



First study of food webs in a large glacial river: the trophic role of invasive trout

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The aim of this study was to determine the food webs structure of a large Patagonian river in two river sections (Upstream and Midstream) and to evaluate isotopic overlap between native and introduced species. We used stable isotope analyses of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ and stomach content. The Upstream section had a more complex food webs structure with a greater richness of macroinvertebrates and fish species than Midstream. Upstream basal resources were dominated by filamentous algae. Lake Trout were found to have a higher trophic position than all other fish species in that area although, the most abundant fish species, were Rainbow Trout. Depending on the life stage, Rainbow Trout shifted from prey to competitor/predator. In the Midstream section, the base of the food webs was dominated by coarse particulate organic matter, and adult Rainbow Trout had the highest trophic level. Isotopic values changed among macroinvertebrates and fish for both areas. The two most abundant native and invasive species — Puyen and Rainbow Trout — showed an isotopic separation in Midstream but did not in Upstream areas. The presence of invasive fish that occupy top trophic levels can have a significant impact on native fish populations that have great ecological importance in the region.

Keywords: Diet, food webs, Mixing models, Salmonids, Stable isotopes analysis.

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El objetivo de este estudio fue determinar la estructura trófica de un gran río de la Patagonia en dos secciones (río arriba y medio) y evaluar la superposición isotópica entre especies nativas e introducidas. Utilizamos análisis de isótopos estables $\delta^{15}\text{N}$ y $\delta^{13}\text{C}$ y contenido estomacal. La sección río arriba tuvo una estructura de trama trófica más compleja, con mayor riqueza de macroinvertebrados y peces respecto de la sección media. Los recursos basales dominantes río arriba fueron las algas filamentosas. En esta área, la trucha de lago tuvo la posición trófica más alta entre los peces, aunque, las especies de peces más abundantes fueron las truchas arcoiris. Dependiendo del estadio, la trucha arcoiris cambió su rol de presa a competidor/depredador. En la sección media del río, la base de la trama trófica estuvo dominada por materia orgánica particulada gruesa y la trucha arcoiris adulta fue el depredador tope. Los valores isotópicos variaron entre zonas para invertebrados y peces. Las dos especies nativas e invasoras más abundantes, Puyen y trucha arcoiris, mostraron una separación isotópica en la sección media, pero no en secciones de río arriba. La presencia de peces invasores que ocupan una posición tope en los niveles tróficos puede tener un impacto significativo sobre las poblaciones de peces nativos de gran importancia ecológica en la región.

Palabras clave: Análisis de Isótopos estables, Dieta, Modelos de mezcla, Salmónidos, Trama trófica.

INTRODUCTION

Species invasion and habitat degradation represent major threats to biodiversity (Vitousek, 1990; Clavero, García-Berthou, 2005; Gallardo *et al.*, 2016). Invasive species can alter fundamental ecological characteristics, such as species composition, the cycling of nutrients and overall ecosystem productivity (Mack *et al.*, 2000; Muñoz *et al.*, 2009; Buoro *et al.*, 2016). The introduction of non-native fish species is considered one of the most significantly negative anthropogenic impacts to aquatic ecosystems (Power *et al.*, 1996; Fausch, 2007; Simberloff *et al.*, 2013). As dominant predators, fish can have an important effect at the individual, population, community, and ecosystem level (Simon, Townsend, 2003; Tagliaferro *et al.*, 2014a; Buoro *et al.*, 2016).

At the community level, food webs can experience different effects from introduced species, both in structural and functional characteristics (Townsend, 2003). Changes in the ecological structure of communities by introducing species can directly modify the flow of energy and matter in the ecosystem (de Ruiter *et al.*, 1995; Chapin III *et al.*, 2000) or indirectly modify abundance or species traits (Power *et al.*, 1996; Milardi *et al.*, 2016). In particular, top predators such as salmonids can alter trophic relationships through cascading effects (Power, 1992; Milardi *et al.*, 2016; Herrera-Martínez *et al.*, 2017). A reduction in native top predators could generate an increase in prey populations and deplete basal resources, generating a cascade of ecological effects (Chapin III *et al.*, 2000; Shelton *et al.*, 2016). Furthermore, a reduction in native species or the presence of non-native assemblages can produce novel species interactions that have not co-evolved (Hobbs *et al.*, 2006; Tagliaferro *et al.*, 2014a) and could lead to a system with unstable

trophic characteristics (Vander Zanden *et al.*, 1999; Cross *et al.*, 2013).

In the Southern Hemisphere, introductions of salmonids were promoted since the beginning of the 20th century by the governments of Chile (Basulto del Campo, 2003), New Zealand (Flecker, Townsend, 1994; McDowall, 2003), and Argentina (Tulian, 1908). Most introductions included anadromous (Atlantic, Chinook, Coho and Sockeye Salmon), partially migratory and resident species (Rainbow Trout), facultative anadromous species (Rainbow and Brown Trout), and freshwater resident species (Brook and Lake Trout) (Pascual *et al.*, 2002; Pascual, Ciancio, 2007). These species were introduced in ecosystems where the fish fauna had originally consisted of small and unique assemblages of native species (Dyer, 2000; López *et al.*, 2003) without prior evaluation of ecological and trophic consequences. Many studies have shown that the introduction of salmonids has been widely implicated in the reduction of different prey species and native biota (Crowl *et al.*, 1992; Greig, McIntosh, 2006; Cussac *et al.*, 2020), reduction of niche breadth in native Galaxiid fish (Townsend, 2003; McHugh *et al.*, 2012), habitat segregation of Galaxiid populations (Penaluna *et al.*, 2009; Cussac *et al.*, 2020), and dietary overlap between native fish species and introduced salmonids (Di Prinzio, Casaux, 2012; Tagliaferro *et al.*, 2014a). However, variations in the impacts on the system would depend on the particular characteristics of the invasive taxa (Arismendi *et al.*, 2014).

One of the main problems of these introductions was the establishment of natural populations of several of these species across freshwater ecosystems in South America (Arismendi *et al.*, 2019). Rainbow Trout *Oncorhynchus mykiss* (Walbaum, 1792) and Brown Trout *Salmo trutta* Linnaeus, 1758 were widely spread and became the most abundant species (Pascual *et al.*, 2002), followed by Chinook Salmon *Oncorhynchus tshawytscha* (Walbaum, 1792) (Ciancio *et al.*, 2005; Becker *et al.*, 2007; Correa, Gross, 2008) and Lake Trout *Salvelinus namaycush* (Walbaum, 1792) (Arismendi *et al.*, 2014; Tagliaferro, 2014). Although salmonids have been classified as visual and opportunistic predators (Elliott, 1973; Hansen *et al.*, 2013), their diet widely changes between species and ontogeny (De Crespín De Billy, Usseglio-Polatera, 2002). For instance, studies in adults indicated that salmonids feed heavily on Galaxiids and silversides (Vila *et al.*, 1999; Macchi, 2004; Alvear *et al.*, 2007), with large Brown Trout being more piscivorous than Rainbow Trout (Pascual *et al.*, 2007). However, both Rainbow and Brown Trout feed on macroinvertebrates during the first year of life (Tagliaferro *et al.*, 2014a). Meanwhile, Chinook Salmon are primarily piscivorous with Galaxiids as the most common prey (Soto *et al.*, 2003; Arismendi *et al.*, 2009) as well as Lake Trout, that although they might feed on macroinvertebrates, it is still considered an apex piscivore (Post *et al.*, 2000; Tronstad *et al.*, 2010; Syslo *et al.*, 2016).

Traditionally, food webs interactions have been studied utilizing stomach content analyses (SCA) and exclusion/forced interaction experiments. Currently, stable isotope analyses (SIA) complement these methodologies because it provides continuous measurements of trophic position and energy flow (DeNiro, Epstein, 1978; Caut *et al.*, 2009; Nielsen *et al.*, 2015). Therefore, SIA provides a robust tool to test theories of trophic connections (Post *et al.*, 2000; Post, 2002) and to evaluate effects of species invasions on trophic structures (Vander Zanden *et al.*, 1999; Collins *et al.*, 2016). This is especially useful for estimating the trophic position of species with diets difficult to quantify (Kling *et al.*, 1992; Bowes, Thorp, 2015). Recently isotopic ratios of carbon

($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) have been utilized in determining the marine diet of introduced salmonids (Ciancio *et al.*, 2008), characterizing food webs of shallow lakes (Lancelotti *et al.*, 2010) and documenting trophic shifts between invasive salmonid and native Galaxiid species in lakes (Correa *et al.*, 2012).

