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1 **Hybridization between native white-spotted charr and nonnative brook trout in**

2 **the upper Sorachi River, Hokkaido, Japan**

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26 Running head: Hybridization between native and nonnative charr

27 Type of paper: Full paper

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29   **Abstract** Invasion status and impacts of nonnative brook trout (*Salvelinus fontinalis*) in

30   a Hokkaido stream were investigated with field surveys and genetic analyses. Nonnative

31   brook trout was detected in nine (41 %) of 22 sampled reaches in three tributaries of the

32   Sorachi River, Hokkaido, Japan. Based on the external pigmentation, twelve putative

33   hybrids between brook trout and native white-spotted charr (*Salvelinus leucomaenis*)

34   were collected in two reaches. Microsatellite and mitochondrial DNA data established

35   that 58% of these hybrids were first generation (F_1) progenies between male brook trout

36   and female white-spotted charr. Our results suggest potential negative impacts of

37   nonnative brook trout on native charr populations in Hokkaido through interspecific

38   interactions.

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40   **Keywords** Brook trout · White-spotted charr · Invasion · Directional hybridization

41

42 **Introduction**

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44 Invasion by nonnative trout is a serious threat to the conservation of freshwater

45 ecosystems (Fuller et al. 1999; Rahel 2002). Rainbow trout (*Oncorhynchus mykiss*),

46 brown trout (*Salmo trutta*), and brook trout (*Salvelinus fontinalis*) are among the most

47 widely introduced fluvial salmonid species worldwide in cool-temperate regions (Elliott

48 1994; Fausch et al. 2001). Evidence suggests these species have negative impacts on

49 native biota through competition, predation, indirect cascade effects, and hybridization

50 (Fausch 1988, 2007; Leary et al. 1993; Townsend 1996; Baxter et al. 2004).

51   In Hokkaido, northern Japan, rainbow trout originated from western North

52 America and brown trout from Europe have rapidly expanded their distributions during

53 the last four decades by both human-mediated introductions and natural dispersal

54 (Takami and Aoyama 1999; Arai et al. 2002). Concerns have consequently emerged

55 over the impacts of rainbow and brown trout on native salmonids, including masu

56 salmon (*Oncorhynchus masou*), white-spotted charr (*Salvelinus leucomaenis*), Dolly

57 Varden (*Salvelinus malma*), and Sakhalin taimen *(Parahucho perri)* (Kitano 2004;

58 Nomoto et al. 2010; Hasegawa et al. 2012a, 2012b). Previous studies have demonstrated

59 competition or niche segregation between masu salmon and rainbow trout (Taniguchi et

60 al. 2000, 2002; Inoue et al. 2009; Hasegawa et al. 2010) and replacement of

61 white-spotted charr by brown trout (Takami et al. 2002; Morita et al. 2004; Hasegawa

62 and Maekawa 2009).

63   Stocking of brook trout in Japan is not as popular and expansion is less evident

64 when compared to rainbow or brown trout (Kitano 2004); yet brook trout originated

65 from eastern North America could be one of concerned invasive species in headwater

66 drainages because of their short life cycle, wider habitat preference, and tendency to

67 overpopulate small streams (Scott and Crossman 1973). In western North America,

68 introduced brook trout have displaced native cutthroat trout (*Oncorhynchus clarki*)

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69   through interspecific competition after the widespread establishment of reproducing

70   populations in headwater streams and lakes (Griffith 1988; Dunham et al. 2002;

71   Benjamin et al. 2007). Moreover, hybridization between native bull trout (*Salvelinus*

72   *confluentus*) and introduced brook trout occurs over a wide geographic area in the

73   western North America (Kanda et al. 2002). For example, Leary et al (1993) described a

74   rapid and almost complete displacement of native bull trout by introduced brook trout in

75   Montana streams, in which initial phases were characterized by frequent hybridization.

76   Although this is not introgressive hybridization forming hybrid swarms mainly due to

77   low fertility of F_1 hybrids, wasted reproductive potential can promote   displacement of

78   bull trout by brook trout since hybridization tends to occur predominantly between

79   female bull trout and male brook trout (Leary et al. 1993, 1995; Kanda et al. 2002). Also

80   in Japanese headwater streams, introduced brook trout and white-spotted charr hybrids

81   have been documented based on appearance in streams in Honshu, central Japan

82   (Suzuki and Kato 1966; EAGJ 1982). Little is known about mechanism of hybridization

83   between these two species due to lacking of genetic analysis, but we should pay

84   attention to interspecific hybridization when brook trout invaded into native charr

85   habitats in Japan.

