



Title	Hybridization between native white-spotted charr and nonnative brook trout in the upper Sorachi River, Hokkaido, Japan
Author(s)	Kitano, Satoshi; Ohdachi, Satoshi; Koizumi, Itsuro; Hasegawa, Koh
Citation	Ichthyological Research, 61(1), 1-8 https://doi.org/10.1007/s10228-013-0362-y
Issue Date	2013
Doc URL	http://hdl.handle.net/2115/57808
Rights	The final publication is available at link.springer.com
Type	article (author version)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	manuscript.pdf



[Instructions for use](#)

1 **Hybridization between native white-spotted charr and nonnative brook trout in**
2 **the upper Sorachi River, Hokkaido, Japan**

3

4 **Satoshi Kitano · Satoshi Ohdachi · Itsuro Koizumi · Koh Hasegawa**

5

6 S. Kitano (✉)

7 Nagano Environmental Conservation Research Institute, Nagano 381-0075, Japan

8 e-mail: kitano-satoshi@pref.nagano.lg.jp

9

10 S. Ohdachi

11 Institute of Low Temperature Science, Hokkaido University, Kita-ku, Sapporo 060-0819,

12 Japan

13

14 I. Koizumi

15 Faculty of Environmental Earth Science, Hokkaido University, Kita-ku, Sapporo

16 060-0810, Japan

17

18 K. Hasegawa

19 Field Science Center for Northern Biosphere, Hokkaido University,

20 Kita-ku, Sapporo 060-0811, Japan

21

22 K. Hasegawa

23 Present Address: Hokkaido National Fisheries Research Institute, Fisheries Research

24 Agency, Nakanoshima, Toyohira, Sapporo, Hokkaido, 062-0922, Japan

25

26 Running head: Hybridization between native and nonnative charr

27 Type of paper: Full paper

28

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 (F₁) 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.

39

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

41

42 **Introduction**

43

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*)

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 F₁ 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.

92

93

94 **Materials and methods**

95

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 langsdorffi*), 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 °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'Reilly 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 MgCl₂, 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 MgCl₂, 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 °C, 9 min followed by 30 cycles of
141 denaturation (94 °C, 0.5 min), annealing (55 °C, 0.5 min) and extension (72 °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.

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.

162

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 F₁ 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

204 phenotypic variability along developmental stage.

205 Of the seven individuals (#1, #118, #122–126) assigned to the F₁ 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.

211

212

213 **Discussion**

214

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

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 F₁ 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 F₁ 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.

296

297 **Acknowledgments** We are indebted to Shinsaku Shibano, Maki Yamamoto for their
298 support in the field survey. We also thank Matthew Hopken for the linguistic editing and
299 helpful comments of the early manuscript. This study was supported by the grant of F-3
300 and S-2 projects, funded by the Ministry of Environment of Japan.

301

302

303 **References**

304

305 Allendorf FW, Leary RF (1988) Conservation and distribution of genetic variation in a
306 polytypic species, the cutthroat trout. *Conserv Biol* 2:170–184

307 Allendorf FW, Leary RF, Spruell P, Wenburg JK (2001) The problems with hybrids:
308 setting conservation guidelines. *Trends Ecol Evol* 16:613–622

309 Anderson EC, Thompson EA (2002) A model-based method for identifying species
310 hybrids using multilocus genetic data. *Genetics* 160:1217–1229

311 Angers B, Bernatchez L, Angers A, Degroiseillers L (1995) Specific microsatellite loci

312 for brook charr (*Salvelius fontinalis* Mitchill) reveals strong population subdivision
313 on a microgeographic scale. *J Fish Biol* 47:177–185

314 Angers B, Bernatchez L (1998) Combined use of SMM and Non-SMM methods to infer
315 fine structure and evolutionary history of closely related brook charr (*Salvelius*
316 *fontinalis*, Salmonidae) population from microsatellites. *Mol Biol Evol* 15:143–
317 159

318 Arai T, Kotake A, Aoyama T, Hayano H, Miyazaki N (2002) Identifying sea-run brown
319 trout, *Salmo trutta*, using Sr:Ca ratios of otolith. *Ichthyol Res* 49:380–383

320 Baxter CV, Fausch KD, Murakami M, Chapman PL (2004) Fish invasion restructures
321 stream and forest food webs by interrupting reciprocal prey subsidies. *Ecology*
322 85:2656–2663

323 Baxter JS, Taylor EB, Hagen J, McPhail JD (1997) Evidence for natural hybridization
324 between Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*)
325 in a northcentral British Columbia watershed. *Can J Fish Aquat Sci* 54:421–429

