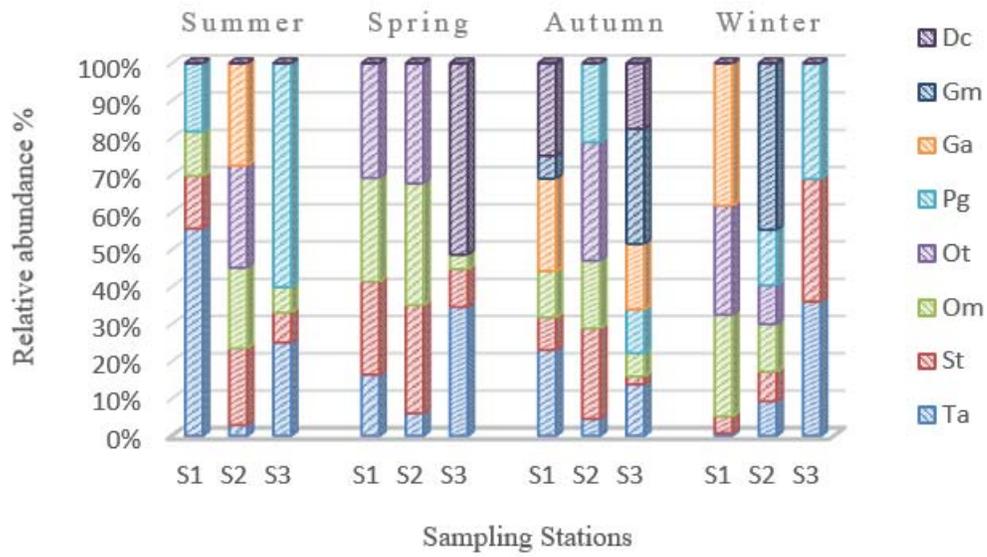


Figure 1.

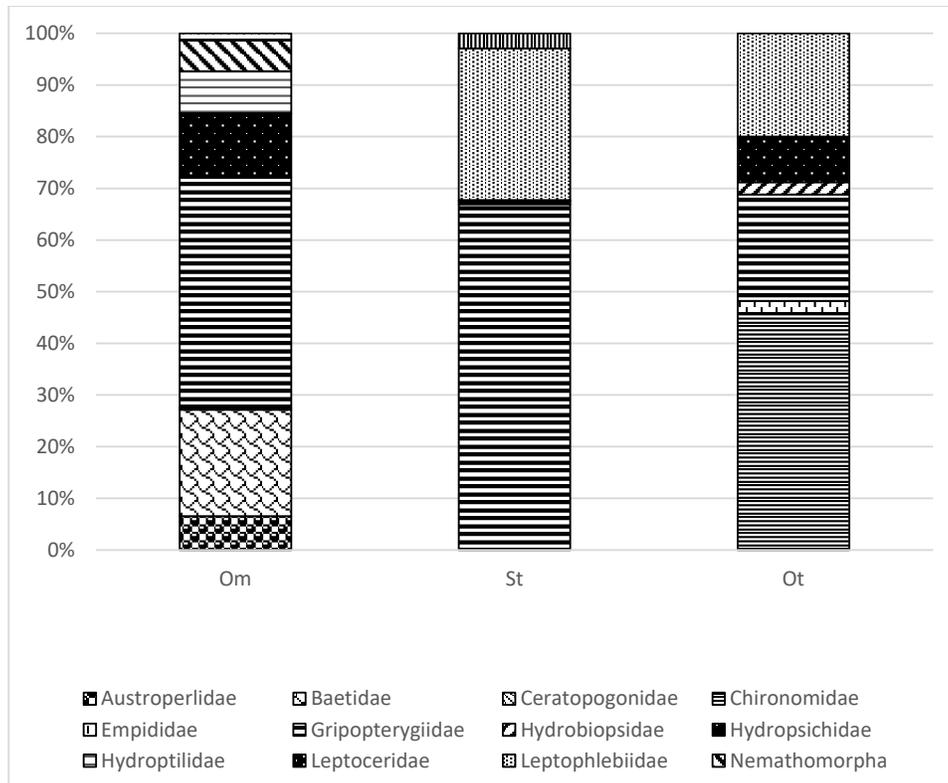


Figure 2.