86   In the present study, we focus on the invasion of brook trout and their potential

87   impacts on native white-spotted charr in upper reaches of the Sorachi River, Hokkaido

88   where brook trout have already been documented (Kondo et al. 2000). We predicted that

89   interspecific hybrids between brook trout and white-spotted charr should be present

90   within this river and utilized genetic analyses to verify putative hybrids identified by

91   external appearance.

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94   **Materials and methods**

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96   *Study area.* The study was conducted during 24–27 June, 2003 at three tributaries of

97   upper Sorachi River in the Sorachi district in central Hokkaido, Japan (Fig. 1).

98   Twenty-two study sites (reach length: ca. 50–100 m) were established over the three

99   streams to investigate fish distribution and abundance. Streams at the study sites were

100   generally small (first to third order), with low to moderate channel gradient (Table 1).

101   The upper Sorachi River is inhabited by white-spotted charr, Dolly Varden, brook trout,

102   rainbow trout, Sakhalin taimen, crucian carp (*Carassius auratus langsdorfii*), Siberian

103   stone loach (*Noemacheilus barbatula toni*), sculpin (*Cottus nozawae*) and brook

104   lamprey (*Lethenteron reissneri*).

105   *Field surveys.* We made one pass electrofishing to estimate the relative

106   abundance of fish using an electrofishing unit (Model 12 Backpack Electrofisher,

107   Smith-Root Inc.). The captured fish were identified to species based on appearance

108   (Nakabo 2000) and standard lengths were measured. Individuals with ambiguous wavy

109   lines on the dorsal fin were marked as putative hybrids between brook trout and

110   white-spotted charr (Suzuki and Fukuda, 1973; Fig. 2). For *Salvelinus* spp. which were

111   well known for interspecific hybridization (e.g., Suzuki and Fukuda 1974; Leary et al.

112   1993), fin clips (less than several square millimeters) were preserved in 99 % ethanol

113   for subsequent DNA analyses.

114   We recorded physical environmental variables (water temperature, reach length,

115   channel width, a maximum depth, and dominant substrate) by measuring at the center of

116   each reach. The channel gradient, expressed as percent change in relative height to

117   reach length, was estimated on 1:25,000 topography maps (Published by Geographical

118   survey institute, government of Japan) by measuring stream length at 10m-incremental

119   changes in elevation.

120   *Genetic analyses.* Total genomic DNA of fish were isolated from fin tissue by

121   Proteinase K/SDS digestion at 55 $^{\circ}$C, and followed by phenol-chloroform extractions.

122   Samples were precipitated in 2.5 volumes of EtOH and 0.1 volume of 2 M NaCl.

123   We used three microsatellite loci (SFO-12, SSA-197, MST-85), which are

124   expected to be diagnostic between brook trout and white-spotted charr, to determine

125   nuclear DNA ancestry of putative hybrids (Angers et al. 1995; O'Relly et al. 1996;

126   Presa and Guyomard 1996; Angers and Bernatchez 1998). Microsatellite amplification

127   was performed on a thermal cycler (Perkin-Elmer Inc.) in a 10 μl reaction containing 50

128   mM KCl, 1.5 mM MgCl$_2$, 10 mM Tris-HCl (pH 8.3), 0.2 mM dNTP, 0.5μM of each

129   primer and 0.25 units of *Taq* DNA polymerase. Microsatellites were analyzed on an ABI

130   310 (Applied Biosystems) automated sequencer. Scoring of allele sizes was performed

131   using Genescan version 2.1 and Genotyper version 2.0 (Applies Biosystems), with

132   reference to the internal standard. We included a reference white-spotted charr sample

133   with known allelic sizes on all runs.

