

Research Article

## The cohabitation of Chinook salmon (*Oncorhynchus tshawytscha*) with trout populations in two important recreational fishing rivers in southern Chile

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**ABSTRACT.** A study was carried out on two important recreational fishing rivers in southern Chile, the Petrohué and the Puelo, to assess the population structure of the salmonid species inhabiting both rivers. Five river sectors were surveyed on four dates between April 2016 and February 2017. In the Petrohué River, 2400 fish were sampled (42.9% rainbow trout *Oncorhynchus mykiss*; 23.8% brown trout *Salmo trutta*, and 33.3% Chinook salmon *Oncorhynchus tshawytscha*), while in the Puelo River, 1972 fish were examined (51.6% rainbow trout; 30.4% brown trout and 18% Chinook salmon). Fry and fingerling stages accounted for the highest proportion of fish collected by electrofishing. In the Petrohué and Puelo rivers, rainbow trout fry and fingerlings represented 96.3 and 99.2% of the fish sampled, respectively; 96.8 and 97.1% in the case of brown trout, while for Chinook salmon, parr and pre-smolts represented 97% of the fish examined in the Petrohué River and 98.3% in the Puelo River. Rainbow and brown trout shared the same habitats and food items in both rivers, and the two oldest trout recorded 5<sup>+</sup> and 6<sup>+</sup> years. Parr and pre-smolt Chinook salmon were present mainly in the winter and spring seasons and were aged 1<sup>+</sup> and 2<sup>+</sup>, respectively. There were no differences in the food items recorded from the juvenile fish sampled in both rivers. The mature, returning adult Chinook salmon aged between 1.5<sup>+</sup> and 1.6<sup>+</sup> years were devoid of food and were recorded over the autumn season with the highest abundance in the Petrohué River. The study did not identify any adverse competition or negative interactions among the three main salmonid species sharing the same habitat.

**Keywords:** *Oncorhynchus tshawytscha*; Chinook salmon; trout species; salmonids population structure; recreational fishing; southern Chile

### INTRODUCTION

Recreational fishing is an important economic activity in southern Chile, based on various salmon and trout species introduced to the southern hemisphere around the 1890s (Basulto 2003). In 1904, a large number of Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), and brown trout (*Salmo trutta*) eggs were imported from Europe (Basulto 2003, Dazarola 2019). This importation formed the basis for introducing salmon species into Chilean rivers and lakes. In 1924, Chinook salmon (*Oncorhynchus tshawytscha*)

eggs were imported from the USA and planted in southern Chile's Maullín, Cochamó, and Puelo rivers. Subsequently, in 1930, sockeye salmon (*O. nerka*) and coho salmon (*O. kisutch*) were also introduced in the USA. However, most attempts to introduce anadromous salmon to waters in Chile failed (Basulto 2003, Dazarola 2019).

Chinook and coho salmon were again introduced between 1982 and 1989 for ranching (Méndez & Munita 1989). In a relatively short time, they have become one of the high-value salmonid species for recreational fishing in both countries. However, only

Chinook adapted successfully to the environmental conditions of the southern hemisphere, with significant annual returns to the main watersheds of southern Chile since 1985 (Bravo et al. 2019). To date, Chinook salmon have a distribution between 39 to 53°S in Chile (Correa & Gross 2008) and between 43 and 54°S in Argentina (Di Prinzio & Pascual 2008).

Despite the initial efforts to establish naturalized populations of coho and Atlantic salmon in Chile, these have, to date, failed to establish self-sustaining populations. (Soto et al. 2001, 2006, Arismendi et al. 2014). Although, over recent years, there is documented evidence that wild coho salmon populations have been recorded at 51°S in southern Patagonian rivers (Górski et al. 2017) and 55°S in the Cape Horn Biosphere Reserve (Maldonado-Márquez et al. 2020). It may indicate that coho salmon have established self-sustaining populations and originated from farmed salmon, at least in these areas, where the environmental conditions appear suitable for establishing this species. By opposite, to date, there is no scientific evidence to show that Atlantic salmon has been naturalized in the southern Chile bodies of water. Even though in 2010-2020, 74.3% of fish escapees corresponded to this salmon species, according to the National Fisheries Service (SERNAPESCA by its Spanish acronym), likely due to the high domestication suffered in their life in captivity (Thorstad et al. 2008).

Rainbow and brown trout were introduced in the early years for recreational fishing purposes. They are now widely spread across lakes and rivers in southern Chile and have shown that they are well adapted to the environmental conditions of the southern hemisphere. This adaptability may well have been supported by the phenotypic plasticity of both trout species, low ecosystem resistance, and propagule pressure (Arismendi et al. 2014). Wild stocks of rainbow trout have also been partially supplemented over recent decades through the escape from aquaculture facilities (Soto et al. 2006, Arismendi et al. 2008, 2014, Sepúlveda et al. 2013).

The occurrence of the brook salmon (*Salvelinus fontinalis*) is very limited in the southern Chile rivers, and its distribution ranges from the Region of Valparaiso (33°S) to the Region of Magallanes (47°S). It was introduced from the USA to Argentina in 1904 and later from Argentina to southern Chile in 1907. Because recreational angling is becoming increasingly important in southern Chile, this study aimed to assess the population structure of the salmonid species inhabiting the Petrohué and Puelo rivers and the interactions between the trout and Chinook salmon populations. Surveys in the Palena River (Chilean

Patagonia) have suggested that only small, very limited brook populations are present in this watershed's upper tributaries. The populations are probably in a very vulnerable state, as during these surveys, only a low abundance of brook trout was recorded, and the mature adults were, on average, very small (16.2 to 17.5 cm) (Bravo et al. 2021).

## MATERIALS AND METHODS

### Study area

Between April 2016 and February 2017 four surveys were carried out in five sections of the Petrohué River (41°23'12" S) and in five sections of the Puelo River (41°39'51" S). Surveys were carried out over the four seasons of the year: autumn, winter, spring, and summer. The local anglers, who collaborated with the study, identified the areas sampled. The sampling sites included section E of the Petrohué River (Fig. 1), which was severely affected by the eruption of the Calbuco Volcano in April 2015.

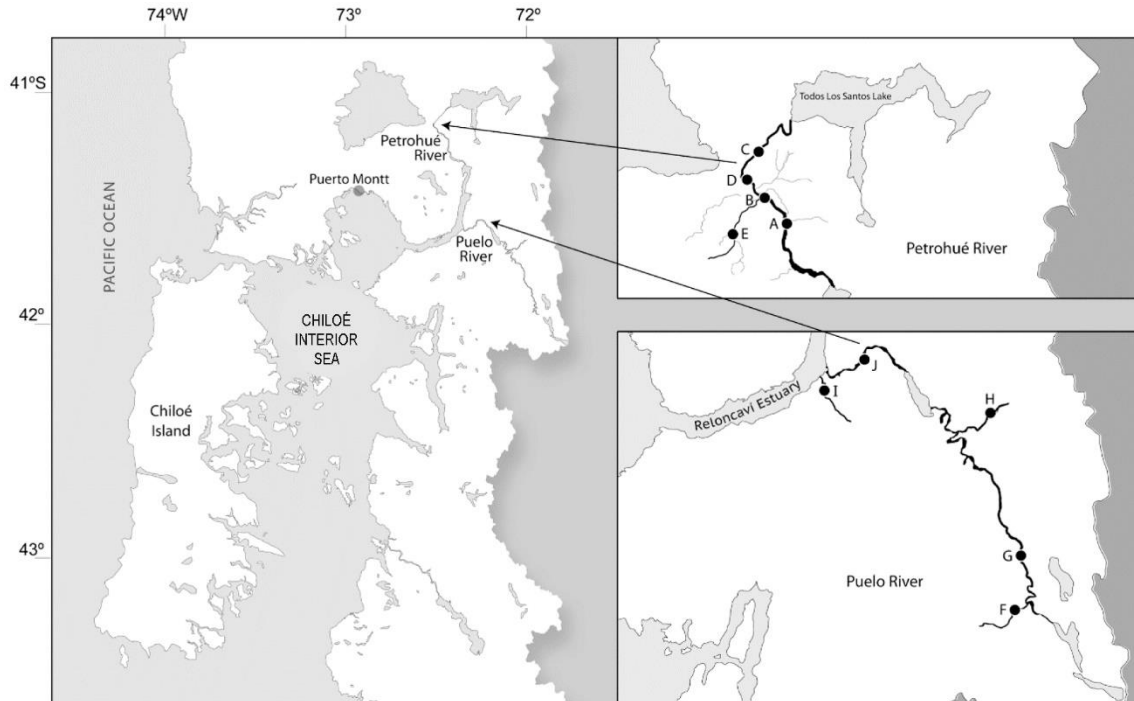
The Petrohué River rises in Todos Los Santos Lake and runs 42 km downstream before discharging into the Reloncaví Estuary. The Puelo River is a watershed shared between Chile and Argentina. It rises in Puelo Lake, located on the border with Argentina, and runs for 100 km downstream until it discharges into the Reloncaví Estuary. Just 14 km separates the discharge points of these two major systems into the Reloncaví Estuary (Fig. 1).

### Sampling methods

Fish were sampled using electrofishing for the smaller fish and angling for the larger fish. The largest fish was caught by local anglers who collaborated in the study. All fish collected by electrofishing were kept alive in large fresh, cool water containers. Fish were anesthetized with benzocaine (10% in ethanol, 1 mL L<sup>-1</sup>) prior to measuring (mm) and weighing (g), and a proportion was retained for subsequent laboratory analysis. The remaining fish were safely returned to the water (Table 1). Trout species were classified as: fry (<10 cm), juveniles (10-24 cm), and adults (>24 cm). Salmon were classified into parr (<5 cm); pre-smolt (5-10 cm); smolt (11-18 cm) and returning adults. A Fulton condition factor (K) was calculated as  $K = 100 \times W \times L^{-3}$ , where W: weight (g) and L: fork length (cm).

### Age analysis

Scales collected from fish were prepared following the methodology recommended by the manual of the Celtic Sea Trout Project Workshop (Poole 2010) to determine



**Figure 1.** Map showing the areas sampled in the Petrohué and Puelo rivers.

the age of each fish from each of the length classes. Six cleaned and suitable scales from each fish were mounted and read using a microfiche projector. Scales were removed from an area below the posterior margin of the dorsal fin and approximately five scale rows above the lateral line (Pascual et al. 2001, Riva-Rossi et al. 2007).

### Sexual maturation

Maturity stages for trout species were defined following the key recommended by Dahl (1917) for trout. Since salmonid is a total spawner, the Manual of Fisheries Science (FAO 1975) was also used to assess the adult fish's maturity.

### Feeding habits

Stomach contents were analyzed with a dissecting microscope, using taxonomic keys to the lowest possible taxa. The stomachs of the fish were removed, and the contents were preserved in 95% ethanol for later examination. In this study, items found in the sampled fish' stomachs were classified at the order level.

Relative abundance was calculated as the number of prey of each item concerning the total number of items recorded in the sample (Macdonald & Green 1983). The Shannon's diversity index ( $H$ ) was calculated as the

proportion of the prey items  $i$  relative to the total number of the prey items ( $p_i$ ) and then multiplied by the natural logarithm of this proportion ( $\ln p_i$ ). The resulting product was summed across the prey items and multiplied by  $-1$  (Zar 1996).

### Data analysis

IBM® SPSS® Statistic 20.0 SPSS and Microsoft Office Professional Plus 2013 software were used to analyze the data. Descriptive statistics were used in tables and graphs. The Mann-Whitney test was used to assess differences in size between males and females of the same age, and the Kruskal Wallis test to assess the condition factor. Results were considered statistically significant at the level of  $P \leq 0.05$ .

## RESULTS

### Abundance and seasonal distribution

#### Petrohué River

Throughout the study, 1030 rainbow trout *Oncorhynchus mykiss* (42.9%); 800 Chinook salmon *O. tshawytscha* (33.3%); and 570 brown trout *Salmo trutta* (23.8%), and were collected, and sampled. The most abundant stage was fry, in the case of rainbow trout (75%) and brown trout (75.4%). Fry was abundant in both trout species' samples in the summer and autumn.

**Table 1.** Seasonal abundance of rainbow trout (*Oncorhynchus mykiss*), Chinook salmon (*O. tshawytscha*) and brown trout (*Salmo trutta*), according to their developmental stage in Petrohué and Puelo rivers. n: number of fishes.

| Species        | Life Stage | Petrohué River |      |        |      |        |      |        |      | Total | Puelo River |      |        |      |        |      |        |      | Total |
|----------------|------------|----------------|------|--------|------|--------|------|--------|------|-------|-------------|------|--------|------|--------|------|--------|------|-------|
|                |            | Autumn         |      | Winter |      | Spring |      | Summer |      |       | Autumn      |      | Winter |      | Spring |      | Summer |      |       |
|                |            | n              | %    | n      | %    | n      | %    | n      | %    |       | n           | %    | n      | %    | n      | %    | n      | %    |       |
| Rainbow trout  | Fry        | 318            | 70.2 | 19     | 36.5 | 7      | 9.6  | 428    | 94.7 | 772   | 45          | 57.7 | 174    | 77.0 | 340    | 85.9 | 217    | 72.3 | 776   |
|                | Fingerling | 121            | 26.7 | 23     | 44.2 | 52     | 71.2 | 24     | 5.3  | 220   | 30          | 38.5 | 51     | 22.6 | 52     | 13.1 | 83     | 27.7 | 216   |
|                | Adult      | 14             | 3.1  | 10     | 19.2 | 14     | 19.2 | 0      | 0.0  | 38    | 3           | 3.8  | 1      | 0.4  | 4      | 1.0  | 0      | 0.0  | 8     |
| Chinook salmon | Parr       | 0              | 0    | 307    | 83.9 | 181    | 48.4 | 2      | 5.4  | 490   | 0           | 0.0  | 10     | 14.9 | 124    | 62.9 | 49     | 60.5 | 183   |
|                | Pre-smolt  | 4              | 17.4 | 59     | 16.1 | 193    | 51.6 | 30     | 81.1 | 286   | 2           | 66.7 | 56     | 83.6 | 70     | 35.5 | 31     | 38.3 | 159   |
|                | Smolt      | 1              | 4.3  | 0      | 0.0  | 0      | 0.0  | 2      | 5.4  | 3     | 0           | 0.0  | 1      | 1.5  | 0      | 0.0  | 1      | 1.2  | 2     |
|                | Adult      | 18             | 78.3 | 0      | 0.0  | 0      | 0.0  | 3      | 8.1  | 21    | 1           | 33.3 | 0      | 0.0  | 3      | 1.5  | 0      | 0.0  | 4     |
| Brown trout    | Fry        | 195            | 77.1 | 13     | 50.0 | 6      | 13.0 | 216    | 88.2 | 430   | 55          | 87.3 | 86     | 56.2 | 52     | 50.5 | 197    | 73.2 | 390   |
|                | Fingerling | 49             | 19.4 | 10     | 38.5 | 35     | 76.1 | 28     | 11.4 | 122   | 6           | 9.5  | 57     | 37.3 | 49     | 47.6 | 69     | 25.7 | 181   |
|                | Adult      | 9              | 3.6  | 3      | 11.5 | 5      | 10.9 | 1      | 0.4  | 18    | 2           | 3.2  | 10     | 6.5  | 2      | 1.9  | 3      | 1.1  | 17    |

The next most abundant stage was fingerlings, collected in autumn and spring for rainbow trout and spring for brown trout. For juvenile Chinook salmon, the highest abundance was recorded for the parr stage (61.2%) in winter and spring, followed by the pre-smolt stage (35.8%), mainly during spring. Local anglers caught only three post-smolts, with lengths varying between 32 and 40.5 cm in the estuarine area of the river. These fish were migrating to the sea during the summer and autumn seasons. Eighteen mature Chinook salmon were collected, returning to sector A in autumn, an important spawning area for Chinook salmon on the Petrohué River (Table 1).

### Puelo River

Throughout the study, 1000 rainbow trout (50.7%), 588 brown trout (29.8%), and 348 Chinook salmon (19.5%) were collected and sampled. In addition, two brook trout were recorded from the summer samples. These fish comprised a male of 15 cm and a female of 17 cm. They were not included in the data analysis.

For rainbow trout, the highest abundance was recorded at the fry stage (77.6%), mainly in samples taken during the spring and summer. The highest abundance recorded was for the fingerling stage in summer. Over the first sampling campaign, carried out in autumn, three adult fish were captured. One was a mature male who escaped from a neighboring commercial fish farm. In the case of brown trout, the highest abundance was recorded at the fry stage (66.3%) and for the fingerling stage in spring. For juvenile Chinook salmon, the highest abundance was recorded at the parr stage (52.6%), followed by the pre-smolt stage

(45.7%). These two life stages were mainly taken in the spring samples. Only two smolts were recorded, one in winter and one in summer. Four adult Chinook salmon were collected by local anglers, three in spring and one in autumn (Table 1).

### Size structure and condition factor

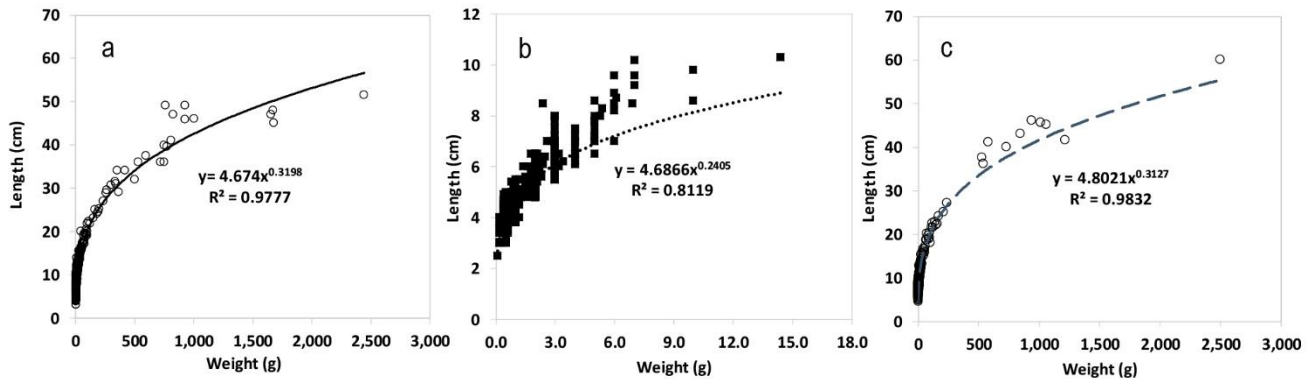
#### Petrohué River

The largest trout recorded were rainbow trout of 51.5 cm, weighing 2.44 kg; a brown trout of 60 cm, weighing 2.5 kg and a Chinook salmon of 120 cm, weighing 14 kg (Table 2). The growth relationship between length and weight for each species is shown (Figs. 2a,c). The rainbow and brown trout showed a similar determination coefficient, with  $R^2 = 0.9777$  and  $R^2 = 0.9832$ , respectively.

The highest average condition factor recorded for rainbow trout was for the fingerling stage (1.12). The fingerlings displayed a significant difference ( $P < 0.05$ ) with the fry (1.03) and adult stages (1.09) when a Kruskal-Wallis test was applied. The Mann-Whitney test ( $P < 0.476$ ) found no significant difference between the fingerling and adult stages. For brown trout, the highest condition factor was recorded for the adult stage (1.14), with significant differences ( $P = 0.002$ ) recorded between the fry (1.01) and fingerling stages (1.10), using the Kruskal-Wallis test. No significant difference was found between fingerlings and adults using the Mann-Whitney test ( $P < 0.883$ ). For Chinook salmon, the highest average condition factor was recorded for the pre-smolt stage (1.07), and there were no significant differences ( $P < 0.001$ ) shown between the fry (0.88) and adult stages (1.00) (Fig. 3).

**Table 2.** Biometric characteristics exhibited by rainbow trout *Oncorhynchus mykiss*, Chinook salmon *O. tshawytscha* and brown trout *Salmo trutta* in the Petrohué River, according to their stage of development (mean  $\pm$  range; SD: standard deviation).

| Species        | Status     | n   | Weight (g)                 |       | Length (cm)         |      |
|----------------|------------|-----|----------------------------|-------|---------------------|------|
|                |            |     | Mean (range)               | SD    | Mean (range)        | SD   |
| Rainbow trout  | Fry        | 349 | 5.2 (0.5 - 12.0)           | 2.7   | 7.7 (3.8 - 9.9)     | 1.4  |
|                | Fingerling | 197 | 25.2 (6.0 - 150.0)         | 22.9  | 12.5 (10.0 - 23.1)  | 2.8  |
|                | Adult      | 22  | 506.0 (162.0 - 1,005.0)    | 288   | 35.3 (24.1 - 49)    | 8.3  |
| Chinook salmon | Parr       | 374 | 0.7 (0.1 - 1.3)            | 0.3   | 4.2 (2.5 - 4.9)     | 0.4  |
|                | Pre-smolt  | 185 | 2.6 (0.8 - 10.0)           | 1.4   | 6.2 (5.0 - 9.8)     | 1    |
|                | Post-smolt | 3   | 607.0 (471.0 - 749.0)      | 139.1 | 36.2 (32.9 - 40.5)  | 4.3  |
|                | Adult      | 20  | 8,921.5 (601.0 - 14,000.0) | 3552  | 93.8 (36.0 - 120.0) | 21.4 |
| Brown trout    | Fry        | 222 | 5.2 (1.0 - 13.2)           | 2.4   | 7.9 (4.5 - 9.9)     | 1.2  |
|                | Fingerling | 101 | 29.4 (8.0 - 167.8)         | 32.3  | 12.9 (10.0 - 24.0)  | 3.5  |
|                | adult      | 11  | 835.2 (212.0 - 2,500.0)    | 622.1 | 40.6 (25.0 - 60.0)  | 9.5  |



**Figure 2.** Growth relationship between length and weight of a) rainbow trout (*Oncorhynchus mykiss*), b) Chinook salmon (*O. tshawytscha*) and c) brown trout (*Salmo trutta*), in the Petrohué River.

### Puelo River

The largest rainbow trout recorded from the Puelo River was 51 cm and weighed 1.5 kg. The largest brown trout was 62.0 cm in length and weighed 2.6 kg, while the largest Chinook salmon recorded was 110 cm in length and weighed 12 kg (Table 3). The growth relationship between length and weight for each of the three species is shown (Figs. 4a,c), where both rainbow and brown trout showed a similar determination coefficient with an  $R^2 = 0.9875$  and  $R^2 = 0.9889$ , respectively.

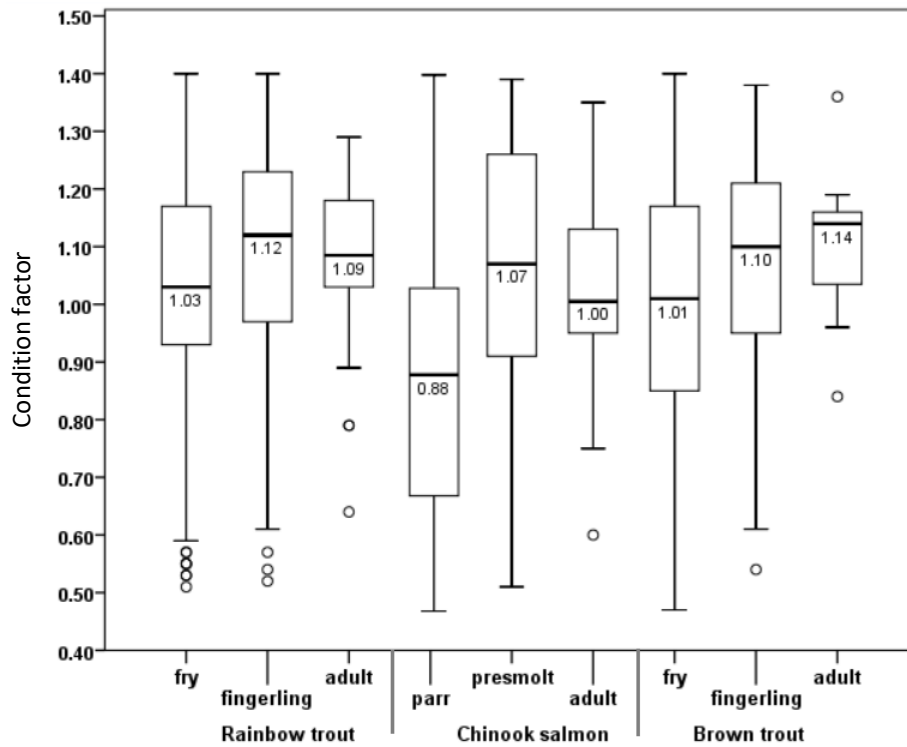
The highest condition factor recorded for rainbow trout was for the fry stage (1.11). The fry stage showed a significant difference ( $P < 0.005$ ) with the fingerling (1.04) and adult stages (0.97) using the Kruskal-Wallis test. For brown trout, the highest condition factor was also recorded for the fry stage (1.07), and significant differences were present ( $P = 0.009$ ) between the fingerling (1.02) and adult stages (1.04) using the

Kruskal-Wallis test. There was no significant difference between the fingerling and adult stages using the Mann-Whitney test ( $P < 0.505$ ). For Chinook salmon, the average condition factor recorded for the pre-smolt stage was 0.94 and 0.95 for the parr stage, respectively (Fig. 5).

### Age structure

#### Petrohué River

Throughout the study, scales of 267 fish were aged, representing 11.1% of the total number of fish sampled from the river. In the rainbow trout, scales of 130 fish (12.6% of the total caught) were used for aging. Female rainbow trout ranged in age between  $0^+$  to  $5^+$ , with a maximum length of 51.5 cm. Males ranged between  $0^+$  and  $3^+$ , with a maximum length of 34.0 cm. No significant differences in length relative to age were recorded between males and females using the Mann-Whitney test for rainbow trout at ages  $0^+$  ( $P = 0.246$ ),



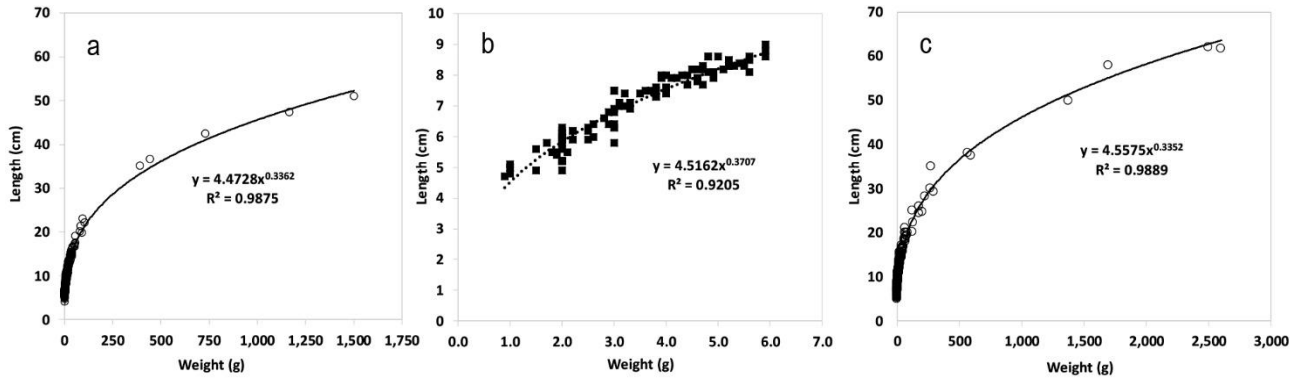
**Figure 3.** Box plot for condition factor, by developmental stage, for rainbow trout *Oncorhynchus mykiss*, Chinook salmon *O. tshawytscha* and brown trout *Salmo trutta*, sampled in the Petrohué River.

**Table 3.** Biometric characteristics exhibited by rainbow trout *Oncorhynchus mykiss*, Chinook salmon *O. tshawytscha* and brown trout *Salmo trutta*, in the Puelo River, according to their stage of development (mean  $\pm$  range, SD: standard deviation).

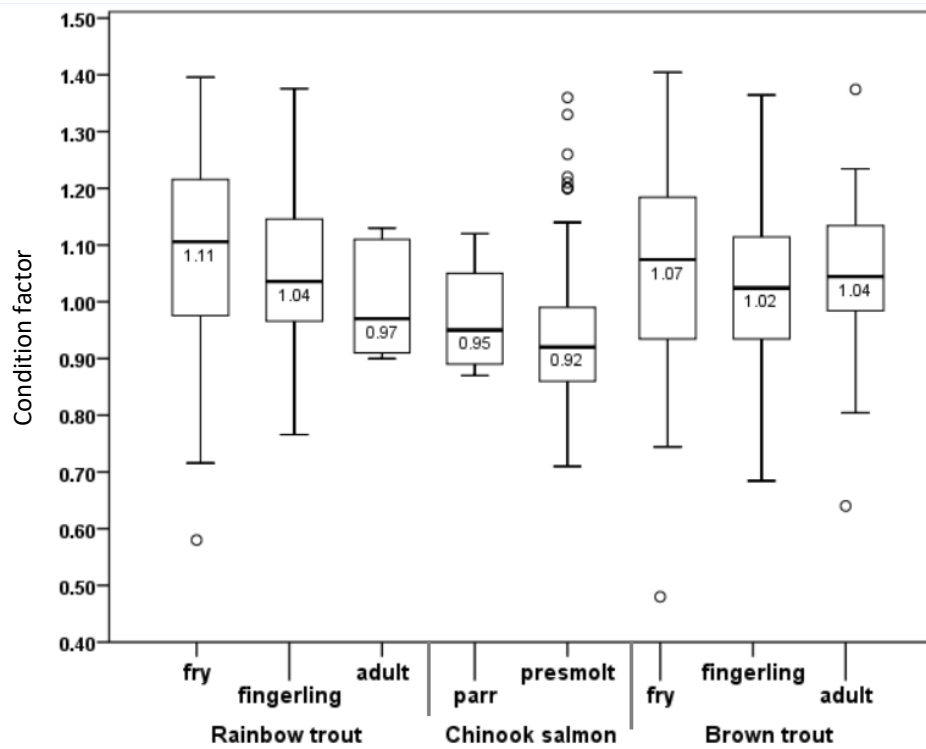
| Species        | Status     | n   | Weight (g)                     |       | Length (cm)           |      |
|----------------|------------|-----|--------------------------------|-------|-----------------------|------|
|                |            |     | Mean (range)                   | SD    | Mean (range)          | SD   |
| Rainbow trout  | Fry        | 303 | 5.0 (0.6 - 11.6)               | 2.4   | 7.5 (4.0 - 9.9)       | 1.2  |
|                | Fingerling | 149 | 22.5 (9.0 - 105.3)             | 16.6  | 12.4 (10.0 - 23.0)    | 2.5  |
|                | Adult      | 5   | 846.8 (393.4 - 1,500.0)        | 477.5 | 42.4 (35.1 - 51.0)    | 6.8  |
| Chinook salmon | Parr       | 129 | 1.1 (0.9 - 1.5)                | 0.3   | 4.8 (4.7 - 4.9)       | 0.1  |
|                | Presmolt   | 105 | 4.0 (1.0 - 8.2)                | 1.7   | 7.4 (5.0 - 9.4)       | 1.2  |
|                | Smolt      | 2   | 10.8 (10.2 - 11.4)             | 0.8   | 10.8 (10.8 - 10.8)    | 0    |
|                | Adult      | 3   | 11,000.0 (10,000.0 - 12,000.0) | 1,000 | 106.7 (100.0 - 110.0) | 5.8  |
| Brown trout    | Fry        | 202 | 5.8 (1.0 - 11.3)               | 2.4   | 8.1 (5.0 - 9.9)       | 1.2  |
|                | Fingerling | 158 | 26.1 (8.6 - 132.0)             | 18.8  | 13.2 (10.0 - 22.3)    | 2.6  |
|                | Adult      | 14  | 792.4 (126.9 - 2,600.0)        | 881   | 37.9 (24.4 - 62.0)    | 14.1 |

1<sup>+</sup> ( $P = 0.637$ ), and 2<sup>+</sup> ( $P = 0.860$ ) (Fig. 6). A total of 97 brown trout scales (17.0% of the total caught) were analyzed. These fish ranged in age between 0<sup>+</sup> to 4<sup>+</sup> for females (max. length 46.0 cm) and between 0<sup>+</sup> to 6<sup>+</sup> for males (max. length 62 cm). Using the Mann Whitney test no significant difference was recorded in terms of length between male and females for brown trout at 0<sup>+</sup>

( $P = 0.447$ ), 1<sup>+</sup> ( $P = 0.632$ ), and 2<sup>+</sup> ( $P = 0.111$ ) stage of development (Fig. 6). No significant differences in length were recorded between rainbow trout and brown trout, sharing the same sampling areas in the Petrohué River, at age 0<sup>+</sup> ( $P = 0.441$ ), 1<sup>+</sup> ( $P = 0.306$ ), and 2<sup>+</sup> ( $P = 0.099$ ).



**Figure 4.** Growth relationship between length and weight of a) rainbow trout (*Oncorhynchus mykiss*), b) Chinook salmon (*O. tshawytscha*) and c) brown trout (*Salmo trutta*), in Puelo River.



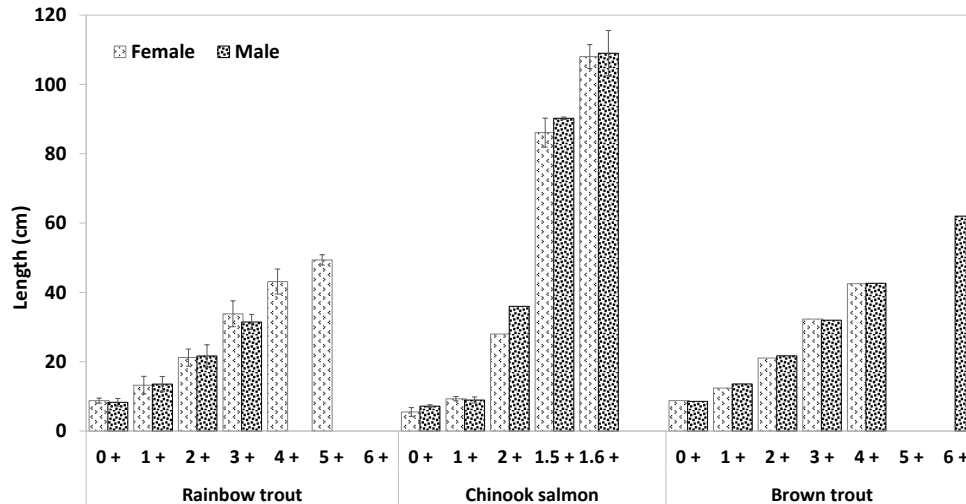
**Figure 5.** Box plot for condition factor, by development stage, of rainbow trout *Oncorhynchus mykiss*, Chinook salmon *O. tshawytscha* and brown trout *Salmo trutta*, sampled in the Puelo River.

In the case of Chinook salmon, scales of 40 fish (5% of the total caught) were analyzed. The parr and pre-smolts ranged in age between 0<sup>+</sup> and 1<sup>+</sup> respectively, while two fish caught in the estuarine area were aged 2<sup>+</sup>. Among the 14 mature Chinook salmon analyzed, three females and two males aged 1.5<sup>+</sup>. Their lengths ranged between 81.3 and 89.0 cm for females and 90.0 to 90.5 cm for males. Three females and six males aged 1.6<sup>+</sup> were recorded. The lengths ranged between 104 and 110 cm for females and 100 to 120 cm for males.

A significant difference was recorded in length relative to age between males and females at age 0<sup>+</sup> ( $P = 0.002$ ), using the Mann-Whitney test, with males larger than females. No significant difference was recorded at age 1<sup>+</sup> ( $P = 0.482$ ) (Fig. 6).

**Puelo River**

Throughout the study, scales from 635 fish were aged, representing 32.8% of the total fish sampled from the river. In the case of rainbow trout, scales from 246 indi-



**Figure 6.** Length (mean  $\pm$  standard deviation) and age of male and female of rainbow trout *Oncorhynchus mykiss*, Chinook salmon *O. tshawytscha* and brown trout *Salmo trutta*, sampled in the Petrohué River.

viduals (24.6% of the total caught) were used for aging. Female rainbow trout ranged in age between 0<sup>+</sup> to 6<sup>+</sup>, with a maximum length of 51.0 cm. Males ranged in age between 0<sup>+</sup> and 3<sup>+</sup>, with a maximum length of 35.1 cm. No significant difference in length relative to age was recorded between males and females, using the Mann-Whitney test for rainbow trout at ages 0<sup>+</sup> ( $P = 0.518$ ), 1<sup>+</sup> ( $P = 0.149$ ), and 2<sup>+</sup> ( $P = 0.808$ ) (Fig. 7). Scales from 298 brown trout were aged (50.7% of the total caught). These fish ranged in age between 0<sup>+</sup> to 5<sup>+</sup> for females (max. length 50 cm) and between 0<sup>+</sup> to 6<sup>+</sup> for males (max. length 62 cm). Using the Mann-Whitney test, no significant difference in length relative to age was recorded between males and females for brown trout at ages 0<sup>+</sup> ( $P = 0.118$ ), 1<sup>+</sup> ( $P = 0.598$ ), and 2<sup>+</sup> ( $P = 0.731$ ) (Fig. 7). Significant differences in length were recorded between rainbow and brown trout, sharing the same sampling areas in the Puelo River at age 0<sup>+</sup> ( $P = 0.0001$ ) and 1<sup>+</sup> ( $P = 0.001$ ), with brown trout larger than rainbow trout. Both wild trout species recorded no significant differences at age 2<sup>+</sup> ( $P = 0.664$ ). The capture of a mature male rainbow trout, which escaped from a commercial farm aged 2<sup>+</sup>, was in line with the production cycle of rainbow trout in captivity.

In the case of Chinook salmon, scales from 91 fish (26.1% of the total caught) were analyzed. The juveniles ranged in age between 0<sup>+</sup> to 1<sup>+</sup>, while the four mature adults were females, aged 1.6<sup>+</sup> with lengths ranging between 100 and 110 cm. Significant differences in size were recorded relative to age between males and females, using the Mann-Whitney

test at age 0<sup>+</sup> ( $P = 0.011$ ), with males larger than females. No differences were found between males and females aged 1<sup>+</sup> ( $P = 0.168$ ) (Fig. 7).

## Maturity stages

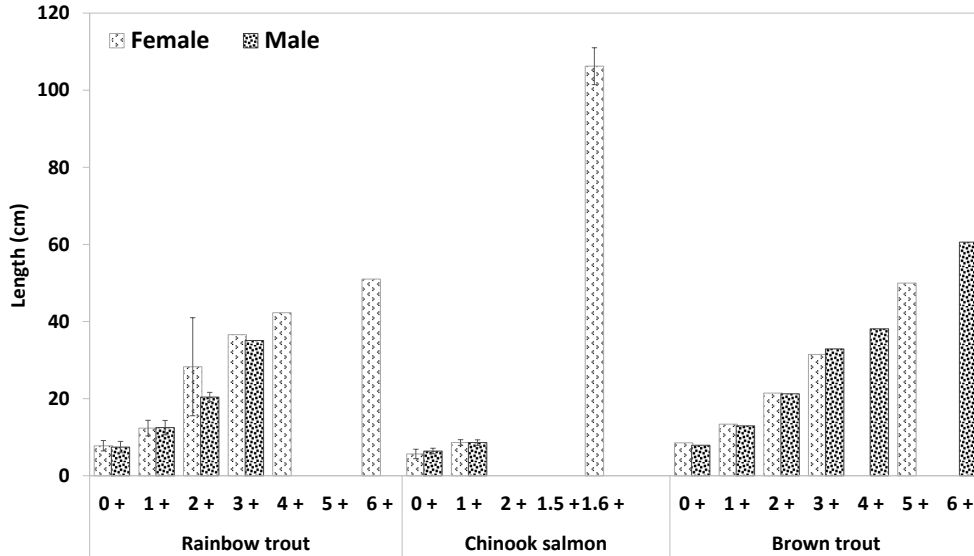
### Petrohué River

Maturity stages in rainbow trout were analyzed for 62 females and 63 males (12.1% of the total caught). For brown trout, 54 females and 37 males were examined (16.0% of the total caught), and in the case of Chinook salmon, 17 females and 14 males were examined (3.9% of the total caught). Maturity stages III, IV, VI, and VII were recorded for rainbow trout females over the period autumn-winter and autumn-winter-spring. For males, maturity stages III, IV, and V were recorded. For brown trout, over the period autumn-winter, females were recorded at maturity stages VI and VII and males at maturity stages III, IV, and V in autumn. Stage VII in spawned females was recorded for rainbow and brown trout, with diminutive eggs and a small number of large eggs free in the body cavity. Chinook salmon at maturity stages V and VI were recorded in autumn, while the three post-smolts caught in the estuary area reached maturity stage II, both for females and males (Table 4).

### Puelo River

Maturity stages in rainbow trout were analyzed for 101 females and 142 males (24.3% of the total caught). In the case of brown trout, 130 females and 169 males were analyzed (50.9% of the total caught), and in the case of Chinook salmon, 43 females and 48 males were





**Figure 7.** Length (mean ± standard deviation) and age of male and female of rainbow trout *Oncorhynchus mykiss*, Chinook salmon *O. tshawytscha* and brown trout *Salmo trutta*, sampled in the Puelo River.

**Table 4.** Maturity stages of trout collected in the Petrohué River, by species (rainbow trout *Oncorhynchus mykiss*, Chinook salmon *O. tshawytscha* and brown trout *Salmo trutta*), according to their gender. n: number of fish, SD: standard deviation.

| Gender | Maturity | Rainbow trout |                    |      | Chinook salmon |                     |      | Brown trout |                    |     |
|--------|----------|---------------|--------------------|------|----------------|---------------------|------|-------------|--------------------|-----|
|        |          | n             | Mean (range)       | SD   | n              | Mean (range)        | SD   | n           | Mean (range)       | SD  |
| Female | I        | 43            | 13.4 (7.5 - 37.5)  | 5.7  | 12             | 6.3 (4.5 - 9.6)     | 1.6  | 46          | 12.0 (6.6 - 27.1)  | 4.4 |
|        | II       | 7             | 19.5 (12.0 - 29.0) | 6.2  | 2              | 38.3 (36 - 40.5)    | 3.2  | 4           | 20.7 (18.5 - 22.5) | 1.7 |
|        | III      | 3             | 38.4 (15.7 - 51.5) | 19.7 | -              | -                   | -    | 1           | 37.5               | -   |
|        | IV       | 2             | 46.5 (46.0 - 47.0) | 0.7  | -              | -                   | -    | -           | -                  | -   |
|        | V        | -             | -                  | -    | 1              | 89                  | -    | -           | -                  | -   |
|        | VI       | 1             | 36                 | -    | 2              | 96.0 (88.0 - 104.0) | 11.3 | 2           | 43.8 (41.5 - 46)   | 3.2 |
|        | VII      | 6             | 39.8 (30.7 - 47.0) | 6.1  | -              | -                   | -    | 1           | 40                 | -   |
| Male   | I        | 47            | 13.5 (6.1 - 34.0)  | 6.6  | 12             | 9.3 (6.9 - 17.0)    | 2.6  | 32          | 10.5 (6 - 18.6)    | 3.2 |
|        | II       | 6             | 12.9 (7.5 - 17.0)  | 3.6  | 1              | 32                  | -    | -           | -                  | -   |
|        | III      | 1             | 31.5               | -    | -              | -                   | -    | 2           | 23.0 (22.0 - 24.0) | 1.4 |
|        | IV       | 8             | 18.2 (14.8 - 27)   | 4    | -              | -                   | -    | 2           | 29.2 (22.3 - 36.0) | 9.7 |
|        | V        | 1             | 34                 | -    | 1              | 90.5                | -    | 1           | 45                 | -   |

examined (23.7% of the total caught). For rainbow trout, maturity stage VII was recorded for females over the period autumn-winter-spring and stages III and IV for males. For brown trout, maturity stages III, IV, V, and VII were recorded for females in winter, for males' stages III and V in winter, and stage VI in autumn. Stage VII in spawned females was recorded for brown and rainbow trout, with diminutive eggs and a small number of large eggs loose in the cavity. For Chinook salmon females, stages V and VI were recorded in spring and autumn, respectively (Table 5).

**Feeding patterns**

**Petrohué River**

Stomach contents from 381 fish were analyzed, corresponding to 15.9% of the total fish sampled. These comprised 175 from rainbow trout (17.0% of the total caught), 132 from brown trout (23.2% of the total caught), and 74 from juvenile Chinook salmon (9.3% of the total caught).

A total of five food items were recorded from the rainbow trout fry. The highest abundance of items found was for Ephemeroptera and Plecoptera. For

**Table 5.** Maturity stages of trout collected in the Puelo River, by species (rainbow trout *Oncorhynchus mykiss*, Chinook salmon *O. tshawytscha* and brown trout *Salmo trutta*), according to their gender. n: number of fish, SD: standard deviation.

| Gender | Maturity | Rainbow trout |                    |     | Chinook salmon |                       |     | Brown trout |                    |      |
|--------|----------|---------------|--------------------|-----|----------------|-----------------------|-----|-------------|--------------------|------|
|        |          | n             | Mean (range)       | SD  | n              | Mean (range)          | SD  | n           | Mean (range)       | SD   |
| Female | I        | 84            | 9.5 (5.0 - 16.4)   | 2.6 | 39             | 6.9 (3.1 - 10.8)      | 1.8 | 103         | 10.6 (4.5 - 22.3)  | 3    |
|        | II       | 13            | 15.0 (6.0 - 23.1)  | 5   | -              | -                     | -   | 16          | 15.3 (9.1 - 30.1)  | 5.4  |
|        | III      | -             | -                  | -   | -              | -                     | -   | 5           | 18.6 (16.2 - 20.1) | 1.6  |
|        | IV       | -             | -                  | -   | -              | -                     | -   | 4           | 27.8 (16.2 - 50.0) | 15.2 |
|        | V        | -             | -                  | -   | 3              | 106.7 (100.0 - 110.0) | 5.8 | 1           | 24.4               | -    |
|        | VI       | -             | -                  | -   | 1              | 105                   | -   | -           | -                  | -    |
|        | VII      | 4             | 44.3 (36.6 - 51.0) | 6.2 | -              | -                     | -   | 1           | 35                 | -    |
| Male   | I        | 113           | 9.8 (4.2 - 22.0)   | 3.5 | 48             | 7.2 (5 - 10.8)        | 1.3 | 129         | 9.5 (5.0 - 29.3)   | 3.2  |
|        | II       | 24            | 13.0 (8.1 - 19.8)  | 2.4 | -              | -                     | -   | 30          | 16.5 (7.3 - 58.0)  | 9.6  |
|        | III      | 1             | 35.1               | -   | -              | -                     | -   | 4           | 20.0 (13.0 - 26.5) | 7.3  |
|        | IV       | 4             | 13.4 (11.6 - 14.6) | 1.4 | -              | -                     | -   | 1           | 17                 | -    |
|        | V        | -             | -                  | -   | -              | -                     | -   | 5           | 49.5 (28.3 - 62.0) | 15.6 |

**Table 6.** Relative abundance of prey items-orders found by salmonid species (rainbow trout *Oncorhynchus mykiss*, Chinook salmon *O. tshawytscha* and brown trout *Salmo trutta*) and by developmental stage in the Petrohué River.

| Order food item | Rainbow trout |            |       | Chinook salmon |           | Brown trout |            |       |
|-----------------|---------------|------------|-------|----------------|-----------|-------------|------------|-------|
|                 | Fry           | Fingerling | Adult | Parr           | Pre-smolt | Fry         | Fingerling | Adult |
| Araneae         | 4.5           | -          | 2.2   | -              | -         | -           | -          | -     |
| Coleoptera      | -             | 1.2        | -     | -              | 11.2      | 3.4         | 1.5        | 6.6   |
| Decapoda        | -             | 6          | 22.2  | -              | -         | -           | 3.1        | 33.3  |
| Diptera         | 9.1           | 4.8        | 2.2   | -              | -         | 13.8        | 4.6        | -     |
| Ephemeroptera   | 36.4          | 20.5       | -     | 19.2           | 33.3      | 27.6        | 38.5       | -     |
| Hemiptera       | -             | 3.6        | -     | -              | -         | 3.4         | 1.5        | -     |
| Hymenoptera     | -             | 1.2        | -     | -              | -         | -           | -          | -     |
| Lepidoptera     | -             | 3.6        | -     | 3.9            | -         | 3.4         | 1.5        | -     |
| Megaloptera     | -             | 1.2        | 2.2   | -              | -         | 3.4         | 1.5        | -     |
| Mesogastropoda  | -             | 9.6        | 35.6  | -              | -         | 6.9         | 12.4       | 33.3  |
| Plecoptera      | 36.4          | 25.3       | 6.7   | 15.4           | 22.2      | 27.6        | 23         | 13.4  |
| Trichoptera     | 13.6          | 22.9       | 2.2   | 61.5           | 33.3      | 10.3        | 12.4       | -     |
| Salmon eggs     | -             | -          | 26.7  | -              | -         | -           | -          | 13.4  |

brown trout fry, nine food items were recorded. Ephemeroptera and Plecoptera were again the most abundant organism recorded. At the fingerling stage, 11 food items were recorded in rainbow trout, and the highest abundance of organisms was Plecoptera, Trichoptera, and Ephemeroptera. Ten food items were present for brown trout, and the highest abundance of food comprised both Ephemeroptera and Plecoptera. Some eight food items were present in the adult rainbow trout sampled. Most frequently present in these fish was a mixture of Mesogastropoda, eggs of Chinook salmon, and Decapoda. In adult brown trout, five food items were identified, comprising Mesogastropoda, Decapoda, and eggs of Chinook salmon (Table 6).

Juvenile Chinook salmon contained four food items at the parr stage. The most abundant organisms were

Trichoptera and Ephemeroptera (Table 6). Trichoptera was the most abundant food item. At the pre-smolt stage, four food items were present. The stomachs of Chinook caught in the Reloncaví Estuary showed abundant seeds of mussels and fingerling trout. The fingerling trout were themselves feeding on a selection of Ephemeroptera. These food items are not included in Table 6.

### Puelo River

Stomach contents from 666 fish were analyzed from the Puelo River, corresponding to 33.8% of the total fish sampled and comprising 249 rainbow trout (24.9% of the total caught), 301 brown trout (51.2% of the total caught), and 114 juvenile Chinook salmon (32.8% of the total caught).