The aim of this study was to reconstruct the trophic relationships within aquatic food webs of the Santa Cruz River using SIA and SCA. This is especially important not only because of the need to identify the impacts of invasive species but also because of imminent changes associated with the construction of dams along glacial rivers in Patagonia, which could also impact aquatic food webs in the region. This research will be the first study of food webs in the Santa Cruz River (the second largest river of Patagonia). This river is a large glacial river, with low human impact, that has a predictable flood pulse with a stable discharge, distinct seasonal cycles, and a high sediment load (Tagliaferro *et al.*, 2013). Besides the interest of knowing how communities are formed in this understudied system, there were two main objectives: 1) determine differences in the food webs structure in two river sections with different habitat structure, 2) evaluate if there are overlaps of isotopic signatures among native species and introduced salmonids. Our hypotheses were: (H1) a more complex food webs will be suitable in upstream sections, (H2) the two most abundant species, Rainbow Trout and the native Galaxiid will experience a different diet and isotopic overlap between the two sections. Since upstream sections represent more suitable environment for Lake Trout and native Perch (Otturi *et al.*, 2016; Arismendi *et al.*, 2019), are widely used for anadromous Rainbow Trout (Liberoff *et al.*, 2015), and have a greater amount of biomass of macroinvertebrates and producers (Tagliaferro *et al.*, 2013). We predict a more diverse food webs in this section; moreover, we predict that diet and isotopic overlap between the two sections we selected will depend on the presence of other salmonids and macroinvertebrate abundance. This study provides evidence for how introduced fish species can significantly alter food webs interactions. Understanding the impacts of introduced species should lead to better management practices that result in greater conservation efforts for native fish populations in these understudied ecosystems.

MATERIAL AND METHODS

Study area. The Santa Cruz River ($50^{\circ}14'S$, $71^{\circ}58'W$ to $50^{\circ}07'S$, $68^{\circ}20'W$) is in one of the least studied areas of Argentina. It originates in two oligotrophic to ultra-oligotrophic large glacial lakes, Viedma and Argentino, and flows uninterrupted for 382 km across the Patagonian plateau to drain into the Atlantic Ocean (Fig. 1; Brunet *et al.*, 2005). The river has an average discharge of $691 \text{ m}^3 \text{ s}^{-1}$ (min. $278.1 \text{ m}^3 \text{ s}^{-1}$ in September and max. $1,278 \text{ m}^3 \text{ s}^{-1}$ in March), which is highly predictable due to a glacially dominated regime (Tagliaferro *et al.*, 2013). The mean water temperature is 9°C with a maximum registered in January (15°C) and a minimum in July (3°C). The sampling sites were located in two river sections: Upstream ($50^{\circ}10'S$, $69^{\circ}55'W$, an area which contains gravel bars and sediment deposits) and Midstream ($50^{\circ}09'S$, $69^{\circ}59'W$, where the river runs through a natural canyon). Downstream areas were not included to avoid the marine influence in trophic webs and possible urban effects. Whereas temperature, slope, dissolved oxygen were homogeneous at large scales, the

two studied sections present different characteristics at the local scale in chlorophyll-a concentrations, inorganic matter, particles substrate size, and depth (S1; Tagliaferro *et al.*, 2013).

In relation to biological characteristics, the Upstream section was previously characterized as areas with higher macroinvertebrate abundance, richness and higher chlorophyll-a biomass. Whereas the Midstream section was associated with lower macroinvertebrate richness and abundance (Tagliaferro *et al.*, 2013; Tagliaferro, Pascual, 2017). Fish assemblages in the Santa Cruz River contain populations of native Perch *Percichthys trucha* (Valenciennes, 1833) (Percichthyidae), Large or Big Puyen *Galaxias platei* Steindachner, 1898 and Puyen *G. maculatus* (Jenyns, 1842) (Galaxiidae), the latter being the most abundant native species (Tagliaferro *et al.*, 2014b). Among the exotic species, the most abundant are Rainbow Trout *Oncorhynchus mykiss*. Other introduced salmonids include Brown Trout *Salmo trutta*, Lake Trout *Salvelinus namaycush* and Chinook Salmon *O. tshawytscha*.

Sampling design. Sampling was done in April 2010 (during average discharge condition of the Santa Cruz River) since (1) large glacial rivers in general experience a high flow during the summer (January–February in Southern hemisphere) due to ice melting, (2) to avoid the spawning period for Rainbow and Brown Trout (around September) in the Santa Cruz River (Riva Rossi *et al.*, 2003). It is important to avoid taking samples for SIA between August–March since during the first month these two adult species are not feeding, and there would be a bias in the stomach content of adults. On the other hand, young of the year (YOY) juveniles can use maternal resources for few months (Liberoff *et al.*, 2013), and the isotopic signal might get confusing results due to maternal effects. Finally, macroinvertebrates tend to experience changes in distribution due to temporal effects. Thus, we selected a mid-flow period which is the most representative scenario with YOY and adult trout feeding, and mid to high macroinvertebrate abundance.

Sampling in the Santa Cruz River included different components of the aquatic community: fish, macroinvertebrates, and basal resources in two distinct areas related to river morphology. Benthic producers (*i.e.*, macrophyte and algae) were estimated by the mean value of three individual visual evaluations of a 10m long transect along the river. Benthic algae were obtained by scraping rocks ($n=9$ and $n=3$ for Upstream and Midstream sections, respectively), whereas planktonic algae ($n=3$ for each section) were collected by filtering river water using a plankton net (15 μm pore-size). Both samples of algae were filtered using sterile glass fiber filters. Macrophytes were cut from the riverside and packed in airtight plastic bags ($n=3$ for each section). Debris samples were taken from macrophytes cover areas. Four to nine benthic macroinvertebrate samples were obtained at each river section with a kick-net of 450 μm mesh covering 0.25 m^2 . Algae, macrophytes, and macroinvertebrate samples were stored in a portable cooler at -18°C in the field. Algae samples were stored in glass fiber filters inside individual aluminum envelopes. Macroinvertebrates were stored in plastic 500ml containers and once in the laboratory were separated and identified to the lowest possible taxonomic level following descriptions from Domínguez, Fernández (2009). Macroinvertebrates were then grouped according to functional feeding group (FFG) (Merritt, Cummins, 1996). Both macroinvertebrates and aquatic plants were dried for 24 h at 60°C . The