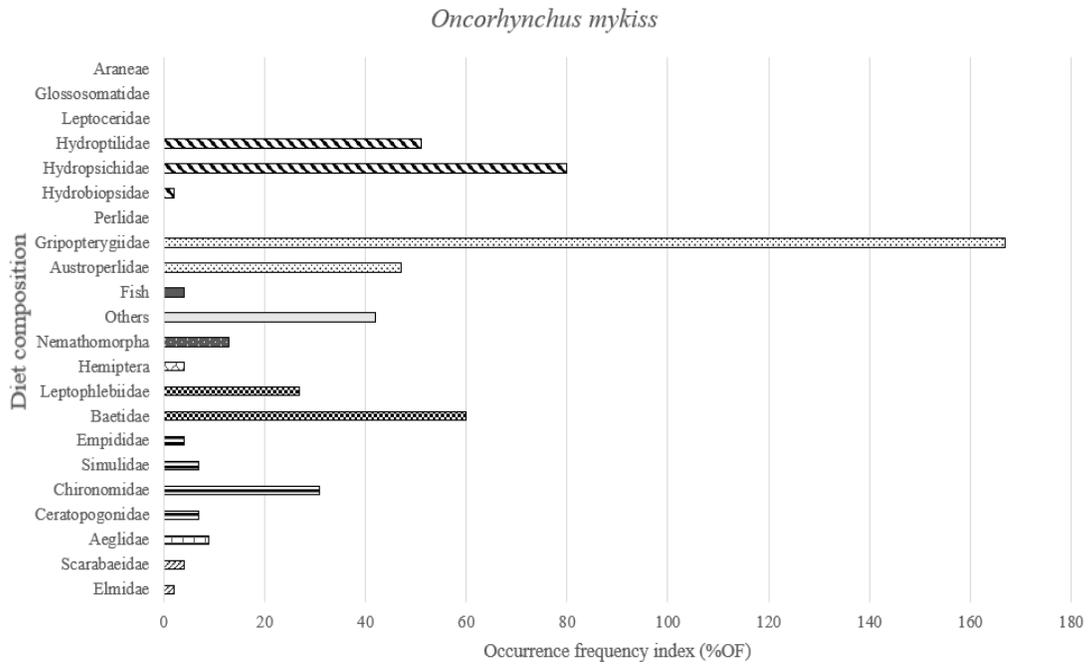


Figure 3.

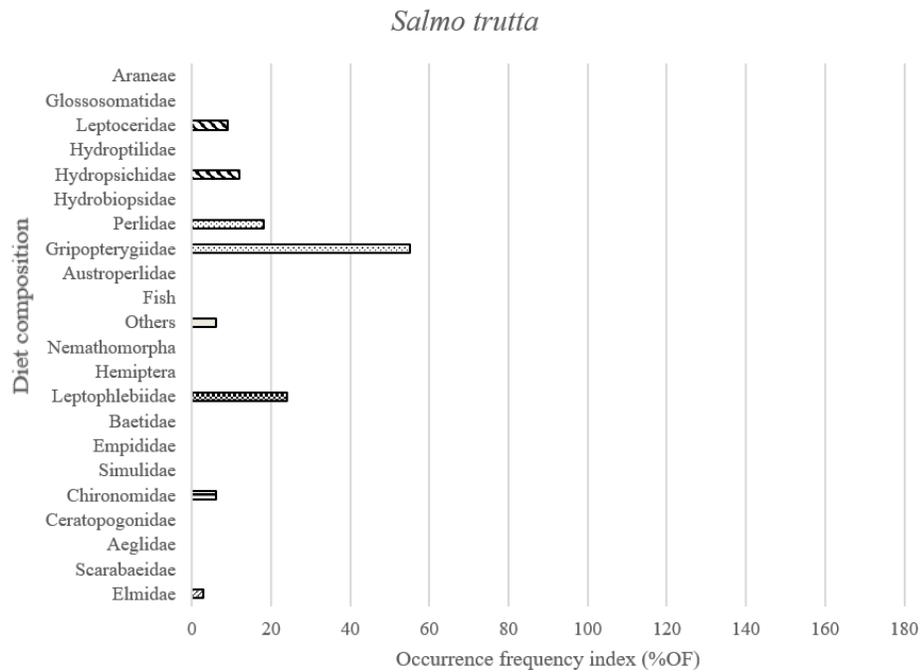


Figure 4.

