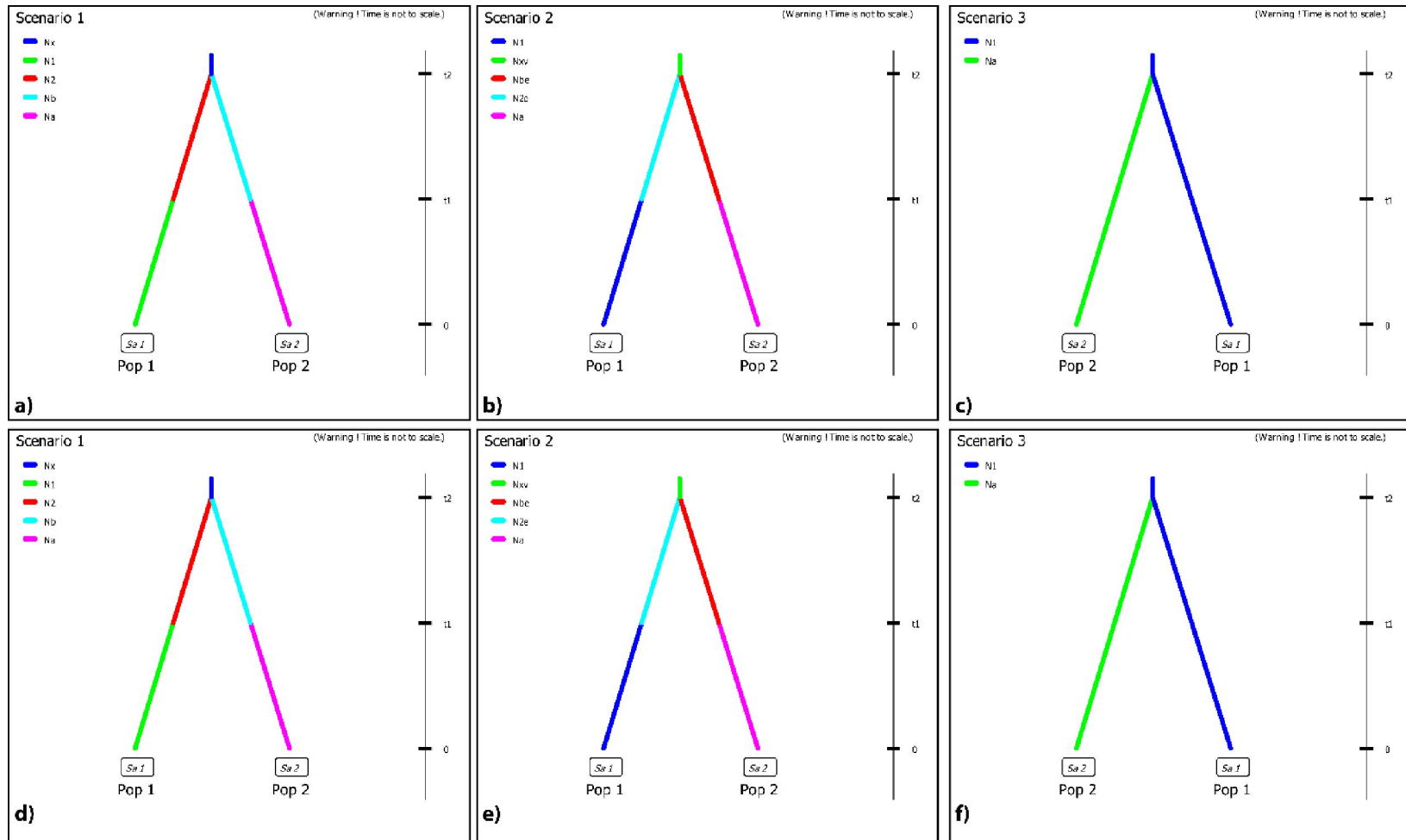


Appendix S1 DNA extraction, PCR protocols and microsatellite scoring

Whole DNA was extracted using a glass milk protocol (Elphinstone et al. 2003) and amplified by PCR using 10 species-specific microsatellite markers (Arias et al. 2012, Vera-Escalona et al. 2014), 8 of which have been used in our previous studies dealing with the Valdivia, Puelo and Serrano river systems using the PCR protocols described in those studies (Vera-Escalona et al. 2015, Vera-Escalona et al. 2018). Amplified microsatellite fragments were observed in polyacrylamide gels with LI-COR[®] sequencers (Biosciences, Lincoln, NE, USA) and a molecular ladder of 65-400 bp. Images were then analyzed with the software SAGA[™] (LI-COR[®]) for microsatellite scoring.

Appendix S2 Scenarios assessed for the *G. platei* population fragmentation between lakes Thompson and Pollux (a-c), and Belgrano and Del Mie (d-f). Scenarios a and d assume population expansion after the division of populations, scenarios b and e assume population size reductions, and scenarios c and f assume no change in population sizes.



Appendix S3 Prior and mean posterior distribution of parameters for two sets of lakes Thompson-Pollux and Belgrano-Del Mie from Patagonia using DIYABC. N = effective population size, t = time

Thompson - Pollux			Belgrano - Del Mie	
Parameter	Prior	Mean posterior (Q0.025-Q0.975)	Prior	Posterior (Q0.025-Q0.975)
$N1$	10 - 10000	2430 (1360 – 2980)	10 – 10000	9110 (6960 – 9970)
$N2$	10 - 10000	1310 (354 – 1970)	10 – 10000	5310 (1380 – 9060)
$N2e$	10 – 10000		10 – 10000	
Na	10 – 10000	3720 (1010 – 9320)	10 – 10000	3960 (1080 – 8970)
Nb	10 – 10000	1090 (167 – 2420)	10 – 10000	4700 (1130 – 9050)
Nbe	10 – 10000		10 – 10000	
Nx	10 – 10000	330 (21 – 1140)	10 – 10000	729 (29 – 2800)
Nxv	10 – 10000		10 – 10000	
$t1$	10 – 10000	750 (176 – 3670)	10 – 10000	968 (32 – 3900)
$t2$	10 – 10000	1240 (121 – 5570)	10 – 10000	1700 (232 – 7190)

Appendix S4 t-test for the number of alleles ($\hat{N}_a$; a), allelic richness ($\hat{A}$; b) and Mann-Whitney U test of the observed heterozygosity ($\hat{H}_o$) for 16 and 21 *G. platei* populations with and without salmonids from Patagonia. N = sample size, SD = Standard deviation, SEM = Standard error of the mean, t = t-value, U = U-value

a) $\hat{N}_a$	Group	N	Mean	SD	SEM	t	p-value
N = 16	With salmonids	10	9.380	1.427	0.451	-2.807	0.014
	Without salmonids	6	11.617	1.733	0.707		
N = 21	With salmonids	11	10.705	1.675	0.505	-2.626	0.017
	Without salmonids	10	12.958	2.242	0.709		

b) $\hat{A}$	Group	N	Mean	SD	SEM	t	p-value
N = 16	With Salmonids	10	4.151	1.225	0.388	-3.559	0.003
	Without Salmonids	6	6.401	0.700	0.499		
N = 21	With Salmonids	11	4.935	1.533	0.462	-3.059	0.006
	Without Salmonids	10	7.034	1.611	0.510		

c) $\hat{H}_o$	Group	N	Median	25%	75%	U	p-value
N = 16	With Salmonids	10	0.606	0.523	0.687	16	0.007
	Without Salmonids	6	0.726	0.700	0.738		
N = 21	With Salmonids	11	0.733	0.530	0.769	3	0.004
	Without Salmonids	10	0.815	0.763	0.838		

Appendix S5 Linear regression analyses for variable lake area and number of alleles ($\hat{N}_a$), Allelic richness ($\hat{A}$), and observed heterozygosity ($\hat{H}_o$) considering 16 and 21 *Galaxias platei* lake populations.