134   PCR-RFLP of NADH dehydrogenase 1 region (ND1: ca. 2,000 bp) of

135   mitochondrial DNA were used for determining maternal ancestry of putative hybrids

136   (Cronin et al. 1993). The ND1 region was chosen because it is expected to have a less

137   intraspecific variation when compared to other mtDNA regions (see Kanda and

138   Allendorf 2001). Amplifications were in 20-μL reaction mixtures containing 50 mM

139   KCl, 1.5 mM MgCl$_2$, 10 mM Tris-HCl (pH 8.3), 0.2 mM dNTP, 0.5 units of *Taq* DNA

140   polymerase. The PCR profile consisted of 95 $^{\circ}$C, 9 min followed by 30 cycles of

141   denaturation (94 $^{\circ}$C, 0.5 min), annealing (55 $^{\circ}$C, 0.5 min) and extension (72 $^{\circ}$C, 2 min).

142   Amplified segments were initially screened for variation with 8 different enzymes:

143   *Hinf*I, *Hpa*II, *Hae*III, *Xba*I, *Taq*I, *Alu*I, *Afa*I (*Rsa*I), and *Dde*I. Digests were performed in

144   12-μL of PCR product and 2–3 units of restriction enzymes. Digested fragments were

145   separated with 6 % polyacrylamide gels and ethidium bromide staining then visualized

146   by ultraviolet transillumination. Based on the results of an initial screening of baseline

147   samples of *Salvelinus leucomaenis* and *S. fontinalis*, *Taq*I was selected as the restriction

148   enzyme with highest interspecific resolution. *Taq*I digested ND1 into six segments (657,

149   525, 510, 208, 90, and 21 bp) for brook trout (DDBJ: AF154850) and six segments (ca.

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150   925, 510, 420, 110, 80, and 20 bp) for white-spotted charr.

151   The software NewHybrids (Anderson and Thompson 2002) was used to

152   estimate the posterior probabilities that each individual belongs to one of six genotypic

153   classes: two parental (P_0, P_1), first generation hybrids (F_1), second generation hybrids

154   (F_2), backcrosses of F_1 with the first parental (B_0), backcrosses of F_1 with the second

155   parental (B_1). Software parameters were set as follows: without individual or allele

156   frequency prior information and independent of "Jeffreys-like" or "Uniform" priors for

157   both mixing proportions and allele frequencies (posterior probabilities were not affected

158   by these priors). Posterior distributions were evaluated after discarding an initial

159   "burnin" of 25,000 sweeps and 10^5 iterations of the Monte Carlo Markov Chain.

160   Individuals were assigned to the class with the highest posterior probability. Individuals

161   with probability under 0.9 were not assigned.

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163

164   **Results**

165

166   **Fish distribution and abundance.** Eight fish species and 12 putative interspecific

167   hybrid individuals were caught in this study (Table 1). Salmonid fish were major taxa

168   occurring in 19 study sites, and native white-spotted charr was the predominant

169   salmonid fish occurring in 13 (59 %) of 22 sites. Dolly Varden was caught in only one

170   headwater site. Nonnative trout were common, brook trout occurred in nine (41 %) sites

171   and rainbow trout in six (27 %) sites. Five (23 %) study sites were comprised only of

172   nonnative salmonids, and in eight (36 %) sites nonnative salmonids were sympatric with

173   native charr. The sites with highest occurrence of brook trout were steeper in gradient

174   ($F_{1,11} = 7.81$, $P = 0.02$ by ANOVA for arcsin-square-root-transformed gradient) than

175   those of rainbow trout. The density of white-spotted charr was not significantly

176   correlated with that of brook trout (Pearson's $r = -0.31$, $P > 0.05$) nor with that of

177   rainbow trout ($r = -0.029$, $P > 0.05$) among 17 sites (F1–3, F5, N1–4, N6–11, T1, T3–4)

178   where either of these salmonid fish (SL > 6 cm) was caught. Putative hybrids between

179   white-spotted charr and brook trout were detected in two sites where these two species

180   co-occurred or inhabited closely. Abundant hybrids were found on the site F5 with the

181   highest salmonid density. The benthic fish abundance differed greatly among streams,

182   rather than among the study sites. For example, stream loaches were abundant in the

183   Furebetsu and Nishitappu streams, but uncommon in the Nunobe stream. Similarly,

184   sculpin commonly occurred in the Nunobe and Nishitappu streams but not in the

185   Furebetsu. Negative effects of invasive nonnative trout on the benthic fish were not

186   clear.

187   Body length distribution of white-spotted charr and brook trout was bimodal

188   with fry (age-0: 4–6 cm in standard length, SL) and older fish (age > 0: SL > 8 cm),

189   indicating self-reproducing populations (Fig. 3). Body lengths of rainbow trout and

190   putative hybrids also varied among individuals, although age-0 cohorts were not clearly

191   identified for them.

192   **DNA analyses of charr.** Genetic variation at microsatellite loci was assessed in

193   63 individuals with >10 cm SL identified by appearance as white-spotted charr, brook

194   trout, and putative hybrids. All three microsatellite loci were polymorphic and variable

195   in both white-spotted charr and brook trout with certain differences in allele size

196   distributions [Table 2, Electronic Supplemental Material (EMS) Table S1].

197   Of these individuals, most were assigned to pure white-spotted charr ($n = 23$),

198   pure brook trout ($n = 29$) or F_1 hybrid category ($n = 7$) with a posterior probability

199   higher than 0.9 (Table 2). The remaining 4 individuals (#119, #120, #121 and #127)

200   could not be assigned to a particular class and may present backcrosses or later

201   generation hybrids (Table 3). Among these, only individual #120 (14.7 cm SL) obtained

202   relatively high support $P = 0.72$ for parental brook trout. One small putative hybrid

203   #117 (12.7 cm SL) was assigned to pure white-spotted charr. These may partly due to

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204   phenotypic variability along developmental stage.

205   Of the seven individuals (#1, #118, #122–126) assigned to the F_1 category with

206   high probability ($P \geq 0.9$) by NewHybrids, white-spotted charr was identified as the

207   maternal parent in all cases, i.e., the first generation hybrids were from mating between

208   male brook trout and female white-spotted charr. Variable genotypes of microsatellite

209   markers also indicate that they were not derived from single clutch, though these

210   hybrids were from almost single study site.

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213   **Discussion**

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215   We found a broad zone of brook trout and rainbow trout invasion in the upper Sorachi

216   River in central Hokkaido. Moreover, we report the first instance of interspecific

217   hybridization between introduced brook trout and native white-spotted charr in

218   Hokkaido. Nonnative trout invasions and following interspecific interactions potentially

219   have negatively impacts on native salmonid species.

220   Nonnative brook and rainbow trout were most likely introduced into the

221   Sorachi River area during the 1950's to 1990's for aquaculture. Based on a

222   questionnaire to a local angler's shop, aquaculture escapees of brook trout had

223   successfully established self-reproducing populations in tributaries of Nishitappu

224   Stream as early as the 1980's (M. Yamamoto, personal communication). Rainbow trout

225   might be occasionally stocked by private anglers since we found some had deformed

226   fins, a common occurrence on aquaculture fish. Water temperature, especially maximum

227   summer temperature, is probably the chief factor determining the success in

228   establishment of nonnative trout (e.g., Dunham et al. 2002; Benjamin et al. 2007). The

229   temperatures recorded in this study (10–15 °C) are consequently conducive to survival

230   of nonnative brook and rainbow trout.

231   Although negative relationship was not clearly observed between nonnative

232   trout and native white-spotted charr abundance, the absence of native salmonids in some

233   study sites might result from strong ecological interactions between natives and

234   nonnatives. The potential impacts of nonnative salmonines on native species are widely

235   reported (e.g., Allendorf and Leary 1988; Dunham et al. 2002). Because nonnative

236   salmonids are ecologically very similar to native salmonids, there is a strong potential

237   for common resource requirements (i.e., niche overlap) and for interspecific competition.

238   Interspecific competition is the most widely recognized mechanism of displacement of

239   native cutthroat trout by nonnative brook trout in western North America (Griffith 1988;

240   Dunham et al. 2002). Furthermore, interspecific hybrids between native white-spotted

241   charr and nonnative brook trout were detected, as we had predicted. However, such

242   hybrids were not caught on all co-occur sites. Therefore, ecological factors such as high

243   density, relatively narrow spawning space or time, may play a role in determining the

244   occurrence of interspecific hybridization. Further studies are necessary to examine the

245   relative importance of each effect of competition, predation, pathogen transmission, and

246   hybridization, which may vary in time and space (Taylor et al. 1984).

247   The genetic analyses of three microsatellite loci and ND1 region RFLPs of

248   mtDNA clearly showed interspecific hybridization between white-spotted charr and

249   brook trout, although possibilities of misidentifying individual fish to each criterion

250   were still remained to some extent based on a limited number of genetic marker (e.g.,

251   Allendorf et al. 2001). The hybrids were comprised of abundant F_1, with the near

252   absence of F_2, when we confined to data with reliable identification. This composition

253   may imply that the hybridization is not introgression. Similar interspecific hybridization

254   has been documented between bull trout and introduced brook trout in North America

255   which produce nearly sterile progeny (Leary et al. 1993, 1995; Kanda et al. 2002). This

256   idea would be supported by the experimental data that survival rates of hybrid progenies

257   between brook trout and white-spotted charr decreased with increased generation of

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258 backcrosses (Suzuki and Fukuda 1974). More diagnostic nuclear loci may help with

259 resolution for this hybridization.

260   Directional hybridization has been implicated in the population decline of

261 endangered species (Leary et al. 1993). Because eggs are generally a crucially limited

262 resource for population growth of a species than sperms, it is possible that the

263 population of white-spotted charr suffers more detrimental effects from hybridization

264 due to reduced egg availability. According to the maternally inherited mtDNA analysis,

265 most F_1 hybrids had white-spotted charr mtDNA. This indicates that the detected

266 hybridization between native white-spotted charr and introduced brook trout is

267 unidirectional, with brook trout males mating with female white-spotted charr in the

268 study streams. Directional hybridization can be caused by various pre- and post-mating

269 factors (Taylor 2004). For post-mating factor of salmonid species, it is often observed

270 that progeny of one direction of hybridization displays higher survival than the

271 reciprocal cross (Suzuki and Fukuda 1974). However, since the rates of survival and

272 growth of F_1 hybrids differ little irrespective of parent combination between brook trout

273 and white-spotted charr (Suzuki and Fukuda 1971), post-mating factors would be less

274 important for hybridization between white-spotted charr and brook trout. Pre-mating

275 factors, such as differences in mating tactics, competitive ability, and/or reproductive

276 timing between parental species, may be the strongest determinants of directional

277 hybridization (Wirtz 1999; Taylor 2004). In cases where there are differences in size at

278 maturity between species, sneaking tactics employed by smaller species have been

279 proposed as an explanation for directional hybridization (Baxter et al. 1997; Taylor

280 2004). However, size range of adult fish was quite similar for white-spotted charr and

281 brook trout in the study area, which also indicates that they have similar competitive

282 potential for mate acquisition. The observed directional hybridization may be due to the

283 asynchronous spawning patterns of these species. In a stream in Honshu, white-spotted

284 charr spawn from late October to early November, whereas brook trout spawn from

285 November to December (Uehara and Yoshida 1984). The reproductively active period

286 of males generally begins earlier than that of females in stream charr (e.g., Kitano 1996).

287 The spawning period of brook trout males is more likely to overlap with white-spotted

288 female charr than *vice versa*. Such hybridization processes have also been suggested in

289 other species of salmonids (e.g., Rosenfield et al. 2000; Kitano et al. 2009).

290   Our results indicate the occurrence of hybridization between native

291 white-spotted charr and nonnative brook trout, which may play a role in the

292 displacement of native charr by nonnative trout in Hokkaido streams. Further ecological

293 studies should attempt to reveal mechanisms and impacts of nonnative trout invasion so

294 that managers can develop effective conservation strategies of native endangered

295 species.