326 Benjamin JR, Dunham JB, Dare MR (2007) Invasion by nonnative brook trout in
327 Panther Creek, Idaho: roles of habitat quality, biotic resistance, and connectivity to
328 source habitats. *Trans Amer Fish Soc* 136:875–888

329 Cronin MA, Sperman WJ, Wilmot RL (1993) Mitochondrial DNA variation in Chinook
330 (*Oncorhynchus tshawytscha*) and chum salmon (*O. keta*) detected by restriction
331 enzyme analysis of polymerase chain reaction (PCR) products. *Can J Fish Aquat*
332 *Sci* 50:708–715

333 Dunham JB, Adams SB, Schroeter RE, Novinger DC (2002) Alien invasion in aquatic
334 ecosystems: Toward an understanding of brook trout invasions and potential
335 impacts on inland cutthroat trout in western North America. *Rev Fish Biol Fisher*
336 12:373–391

337 EAGJ (Environmental Agency, Government of Japan) (1982) Report on conservation of
338 charrs in the headwater drainage of Azusa River. Japan Environmental Division,

339 Tokyo

340 Elliott JM (1994) Quantitative Ecology and the Brown Trout. Oxford Univ Press,
341 Oxford

342 Fausch K D (1988) Tests of competition between native and introduced salmonids in
343 streams: what have we learned? *Can J Fish Aquat Sci* 45:2238–2246

344 Fausch KD, Taniguchi Y, Nakano S, Grossman GD, Townsend CR (2001) Flood
345 disturbance regimes influence rainbow trout invasion success among five holarctic
346 regions. *Ecol Appl* 11:1438–1455

347 Fausch KD (2007) Introduction, establishment and effects of non-native salmonids:
348 considering the risk of rainbow trout invasion in the United Kingdom. *J Fish Biol*
349 71:1–32

350 Fuller PL, Nico LG, Williams JD (1999) Nonindigenous fishes introduced into inland
351 waters of the United States. *Amer Fish Soc Spec Publ* 27, Bethesda, Maryland

352 Griffith JS (1988) A review of competition between cutthroat trout and other salmonids.
353 In: Gresswell RE (ed) Status and management of interior stocks of cutthroat trout.
354 *Amer Fish Soc Symp* 4, Bethesda, Maryland, pp 134–140

355 Hasegawa K, Maekawa K (2009) Role of visual barriers on mitigation of interspecific
356 interference competition between native and non-native salmonid species. *Can J*
357 *Zool* 87:781–786

358 Hasegawa K, Yamamoto T, Kitanishi S (2010) Habitat niche separation of the nonnative
359 rainbow trout and native masu salmon in the Atsuta River, Hokkaido Japan. *Fish*
360 *Sci* 76:251–256

361 Hasegawa K, Yamazaki C, Ohta T, Ohkuma K (2012a) Food habits of introduced brown
362 trout and native masu salmon are influenced by seasonal and locational prey
363 availability. *Fish Sci* 78:1163–1171

364 Hasegawa K, Yamazaki C, Ohkuma K, Ban M (2012b) Evidence that ontogenetic shift
365 by native masu salmon facilitates invasion by nonnative brown trout. *Biol*

366 Invasions (22 Mar 2012), pp1–8

367 Inoue M, Miyata H, Tange Y, Taniguchi Y (2009) Rainbow trout (*Oncorhynchus mykiss*)
368 invasion in Hokkaido streams, northern Japan, in relation to flow variability and
369 biotic interactions. *Can J Fish Aquat Sci* 66:1423–1434

370 Kanda N, Allendorf FW (2001) Genetic population structure of bull trout from the
371 Flathead River basin as shown by microsatellites and mitochondrial DNA markers.
372 *Trans Amer Fish Soc* 130:92–106

373 Kanda N, Leary RF, Allendorf FW (2002) Evidence of introgressive hybridization
374 between bull trout and brook trout. *Trans Amer Fish Soc* 131:772–782

375 Kitano S (1996) Size-related factors causing individual variation in seasonal
376 reproductive success of fluvial male Dolly Varden (*Salvelinus malma*). *Ecol*
377 *Freshw Fish* 5:59–67

378 Kitano S (2004) Ecological impacts of rainbow, brown and brook trout in Japanese
379 inland waters. *Glob Environ Res* 8:41-50

380 Kitano S, Hasegawa K, Maekawa K (2009) Evidence for interspecific hybridization
381 between native white-spotted charr and nonnative brown trout on Hokkaido Island,
382 Japan. *J Fish Biol* 74:467–473

383 Koizumi I, Kobayashi H, Maekawa K, Azuma N, Nagase T (2005) Hybridization
384 between endemic Miyabe charr (*Salvelinus malma miyabei*) and introduced masu
385 salmon (*Oncorhynchus masou*) in Shikaribetsu Lake. *Ichthyol Res* 52:83–85

386 Kondo T, Yamada M, Kusano Y, Sakai K (2000) Fish hosts of the freshwater mussel
387 *Margaritifera laevis* (Bivalvia: Margaritiferidae) in the Furebetsu River, Hokkaido.
388 *Venus* 59:177–179

389 Leary RF, Allendorf FW, Forbes SH (1993) Conservation genetics of bull trout in the
390 Columbia and Klamath River drainages. *Cons Biol* 7:856–865

391 Leary RF, Allendorf FW, Sage G (1995) Hybridization and introgression between
392 introduced and native fish. *Amer Fish Soc Symp* 15:91–101

393 Morita K, Tsuboi J, Matsuda H (2004) The impact of exotic trout on native charr in a
394 Japanese stream. *J Appl Ecol* 41:962–972

395 Nakabo, T (ed) (2000) *Fishes of Japan with pictorial keys to the species*, second edition.
396 Tokai University Press, Tokyo

397 Nomoto K, Omiya H, Sugimoto T, Akiba K, Edo K, Higashi S (2010) Potential negative
398 impacts of introduced rainbow trout on endangered Sakhalin taimen through redd
399 disturbance in an agricultural stream, eastern Hokkaido. *Ecol Freshw Fish* 19:116–
400 126

401 O'Reilly PT, Hamilton L, McConnell S, Wright JM (1996) Rapid analysis of genetic
402 variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide
403 and tetranucleotide microsatellites. *Can J Fish Aquat Sci* 53:2292–2298

404 Presa P, Guyomard R (1996) Conservation of microsatellite in three species of
405 salmonids. *J Fish Biol* 49:1326–1329

406 Rahel FJ (2002) Homogenization of freshwater faunas. *Annu Rev Ecol Syst* 33:291–
407 315

408 Rosenfield JA, Todd T, Greil R (2000) Asymmetric hybridization and introgression
409 between Pink salmon and Chinook salmon in the Laurentian Great Lakes. *Trans*
410 *Amer Fish Soc* 129:670–679

411 Scott WB, Crossman EJ (1973) *Freshwater fishes of Canada*. *Bull Fish Res Board*
412 *Canada* 184

413 Suzuki R, Fukuda Y (1971) Survival potential of F₁ hybrids among salmonid fishes.
414 *Bull Freshw Fish Res Lab* 21:69–83

415 Suzuki R, Fukuda Y (1973) Appearance and numerical characters of F₁ hybrids among
416 salmonid fishes. *Bull Freshw Fish Res Lab* 23:5–32

417 Suzuki R, Fukuda Y (1974) Intercrossing and backcrossing of F₁ hybrids among
418 salmonid fishes. *Bull Freshw Fish Res Lab* 24:10–30

419 Suzuki R, Kato T (1966) Hybridization in nature between salmonid fishes, *Salvelinus*
420 *pluvius* × *Salvelinus fontinalis*. Bull Freshw Fish Res Lab 17:83–90

421 Takami T, Aoyama T (1999) Distribution of rainbow trout and brown trout in Hokkaido,
422 Japan. Wildl Cons 4:41–48

423 Takami T, Yoshihara T, Miyakoshi Y, Kuwabara R (2002) Replacement of the
424 white-spotted charr *Salvelinus leucomaenis* by brown trout *Salmo trutta* in a
425 branch of the Chitose River, Hokkaido. Nippon Suisan Gakkaishi 68:24–28

426 Taniguchi Y, Miyake Y, Saito T, Urabe H, Nakano S (2000) Redd superimposition by
427 introduced rainbow trout on native charrs in a Japanese stream. Ichthyol Res
428 47:149–156

429 Taniguchi Y, Fausch KD, Nakano S (2002) Size structured interactions between native
430 and introduced species: can intraguild predation facilitate invasion by stream
431 salmonids? Biol Invas 4:223–233

432 Taylor EB (2004) Evolution in mixed company: evolutionary inferences from studies of
433 natural hybridization in Salmonidae. In: Hendry AP, Stearns S (eds) Evolution
434 Illuminated. Salmon and their relatives. Oxford University Press, Oxford. pp 232–
435 263

436 Taylor JN, Courtenay WR, McCann JA (1984) Known impact of exotic fishes in the
437 continental United States. In: W. R. Courtenay WR, Stauffer JR (eds) Distribution,
438 Biology, and Management of Exotic Fish. John Hopkins University Press,
439 Baltimore, Maryland, pp 322–373

440 Townsend CR (1996) Invasion biology and ecological impacts of brown trout *Salmo*
441 *trutta* in New Zealand. Biol Cons 78:13–22

442 Uehara T, Yoshida T (1984) Charr in the upper Azusa stream. In: Environmental Agency,
443 Government of Japan (ed) Nature of Kamikochi, Tokyo, pp 83–103

444 Wirtz P (1999) Mother species-father species: unidirectional hybridization in animals
445 with female choice. Anim Behav 58:1–12
446

Figure Captions

447

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·100m ⁻²)	Fish species								
								<i>SM</i>	<i>SL</i>	<i>SF</i>	<i>OM</i>	<i>HB</i>	<i>CA</i>	<i>BT</i>	<i>CN</i>	<i>LR</i>
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* *Oncorhynchus 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 <i>SL</i>	Pure <i>SF</i>	F ₁ hybrid	<i>SL</i>	<i>SF</i>
<i>SL</i>	199–235	114–120	138–150	22	0	0	22	0
<i>SF</i>	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	$B_{SF} = 0.53$; $F_1 = 0.25$; $F_2 = 0.20$	<i>SL</i>
#120	$P_{SF} = 0.72$; $B_{SF} = 0.22$	<i>SF</i>
#121	$B_{SF} = 0.46$; $P_{SF} = 0.22$; $F_1 = 0.18$; $F_2 = 0.14$	<i>SF</i>
#127	$F_1 = 0.66$; $B_{SF} = 0.22$	<i>SF</i>