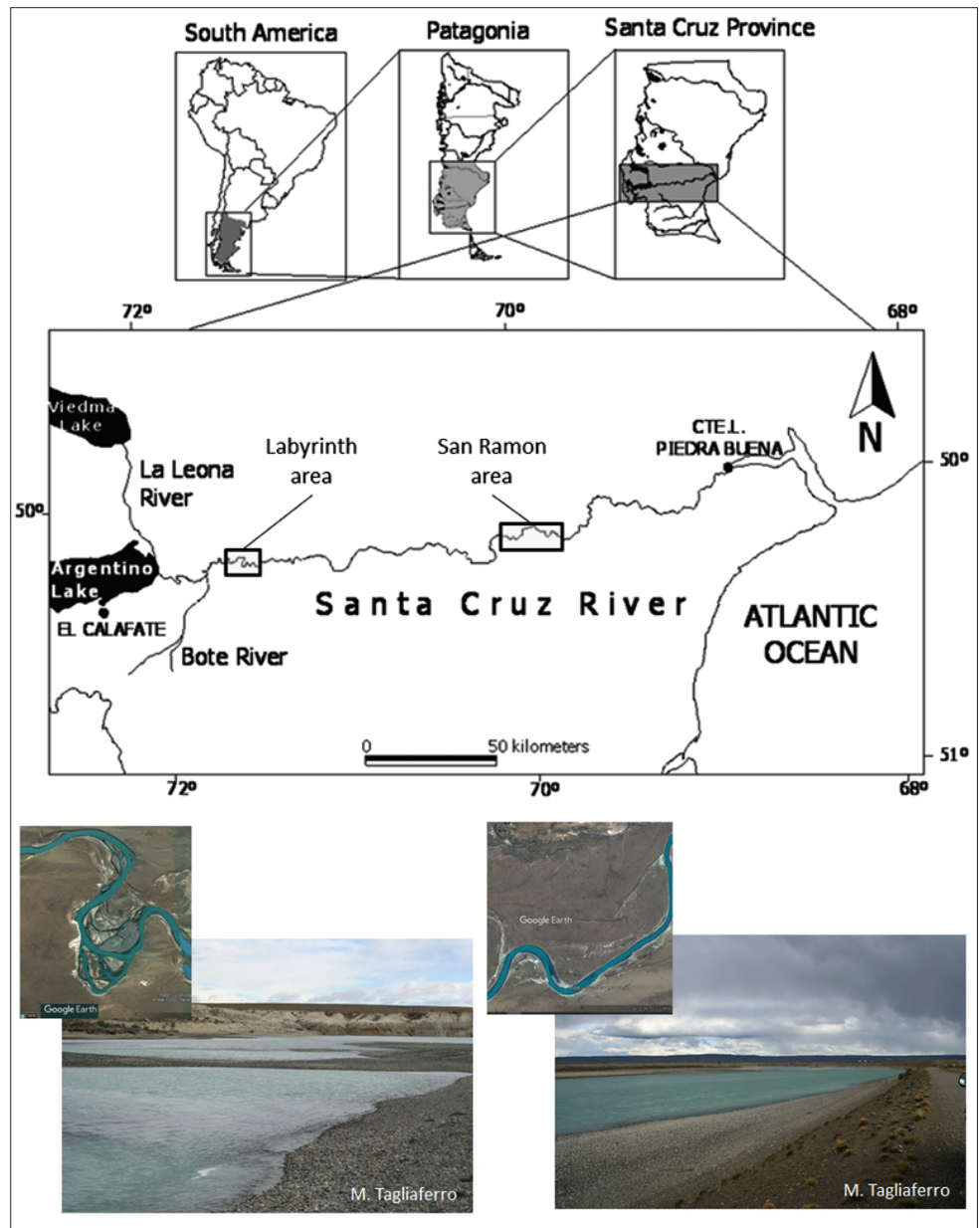


FIGURE 1 | Sampling areas in the Santa Cruz River, Argentina. Upstream area corresponds to the locally known “Labyrinth”, and Midstream area correspond to “Estancia San Ramon”. Map created by the authors, upper picture taken from Google Earth (R).

most abundant macroinvertebrates, along those with sufficient biomass were used for SIA.

Small fish (*i.e.*, total length range: 50 to 140 mm) were caught by using standard three-pass electrofishing methods along 100 m transects at each site from the littoral zone to depths of 0.6 m (Jones, Stockwell, 1995; Meador *et al.*, 2003) using a Smith–Root LR–24 electrofisher set to a frequency of 90 Hz and a pulse width of 3 m/s. This data was then used as an indirect measurement of abundance (CPUE). Due to the morphology

of the river and water velocity at the time of the study, as well as following work safety protocols, the sampling was restricted to a narrow width of the main stem of the river. Larger fish (length range > 180 mm) were captured by using gillnets of 15, 20, 30, 50, 60 mm. Captures were related to gillnet effort (CPUE). All fish were measured for total length with a digital caliper (0.01mm nearest unit) and weighed on a Mettler PC 440 Delta Range balance (0.003 g nearest unit). A portion of the posterior dorsal muscle was excised and dried at 60°C. Fish stomach contents were removed and stored in 70° ethanol for further separation and identification using the same procedure previously mentioned for macroinvertebrate samples.

Once dried, all samples were ground into a homogeneous powder using a hand mortar and pestle. Three replicates of macroinvertebrate and aquatic plants were used for stable isotopes analyses. In each stream area (*i.e.*, Upstream and Midstream), we used replicate samples for Puyen (n=8-10), Chinook Salmon (n=4), and Rainbow Trout (n=18, n=6 for each life stage). In Upstream areas, we analyzed replicate samples for Brown Trout (n=3), Perch (n=4), Big Puyen (n=3) and Lake Trout (n=3). A subsample of each individual or group of individuals in case of very small species (*e.g.*, chironomids) was weighed on a precision balance Shimadzu (error 0.001 mg), placed in a tin capsule for further analysis at the Stable Isotopes laboratory at the University of California, Davis: 2-3 mg in the case of plants and 1 ± 0.2 mg samples for animal tissue. Samples were analyzed for ¹³C and ¹⁵N isotopes using an elemental analyzer PDZ Europa ANCA-GSL interface with a mass spectrometer PDZ Europa 20-20 isotope ratio (Sercon Ltd., Cheshire, UK). The long-term standard deviation of these analyses was 0.2 ‰ to 0.3 ‰ for ¹³C to ¹⁵N. The stable isotope ratios are expressed as δ values of ‰: $\delta X = 10^3 [(R_{\text{sample}}/R_{\text{standard}}) - 1]$, where X is ¹³C or ¹⁵N and R is the corresponding ratio ¹³C:¹²C or ¹⁵N:¹⁴N. The values of final “δX” were expressed relative to international standards V-PDB (Vienna PeeDee Belemnite) and N₂ from air for carbon and nitrogen, respectively.

Data Analysis. A two-way PERMANOVA test was performed using the statistical program PAST (version 3.14.) to evaluate possible differences in isotopic values of carbon and nitrogen between the two selected areas of the river for the two most abundant fish species (Rainbow Trout and Puyen) and dominant macroinvertebrate FFGs. For the most abundant species, a one-way PERMANOVA was performed to evaluate possible local differences.

Isotopic fractionation values for Rainbow Trout were Δ¹³C 1.9 ± 0.5 and Δ¹⁵N 3.2 ± 0.2 (McCutchan Jr *et al.*, 2003), and Δ¹³C 1.6 ± 0.5 and Δ¹⁵N 3.5±0.7 were applied for macroinvertebrates and plants (DeNiro, Epstein, 1980; Rounick, Hicks, 1985; McCutchan Jr *et al.*, 2003). The trophic position was calculated for fish and macroinvertebrates using the isotopic variation in nitrogen (Post, 2002) and possible variants of fractionation as follow:

$$TP = \lambda + \frac{(\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{base}})}{\Delta_n}$$

where TP indicates the trophic position, λ represents the trophic position of the prey (possible prey items from diet), δ¹⁵N_{consumer} are the stable isotope ratios of the organism of which the trophic position is being calculated and δ¹⁵N_{base} is the ratio for primary producers. Finally, Δ_n indicates the fractionation in ¹⁵N between the consumer and its

diet. The baseline for each trophic position in each stream zone was estimated using mean value of possible primary producers considering the fractionation factors (DeNiro, Epstein, 1980; Rounick, Hicks, 1985; McCutchan Jr *et al.*, 2003). In the Midstream section only Debris and Debris associated to *Myriophyllum* sp. were used to calculate trophic positions of preys since the fractionation did not exceed the $\Delta^{13}\text{C}$ 1.6 ± 0.5 and $\Delta^{15}\text{N}$ 3.5 ± 0.7 ; algae were not used since there was a $\Delta^{13}\text{C} > 15$. Similarly, in Upstream sections, the macrophytes and the planktonic algae and *Nostoc* sp. were excluded from the estimation.

A total of 432 stomach contents of fish were analyzed in terms of biomass to evaluate the contribution of prey to diet. After the selection of possible isotopic sources according to SCA, Bayesian isotopic mixing models were applied by using V4.0 SIAR (Stable Isotope Analysis in R) (Parnell *et al.*, 2010) using R software (R –version 3.2.5 2016) to assess the relative contributions of prey to the diet of fish. SIAR mixing model results were calculated with credibility intervals of 5, 25, 75 and 95%.

RESULTS

General Pattern. Basal resources were represented by macrophytes (mainly *Myriophyllum* sp.) and algae (mainly *Cladophora* sp., but also *Nostoc* sp. and *Batrachospermum* sp.) (Tab. 1). Debris was constituted by dead macrophytes and *Coiron* sp. grasses. Both macrophytes and benthic visual algae cover were very low along the two sections (< 1.5–3% and < 4–5%, respectively), with algae patches being in the Upstream section and macrophytes in the Midstream section. Macroinvertebrate FFGs included scraper-grazers, shredders, filterer-collectors, collector-gatherers, and predators. Most abundant FFGs in Upstream areas were scraper-grazers ($47.5 \pm 22.9\%$), filterer-collectors ($24.3 \pm 33.0\%$), and shredders ($19.7 \pm 6.0\%$); in Midstream areas shredders were the most abundant FFG ($41.0 \pm 4.4\%$), followed by scraper-grazers ($33.7 \pm 12.6\%$) and collector-gatherers ($17.4 \pm 22.6\%$). Fish taxa in Upstream areas were dominated by top predators, including Lake Trout, Brown Trout, Rainbow Trout, Chinook Salmon, Perch, Puyen and Big Puyen, with Rainbow Trout being the most abundant species (Tab. 2). Moreover, different ontogenetic stages of Rainbow Trout were captured (yearling, juveniles and adults). In Midstream areas only four fish species were captured: Rainbow Trout (different ontogenetic stages), Chinook Salmon (ocean type), Perch and Puyen (Tab. 2), with Puyen being the most abundant species.

Stomach Content Analyses. Stomach contents for small fish (Puyen and yearling Rainbow Trout) were composed nearly entirely of benthic macroinvertebrates, mainly shredders and collector-filterers, with less than 2% being attributed to terrestrial inputs (Tab. 3). Juvenile and adult Rainbow Trout (in both river areas) were found to consume Puyen, along with macroinvertebrates of different FFGs (Tab. 3). Brown Trout, Chinook Salmon, and Perch consumed juvenile Trout. Lake Trout fed exclusively on fish of any size including both Trout and Puyen species (Tab. 3).

Stable Isotope Analyses. Biplots for carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) showed a clear pattern for an autotrophic base of $\delta^{13}\text{C}$ values (Fig. 2). The Midstream area

TABLE 1 | Primary producer cover (%) along a sampling line of 10 m and macroinvertebrates abundance in each kick-net sample (0.25 m²).

| | Upstream | Midstream |
|------------------------------------|------------|-------------|
| Primary producers (% cover) | | |
| Filamentous algae (fil) | 0.8 ± 0.8 | < 0.1 |
| <i>Cladophora</i> algae (Ch), | 17.1 ± 6.9 | < 0.1 |
| <i>Nostoc</i> sp. | < 0.1 | < 0.1 |
| <i>Myriophyllum</i> (Plant-My) | 1.2 ± 0.9 | 5.7 ± 4.9 |
| Debris | < 0.1 | 1.4 ± 1.0 |
| Macroinvertebrates | | |
| Shredder | 3.7 ± 1.0 | 0.9 ± 0.9 |
| Scraper/grazer | 15.5 ± 1.8 | 9.3 ± 2.7 |
| Collector-Gatherer | 6.3 ± 1.3 | 15.8 ± 11.2 |
| Collector-filterer | 3.8 ± 1.3 | 2.6 ± 1.7 |
| Predator | 5.8 ± 1.0 | 2.1 ± 0.9 |

TABLE 2 | Fish captures along the studied areas in the Santa Cruz River. YRT refers to Yearling Rainbow Trout, JRT to juvenile Rainbow Trout, and ART to Adult Rainbow Trout. Puyen and YRT captures were related to the three pass electrofishing method and the rest of the fish species and stages were related to the use of gillnets.

| | Proportion of captures (%) | |
|-------------|----------------------------|-----------|
| | Upstream | Midstream |
| Lake Trout | 1.4 | 0 |
| Brown Trout | 1.9 | 0 |
| YRT | 42.1 | 13.9 |
| JRT | 21.5 | 21.3 |
| ART | 15.8 | 1.9 |
| Chinook | 2.4 | 1.9 |
| Big Puyen | 1.4 | 0 |
| Puyen | 10.0 | 58.3 |
| Perch | 3.3 | 2.8 |

TABLE 3 | Stomach content of fish species found along the Santa Cruz River. Relative contribution (% range) of collector-filterer, grazer, shredder and scraper benthic macroinvertebrates (functional feeding groups), predator invertebrates and fish items.

| | Lake Trout | Brown Trout | Rainbow yearling trout | Rainbow juvenile trout | Rainbow adult trout | Chinook Salmon | Puyen | Perch | Big/Large Puyen |
|-----------------------|------------|-------------|------------------------|------------------------|---------------------|----------------|-------|-------|-----------------|
| Fish | 98-100 | 20-35 | 0-1 | 2-4 | 35-48 | 18-35 | | 10-19 | 0-1 |
| Invertebrate predator | | 2-6 | 6-11 | 28-35 | 14-21 | 9-13 | 32-48 | 0-3 | 28-37 |
| Collector-filterer | | 24-32 | 32-46 | 3-11 | 1-4 | 2-8 | 4-9 | 9-19 | 1-5 |
| Grazer | | 4-7 | 16-22 | 27-39 | 7-14 | 0-1 | 0-2 | 46-65 | 47-69 |
| Shredder-scraper | 0-2 | 38-44 | 32-37 | 25-37 | 21-33 | 62-71 | 59-72 | 15-22 | 4-8 |

showed a narrower range at the base of the trophic web with debris (CPOM) and parts of *Myriophyllum* sp. being the primary basal sources. The Upstream area showed a wider range of basal sources with several different species of algae (mainly *Cladophora* sp. and *Batrachospermum* sp.) (Fig. 2A, B). Isotopic values also showed a grouping of herbivorous macroinvertebrates enriched in ^{15}N , and a grouping of fish enriched in both ^{13}C and ^{15}N (Fig. 2A, B). Isotopic values in Midstream areas (Fig. 2A) tended to be enriched in ^{15}N for all groups in comparison with Upstream areas (Fig. 2B). Although the general pattern of isotopic composition was similar for both study areas, there were statistically significant differences between sites in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (Two-ways PERMANOVA, $F_{\text{river area}} = 4.361$; $p = 0.007$; $F_{\text{species}} = 41.329$; $p = 0.0001$). Among the most abundant fish species, Puyen showed significant differences in isotopic signature between Upstream and Midstream areas ($p = 0.0001$). Due to the presence of different ontogenetic stages of Rainbow Trout, the isotopic signature was analyzed separately, and differences were found depending on life stage. Only juvenile Rainbow Trout of the first year showed significant differences between Mid and Upstream areas ($p = 0.0001$). Adult Rainbow Trout and older juveniles showed no significant differences ($p = 0.12$, and $p = 0.834$, respectively).

In the Upstream section, Lake Trout showed significantly higher $\delta^{15}\text{N}$ values than the rest of the fish species. The most abundant species was Puyen, followed by Rainbow Trout. Although differences were found in isotopic signatures ($F = 21.174$, $p = 0.001$), the “a-posteriori” comparisons showed no significant differences between Puyen and

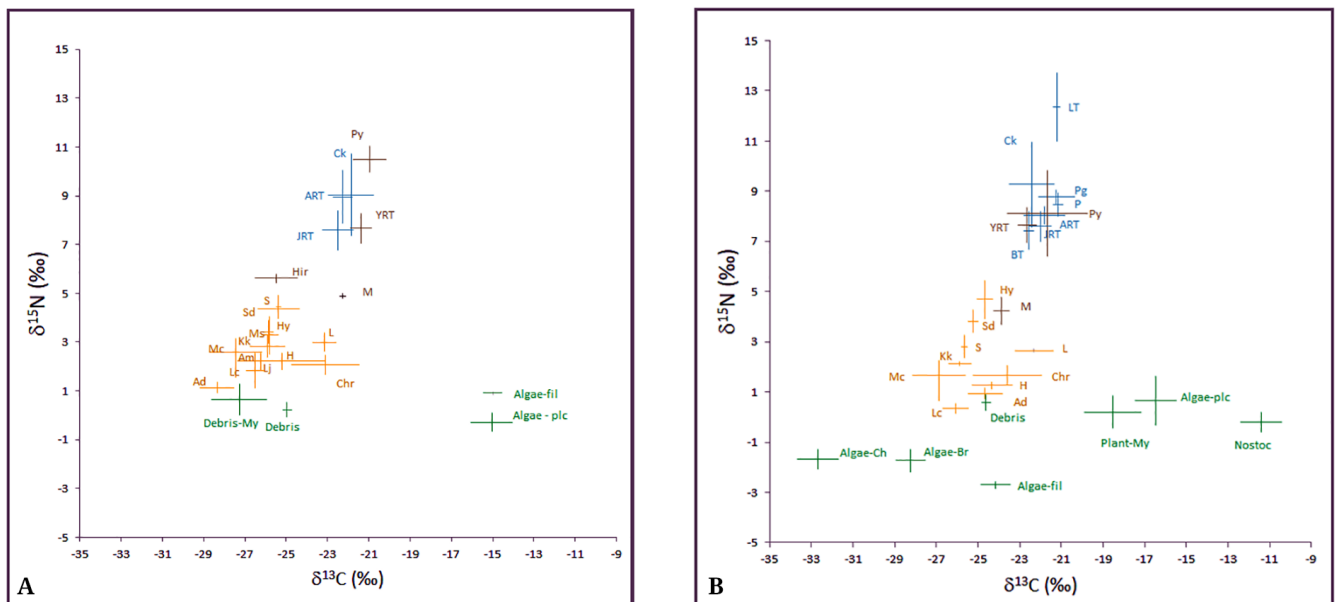


FIGURE 2 | Values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ found in the Midstream areas (A) and Upstream areas (B). Error bars correspond to standard deviation. Abbreviations: Hirudinea (Hir), Muscidae (M), Simuliidae (S), *Smicridea dythira* (Sd), Hydrobiosidae (Hy), *Mastigoptila* spp. (Ms), *Klapopteryx kuscheli* (Kk), *Lymnaea* (L), *Meridialaris chiloensis* (Mc), *Antarctoperla michaelsoni* (Am), *Hyaella* sp. (H), *Luchoelmis cekalovici* (Lc), *Limnoperla jaffuelli* (Lj), *Andesiops* sp. (Ad), Chironomidae (Chr), Filamentous algae (fil), *Batrachospermum* sp. algae (Br), planktonic algae (plc), Debris associated to *Myriophyllum* sp. (My), *Cladophora* algae (Ch), macrophyta of genus *Myriophyllum* (plant-My). Colors indicate primary producers (green), herbivores (orange), non-piscivores predators (brown) and general predators (blue).

the rest of the fish species, except for Lake Trout and juvenile Rainbow Trout (Tab. 4). Rainbow Trout yearlings showed significant differences with Perch, Chinook Salmon, and Lake Trout. However, older juveniles (>1 year) were significantly different from Perch and Lake Trout, while adult specimens only differed from Lake Trout (Tab. 4). In Midstream areas significant differences in isotopic signature were found (F= 87.185, p=0.001). Puyen was the most abundant fish species and showed significant differences (a posteriori test) with all Rainbow Trout ontogenetic stages (Tab. 5). Among Rainbow Trout, ontogenetic stages differed between yearling Rainbow Trout and juveniles and adults, but no significant differences were found between juveniles and adult specimens (Tab. 5). Perch had low abundances and showed no significant differences in isotopic values with Puyen or Rainbow Trout (Tab. 5).

Juvenile and adult Rainbow Trout showed no significant differences in isotopic values between river sections, but there were significant differences with yearling stages between Mid and Upstream areas (F= 5.201, p=0.012). However, in the SCA, the contribution of different prey biomass in the diet of Rainbow Trout showed differences

TABLE 4 | PERMANOVA analysis results of Upstream area and pairwise comparisons. CF= collector-filterer, CG= collector-gatherer, SCR=scrapers, SHR= shredders, PRED= predator, L. Trout= Lake Trout, B. Trout= Brown Trout, R. Trout= Rainbow Trout.

| Pairwise | CF | CG | SCR | SHR | PRED | Puyen | Perch | Chinook | Lake | Brown | A R. Trout | J R. Trout |
|------------|-------|-------|-------|-------|-------|--------------|--------------|--------------|--------------|-------|------------|------------|
| CG | 0.274 | | | | | | | | | | | |
| SCR | 0.009 | 0.001 | | | | | | | | | | |
| SHR | 0.011 | 0.447 | 0.002 | | | | | | | | | |
| PRED | 0.009 | 0.002 | 0.008 | 0.005 | | | | | | | | |
| Puyen | 0.000 | 0.000 | 0.001 | 0.000 | 0.002 | | | | | | | |
| Perch | 0.003 | 0.001 | 0.007 | 0.002 | 0.009 | 0.681 | | | | | | |
| Chinook | 0.003 | 0.001 | 0.008 | 0.002 | 0.016 | 0.301 | 0.173 | | | | | |
| L. Trout | 0.003 | 0.001 | 0.007 | 0.002 | 0.008 | 0.000 | 0.007 | 0.008 | | | | |
| B. Trout | 0.004 | 0.010 | 0.007 | 0.008 | 0.096 | 0.308 | 0.024 | 0.109 | 0.009 | | | |
| A R. Trout | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.381 | 0.084 | 0.107 | 0.001 | 0.161 | | |
| J R. Trout | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.673 | 0.030 | 0.159 | 0.000 | 0.076 | 0.505 | |
| Y R. Trout | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.366 | 0.003 | 0.027 | 0.000 | 0.057 | 0.106 | 0.628 |

TABLE 5 | PERMANOVA analysis results of Midstream area and pairwise comparisons. CF= collector-filterer, CG= collector-gatherer, SCR=scrapers, SHR= shredders, PRED= predator, R. Trout= Rainbow Trout.

| Pairwise | CF | CG | SCR | SHR | PRED | Puyen | Perch | A R. Trout | J R. Trout |
|------------|-------|-------|-------|-------|-------|--------------|-------|--------------|--------------|
| CG | 0.000 | | | | | | | | |
| SCR | 0.009 | 0.001 | | | | | | | |
| SHR | 0.030 | 0.009 | 0.007 | | | | | | |
| PRED | 0.236 | 0.000 | 0.032 | 0.004 | | | | | |
| Puyen | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | | | | |
| Perch | 0.029 | 0.011 | 0.006 | 0.011 | 0.044 | 0.180 | | | |
| A R. Trout | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.112 | | |
| J R. Trout | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.001 | 0.082 | 0.056 | |
| Y R. Trout | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.038 | 0.061 | 0.038 | 0.038 |

between ontogenetic stages and river areas for the period under study (Tab. 3). When comparing the contribution of prey to stomach contents and the possible sources for the mixing model, it was not possible to create a virtual polygon of resources that included the predator, and therefore mixing models were not employed for yearling Rainbow Trout.

Trophic Position. Trophic positions for yearling Rainbow Trout and Puyen were not included due to large differences in isotopic values of their prey, which could cause an incorrect positioning. Herbivores, independently of their FFG, were placed in a lower secondary trophic position and close to one as would be expected for their feeding habits (Tab. 6). Collector-filterers showed a greater isotopic enrichment value for $\delta^{15}\text{N}$, which resulted in a higher trophic level. The trophic position for predatory macroinvertebrates was based on other macroinvertebrate isotopic signals and the trophic level varied between 2.5 and 3 (Tab. 6). Exclusively piscivorous fish, such as Lake Trout, showed a trophic level of 4, while fish that had mixed diets of fish and macroinvertebrates were 2.5 (Tab. 6).