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446

447   **Figure Captions**

448

449   **Fig. 1** Study area location in the Sorachi River with relative percent composition of

450   salmonid species (pie chart). Site and species codes correspond to Table 1

451

452   **Fig. 2** Appearances of *Salvelinus fontinalis* (top: 18.4 cm SL), a putative hybrid

453   (middle: 25.3 cm SL), and *Salvelinus leucomaenis* (bottom: 15.3 cm SL)

454

455   **Fig. 3** Size-frequency distribution of salmonid fishes. *OM Oncorhynchus mykiss*, *HB*

456   putative hybrids between *Salvelinus leucomaenis* and *Salvelinus fontinalis*, *SF*

457   *Salvelinus fontinalis*, *SM Salvelinus malma*, *SL Salvelinus leucomaenis*

Table 1 Habitat variables and fish data of each sampling site in Furebetsu stream (F1–5), Nunobe Shimonosawa stream (N1–5), Nunobe main stream (N6-12), and Nishitappu stream (T1–5)

Sites	Elevation (m)	Gradient (%)	Width (m)	Depth (cm)	Substrate	WT (°C)	Salmonids density[a] ($N \cdot 100m^{-2}$)	SM	SL	SF	OM	HB	CA	BT	CN	LR
F1	185	0.8	5.0	80	Boulder/Sand	11.9	0.5		2		2		1	48		1
F2	260	0.8	4.0	50	Pebble/Sand	11.3	1.3		14	1				8		
F3	270	1.5	3.0	60	Boulder/Pebble	11.7	4.5		8					12		
F4	290	4.0	3.0	40	Pebble/Sand	—	0.0		4					1		
F5	320	1.5	2.5	50	Boulder/Pebble	11.5	29.6		22	4		11		19		
N1	260	0.7	3.5	80	Boulder/Sand	11.5	6.9		2	10				15		
N2	290	1.3	3.0	50	Pebble	9.9	2.0			6	2			1	28	1
N3	300	1.0	1.5	40	Pebble/Sand	10.1	3.3			7	1				16	
N4	310	2.0	2.0	40	Pebble/Sand	10.1	6.9			12					12	
N5	300	2.9	1.0	40	Pebble/Sand	11.5	0.0								23	
N6	310	2.0	4.0	40	Boulder/Pebble	10.3	0.5		1						8	
N7	310	1.7	5.0	80	Bedrock/Pebble	12.2	1.3		4			1				
N8	310	1.5	2.0	50	Bedrock/Boulder	18.3	5.0		1	1			11	1	2	
N9	315	1.5	2.0	60	Pebble/Sand	—	2.7		1	1					1	
N10	350	4.0	2.0	20	Pebble	8.5	2.5			5					1	
N11	320	1.5	5.0	80	Boulder/Pebble	15.5	0.4			2				29		
N12	670	6.7	8.0	150	Bedrock/Boulder	11.0	0.1	1								
T1	330	2.2	4.0	40	Bedrock/Pebble	14.3	0.8		2	1				12	3	1
T2	310	7.5	1.0	30	Boulder/Pebble	11.1	0.0							14	2	
T3	330	1.3	2.0	30	Pebble/Sand	10.8	4.2			5				10	6	1
T4	340	1.0	2.5	50	Pebble/Sand	11.1	12.0			9		9		6	13	4
T5	360	1.5	7.0	120	Boulder	9.3	0.0								7	

SM Salvelinus malma, *SL Salvelinus leucomaenis*, *SF Salvelinus fontinalis*, *OM Oncorhyncus mykiss*, *HB* putative hybrids between *SL* and *SF*, *CA Carassius auratus langsdorfii*, *BT Noemacheilus barbatulus toni*, *CN Cottus nozawae*, *LR Lethenteron reissneri*

a An underestimate, because based on number of salmonids (SL > 6 cm) caught by one pass electrofishing

Table 2 Results of hybrid analyses of two salmonid fish (*SL Salvelinus leucomaenis*, *SF Salvelinus fontinalis*) implemented by NewHybrids (Anderson and Thompson 2002), with associated species identification based on appearance