Oncorhynchus tshawytscha

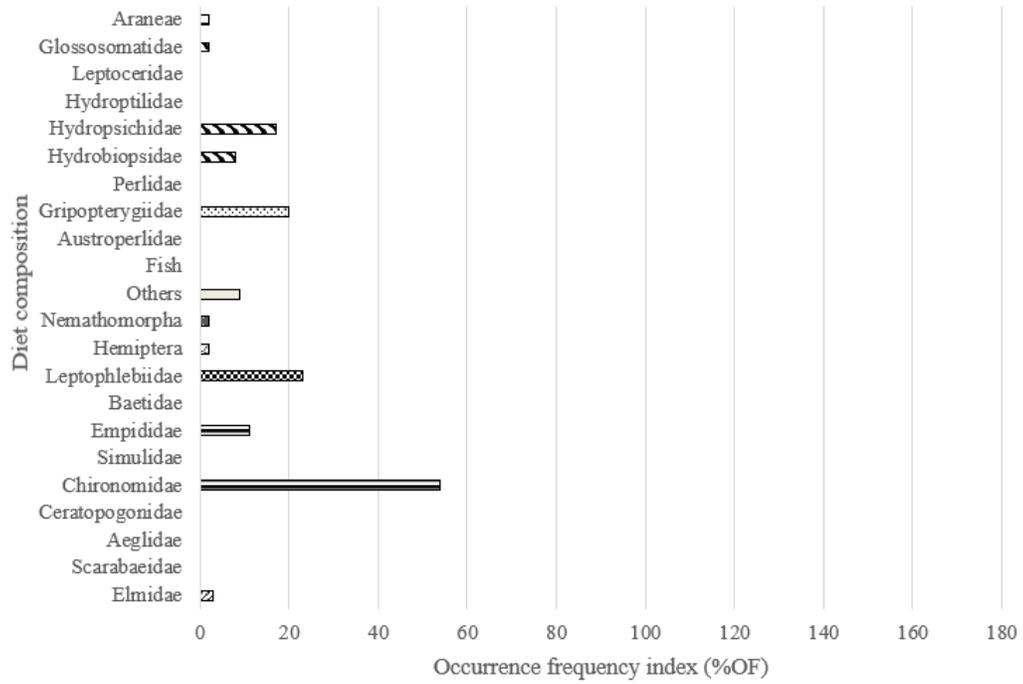


Figure 5.

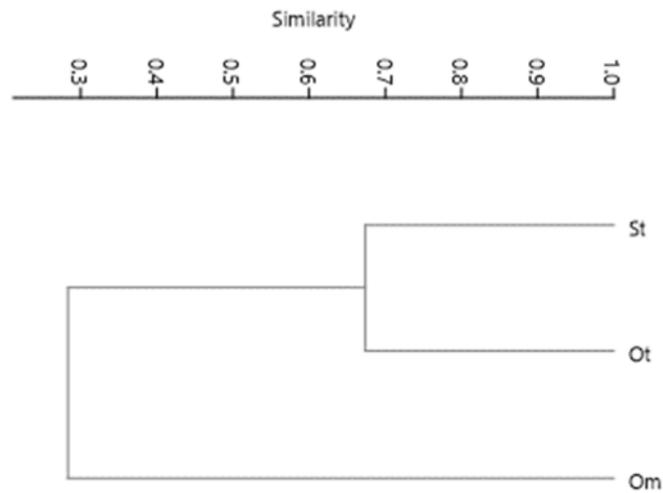


Figure 6.

Table 1.

<i>Oncorhynchus mykiss</i> (9,9 ± 2,3 LT/DS) n: 45						
Item-Prey	N	%N	%OF	%V	RII	Ivlev
Austroperlidae	21	53	47	7,01	3,3	0,8
Gripopterygiidae	75	190	167	13,47	22,5	-0,1
Baetidae	27	68	60	17,36	10,4	-0,7
Leptophlebiidae	12	30	27	0,77	0,0	-0,9
Ceratopogonidae	3	8	7	0,58	0,2	1,0
Chironomidae	14	35	31	0,08	0,0	-1,0
Simulidae	3	8	7	8,41	0,6	-0,9
Empididae	2	5	4	0,49	0,0	-0,4
Aeglididae	4	10	9	2,02	0,0	-0,5
Elmidae	1	3	2	0,02	0,0	-1,0
Hemiptera	2	5	4	0,71	0,0	0,4
Hydrobiopsidae	1	3	2	0,04	0,0	-0,7
Hydropsichidae	36	91	80	7,85	6,3	-0,9
Hydroptilidae	23	58	51	7,98	4,1	0,8
n.d	6	15	13	25,24	3,0	0,8
Nematomorpha	2	5	4	0,01	0,1	-1,0
Scarabaeidae	2	5	4	3,00	0,0	1,0
Otros	19	48,07	42,2	4,95	2,1	n/a
<i>Salmo trutta</i> (7,3 ± 2,4 LT/DS) n: 33						
Item-Prey	N	%N	%FO	%V	IIR	Ivlev
Chironomidae	31	72	6	0,71	0,0	-0,9
Elmidae	1	2	3	0,02	0,0	-1,0

Gripopterygidae	128	297	55	45,93	25,1	-0,7
Perlidae	30	70	18	5,90	1,1	0,5
Hydropsichidae	13	30	12	1,52	0,2	-1,0
Leptoceridae	5	12	9	0,71	0,1	-0,2
Leptophlebiidae	22	51	24	45,18	11,0	-0,9
Others	2	5	6	0,00	0,0	n/a