		Beta	SE Beta	t	p-value
$\hat{N}_a$ 16	Intercept	10.574	0.604	17.501	<0.001
	Slope	-0.014	0.015	-0.929	0.369
$\hat{N}_a$ 21	Intercept	12.028	0.646	18.616	<0.001
	Slope	-0.010	0.016	-0.603	0.554
$\hat{A}$ 16	Intercept	5.196	0.538	9.649	<0.001
	Slope	-0.007	0.014	-0.590	0.569
$\hat{A}$ 21	Intercept	6.218	0.536	11.596	<0.001
	Slope	-0.011	0.014	-0.823	0.420
$\hat{H}_o$ 16	Intercept	0.648	0.029	21.832	<0.000
	Slope	-8.891e ⁻⁵	<0.000	-0.120	0.906
$\hat{H}_o$ 21	Intercept	0.738	0.036	20.731	<0.001
	Slope	<-0.001	<0.001	-0.535	0.599

Appendix S6 t-tests (a) and Mann-Whitney U tests (b) for parameters number of alleles ($\hat{N}_a$), allelic richness ($\hat{A}$), and observed heterozygosity ($\hat{H}_o$) of *Galaxias platei* populations from lakes with and without salmonids after correcting for lake area. N markers = number of microsatellite markers, N = sample size, SD = Standard deviation, SEM = Standard error of the mean, t = t-value, U = U-value

a)

Parameter	N markers	Group	N	Mean	SD	SEM	t	p-value
$\hat{N}_a$	N = 21	With salmonids	11	-0.136	-1.439	0.145	145	0.015
		Without salmonids	10	0.848	<0.001	1.221		
$\hat{A}$	N = 16	With salmonids	10	-0.470	0.761	0.241	-3.194	<0.001
		Without salmonids	6	0.784	0.759	0.310		

b)

Parameter	N markers	Group	N	Mean	25%	75%	U	p-value
$\hat{N}_a$	N = 16	With salmonids	10	-0.279	-0.435	0.150	72	0.026
		Without salmonids	6	0.825	0.365	1.502		
$\hat{A}$	N = 21	With salmonids	11	-0.136	-1.439	0.145	145	0.015
		Without salmonids	10	0.848	<0.001	1.221		
$\hat{H}_o$	N = 16	With Salmonids	10	-0.437	-1.347	0.442	78	0.004
		Without Salmonids	6	0.871	0.589	0.989		
$\hat{H}_o$	N = 21	With Salmonids	11	0.004	-1.636	0.408	145	0.015
		Without Salmonids	10	0.628	0.392	0.881		

Appendix S7 Presence of salmonids and native species in 21 sampled lakes from Patagonia. At = *Aplochiton taeniatus*, Az = *Aplochiton zebra*, Ba = *Basilichthys australis*, Cha = *Cheirodon australe*, Gm = *Galaxias maculatus*, Gp = *Galaxias platei*, Omau = *Oncorhynchus masou*, Pt = *Percichthys trucha*,

Basin	Lake	Salmonids	Native
Valdivia	Pellaiifa	1 ¹	Pt,Gp ¹
	Pullinque	1 ¹	n.i.
	Panguipulli	1 ¹	Ba,Gm,Omau,Gp ¹
	Riñihue	1 ¹	Az,Ba,Gm,Omau,Pt,Cha,Gp ¹
	Neltume	1 ¹	At,Ba,Gm,Gp,Pt,Gp ¹
Puelo	Puelo	1 ¹	Az,Gp,Oh,Pt ¹
	Inferior	1 ^{1,2}	Gp,Pt ^{1,2}
	Las Rocas	1 ^{1,2}	Gp ^{1,2}
	Azul	1 ^{1,2}	Gp,Pt ^{1,2}
Cuervo	Yulton	0 ^{1,2}	Gp ¹²
Aysén	Thompson	0 ^{1,2}	Gp ¹²
	Pollux	1 ¹	Gp ¹
Nansen	Belgrano	0 ³	Gp ³
Chico	Del Mie	0 ³	Gp ³
Serrano	Dickson	0 ^{1,2}	Gp ^{1,2}
	Paine	0 ^{1,2}	Gp ^{1,2}
	Azul	0 ^{1,2}	Az,At,Gp ^{1,2}
	Mellizas	0 ^{1,2}	Gp ^{1,2}
	Nordenskjold	0 ^{1,2}	Gp ^{1,2}
	Pehoe	0 ^{1,2}	Gp ^{1,2}
	Porteño	1 ^{1,2}	Az,At,Gm,Gp ^{1,2}