Appearance	Range of microsatellite loci (bp)			No of individuals assigned by NewHybrids			No of individuals with each mtDNA	
	SFO-12	SSA-197	MST-85	Pure *SL*	Pure *SF*	F$_1$ hybrid	*SL*	*SF*
SL	199–235	114–120	138–150	22	0	0	22	0
SF	269–273	146–160	174–190	0	29	0	0	29
Putative hybrids*	199–273	114–158	138–176	1	0	7	8	0

* Four individuals with low posterior probabilities ($P < 0.90$) were not included in this Table

Table 3 Individual posterior probabilities output by NewHybrids which was not able to be assigned to a particular class with $P \geq 0.90$ and mtDNA type

Individual	Output by NewHybrids	mtDNA
#119	$B_{SF} = 0.53$; $F_1 = 0.25$; $F_2 = 0.20$	*SL*
#120	$P_{SF} = 0.72$; $B_{SF} = 0.22$	*SF*
#121	$B_{SF} = 0.46$; $P_{SF} = 0.22$; $F_1 = 0.18$; $F_2 = 0.14$	*SF*
#127	$F_1 = 0.66$; $B_{SF} = 0.22$	*SF*

P_{SF} parental *S. fontinalis*, B_{SF} backcross $F_1 \times$ *S. fontinalis*, F_1 first generation hybrid, F_2 second generation hybrid
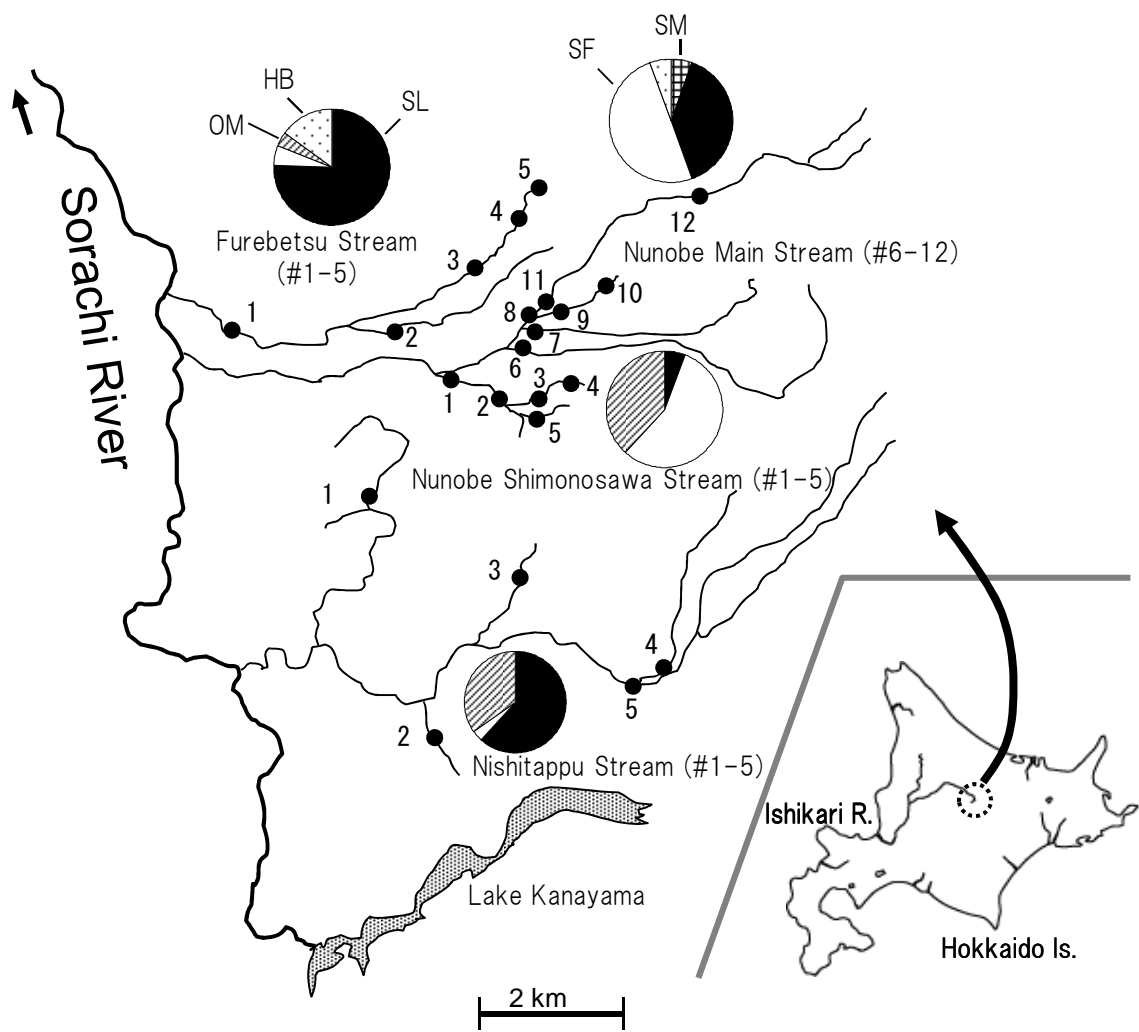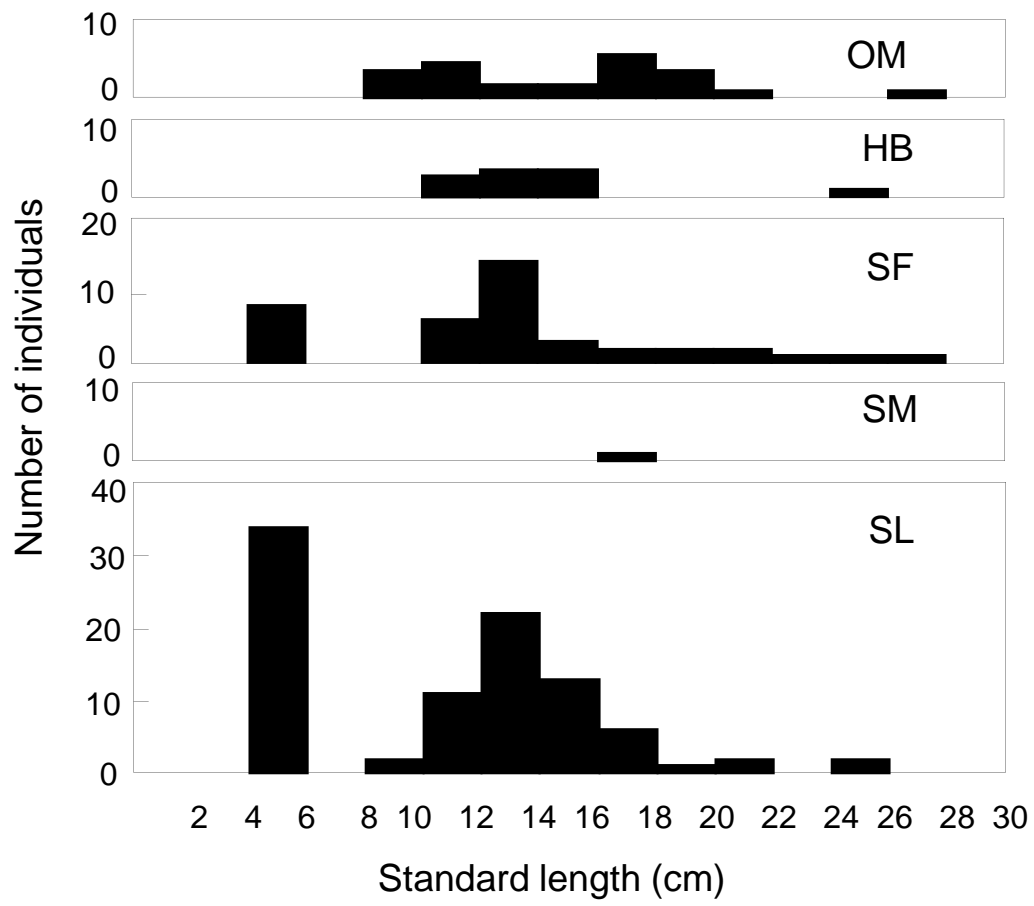
Fig 1 Kitano et al

Fig 2   Kitano et al

Fig 3   Kitano et al