Oncorhynchus tshawytscha (4,6 ± 1,3 LT/DS) n: 86

Item-Prey	N	%N	%FO	%V	IIR	Ivlev
Araneae	1	2	2	0,29	0,0	-0,5
Chironomidae	121	267	54	18,86	7,8	-0,6
Empididae	12	27	11	4,49	0,4	0,4
Hemiptera	1	2	2	0,87	0,0	-0,2
Elmidae	2	4	3	0,58	0,0	-1,0
Leptophlebiidae	28	62	23	19,33	3,4	-0,8
Glossosomatidae	1	2	2	1,74	0,0	-0,5
Hydropsichidae	14	31	17	11,79	1,5	-1,0
Hydrobiosidae	15	33	8	6,95	0,4	0,6
Gripopterygidae	17	38	20	23,01	3,5	-0,9
Nematomorpha	2	4	2	0,58	0,0	0,6
Others	7	15	9	12,08	0,9	n/a

CHAPTER VI

General Discussion and Conclusions

GENERAL DISCUSSION

The study of the food chain and its interaction with other components of the ecosystem with their environment, through the description and quantification of the diet of aquatic organisms, is the basis for understanding how energy cycles occur (Silva *et al.*, 2014; Trujillo *et al.*, 2016). We analyzed the stomach contents of the salmon and trout present in the Allipen river, finding a total of 12 food groups: insects (Diptera, Hemiptera, Coleoptera, Ephemeroptera, Plecoptera, and Trichoptera), crustaceans (Decapoda), fish, plant materials, nematodes, detritus, and stones. Nematode and stones that are part of the stomach contents are considered casual or occasional, as they would be consumed at the time of ingesting other prey, or being contained within them (Trias *et al.*, 2005; Bashirullah *et al.*, 2005). Insecta was the most represented prey group; it appeared most frequently in the diets of the studied species. Within the insects, the most important groups, in terms of abundance, were Gripopterygidae and Chironomidae families. These results are in agreement with those reported by Figueroa *et al.*, (2010), showing a marked preference for the prey Chironomidae in all fish species from the Chillán river. In addition, a total of 714 preys were counted, of which 93.4% were autochthonous items (zooplankton, insect larvae and nematomorphs), 6.3% were of allochthonous origin (adult insects, crustaceans, arachnids and gastropods), and 0.3% were fish items. These results are consistent with those described for salmonids by diverse authors, Coche & Muir, (1998) noted that natural fish feeds are very diverse and usually consist of a complex mixture of plants and animals, depending on the size of the fish, they can be microscopic in size such as phytoplankton, zooplankton to a relatively large size aquatic insects, and land insect larvae. Escobar (2014) described different Chinook prey, mainly including insects, arachnids, crustaceans, and fish. Furthermore, freshwater crabs of the genus *Aegla* and aquatic insects can be an important prey for *Oncorhynchus mykiss* (Palma *et*

al., 2002; Pascual & Ciancio 2007; Penaluna *et al.*, 2009). The results agree with the food supply determined for the Allipen river, where it was pointed out that the benthic macroinvertebrate community along the river possesses 96% of organisms of autochthonous origin (larvae, pupae and nymphs).

The three trophic groups with the greatest contribution to the diet of the three species studied correspond to Gripopterygiidae, Leptophlebiidae, and Baetidae. This is consistent with what was indicated by Palma *et al.*, (2002) for *Oncorhynchus mykiss*, showing that prey items Leptophlebiidae and Baetidae (Ephemeroptera) are more important in a similar basin (Figueroa *et al.*, 2010). As for the degree of prey selection, *Oncorhynchus mykiss* was more heterogeneous, with a wider trophic spectrum of other species, where it highlights the preference for families such as Austroperlidae, Ceratopogonidae, Hydroptilidae, and Scarabaeidae. This could suggest that this species are the most competitive in terms of a broad trophic niche, as the number of prey increases (Soto *et al.*, 2006), while *Oncorhynchus tshawytscha* presented a higher degree of selectivity by the Hydrobiosidae and Nematomorpha. Whereas it *Salmo trutta* presented a higher degree of selectivity by the families Perlidae, Elmidae it is scarcely present in the diet of the three species even though there is a high availability of these in the environment. These results are consistent with the high degree of ecological plasticity of salmonids concerning their feeding behavior (Arenas 1978; Zama 1987; Villalobos *et al.*, 2003; Figueroa *et al.*, 2010; Arismendi *et al.*, 2012; Arismendi *et al.*, 2014).

The level of utilization of food resources that may be common to the species studied by coexisting in the same habitat was evaluated quantitatively with the degree of trophic interaction between the species through the superposition of their diets using the Morisita index (Morisita 1959). Overlap of the trophic niches, according to the Morisita Index, is elevated for *Salmo trutta* and *Oncorhynchus tshawytscha*, but not for

Oncorhynchus mykiss, has a greater variety of food items, showing that there is a strong association, and suggesting that there is interspecific competition between the *S. trutta* and *O. tshawytscha*, but not with *O. mykiss*. In this case, the trophic overlap acts as an indirect evaluation of competition for resources, as already indicated by some authors (Ferriz 1988; Bocanegra-Castillo *et al.*, 2002; Figueroa *et al.*, 2010). In addition, significant differences were determined ($H=102.57$, $p < 0.05$ /Dunn test) between the food "items" of *O. mykiss* compared to *S. trutta* and *O. tshawytscha*. This is in line with the feeding habits described of this species, where several authors show that *O. mykiss* does not have a preference for a specific stage, feeding on all kinds of insects at any stage of their life cycle (Medina & Paggi 2004; Castro *et al.*, 2004; Soto *et al.*, 2007; Villalba 2009; Arismendi *et al.*, 2014).