TABLE 6 | Trophic position for selected taxa in Upstream and Midstream areas. FFG: Functional feeding groups. Rainbow Trout includes both juvenile and adult individuals.

| FFG | Taxa | Study Area | |
|--------------------|----------------------------------|------------|-----------|
| | | Upstream | Midstream |
| Scraper-grazer | <i>Lymnaea</i> sp. | 1.50-1.65 | 1.55-1.71 |
| Scraper-grazer | <i>Luchoelmis cekalovici</i> | 1.13-1.24 | 1.20-1.36 |
| Scraper-grazer | <i>Andesiops</i> sp. | 1.30-1.43 | 1.00-1.14 |
| Scraper-grazer | <i>Meridialaris chiloeensis</i> | 1.50-1.65 | 1.46-1.62 |
| Collector-gatherer | <i>Hyaella</i> sp. | 1.38-1.53 | 1.30-1.48 |
| Collector-gatherer | <i>Limnoperla jaffueli</i> | - | 1.22-1.35 |
| Collector-gatherer | Chironomidae | 1.39-1.60 | 1.30-1.45 |
| Shredder | <i>Antarctoperla michaelsoni</i> | - | 1.34-1.38 |
| Shredder | <i>Klapopteryx kuschelli</i> | 1.60-1.80 | 1.50-1.67 |
| Collector-filterer | <i>Mastigoptila</i> sp. | - | 1.70-1.85 |
| Collector-filterer | <i>Smicridea dithira</i> | 2.10-2.33 | 1.95-2.18 |
| Collector-filterer | Simuliidae spp. | 1.82-2.02 | 1.97-2.11 |
| Predator | Glossiphoniidae spp. | - | 2.80-3.19 |
| Predator | Hydrobiosidae spp. | 2.74-3.03 | 2.23-2.52 |
| Predator | Muscidae spp. | 2.52-2.90 | 2.68-2.97 |
| Predator | Lake Trout | 4.24-4.60 | - |
| Predator | Brown Trout | 2.89-4.58 | - |
| Predator | Rainbow Trout | 2.65-3.42 | 1.97-3.22 |
| Predator | Chinook Salmon | 3.33-4.60 | 2.57-3.03 |
| Predator | Big Puyen | 3.18-3.40 | - |
| Predator | Puyen | 3.24-3.72 | 2.92-3.51 |
| Predator | Perch | 3.11-4.12 | 2.70-3.70 |

Mixing Models. Only the most abundant prey items found during the SCA were selected to be included as possible sources in the subsequent mixing models for calculating the proportion of each group to the diet of native and introduced fish species. The results of Chinook Salmon mixing models showed a high contribution (95% Confidence Interval: 95%-CI) of Puyen (43.4 ± 19.7%), followed by simuliid larvae (35.4 ± 20.5%), and amphipod *Hyaella* sp. (21.2 ± 18.6%). The estimated mixing model for adult Brown Trout diet showed a major contribution from *Hyaella* sp. (5%-CI= 29.5 ± 11.3%), *Klapopteryx kuscheli* (5%-CI 33.4 ± 15%), Puyen (5%-CI 15.8 ± 13%) and juvenile Rainbow Trout (5%-CI 21.3 ± 15.1%). The fit of the data for Lake Trout was concordant with the results of stomach contents, showing a comparable contribution from Puyen (5%-CI 46.6 ± 16.8%) and Rainbow Trout (5%-CI 53.4 ± 13.6%). Perch showed a dominantly piscivorous diet, mainly composed of Puyen and juvenile Trout, followed by a variety of predatory (e.g., Hydrobiosidae, *Lancetes* sp.) and herbivorous (e.g., *Hyaella* sp., Chironomidae) macroinvertebrates. Prey that fit the diet model for Perch were amphipods (37.5 ± 10.1%), juvenile Rainbow Trout (5%-CI 32.1 ± 11.6 %), and Puyen (5%-CI 30.4 ± 14.9%).

In the Midstream section, due to differences in diets seen in SCA of juvenile and adult Rainbow Trout, mixing models were performed using *Lymnaea* sp., Simuliidae spp. larvae and Puyen as sources for juvenile Trout; and for adult Trout, we included Puyen and the stoneflies *K. kuscheli* and *Antarctoperla michaelsoni*. Juvenile Rainbow Trout showed a low contribution of Puyen in their diet (5%-CI 10.5 ± 8.5%); while Simuliidae spp. and gastropods contributed 49.5 ± 12% and 40 ± 23.1% (5%-CI), respectively. The model for adult Trout showed, unlike the Upstream areas, an important contribution of Puyen (5%-CI 39.2 ± 10.1%), followed by *A. michaelsoni* (5%-CI 30.7 ± 22.5%) and *K. kuscheli* (5%-CI 30.1 ± 19.8%) (Fig. 3B).

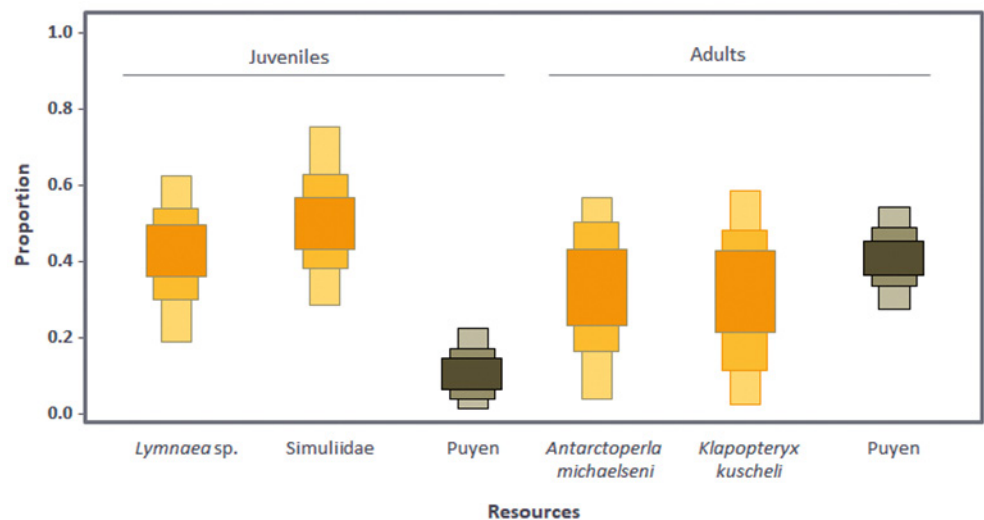


FIGURE 3 | Mixing models adjusted for juvenile and adult Rainbow Trout in Midstream areas. Herbivores are in orange and predators in brown color.

In Upstream section, mixing models for Rainbow Trout showed Puyen, the stonefly nymph *K. kuscheli*, the gastropod *Lymnaea* sp., and caddisfly larvae Hydrobiosidae as possible resources. Mixing models for juvenile Rainbow Trout showed the contribution of *K. kuscheli* (5%-CI $49.7 \pm 16.4\%$), followed by Puyen (5%-CI $27.4 \pm 8.3\%$) and *Lymnaea* sp. (5%-CI $22.9 \pm 16\%$) (Fig. 4A). The model for adult Rainbow Trout showed a similar contribution of *Lymnaea* sp. (5%-CI $23.9 \pm 13.1\%$), *K. kuscheli* ($25.4 \pm 17.8\%$), the Hydrobiosidae spp. (5%-CI $26.9 \pm 15.7\%$), and Puyen (5%-CI $25.1 \pm 7.9\%$) (Fig. 4B).

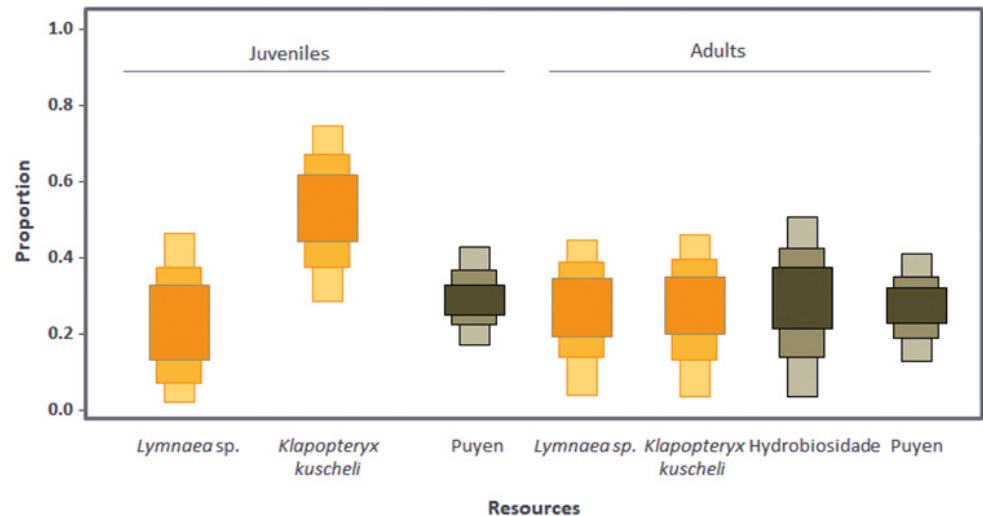


FIGURE 4 | Mixing models adjusted for juvenile and adult Rainbow Trout in Upstream areas. Herbivores are in orange and predators in brown color.

