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](#)

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

Satoshi Kitano · Satoshi Ohdachi · Itsuro Koizumi · Koh Hasegawa

S. Kitano (✉)

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

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

S. Ohdachi

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

I. Koizumi

Faculty of Environmental Earth Science, Hokkaido University, Kita-ku, Sapporo
060-0810, Japan

K. Hasegawa

Field Science Center for Northern Biosphere, Hokkaido University,
Kita-ku, Sapporo 060-0811, Japan

K. Hasegawa

Present Address: Hokkaido National Fisheries Research Institute, Fisheries Research
Agency, Nakanoshima, Toyohira, Sapporo, Hokkaido, 062-0922, Japan

Running head: Hybridization between native and nonnative charr

Type of paper: Full paper

Abstract Invasion status and impacts of nonnative brook trout (*Salvelinus fontinalis*) in a Hokkaido stream were investigated with field surveys and genetic analyses. Nonnative brook trout was detected in nine (41 %) of 22 sampled reaches in three tributaries of the Sorachi River, Hokkaido, Japan. Based on the external pigmentation, twelve putative hybrids between brook trout and native white-spotted charr (*Salvelinus leucomaenis*) were collected in two reaches. Microsatellite and mitochondrial DNA data established that 58% of these hybrids were first generation (F₁) progenies between male brook trout and female white-spotted charr. Our results suggest potential negative impacts of nonnative brook trout on native charr populations in Hokkaido through interspecific interactions.

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

Introduction

Invasion by nonnative trout is a serious threat to the conservation of freshwater ecosystems (Fuller et al. 1999; Rahel 2002). Rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), and brook trout (*Salvelinus fontinalis*) are among the most widely introduced fluvial salmonid species worldwide in cool-temperate regions (Elliott 1994; Fausch et al. 2001). Evidence suggests these species have negative impacts on native biota through competition, predation, indirect cascade effects, and hybridization (Fausch 1988, 2007; Leary et al. 1993; Townsend 1996; Baxter et al. 2004).

In Hokkaido, northern Japan, rainbow trout originated from western North America and brown trout from Europe have rapidly expanded their distributions during the last four decades by both human-mediated introductions and natural dispersal (Takami and Aoyama 1999; Arai et al. 2002). Concerns have consequently emerged over the impacts of rainbow and brown trout on native salmonids, including masu salmon (*Oncorhynchus masou*), white-spotted charr (*Salvelinus leucomaenis*), Dolly Varden (*Salvelinus malma*), and Sakhalin taimen (*Parahucho perri*) (Kitano 2004; Nomoto et al. 2010; Hasegawa et al. 2012a, 2012b). Previous studies have demonstrated competition or niche segregation between masu salmon and rainbow trout (Taniguchi et al. 2000, 2002; Inoue et al. 2009; Hasegawa et al. 2010) and replacement of white-spotted charr by brown trout (Takami et al. 2002; