Research Article



Size structure, age, and diets of introduced Chinook salmon (*Oncorhynchus tshawytscha*) inhabiting the Palena River, Chilean Patagonia

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ABSTRACT. Chinook salmon (*Oncorhynchus tshawytscha*) were recorded in two extensive areas during a survey conducted between February 2012 and June 2013 to obtain information regarding the seasonal distribution and population structure of a self-sustained salmonid population inhabiting the Palena River. A total of 83 Chinook salmon were captured, which corresponded to 8.3% of the total salmonids collected in both areas. Juveniles of less than 15 cm were recorded in the upstream section of the river, from which fry-parr were less than 5 cm, and pre-smolt ranged between 5 and 10 cm aged 0+. Smolts displaying the characteristic silver coloration with an absence of parr-markings ranged between 10 and 15 cm and were aged 1+. The diet of the juvenile salmon was composed mainly of aquatic insects. Mature salmon returning from the ocean were collected in the middle section of the river, with weights ranging between 4.6 and 28 kg and their age ranged from 4+ to 6+ years in the ocean, plus 1+ year's growth in freshwater (stream type ecotype). All the adult salmon were devoid of food. Immature adults, with ages between 3+ and 4+, were collected from a small lake (Claro Solar), displayed a piscivorous diet mainly based on native Galaxid species. Adult salmon returning from the ocean were infested with the cestode *Hepatoxylon trichuri*, attached to the pyloric caeca.

Keywords: invasive salmon; spawning migration; population structure; feeding habits; recreational fishing; Chilean Patagonia

INTRODUCTION

Salmon and trout species are not native to the Southern Hemisphere. With the intention of establishing recreational fisheries, the first salmon eggs were brought to Chile and Argentina around the turn of the last century (Basulto, 2003). In 1924, Chinook salmon eggs (Oncorhynchus tshawytscha) were imported from the United States and were planted in the Maullín, Cochamó, and Puelo rivers in southern Chile. In 1930, eggs of Sockeye salmon (Oncorhynchus nerka) and Coho salmon (Oncorhynchus kisutch) were also introduced from the United States. However, most of the attempts to introduce anadromous salmon failed, until the onset of marine-based aquaculture in the 1980s (Soto et al., 2006; Arismendi et al., 2009). In the 1970s, Dom Sea Farms carried out experimental ocean ranching programmes using both Coho and Chinook

salmon. The ranching programme was based on the release of 170,000 Chinook salmon smolts from Curaco de Vélez, Chiloé, southern Chile in 1977. The first ranched return was recorded in 1979; returns continued intermittently until 1991 when they apparently ceased (Mendez & Munita, 1989).

Since 1995, yearly runs of returning Chinook salmon have been recorded to the middle and upper reaches in several rivers in both, southern Chile and Argentina (Soto, 1997; Ciancio *et al.*, 2005; Becker *et al.*, 2007). In the Palena River Basin, local recreational fishermen reported the first run of adult spawning Chinook salmon occurred in 1985 (Rolf Shilling, *pers. comm.*), while in the Argentinian part of Patagonia the first reports of wild spawning Chinook salmon date back to 1986 (Di Prinzio & Pascual, 2008). These runs have been attributed to escapees from Chilean salmon farms (Soto *et al.*, 2001, 2007; Astorga *et al.*, 2008),

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however, evidence from molecular markers suggests that the population of the nearby Futaleufú Basin corresponds to an admixture between Chinook salmon originated by the ranching programmes and escapees from salmon farming (Riva-Rossi *et al.*, 2012; Di Prinzio *et al.*, 2015).

The current distribution of Chinook salmon in Chile is from 39° to 53°S (Correa & Gross, 2008), and from 43° to 54°S in Argentina (Di Prinzio & Pascual, 2008; Fernandez et al., 2010). The above includes the transnational Pacific Puelo River Basin, and the Futaleufú, Corcovado and Pico rivers. Chile and Argentina share these rivers, and flow into the Pacific Ocean (Fig. 1). Although Chinook salmon have constituted less than 0.5% of the total farmed salmonids in Chile and the last production was recorded in 2013 (SERNAPESCA, 2013), it is the only exotic salmon species that has successfully established naturalized anadromous populations in Patagonian rivers with a Pacific outlet (Soto et al., 2001, 2007; Di Prinzio & Pascual, 2008; Sepúlveda et al., 2013; Arismendi et al., 2014). However, Chinook salmon populations have also been reported in the Atlantic basins such as the Argentinean Santa Cruz River (Ciancio et al., 2005). The populations resulting from this colonization constitute the establishment of the largest exotic anadromous salmon populations to date, on a worldwide basis (Ciancio et al., 2015).

This paper provides the first description of the Palena River Chinook salmon population regarding adult spawning and juvenile recruitment, and we expect that our findings may serve as a baseline for the development of a management programme for the sustainability of the Chinook salmon population in the Palena River and its tributaries.

MATERIALS AND METHODS

Study area

The study was carried out on the Palena River Basin (43°14′-44°35′S, 71°07′-72°58′W). The Palena River (Fig. 1), originates in Argentina, where it is named as the Corcovado River. It flows from Palena-Vinter Lake, a border lake, shared by both Chile and Argentina, to the Pacific Ocean. The outflow is located in the southern shoreline of Chile, northwest of Termas de Puyuhuapi and southeast of Chiloé Island. Its total watershed area comprises 12,867 km², 5,606 km² (43.5%) of which lies in Argentina and 7,281 km² (56.5%) in Chile.

Sampling sites

Between February 2012 and June 2013, six surveys covering the four seasons of the year were undertaken

(Table 1). Fish were collected from two extensive areas of the Palena River, one situated in the upper reach of the river (upstream), where six sites were sampled, and the other in the middle reach of the river (midstream), where five sites were sampled, one of which corresponded to the small Claro Solar Lake (Fig.1), where adult Chinook salmon were recorded.

Capture methods and fish characteristics

Fish were sampled using both gillnets for the larger fish and electrofishing for the smaller fish. Gillnet sites were carefully selected and sampled using nylon gillnets of 100 m length and 3 m height. The nets with a mesh size of 3" (76 mm) and 7" (180 mm), were set for periods of 24 h and checked for the presence of fish every 12 h. Electrofishing was carried out in the shallower riffles. All fish collected by electrofishing were kept alive in a container with fresh water and were anesthetized with benzocaine (10% in ethanol, 1 ml L⁻¹) before measuring (mm) and weighing (g). A proportion of the fish (47%) was retained for subsequent laboratory analysis, and the others were safely returned to the water. No fishing effort was calculated because the initial goal of the surveys did not focus on Chinook salmon.

Fish were classified according to its development stage as fry-parr, pre-smolt, smolt, adult, and mature adult. Fulton's Condition Factor (K) was calculated as $K = 100 \times W \times L^{-3}$, where W: weight in grams, and L: fork length in centimeters.

Age analysis

Scales from a subsample of 29 fish were collected and prepared following the methodology recommended by the Manual of Fisheries Science (FAO, 1975a), to determine the age of each fish from each of the length classes. Chinook was classified as "stream type" when the freshwater growth of the scale contained more than 10 circuli, otherwise were classified "ocean type" (Ciancio et al., 2005). For fish returning from the sea, the age designation was made according to Mosher (1969) recommendations. For example, a fish recorded as 1.4+ indicates that the fish had spent one winter in freshwater before smoltification; the decimal point indicates the separation between the river and marine life phases, and the number after the decimal point records the number of complete post-migration winters spent at sea.

Maturity stages

Chinook spawner maturity stages were defined following the key recommended by the Manual of Fisheries Science (FAO, 1975b), for total spawner, adapted for Chinook salmon.