DISCUSSION

The present research is the first study of food webs in the Santa Cruz River, a river that is about to change due to damming without information regarding the trophic structure and with poor information about the influence of introduced species on aquatic food webs. Findings in this study support that native Puyen is more abundant in Midstream areas and exotic salmonids are more abundant in Upstream areas, consistent with previous studies of juvenile fish distributions in this river (Tagliaferro *et al.*, 2014a) and with seasonal studies on fish assemblages over three years (Tagliaferro, 2014).

This study supports the prediction of having a more complex food webs with a wider base and an extra trophic level (due to the presence of Lake Trout) in Upstream sections. This section presents a more heterogeneous habitat structure (Tagliaferro *et al.*, 2014b; Quiroga *et al.*, 2015) where the river runs through gravel bars and small gravel islands, which were associated with a more complex macroinvertebrate community structure with higher richness and biomass (Tagliaferro *et al.*, 2013; Tagliaferro, Pascual, 2017). Moreover, using SIA, we noted that in Upstream sections, food webs are based on algae as the basal energy source; while in Midstream sections the main resource is fine debris

(mainly parts of *Myriophyllum* sp.) that might have a lower energetic value and not be able to sustain complex food webs. Also, the two river sections differ in the trophic position and role of the most abundant invasive species, the Rainbow Trout. Previous research by Tagliaferro *et al.* (2014a) showed that 25% of fish captured in the lower part of the watershed were Rainbow Trout and 75% Puyen; in that case we expect Rainbow Trout to be the apex predator, without top down controls from larger fish (*e.g.*, Lake Trout) feeding on yearlings, juveniles or adult Trout. More suitable interactions between small fish of the two species would be competition, and predation on Puyen by larger Rainbow Trout. However, in Upstream sections, the role of Rainbow Trout might change from prey and competitor to top predator depending on the abundance of other piscivorous fish taxa such as, Brown Trout, Perch, and Lake Trout.

Regarding our second hypothesis, where we proposed that Rainbow Trout and the native Galaxiid will experience a higher diet and isotopic overlap in Midstream sections we found that, although in Upstream sections a greater diversity of prey contributed to Rainbow Trout diet and in Midstream sections, larger stoneflies and fish had a greater contribution, similar isotopic signatures were found in both river sections. Thus, Rainbow Trout might have a stronger effect on Puyen populations in Upstream sections due to predatory effect and possible competition. Since Puyen and Rainbow Trout did not have significant differences in isotopic values in Upstream sections, but changes in feeding behavior of Galaxiids occur in the presence of Trout (Elgueta *et al.*, 2013; Cussac *et al.*, 2020), we propose that these species might be feeding in different areas (*i.e.*, deeper or littoral areas) of the river to reduce possible competition.

There is wide support for all salmonids having certain degrees of piscivory (Pascual *et al.*, 2007), with Lake Trout being a top predator (Post *et al.*, 2000; Tronstad *et al.*, 2010; Syslo *et al.*, 2016). There is also evidence of the predation of Brown Trout, Chinook and Coho Salmon on Puyen (Vila *et al.*, 1999; Penaluna *et al.*, 2009). While in much of the work studying the diet of introduced salmonids, selectivity (Di Prinzio, Casaux, 2012; Tagliaferro *et al.*, 2014a), size of prey (Di Prinzio *et al.*, 2015), overlap with native species (Kusabs, Swales, 1991; Tagliaferro *et al.*, 2014a; Horká *et al.*, 2017) are evaluated, this work adds the interaction with other introduced species of salmonids. Thus, we could observe that in areas where several introduced species coexist, natural interactions such as competition and predation by other salmonids occur and less pressure could be exerted on native species. For instance, the presence of Lake Trout have been associated to the decline of both native and invasive fish species (Tronstad *et al.*, 2010). In addition, this study highlights the differences in distribution of some native species such as Perch and Puyen. Thus, the interaction between yearling Rainbow Trout and Puyen feeding on macroinvertebrates, and juvenile and adult Rainbow Trout feeding also on Puyen in Midstream section, get more complex in the Upstream section. In Upstream section, yearling Rainbow and Brown Trout together with juvenile Chinook Salmon and Puyen are feeding on macroinvertebrates, and adults are preying on Puyen and also on yearling Trout. In conclusion, whereas one invasive salmonid species can generate negative effects on native species on a new environment, when new invasive species are established, the associated changes are much more complex; for instance, the establishment of other invasive species may have opposite effects on native fauna since they might release or increase pressure on native species, for example, controlling the population abundance of other introduced species.

Two main effects have been highlighted throughout studies of the ecology of salmonid invasion: (1) the use of habitat and timing (Glova *et al.*, 1992; McIntosh *et al.*, 1992; Stuart-Smith *et al.*, 2008; Correa *et al.*, 2012; Sowersby *et al.*, 2016), and (2) the use of food resources and possible interactions with native species (Glova *et al.*, 1992; Shelton *et al.*, 2016; Milardi *et al.*, 2020). Differential selection of habitat or time of the day using a certain space could help reducing unnatural interactions between species (Stuart-Smith *et al.*, 2008; Otturi *et al.*, 2016). Food webs can be altered in their structure and function through top-down or bottom-up mechanisms (Gozlan *et al.*, 2010). On the other hand, by reducing native species, introduced fish can also change the ecofunctional diversity of a community (Milardi *et al.*, 2020). Recent studies found that invasive fish species can diminish the relative diversity of native fish communities (Milardi *et al.*, 2016; 2020), and alter their functional traits (Shuai *et al.*, 2018). Although most abundant juvenile fish species in the Santa Cruz River, independently from their origin are considered generalized benthic predators (Lattuca *et al.*, 2008; Di Prinzio *et al.*, 2013; Tagliaferro *et al.*, 2014a; Hertz *et al.*, 2017), they might feed on different functional feeding groups, changing food webs structures. For example, the replacement of native fish by non-native Trout has been shown to reduced top-down control over collector-gatherer (Shelton *et al.*, 2017). On the other hand, predator pressure over Galaxiid by native Perch and adult salmonids might indirectly affect macroinvertebrate abundances. The reduction of Galaxiids due to salmonids was also associated with changes in insect behavior and algal standing crops (Flecker, Townsend, 1994; Herrera-Martínez *et al.*, 2017).

In the present study, we were able to determine trophic interactions and identify differences in trophic structure depending on the river section by using the two alternative techniques of stomach content and stable isotope analyses (Fig. 5). While SCA results in partially or completely digested organisms creating difficulties in the identification process, stable isotope techniques allow an easier way of integrating information from all components of the food webs. Puyen was the most abundant native species in both river areas with similar roles in the food webs, but the stable isotopes analyses indicated a higher trophic position in midstream areas, which might be due to the presence of fewer fish species allowing it to have a broader diet. Also, SIA integrate information over a greater time span (months to years), which is especially important to assess the trophic role when organisms are slow-growing fish (Hesslein *et al.*, 1993; McCarthy *et al.*, 2004) or spend several days without feeding (*e.g.*, spawning Steelhead Rainbow Trout or Lake Trout). The time span was important to take into consideration with Lake Trout, Chinook Salmon, and Big Puyen, since the number of stomach content samples were low and the integration of time in the SIA support the same diet over several months. On the other hand, SCA has the advantage of providing taxonomic information for food items, which is not possible with SIA (Power *et al.*, 2002).