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

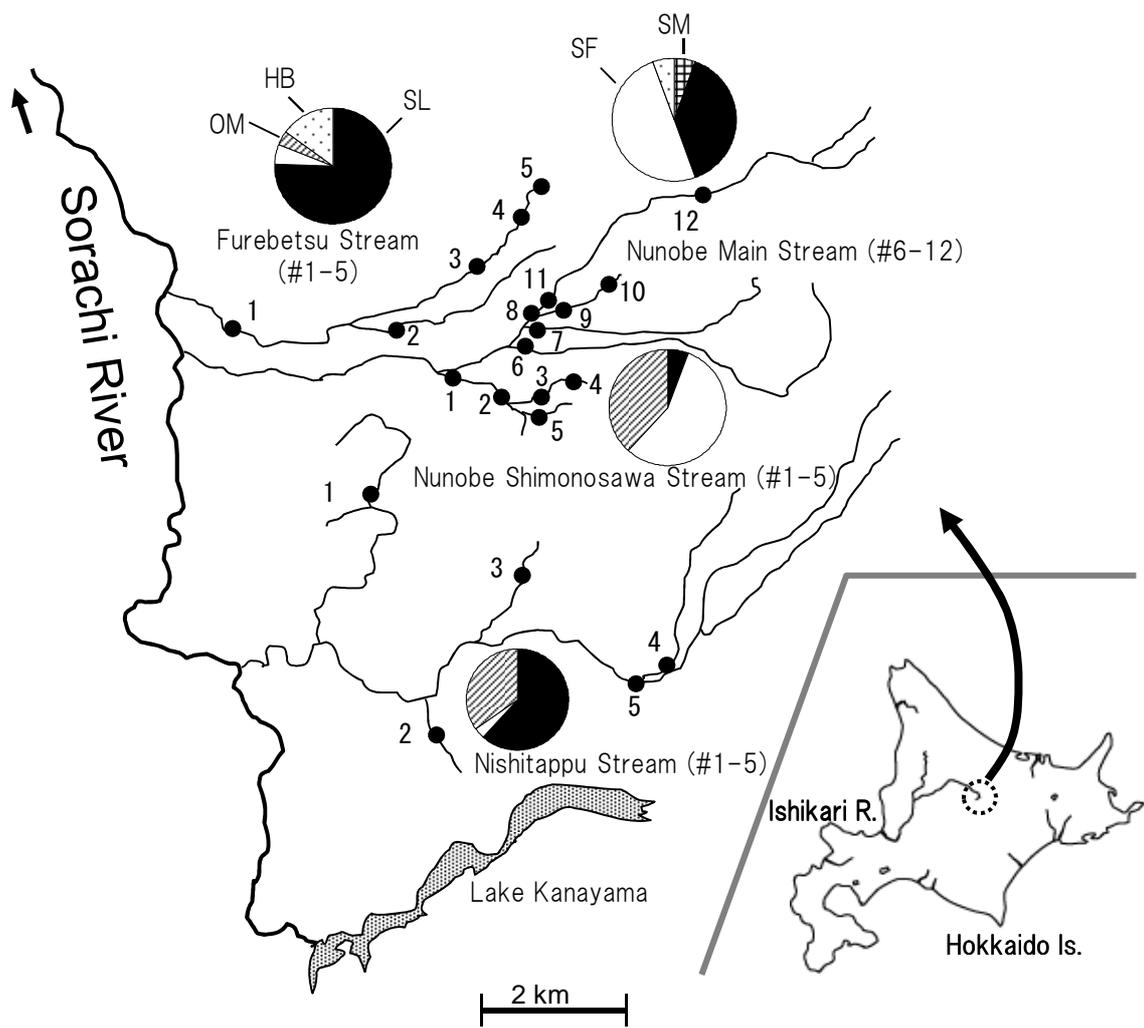


Fig 1 Kitano et al



Fig 2 Kitano et al

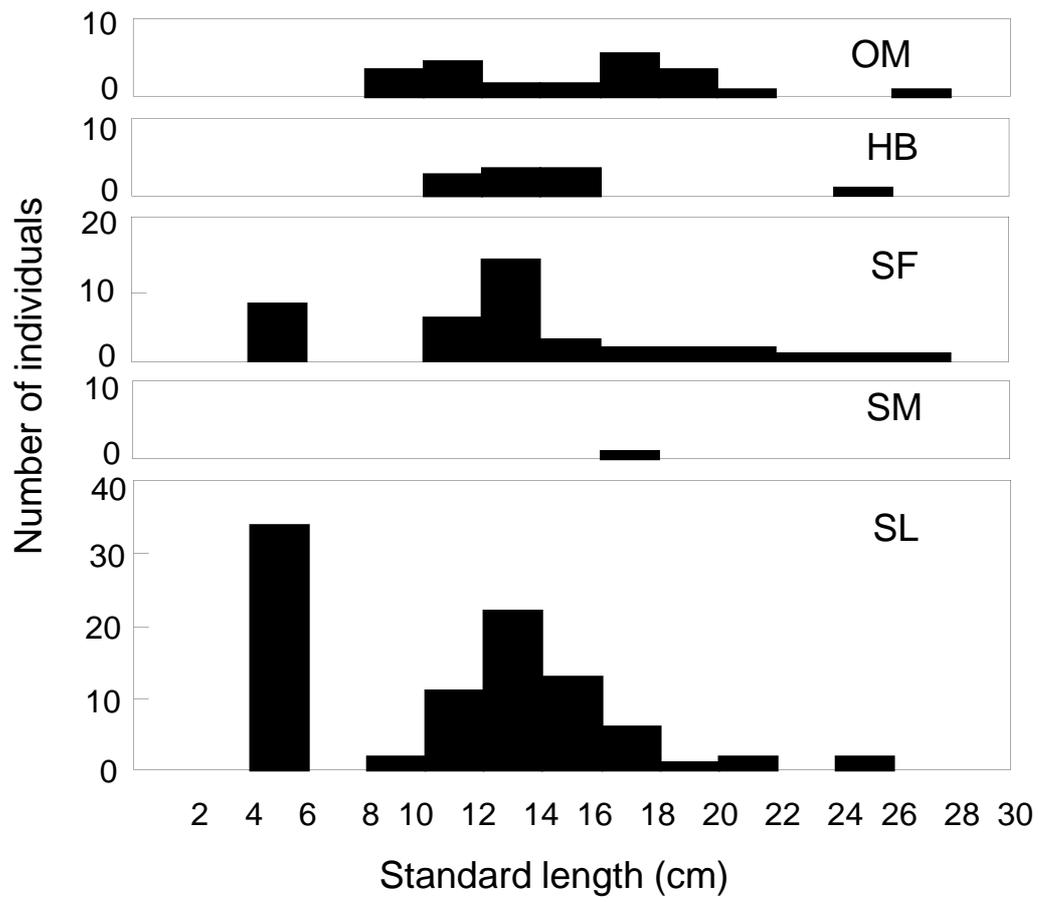


Fig 3 Kitano et al