	Season	Parr			Pre-smolt			Smolt		
Campaing		n	Lenght (cm) Mean (Range)	Weight (g) Mean (Range)	n	Lenght (cm) Mean (Range)	Weight (g) Mean (Range)	n	Lenght (cm) Mean (Range)	Weight (g) Mean (Range)
Upstream										
C - 1	summer 12	-	-	-	1	9.8	9.2	1	11,1	10,0
C - 2	autumn 12	-	-	-	4	8.3 (6.2 - 9.7)	8.1 (2.6 - 15.0)	6	11.7 (10.4 - 13.5)	11.7 (10.0 - 15.0)
C - 3	winter 12	22	4.3 (3.7 - 4.9)	0.6 (0.3 - 1.3)	3	9.0 (8.7 - 9.3)	7.4 (6.5 - 8.0)	-	-	-
C - 4	spring 12	-	-	-	-	-	-	-	-	-
C - 5	autumn 13	1	4.4	0.9	5	7.9 (6.9 - 8.4)	4.5 (3.3 - 5.5)	1	13,7	24,1
C - 6	winter 13	-	-		1	7	3.2	-	-	-
Subtotal		23	4.3 (3.7 - 4.9)	0.6 (0.3 - 1.3)	14	8.3 (6.2 - 9.8)	6.4 (2.6 -15.0)	8	11.9 (10.4 - 13.7)	13.0 (10.0 - 24.1)
Midstream										
C - 1	summer 12	17	4.1 (3.6 - 4.8)	0.8 (0.6 - 1.2)	9	6 (5.0 - 6.9)	2.4 (1.4 - 3.4)	-	-	-
C - 2	autumn 12	-	-	-	-	-	-	-	-	-
C - 3	winter 12	-	-	-	1	9.7	8.3	-	-	-
C - 4	spring 12	-	-	-	-	-	-	-	-	-
Т	otal	17	4.1 (3.6 - 4.8)	0.8 (0.6 - 1.2)	10	6.4 (5.0 - 9.8)	3.0 (1.4 - 8.3)	-	-	-

Table 1a. Biometric characteristics exhibited by Chinook salmon caught by electrofishing in the Palena River.

Table 1b. Biometric characteristics exhibited by Chinook salmon caught by gillnet in the Palena River.

		Adult (River)				Adult (Lake)			
Campaing	Season	n Lenght (cm) Mean (Range)		Weight (g) Mean (Range)		Lenght (cm) Mean (Range)	Weight (g) Mean (Range)		
Midstream				-					
C - 1	summer 12	1	84	7,910.00	1	37.7	420		
C - 2	autumn 12	4	102.8 (71.4 - 123.3)	15,453.7 (4,610.0 - 27,955.0)	-	-	-		
C - 3	winter 12	-	-	-	1	49.4	840		
C - 4	spring 12	-	-	-	4	39.3 (35.0 - 45.2)	502.7 (359.7 - 687.3)		
Total		5	99.0 (71.4 - 123.3)	13,945.0 (4,610.0 - 27,955.0)	6	40.6 (35.0 - 49.4)	545.1 (359.7 - 840.0)		

Stage I (virgin): sexual organs very small, situated close to the vertebral column. Testis and ovary transparent, colorless or grey. Eggs not visible to naked eye.

Stage II (maturing virgin): testis and ovary translucent, grey-red. Length of gonads 1/2, or slightly more, of the length of the ventral cavity. Individual eggs can be seen with a magnifying glass.

Stage III (developing): testis and ovary opaque, reddish with blood capillaries. Occupy about 1/2 of ventral cavity. Eggs visible to the naked eye as a whitish granular material.

Stage IV (developed): testis reddish-white, no milt produced under pressure. Ovary orange-red. Eggs clearly discernible, opaque. Testis and ovary occupy about 2/3 of ventral cavity.

Stage V (gravid): sexual organs fill the ventral cavity. Testis white. Drops of milt produced under pressure. Eggs completely round, some already translucent and ripe.

Stage VI (spawning): roe and milt run under slight pressure. Most eggs are translucent with few opaque eggs left in the ovary.

Feeding habits

Stomachs from a subsample of 39 fish were removed and contents preserved in 95% ethanol for later examination. Stomach contents were analyzed with a dissecting microscope, using taxonomic keys to the lowest possible taxa. Prey was weighed and counted. The remaining food items, which were not identified, because its high level of decomposition, were classified as "other."

Relative abundance was calculated as the number of preys of each item in relation of the total items recorded in the sampled, while frequency of occurrence (FO%) was calculated as the number of stomachs containing a prey item divided by the total number of non-empty stomachs, expressed as a percentage (Mac-Donald & 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

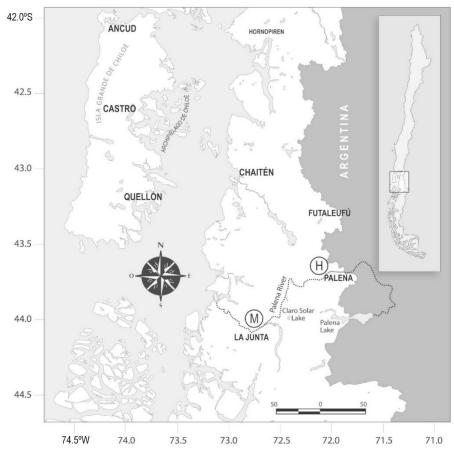


Figure 1. Map showing the two section of the Palena River from where Chinook salmon individuals were sampled. H: upstream area, M: midstream area.

multiplied by the natural logarithm of this proportion $(\ln p_i)$. The resulting product is summed across the prey items and multiplied by -1.

Fish health

Fish were externally and internally examined in order to detect parasites or any abnormality which might indicate the presence of diseases. In addition, samples of kidney were sent to a specialist fish disease laboratory (ETECMA, Puerto Montt, Chile) to test, using PCR (polymerase chain reaction), for the presence of the ISA virus, pathogen widely spreading in farmed Atlantic salmon in Chile since 2007.

RESULTS

Population spatial distribution

A total of 83 Chinook salmon were collected during the six surveys, 11caught by gillnet and 72 by electrofishing. In the upstream section, 45 Chinook salmon were collected which corresponded to 6.4% of

the total salmonids collected in that area (57% corresponded to rainbow trout (*Oncorhynchus mykiss*); 27.3% to brown trout (*Salmo trutta*) and 9.3% to brook trout (*Salvelinus fontinalis*). In the midstream section, 38 Chinook salmon were collected which corresponded to 13.1% of the total salmonids collected in that area (41% corresponded to rainbow trout; 38.2% brown trout and 7.8% to brook trout).

In the upstream area juveniles of less than 15 cm caught by electrofishing, were recorded in five of the six sites. The highest abundance (34.7% of the total) was recorded in winter 2012 (Table 1a), with a higher proportion of fry displayed the characteristic parrmarkings, and measured less than 5 cm. Pre-smolts with size between 5 to 10 cm and displaying a distinct silver colouration and diffuse parr-markings, were collected in all five surveys, while the smolts with the characteristic silver coloration and the absence of parrmarkings, with a length between 10 to 15 cm, were recorded only in this section of the river, with the highest abundance in autumn 2012 (Table 1a).

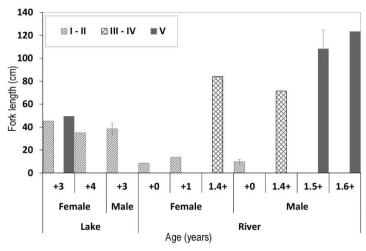


Figure 2. Length according to the age structure of Chinook salmon collected throughout the study, by gender and stage of maturity (stages I to V).

In the midstream area, Chinook salmon caught by electrofishing was only recorded from two of the five sampling sites, and the highest abundance (36.1% of the total) were recorded in summer 2012 (Table 1a). Fish collected in the midstream section were dominated by fry-parr and pre-smolt stages of less than 10 cm (Table 1a).

Maturing adult salmon greater than 70 cm were collected using gillnets in summer and autumn 2012, only in the midstream section (Table 1b). Also, six adults Chinook salmon were collected in the Claro Solar Lake in three of the four surveys, displaying the characteristic silver coloration, with a length ranging between 35 and 49.4 cm (Table 1b).

Population structure and ecotype

The scales of 29 fish analyzed showed that all mature fish collected during the autumn and summer of 2012 belonged to the stream type and had ages 1.4+, 1.5+, and 1.6+. The fry-parr aged 0+ while pre-smolts and smolts aged 1+. In the Claro Solar Lake, collected females had ages between 3+ and 4+, while males aged 3+ (Fig. 2).

Maturity stages

Returning adult salmon displaying typical brown spawning colours and characteristics of sexual maturity (stage V) were recorded in summer and autumn 2012, ranged in age between 1.5+ and 1.6+. Fish aged 1.4+ were in stages III and IV of sexual maturity. Only one mature female salmon was recorded in Claro Solar Lake (stage V), while the other fish (both male and female) displayed a silver coloration, with the gonads at stage II of maturity (Fig. 2).