When analyzing these results based on the food supply present in the Allipen river we can see that the greater frequency of the items indicated in the diet agrees with the high availability of these in the environment since the community of macroinvertebrates of the Allipen river is represented by high abundances mainly of the taxa Elmidae, Hydropsychidae, Gripopterygidae, and Chironomidae. The piscivorous behavior, measured as the number of fish per stomach and prey biomass, was considerably low in the present study, which could be due to the size range of the species found in the Allipen river, which are smaller than the sizes reported as potentially piscivorous for *O. mykiss* and *S. trutta* species, in which individuals < 13 cm LT (Total length) do not possess this feeding habit (L'Abéc-Lund *et al.*, 1992; Mittelbach & Persson 1998). In the case of the *O. tshawytscha*, studies carried out in Patagonia indicate that individuals > 8 cm LT (Total length) would present piscivorous diets mainly of native Galaxiids, while the diet of individuals younger than 1 year would be based on insects of allochthonous origin (Ibarra *et al.*, 2011).

Based on our results, the Allipen river basin has a low diversity of benthic macroinvertebrates ($H' \leq 1.5$ unit), twenty eight families were identified throughout the river. In terms of richness, Trichoptera and Diptera followed by Plecoptera were the orders showing the greatest diversity. These taxonomic compositions are characteristic of rivers with permanent regimes (Moya *et al.*, 2009) such as the Allipen river basin. In addition, our results are consistent with the larval stages of Diptera, Trichoptera, and Ephemeroptera described for the Toltén river basin (Figueroa 2000; Figueroa *et al.*, 2006, 2007). The results indicate that in upper zone of basin Allipen river, the number of taxa of the Ephemeroptera, Plecoptera and Trichoptera orders remained relatively constant during the four seasons of the year, being replaced by the Diptera order taxa, in the downstream sites. This would suggest that the waters of the Allipen river in this area are well oxygenated water and in appropriate conditions for the development of these organisms due do Ephemeropters prefer to live in good oxygenation places, in stone and sand substrates (Baptista *et al.*, 2006; Romero *et al.*, 2006; Moya *et al.*, 2009; Oyanedel *et al.*, 2008) and individuals of the Trichoptera order, in their larval phase are very well adapted to rapid water courses and stony substrates in high-speed rivers, and are distributed across all types of habitats, being very well represented in all river flows, except in highly polluted areas (Basaguren, 1990). Besides, the results point out that the resident species *Oncorhynchus mykiss* exert more predation than other introduced species on the native fauna of the Allipen river, mainly on macroinvertebrates belonging to Gripopterygiidae, Baetidae, and Hydropsichidae families from the orders Plecoptera, Ephemeroptera, and Trichoptera, respectively. Coche & Muir, (1998) noted that natural fish feeds are very diverse and usually consist of a complex mixture of plants and animals, depending on the size of the fish.

Benthic macroinvertebrates are one of the most important components of freshwater ecosystems (Fierro *et al.*, 2015). In the first site of the Allipen river, Melipeuco, the number of taxa of the Ephemeroptera, Plecoptera, and Trichoptera orders remained relatively constant during the four seasons of the year, being replaced by the Diptera order taxa, in the downstream stations. The Chironomidae family, which prevailed in all sampling sites during the four seasons of the year, included individuals with a high capacity to tolerate high organic load concentrations, and long periods with low oxygen concentrations (Kay *et al.*, 2001; Newall & Tiller 2002; rivera *et al.*, 2004). This dominance of Chironomidae along the river could be a warning sign for changes in the structure and abundance of macroinvertebrate communities since these organisms have been described by various authors as tolerant to organic pollution (Figueroa 2000; Oscoz *et al.*, 2006; Oyanedel *et al.*, 2008; Moya *et al.*, 2009; Oyanedel *et al.*, 2011). Also, analyzing the antecedents given by Figueroa *et al.*, (2003) for the fluvial ecosystems of South Central Chile, only in the lower part, these taxa reach high abundance and biomass, and the Allipen river is one of the main tributaries of the Toltén river basin; hence, it does not correspond to a "low" zone of the river. These higher organic load concentrations could be associated with both urban centers and fish farms discharges, near the study area (FIP 2017).