Stable isotope analyses were found to be a useful tool in evaluating possible energy sources according to $\delta^{13}\text{C}$ values and trophic positions with $\delta^{15}\text{N}$ values. The fractionation of ^{15}N , usually assumed to be 3.2–3.4‰, in an animal in relation to its diet (Peterson, Fry, 1987; Post, 2002; Baeta, 2018) depends on environmental and individual conditions (Minagawa, Wada, 1984; Peterson, Fry, 1987; Wiederhold, 2015). Some factors affecting the fractionation of nitrogen are tissue type (Pinnegar *et al.*, 2000;

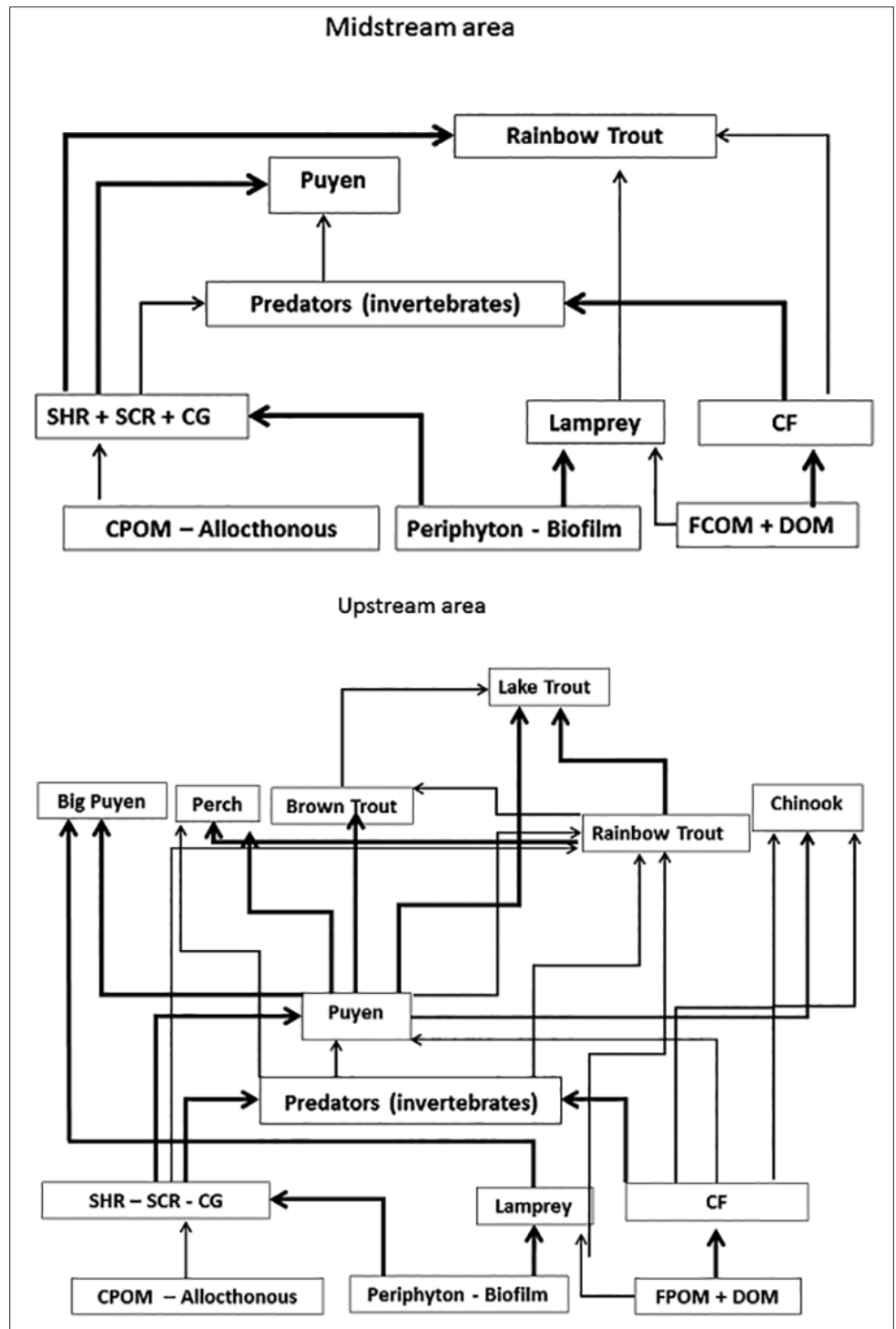


FIGURE 5 | Midstream and Upstream areas food webs scheme done considering stable isotopes and stomach content analysis. Dark arrows indicate a higher contribution to diet. Rainbow Trout is placed above Perch, Chinook, and Brown Trout since when analyzing stomach content, its trophic role depends on the ontogenetic stage. CF= collector-filterer, CG= collector-gatherer, SCR=scrapers, SHR= shredders, PRED= predator.

Vanderklift, Ponsard, 2003), quality of the diet (McCutchan Jr *et al.*, 2003; Cashman *et al.*, 2016), species being studied (DeNiro, Epstein, 1980; Arcagni *et al.*, 2015; Sánchez-Carrillo, Álvarez-Cobelas, 2018), and transgenerational effects (Liberoff *et al.*, 2013). Moreover, methods for understanding the results of SIA are still developing (Phillips, Gregg, 2003; Moore, Semmens, 2008; Parnell *et al.*, 2010) and therefore the sources of variability that contribute to these methods have not yet been fully explored (Bond, Diamond, 2011). In the case of mixing models, the fractionation factor (or isotopic enrichment) is cited as one of the weakest points for the reconstruction of diets (Wolf *et al.*, 2009). Statistical programs developed for analyzing food webs and diets such as SIAR (Parnell *et al.*, 2010) have the possibility of incorporating fractionation values for each species, the concentration of ^{13}C and ^{15}N and values of standard deviation; however, the absence of some of these estimates may give erroneous results. In their study, Bond, Diamond (2011) showed that in most studies diet reconstruction with no information on species-specific fractionation values, generates studies where these values are considered fixed following the widely cited work of Post (2002), or were selected from taxonomically similar groups. The results of these investigations had a bias in the estimation of the diet, which should be checked and corroborated by other methods.

Stable isotope analyses also showed that Rainbow Trout involved in the present study corresponded to the resident type. Even though we used non-selective fishing techniques, the isotopic ranges for the most abundant invasive species were concordant with previously published values: $\delta^{15}\text{N} = 8.8 \pm 1.1\text{‰}$ and $\delta^{13}\text{C} = 23.2 \pm 2.5\text{‰}$ (Ciancio *et al.*, 2008). Even though it has been reported that the probability of capturing the offspring of anadromous mothers might increase towards Upstream sections due to the suitability of the environment (Liberoff *et al.*, 2015), the isotopic signature of Rainbow Trout in Mid and Upstream sections were concordant with resident types. In the present research, all relevant prey present in fish diets were sampled (except for the rare contribution of terrestrial prey), though an inconsistency in the isotopic enrichment between the value of $\delta^{15}\text{N}$ of prey and Puyen and yearling Rainbow Trout was found. The absence of the isotopic value of terrestrial prey, mainly arthropods, could be generating a deficiency in the necessary sources for the use of mixing models in Puyen and yearling Rainbow Trout; however, the enrichment of the latter species varied up to $\sim 6\text{‰}$ units in $\delta^{15}\text{N}$ and we expect another source to be contributing to this variation. In the absence of experimental studies on the fractionation of Puyen, or other Galaxiids, many questions arise: is it possible that the terrestrial contribution accounts for this difference between diet and isotopic values in Puyen? Secondly, might Puyen have a higher isotopic fractionation to improve the utilization of their prey in the Santa Cruz River? Regarding yearling Rainbow Trout, might the fractionation change between different life stages?

In conclusion, the information presented in this study shows the importance of the spatial pattern in aquatic food webs and species distribution in the Santa Cruz River. This data will be relevant when considering possible dam management in each section of the river where recreational and economical activities related to salmonids will be affected.

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Marina Tagliaferro: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing-original draft, Writing-review & editing.

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COMPETING INTERESTS

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