Condition factor

The condition factor for fish collected from Claro Solar Lake ranged from 0.72 to 0.85. In contrast, the mature adult Chinook salmon (>45 cm) returning from the sea, showed a mean condition factor of 1.27. The average condition factor for the fry-parr stages was 0.96; for the pre-smolt 1.03 and 0.76 for the smolt stage (Fig. 3).

Diet

The stomachs of mature fish returning from the ocean were empty. The food items recorded in fry-parr stage included the orders Ephemeroptera, Plecoptera, Diptera, and unidentified items classified as others (Fig. 4). Presmolt stage diet was constituted by a wide variety of items, which showed the highest abundance for Ephemeroptera, Diptera, and Plecoptera, and low abundance of unidentified food items (others) (Fig. 4). The predominant food items for the smolt stage were Plecptera

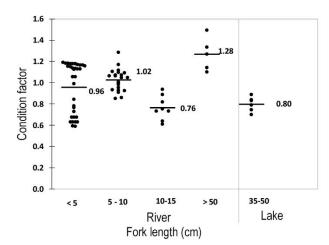


Figure 3. Condition factor, relative to the length of Chinook salmon collected from the river and lake areas in the Palena River watershed.

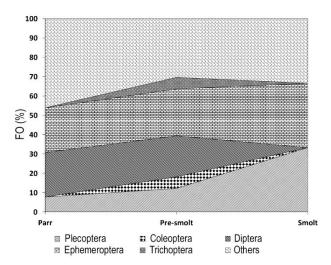


Figure 4. Relative abundance (%) of food items in sampled Chinook salmon stomachs, according to the developed stages.

and Ephemeroptera, with a low abundance of unidentified items (Fig. 4). The only food item recorded in the sampled fish in the Lake Claro Solar was *Galaxias maculatus*, with up to 189 fish by the stomach.

The highest frequency of occurrence in the fry-parr stage was recorded for the item "other" followed by Diptera and Ephemeroptera. For pre-smolt the highest frequency of occurrence was recorded for the item "others" followed by Ephemeroptera and Diptera and for the smolt stage was recorded for the items "othes," Plecoptera and Ephemeroptera in the same level (Fig. 5).

The lowest diversity was recorded for the smolt stage (0.8), followed by the fry-parr stage (1.3) and the pre-smolt stage (1.4) (Fig. 6).

Fish health

The sampled fish showed good health condition, and the results provided by the laboratory of fish diseases were negative for the ISA virus, both, for the adult and the juvenile stages. The cestode *Hepatoxylon trichuri* was recorded attached to the pyloric caeca of returning adult Chinook salmon in the surveys carried out in summer and autumn 2012, recording up to 31 larvae per fish.

DISCUSSION

Returning Chinook salmon to Palena River from the ocean-aged 1.4+ to 1.6+ years, showing similar age profiles described for Chinook salmon returns in the Northern Hemisphere after spending 3 to 8 years in the ocean (Healey, 1991). Chinook salmon is the largest of

the Pacific salmon species (Healey, 1991). Returning

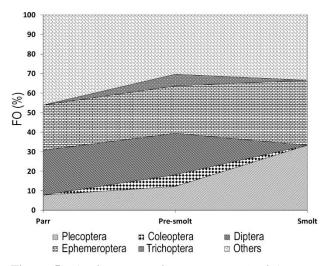


Figure 5. The frequency of occurrence (%) of the prey items found in the juvenile Chinook salmon stomachs sampled in Palena River during the six surveys.

fish collected during this study reached up to 28 kg (123 cm), which is similar to sizes reported from other rivers in southern Chile (Soto *et al.*, 2007; Correa & Gross, 2008). Juveniles Chinook salmon were collected in the mainstream, both in the upstream as in the midstream section. Smolts ranged between 10-15 cm (Table 1), which also is similar to sizes reported for smolt in other rivers of the Chilean Patagonia (Soto *et al.*, 2007; Correa & Gross, 2008; Ibarra *et al.*, 2011). Variations in condition factor were attributable to the differences in metabolism and physiological behaviour at each stage of development.

The analysis of juvenile Chinook scales suggests that the watershed is mainly populated by a typical stream-type strain, which migrates to the sea at between one and two years of age (1+). On the contrary, juvenile Chinook from the Petrohué River, located north of the Palena Watershed (41°-41°25'S, 72°5'-72°25'W), have a dominant ocean-type ecotype (Soto et al., 2007). The length of the parent river could drive this difference. In this sense, the Palena River has a larger watershed (12,867 km²), and the main stem is far longer than the Petrohué River (2,704 km²). The distance from the juvenile feeding areas to the sea is also longer. However, the same river could produce ocean type fish in spawning beds close to the estuary and stream type in the river headwaters. In this study, juveniles were recorded in five of the six surveys of the upstream area; while in the middle reaches, they were recorded in two of the six surveys (Table 1). According to Healy (1991), freshwater residence often co-varies with the timing of the adult return to freshwater, and populations that

produce stream type juveniles, tend to have adults returning earlier in the year than ocean type populations.

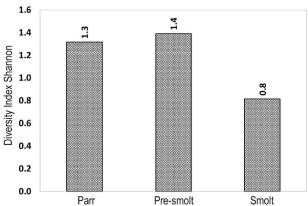


Figure 6. Shannon Diversity Index of the prey items found in the juvenile Chinook salmon stomachs sampled in Palena River.

The fact that most of the adult fish sampled displayed a stream ecotype and most of the juveniles were captured in the upper basin of the river suggests that environmental factors greatly influence the life history strategy of these fish environmental factors.

The diet of the juvenile stages (fry/parr, pre-smolts, and smolts) analyzed in this study, was mainly based on insects of aquatic origin, which are in turn important food and energy sources for native fish species (Vargas et al., 2010). Some authors have reported that in oligotrophic systems such as in Patagonia, the spread of Chinook salmon, with the resultant high densities of juveniles, may lead to competition for food with resident fish stocks (Soto et al., 2006, 2007; Ibarra et al., 2011). A low diversity of food items was recorded in this study, mainly constituted by Ephemeroptera, Diptera, and Plecoptera, which also have been reported for juveniles' stages of rainbow trout and brown trout (Arismendi et al., 2012). Chinook salmon collected from the Claro Solar Lake displayed a low condition factor, which suggests poor food availability for this carnivorous species in this small lake dominated by populations of small native fish and snails. Its diet comprised mainly the native fish Galaxias maculatus. A similar situation has been reported for landlocked Chinook salmon from other lakes of southern Chile (Ibarra et al., 2011). Chinook salmon predation on either native fish or aquatic insects suggests a potential negative interaction between the native fish species and the invading Chinook salmon inhabiting the oligotrophic lakes of southern Chile.

It is encouraging to note that no pathogens were recorded from the juvenile Chinook salmon examined from the Palena River, indicating a good fish health status in the river itself and good water quality. The presence of *Hepatoxylon trichuri* in the body cavity of the returning adult salmon in the Palena River shows

that these stocks were infested in the marine environment. Because this parasite has been reported for Patagonian toothfish (*Dissostichus eleginoides*), rays and sharks, in Chile (Rodríguez & George-Nascimento, 1996; Pardo-Gandarillas *et al.*, 2007, 2009), it may allow concluding that Chinook salmon returning to Palena River could have fed on similar food items, which act as inter-mediate host of *Hepatoxylon trichuri*.

Anadromous salmon runs function as conveyor belts that transport marine-derived nutrients (MDN) to the river basins from the ocean which results in a significant enrichment of the freshwater ecosystem (Kline et al., 1993; Naiman et al., 2002). Studies carried out by Arismendi & Soto (2012) suggested that MDN from salmon carcasses are most likely to be incorporated into stream food webs of an invaded river where Chinook salmon is well established. This surplus of energy and organic matter could disturb both aquatic and terrestrial natural cycles and balances and food web structures. However, this natural fertilization alternatively could be considered as an important source of energy in oligotrophic systems, enhancing the productivity of the waters and creating conditions more favourable to the establishment of sustainable stocks of Chinook salmon and other resident salmonids such as brown trout and rainbow trout, favouring, in turn, the establishment of sports fisheries in the Palena River watershed.

This study has contributed to a baseline, which will increase our understanding of the role of this invasive species in the Palena River watershed. More longer-term and intensive studies are required in order to clarify the long-term impacts, both positive and negative, of the establishment of Chinook salmon populations in the pristine waters of southern Chile.

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