On the other hand, Ephemeroptera order had representatives all over the Allipen river, with the Gripopterygidae family prevailing and the Leptophlebiidae family; nevertheless, their abundance remained low at all season of the year, as compared to Chironomidae family, which could be because Ephemeroptera, in general, prefer to live in well-oxygenated places, in stone and sand substrates (Baptista *et al.*, 2006; Romero *et al.*, 2006). Also, the larval form lives in both running (Iotic) and backwaters (Ientical), but generally clean, well-oxygenated waters (Alba-Tercedor 2015). Furthermore, in

Huerere and Las Hortensias sites, downstream of the river, partial elimination of riverine vegetation was detected, producing instability in the thermal characteristics of the water column, and an increase of the sediment's frequency and aquatic macrophytes, facilitating the pollutants to entry into water bodies (Scalley & Aide 2003). This produces important impacts on the ecosystems such as water quality deterioration and biodiversity reduction (Corbacho *et al.*, 2003; Bonada *et al.*, 2006). Over the past decade, studies have shown that the changes in land use have been one of the main causes of biodiversity loss (Diggelen *et al.*, 2005). Some studies (Yam & Dudgeon 2005) have confirmed the dependence of aquatic invertebrates on allochthonous material that reaches rivers, such as leaves and pieces of wood (detritus) that are consumed as food, and their influence on physical and biological processes, especially in habitat diversification (Nakano *et al.*, 1999; Thompson & Townsend 2004; Lyon & Gross 2005; Ríos & Bailey 2006).

Out of the twenty eight families of macroinvertebrates present in the Allipen river, fifteen are shared by the 3 sampled sites, corresponding to Gripopterygiidae and Perlidae (Plecoptera order), Baetidae and Leptophlebiidae (Ephemeroptera), Hydropsychidae and Leptoceridae (Trichoptera), Elmidae (Coleoptera), Athericidae, Blephariceridae, Chironomidae, Empididae, Simuliidae and Tipulidae (Diptera), Hyalellidae, and Chiliniidae (Amphipoda and Mollusca respectively). This could be due to the fact that the Allipen river presents typical characteristics of rithron along its whole course, with predominant big boulders and alternating rapids and pools, so that the spatial distribution of the biota is relatively homogeneous (Whiles *et al.*, 2000; Rice *et al.*, 2001; Figueroa *et al.*, 2003). Many of these groups are currently endangered due to human-induced alterations in their habitats and the introduction of exotic salmonids species (Jara *et al.*, 2006; Encina *et al.*, 2017; Vega *et al.*, 2017). The Chironomidae, which prevailed in all Allipen river during the four seasons of the year, included individuals with a high capacity

to tolerate high organic load concentrations, and long periods with low oxygen concentrations, facilitating high reproduction rates in these environments (Kay et al., 2001; Newall & Tiller, 2002; rivera et al., 2004), this higher organic load concentrations could be associated with both urban centers and fish farming discharges, near the study area (FIP, 2017). According to studies by Figueroa et al., (2003, 2007, 2010), for river ecosystems in central southern Chile, only in the lower part, these taxa reach high abundance and biomass, being favored by their tolerance and the high organic detritus availability, which is part of their diet.

On the other hand, the Chilean freshwater fish fauna is composed by 45 native species and high endemism (80%) [Vila et al., 1999; Dyer, 2000; Vila & Quezada-Romegialli 2018). This richness increases with latitude, from 1 to 18 species per basin up to 40° S, and then it decreases to 6 species per basin (Habit et al., 2006; Vila & Quezada-Romegialli 2018). The fish community of the Allipen river is represented by a total 9 species of fish, from those, 6 correspond to native fish species *Percilia gillissi* (Girard, 1855), *Diplomystes camposensis* (Arratia, 1987), *Galaxias maculatus* (Jenyns, 1842), *Geotria australis*, *Basilichthys microlepidotus* (Jenyns, 1841), and *Trichomycterus areolatus* (Valenciennes, 1840); and three to introduced or exotic species *Oncorhynchus mykiss*, *Oncorhynchus tshawytscha*, and *Salmo trutta*, the most abundant being the native catfish *Trichomycterus areolatus* species. These is consistent with the poor native richness described (1–6 species per basin), and the presence of four widely distributed exotic species in Chile (*G. affinis*, *G. holbrooki*, *O. mykiss*, and *S. trutta*) (Castro et al., 2020). Furthermore, generally, in Chile the rithron of the rivers (areas of greater slope) are home to 3 to 5 fish native species (Habit et al., 2006b; Habit et al., 2012); in so doing the Allipen river is a singular river ecosystem from the point of view of its fish biodiversity. These results are consistent with what has been described by there are few