**Table 7.** Relative abundance of prey items-orders found by salmonid species (rainbow trout *Oncorhynchus mykiss*, Chinook salmon *O. tshawytscha* and brown trout *Salmo trutta*) and by developmental stage in the Puelo River.

| Order food item | Rainbow trout |            |       | Chinook salmon |           |       | Brown trout |            |       |
|-----------------|---------------|------------|-------|----------------|-----------|-------|-------------|------------|-------|
|                 | Fry           | Fingerling | Adult | Parr           | Pre-smolt | Smolt | Fry         | Fingerling | Adult |
| Araneae         | -             | 1          | -     | -              | -         | -     | -           | 1.7        | -     |
| Coleoptera      | 4.9           | 6.8        | -     | -              | -         | .     | 4           | 9.6        | -     |
| Decapoda        | -             | 2.9        | 16.7  | -              | -         | -     | 3           | 0.9        | 14.3  |
| Diptera         | 7.3           | 2.9        | -     | -              | 4         | -     | 8           | 5.2        | -     |
| Ephemeroptera   | 35.4          | 28.2       | -     | 25.0           | 20        | -     | 26          | 20.9       | 9.5   |
| Hemiptera       | -             | 1          | -     | -              | -         | -     | -           | 0.9        | -     |
| Hymenoptera     | -             | 1.9        | 16.7  | -              | -         | -     | -           | 3.5        | -     |
| Lepidoptera     | 2.4           | -          | -     | -              | -         | -     | 2           | 0.9        | -     |
| Megaloptera     | -             | 1          | -     | -              | -         | -     | 2           | 2.6        | -     |
| Mesagastropoda  | -             | 5.8        | 50    | -              | -         | -     | -           | 17.4       | 66.7  |
| Plecoptera      | 26.8          | 25.2       | -     | 25             | 72        | 100   | 24          | 17.4       | -     |
| Trichoptera     | 23.2          | 23.3       | -     | 50.0           | 4         | -     | 31          | 19.1       | 4.8   |
| Salmon eggs     | -             | -          | 16.7  | -              | -         | -     | -           | -          | 4.8   |

In the case of rainbow trout, six food items were recorded at the fry stage-the highest abundance of items comprised: Ephemeroptera, Plecoptera, and Trichoptera. Eight food items were recorded in the case of brown trout, and the highest abundance of organisms present was represented by the orders Trichoptera, Ephemeroptera, and Plecoptera. At the fingerling stage, 11 food items were recorded in rainbow trout, and the highest abundance of items comprised the Ephemeroptera, Plecoptera, and Trichoptera. In the case of brown trout, 12 food items were recorded in the fish. The highest abundance of food items presented comprised Ephemeroptera, Trichoptera, Plecoptera, and Mesagastropoda. Some four food items were present in adult rainbow trout, and five food items were identified from the brown trout examined. The most common prey was Mesagastropoda, for both trout species and some Chinook salmon eggs were also recorded (Table 7). The escaped adult rainbow trout showed much visceral fat. Its stomach was empty of food, containing only leaves and pieces of branches.

In the case of juvenile Chinook salmon, three food items were recorded from the parr examined. The highest abundance of food items was Trichoptera. A total of four food items were present in the pre-smolts, and the highest abundance of items was Plecoptera. The two smolts only contained Trichoptera (Table 7).

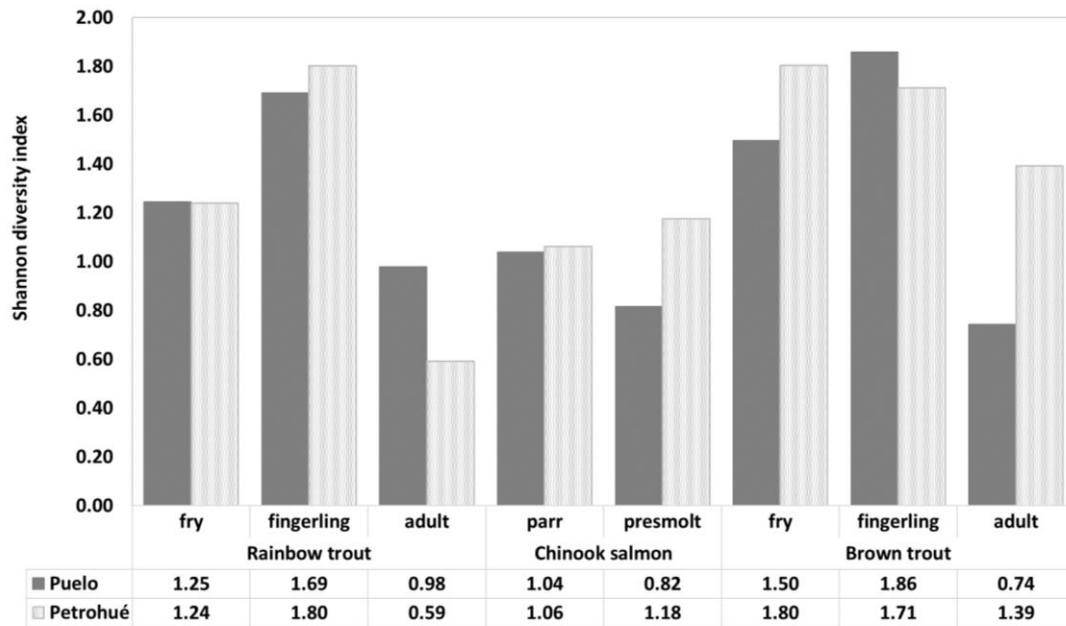
For rainbow trout, there were no significant differences in the overall diversity of food found in the stomachs of fish from the Petrohué and Puelo rivers at the fry ( $P = 0.454$ ) or the fingerling stage ( $P = 0.117$ ). Significant differences ( $P = 0.008$ ) were recorded between the food of adult rainbow and brown trout. In

the case of brown trout, significant differences were recorded between both rivers at the fry ( $P = 0.005$ ) and adult ( $P < 0.001$ ) stages, while in fingerlings, no significant differences ( $P = 0.065$ ) were observed. For juvenile Chinook salmon, no significant differences were recorded between the food of parr ( $P = 0.876$ ) and pre-smolts ( $P = 0.145$ ) occupying the two rivers (Fig. 8).

## DISCUSSION

This study focused on the salmonid species inhabiting the Petrohué and Puelo rivers. Rainbow *Oncorhynchus mykiss* and brown trout *Salmo trutta* were the most abundant species, sharing the same habitat as Chinook salmon *O. tshawytscha* juveniles. Two brook trout *Salvelinus fontinalis* were also recorded in the Puelo River, and no coho *O. kisutch* nor Atlantic salmon *Salmo salar* were recorded in any of the sampled sites during the study period. Although coho salmon apparently have adapted successfully to life in the wild, recently in southern Chile (Niklistcheck et al. 2013, Górski et al. 2017, Chalde et al. 2019, Maldonado-Marquez et al. 2020), and despite the reports of very large numbers of Atlantic salmon escaping into the wild in Chile, there is no evidence that self-sustaining populations have been established by these fish (Soto et al. 2001, 2006, Bravo et al. 2019).

The widespread presence of different life stages of Chinook salmon, including mature, pre-spawning adults, indicates that this non-native species of salmon has successfully colonized these two major river systems, which support important sports fisheries. The



**Figure 8.** Shannon diversity index of the prey items found by salmonid species (rainbow trout *Oncorhynchus mykiss*, Chinook salmon *O. tshawytscha* and brown trout *Salmo trutta*) and by developmental stage.

present study has further shown that there is no evidence to date that the colonization of these rivers by Chinook salmon has negatively impacted the resident populations of rainbow trout and brown trout. The presence of the salmon may enhance the productivity of nutrient-poor oligotrophic systems, such as the Puelo River, due to the release of additional marine nutrients, by decomposing the salmon carcasses following spawning. This enrichment may enhance the systems' overall productivity, improving food availability for the trout and other native fish populations and increasing the abundance and resilience of the fish stocks in these waters.

Different authors have attributed negative impacts on the waters that Chinook salmon have colonized. Montory et al. (2020) reported that persistent organic pollutants, bio-transported by the returning adult Chinook salmon, could harm Patagonian inland waters. The authors noted that the mechanisms through which such bio-transport works and how these phenomena affect Patagonian ecosystems are still unknown. However, previous research has shown that runs of anadromous fish species function as a conveyor belt, transporting marine-derived nutrients (MDN) to river basins from the ocean, which can significantly enrich freshwater ecosystems (Kline et al. 1993, Naiman et al. 2002). Arismendi & Soto (2012) noted that it is now well established that, following colonization by

invading Chinook salmon, MDN from salmon carcasses are directly assimilated into the food webs of streams and rivers, hosting runs of these exotic species of anadromous fish.