¹ Habit et al. 2012, ² Ortiz-Sandoval et al. 2016, ³ Aigo et al. 2008

Appendix S8. Linear regression analyses for number of native species (other than *G. platei*) and number of alleles ($\hat{N}_a$), Allelic richness ($\hat{A}$), and observed heterozygosity ($\hat{H}_o$) considering 16 and 21 *Galaxias platei* lake populations.

		Beta	SE Beta	t	p-value
$\hat{N}_a$ 16	Intercept	10.772	0.651	16.552	<0.001
	Slope	-0.327	0.259	-1.266	0.228
$\hat{N}_a$ 21	Intercept	12.095	0.630	19.203	<0.001
	Slope	-0.253	0.289	-0.874	0.394
$\hat{A}$ 16	Intercept	5.173	0.600	8.626	<0.001
	Slope	-0.105	0.238	-0.442	0.666
$\hat{A}$ 21	Intercept	6.123	0.533	11.497	<0.001
	Slope	-0.149	0.244	-0.609	0.550
$\hat{H}_o$ 16	Intercept	0.650	0.033	19.831	<0.001
	Slope	-0.002	<0.013	-0.186	0.856
$\hat{H}_o$ 21	Intercept	0.728	0.035	20.617	<0.001
	Slope	-0.002	0.016	-0.161	0.874

Appendix S9 Introduced salmonids species in 21 Patagonian lakes including year of introduction.

Basin	Lake	Species	Year
Valdivia	Pellaifa	<i>Salmo trutta</i>	1912 ⁴
		<i>Oncorhynchus mykiss</i>	1907 ⁴
	Pullinque	<i>Salmo trutta</i>	1912 ⁴
		<i>Oncorhynchus mykiss</i>	1907 ⁴
	Panguipulli	<i>Salmo trutta</i>	1912 ⁴ , 1972 ⁴
		<i>Oncorhynchus mykiss</i>	1907 ⁴
	Riñihue	<i>Salmo trutta</i>	1912 ⁴
		<i>Oncorhynchus mykiss</i>	1907 ⁴
	Neltume	<i>Salmo trutta</i>	1912 ⁴
		<i>Oncorhynchus mykiss</i>	1907 ⁴
Puelo	Puelo	<i>Salmo trutta</i>	1927 ⁴
		<i>Oncorhynchus mykiss</i>	1927 ⁴
		<i>Oncorhynchus tshawytscha</i>	1924 ⁴⁵
		<i>Oncorhynchus kisutch</i>	1961 ⁴
		<i>Salvelinus fontinalis</i>	1932 ⁴
		<i>Salmo salar</i>	1927 ⁴
	Inferior	<i>Salmo trutta</i>	1927 ⁴
		<i>Oncorhynchus mykiss</i>	1927 ⁴
	Las Rocas	<i>Oncorhynchus mykiss</i>	1927 ⁴
	Azul	<i>Oncorhynchus mykiss</i>	1927 ⁴
Cuervo	Yulton		
Aysén	Thompson	<i>Oncorhynchus mykiss</i>	2002 ⁶
	Pollux	<i>Salmo trutta</i>	n.i
Nansen	Belgrano		
Chico	Del Mie		
Serrano	Dickson		
	Paine		
	Azul		
	Mellizas		
	Nordenskjold		
	Pehoe		
	Porteño	<i>Salmo trutta</i>	1927 ⁴

⁴ Basulto 2003, ⁵ Soto et al. 2007, ⁶ Evelyn Habit pers.comm. n.i. no information available