previous studies of the Allipen river basin, Gomez-Huchida *et al.*, (2016) indicates the presence of seven species of fishes; three introduced or exotic species *O. mykiss*, *O. tshawytscha*, and *S. trutta*; and four native species with low abundances, identified only at the genus level, *Galaxias* sp., *Cheirodon* sp., *Diplomystes* sp., and *Geotria australis* (Gray, 1851). According to Vila & Quezada-Romegialli (2018), the Allipen river belongs to the ichthyogeographic region of the “Valdivian Lakes” Province would be inhabited by *Aplochiton zebra* (Jenyns, 1842), *Trichomycterus areolatus*, *Percilia gillissi*, and *Galaxias platei* (Steindachner, 1898). Another singularity of this ecosystem is the presence of *Diplomystes camposensis*, as fishes of the family Diplomystidae are considered to be basal or a primitive parent group of all other catfish (Arratia 1992; dePinna 1998; Sullivan *et al.*, 2006; Habit *et al.*, 2012), with the genus *Diplomystes* endemic to Chile (Arratia & Quezada-Romegialli 2017; Vila & Quezada-Romegialli 2018). According to recent morphological evidence, *D. camposensis* would also be present (Arratia & Quezada-Romegialli 2017).

In addition, our results indicate that there is a characteristic assemblage of salmonids with two native species, *Trichomycterus areolatus* and *Percilia gillissi*, these results are consistent with those noted by Vargas *et al.*, (2010) for the Allipen river, suggesting a high niche overlap between juvenile salmonids of Chinook and native catfish due to microhabitat preferences. Because of its biology and life cycle, it is very difficult for anadromous species of salmonids to be established in environments that are not native; however, the oceanic and freshwater characteristics present in Southern Chile, similar to the Northern Hemisphere, offer a low ecological resistance (Soto *et al.*, 2006, 2007; Arismendi *et al.*, 2014). Hence, the great genetic plasticity and diversity of life histories of the Chinook salmon, makes it easier to adapt to different situations, and finally its colonization and establishment (Soto *et al.*, 2007; Correa & Gross 2008; Arismendi *et al.*,

2014). This is reflected in the fish fauna captured in site 1 that presents a greater abundance of species of salmonids with 64% of the total captured, and at the same time, *Oncorhynchus tshawytscha* (Chinook salmon) presented the greatest abundance in this site, with 34% of the total amount.

Previous records of salmonids in the Allipen river only included adult specimens, due to its morphological similarity with its salmonid congeners, in this study we use *microsatellite DNA markers* to identify juvenile *Oncorhynchus tshawytscha*, DNA markers are particularly useful because samples can be collected without killing the organism and many markers (e.g., microsatellites) show a high level of polymorphism (Docker & Heath 2002). In addition, DNA markers are currently recognized as attractive tools in salmon management, due to their less stringent storage requirements and potentially higher resolution (Rasmussen et al., 2003). In relation to *microsatellite DNA markers*, these are characterized by being highly polymorphic, providing greater statistical power to detect kinship than alloenzymes previously provided. (Banks et al., 2000); in addition, they have a high power of discrimination to solve the complex genetic structure of salmon populations within a hydrographic basin (Banks et al., 2000). Another characteristic that highlights the importance of their use is the technique by which they are analyzed, which corresponds to PCR amplification, which stands out for being a fast method that requires only small amounts of tissue that can be obtained in a non-lethal way, which is favorable for the work with juvenile fish (Rasmussen et al., 2003). Moreover, there is an evident genetic differentiation between populations of Chinook salmon in the different geographical regions (Beacham et al., 2006a; Garza et al., 2008; Gómez-Uchida et al., 2018). Where of twenty markers used in this study, only 5 microsatellite loci that presented the highest levels of polymorphism were taken into account, this did not represent a problem for obtaining reliable results, since according to

Beacham et al., (2003), when more than 8 loci are used to estimate stock compositions, the increase in precision is marginal and the least effective loci provides only a modest increase in accuracy. Furthermore, not all loci can be amplified in all species under the given set of PCR conditions, and the degree of polymorphism at each locus differs between species (Williamson et al., 2002). The results of this work, although preliminary, indicate that these DNA markers can be highly effective for the identification of populations of juveniles of *Oncorhynchus tshawytscha* in the Allipen river, since they allow them to be identified with greater certainty than is currently achieved due to morphological characteristics. The potential for discrimination that is achieved with microsatellite loci to identify juvenile populations of Chinook salmon is of obvious importance for the management and handling of this species introduced into the Allipen river. In countries like the United States, this species is threatened in California Valley due to massive habitat destruction, exploitation, and artificial propagation (Banks et al., 2000), therefore it is also important to have valid reports of its presence elsewhere in the world.