### Population structure

The Petrohué and Puelo rivers showed a similar salmonid population structure comprising rainbow trout, brown trout, and Chinook salmon. Fry and fingerling were the most abundant stages recorded during the study. This can be explained because sampling in these rivers was generally carried out using electrofishing in the shallower runs and pools, which accounts for the high proportion of juvenile stages in the fish examined. Anglers caught the larger trout from local angling associations. Overall, rainbow and brown trout shared the same habitat in the two rivers, and there was no evidence of habitat segregation between the two species. However, the proportion of rainbow trout was higher than brown trout in both rivers, and the same species composition was found in the Palena River over the period 2012-201, where 63% of the trout sampled were rainbow trout, and 37% were brown trout (Bravo et al. 2021). As expected, fry and fingerlings were recorded in the shallower waters of the main tributaries. In contrast, the adults were recorded in deeper, well-shaded areas, which often displayed an abundance of bankside vegetation.

Only two brook trout were recorded in the Puelo River, one male of 15 cm and one female of 17 cm, both aging 2<sup>+</sup> years and displaying stage III of sexual maturation. That would indicate that this species' abundance and distribution are even lower and more confined in the Puelo River watershed than in the Palena River. It was concluded that the overall low abundance and the small size of the mature adults in the Palena River were indicators of high vulnerability for this trout species (Bravo et al. 2021).

Parr and pre-smolt Chinook salmon were recorded mainly over the winter and spring seasons in the tributaries where spawning had previously occurred. Local anglers provided the four adult salmon examined from the Puelo River. Only two Chinook salmon smolts were recorded from the Puelo River. The low number of smolts encountered during the survey may be due to the characteristics of the selected sampling areas, which were mainly located in the shallower reaches of the main tributaries of each river. Results from the earlier Palena River survey indicate that salmon smolts are mainly located in the mainstem of the parent river, flowing towards its estuary (Bravo et al. 2019).

### **Maturity stages**

In the survey carried out on the Palena River (2012/2013), mature rainbow and brown trout were recorded in the same seasons observed in Petrohué and Puelo rivers (Bravo et al. 2021). Mature rainbow trout were recorded over the autumn-winter-spring period in both rivers, while mature brown trout were recorded in the autumn-winter period. The oldest female trout encountered throughout the survey, aged 5<sup>+</sup> (49 cm) from the Petrohué River and 6<sup>+</sup> (51 cm) from the Puelo River, while males were aged 6<sup>+</sup> (60 cm) for both rivers. These results were similar to those recorded from the Palena River (2012/2013), where trout aged 6<sup>+</sup> years were recorded for both trout species (Bravo et al. 2021). The results from the two studies indicate that brown trout would appear to have a narrower spawning period than rainbow trout.

The mature male rainbow trout recorded from the Puelo River was most likely seeking a spawning area and was an escapee from a marine fish farm. The discovery of a mature male rainbow trout during this study supports earlier evidence that spawning rainbow trout populations in the wild are, in part, supported by the occurrence of escaped farmed trout (Soto et al. 2006, Arismendi et al. 2011, 2012, 2014, Sepúlveda et al. 2013). Even though trout species are non-native in Chile, the escaped farmed rainbow trout are considered a threat to the naturalized populations. Interbreeding

between escaped farmed trout and wild trout could be a risk to the genetic diversity, integrity, fitness, and viability of wild trout populations; such as has been reported for Atlantic salmon in the northern hemisphere (Naylor et al. 2005, Thorstad et al. 2008, Glover et al. 2017), with a negative impact on the recreational fishing.

Mature, returning adult Chinook salmon were recorded over the autumn season. The highest abundance was in sector A of the Petrohué River (Fig. 1). This area is locally considered an important spawning area for Chinook salmon. The three post-smolts, aged 2<sup>+</sup>, recorded in the estuarine area of the Petrohué River in summer and autumn, classified as stream type, showed evidence of maturity stage II, which could be an indicator of early sexual maturation. The mature, returning adult Chinook salmon were aged 1.5<sup>+</sup> and 1.6<sup>+</sup> years in the Petrohué and Puelo rivers. These fish displayed an ocean ecotype life history, migrating as 1<sup>+</sup> smolts and feeding at sea for five to six winters before returning to the river to spawn (Healey 1991). This finding agrees with Soto et al. (2007), earlier work on salmon from the Petrohué River. In contrast, returning Chinook salmon in the Palena River was classified as a stream ecotype from 2012-2013. The differences between the two populations may be attributed to the length of the main river and the distance that smolts must travel to reach the ocean (Bravo et al. 2019).

### **Feeding patterns**

The condition factor values for both trout species and Chinook salmon in Petrohué and Puelo rivers align with those recorded from the earlier study on the Palena River (Bravo et al. 2019, 2021). This result may reflect the good ecological status of both rivers in terms of salmonid habitat and the abundance of food items available to the fish communities in these systems.

For the two trout species, which shared the same habitats, the food items were, not surprisingly, very similar for the different stages of development in both rivers. The highest food items diversity was recorded for the fingerling stage (Tables 6-7, Fig. 8). However, the number of food items in the rainbow trout fry stage was lower in both rivers than in brown trout (Table 6, Fig. 8). Interestingly, the eggs of Chinook salmon were the dominant food item in the stomachs of adult trout taken during the spawning season. Particularly true in the case of the Petrohué River. This finding may reflect Chinook salmon's contribution to the sustainability of the trout populations in these two rivers. Both rivers recorded no significant differences in the Shannon

index in the fry and fingerling stages for rainbow trout. For brown trout, differences were recorded for the fry and adult stages between both rivers (Fig. 8).

The diversity of food items in Chinook salmon was significantly lower in the two rivers compared to the two trout species. This result differs from earlier studies which showed that in oligotrophic systems, such as in the Patagonian rivers, the spread of Chinook salmon, with the resultant high densities of juveniles, may lead to direct competition for food with the resident fish stocks (Soto et al. 2006, 2007, Ibarra et al. 2011). A similar situation was reported by Perez et al. (2021) in a study in southern Patagonia (49-52°S). The study showed that in estuaries, the diet of *Genypterus maculatus* and non-native salmon species (coho and Chinook) overlap, indicating that these salmon species are a major competitor for food in such areas.

This study recorded four dominant food items for the parr stage in the Petrohué River. Three dominant food items were recorded from parr in the Puelo River. The highest abundance was found for the order Trichoptera. In the pre-smolt stage, four food items were dominant in each river. The highest abundance was recorded for Trichoptera and Ephemeroptera in the Petrohué River and the order Plecoptera in the Puelo River. Plecoptera was the only food item recorded in the two smolts collected from the Puelo River (Table 6). No significant differences were recorded in food items diversity of parr and pre-smolt stages between both rivers (Fig. 8).

In contrast, in the Reloncaví Estuary, the stomachs of Chinook showed an abundance of mussel seeds and fingerling trout, which contained Ephemeroptera (not included in Table 6).

In the surveys carried out in Petrohué and Puelo rivers, native fish species were not recorded in the food items identified from the various life history stages of the sampled Chinook salmon and the two trout species. This result is similar to that obtained during the earlier survey of the Palena River (2012/2013), in which aquatic insects (Ephemeroptera, Diptera, and Plecoptera) dominated the diet of all juvenile stages (parr, pre-smolts, and smolts). In line with previous research in the northern hemisphere, it was found that benthic macroinvertebrates and aquatic insects also dominated the diet of juvenile Chinook salmon (Limm & Marchetti 2009). In contrast, Ibarra et al. (2011) reported that juveniles aged 1+ years and over 8.7 cm in length, sampled in Yelcho Lake (Palena, southern Patagonia), showed high condition factors and piscivorous diets based predominantly on native galaxids. A similar finding was recorded by Bravo et al.

(2019) from the diet of landlocked Chinook salmon collected from a small lake in southern Patagonia (Claro Solar, Palena). These results may indicate that, in enclosed systems, the Chinook salmon rely more on a piscivorous diet, which could negatively impact the native wild fish species in these waters. In general, salmonid invasions are considered a serious threat to the freshwater ecosystems of Patagonia (Arismendi et al. 2012, Habit et al. 2012, Pérez et al. 2021). In this study, it was further found that the stomachs of the returning adult Chinook salmon in both rivers (Petrohué and Puelo) were devoid of food. Similar results were recorded from the Palena River (2012/2013).

## CONCLUSIONS

The study did not identify any adverse competition or negative interactions among the three main salmonid species sharing the same habitat. Chinook salmon did not constitute a threat to the trout species in the two rivers studied. The presence of the salmon may enhance the productivity of nutrient-poor oligotrophic systems, as a result of the release of additional marine nutrients, through the decomposition of the salmon carcasses following spawning. This enrichment may enhance the systems' overall productivity, improving food availability for the trout and other native fish populations and increasing the abundance and resilience of the fish stocks in these waters.

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