Among the studied potential effects of exotic species, there are competition for food, competition for space, and predation, among others (Arthington 1991; Crowl *et al.*, 1992). This strongly determines the predator/prey relationship that could potentially be generated between the different species. Penaluna *et al.*, (2009) suggest the existence of microhabitat segregation of the trout, *Oncorhynchus mykiss* and *Salmo trutta*, towards the catfish *Trichomycterus areolatus*. That relationship could explain the highest abundances of native species occurred on site 3, where there is a low abundance of exotic species. Furthermore, given the aggressiveness and voracity of Chinook salmon (Scott & Grossman 1973; Healey 1991) there is a potential displacement of the catfish towards lower quality habitats, which would limit the condition and health of native populations

in places where both species coexist (Vargas *et al.*, 2010). In addition, Arismendi *et al.*, (2009) observed an increase in predation by some salmonids (including Chinook) on native species (i.e. *Galaxias platei*, *G. maculatus* and *Aplocheilichthys zebra*). The introduced salmonids, specifically, constitute non-selective, active predators (Soto *et al.*, 2006, 2007; Piedra *et al.*, 2012; Valdovinos *et al.*, 2012; Vargas *et al.*, 2010, 2015; Fierro *et al.*, 2015, 2016), presenting effects by trophic interactions with other components of the ecosystem (Vargas *et al.*, 2010; Sobenes *et al.*, 2013; Habit *et al.*, 2015).

The relationships indicated between the different components of the studied ecosystem would have a potentially negative effect above the biotic community of the Allipen river considering the trophic interactions between benthivores fishes, and top predator fishes that would affect the low trophic levels on which also the macroinvertebrates are important (Soto *et al.*, 2006, 2007; Arismendi *et al.*, 2009; Penaluna *et al.*, 2009). Finally, to conserve properly, it is necessary to know exactly how many species we have, how we properly identify them and what its distribution is. (Vila & Quezada-Romegialli 2018). Furthermore, this background is of great importance for the adequate management and conservation of the biological resources of the Toltén river basin since it corresponds to unpublished information on the components of this river ecosystem and their interaction.

CONCLUDING REMARKS

What has been exposed in this investigation allows us to come to the following conclusions:

- Identification of species feeding habits helps to understand their life cycles developments. The species *Oncorhynchus mykiss* exert predation greater than other introduced species in the native fauna of the Allipen river, mainly on macroinvertebrates belonging to the families Gripopterygiidae, Baetidae, and Hydropsichidae of Plecoptera, Ephemeroptera, and Trichoptera orders respectively.
- This is the first report that describes *Oncorhynchus tshawytscha* (Chinook salmon) juvenile stages present in the Allipen river basin, it also provides an unprecedented fish community description, composed of nine fish total species, of which six correspond to native fish; *Percilia gillissi*, *Galaxias maculatus*, *Geotria australis*, *Trichomycterus areolatus*, *Diplomystes camposensis*, and *Percichthys trucha* and three introduced or exotic species *Salmo trutta*, *Oncorhynchus mykiss*, and *Oncorhynchus tshawytscha*.
- Allipen river macroinvertebrate community is mainly composed of about fifteen families corresponding to Plecoptera order, Ephemeroptera, Trichoptera, Coleoptera, Diptera, Amphipoda, and Mollusca orders.
- The results conclude the three trophic groups with the greatest contribution to the diet of the three species studied correspond to Gripopterygiidae, Leptophlebiidae, and Baetidae, being piscivory unusual.
- The potential for discrimination that is achieved with microsatellite loci to identify juvenile populations of Chinook salmon is of obvious importance for the management and handling of this species introduced into Allipen river. These microsatellite loci are expected to be useful in a variety of studies, given their efficacy

in identifying juveniles of the species, the low cost of PCR assays, and their high performance relative to other DNA-based methods.

- Finally, with this information, the hypothesis of the thesis is accepted, as the Allipen river fish community is dominated by juvenile salmonids which feed preferentially on benthic macroinvertebrates in a selective way and without a wide dietary overlap.

FUTURE DIRECTIONS

In general, there is scarce study and information about the Allipen river ecosystem. The background given in this doctoral thesis is a first concrete contribution to the knowledge of this ecosystem and its components. However, it is necessary to carry out similar studies in the Allipen river tributaries for establishing interaction that may be occurring in the ecosystem and its components.

The information provided in this document is an important background for future programs for the management of hydrobiological resources in the Allipen river Basin. Following, some guidelines are provided in this regard.

- Currently, this zone has a fishing prohibition, protected by the General Fishing and Aquaculture Law, which states in its article 70 points out that *"it prohibits the capture of anadromous and catadromous species, coming from open crops, in land waters, internal waters and territorial sea, in those areas where such species begin or end their migratory cycle, either as a fry or juvenile, or in their appropriate maturity stage for their commercial exploitation"*. Therefore, the actions or management measures that are established must have as their main objective "To contribute to the conservation and protection of the ecosystem".
- The specific objectives to be developed are proposed: 1) Develop research for the conservation and protection of the ecosystem, 2) Educate, divulge and promote the conservation and protection of the Allipen river's fish fauna and 3) Increase the skills of traditional fishermen, recreational fishermen and fishing guides in aspects of biology and ecology of the resource.

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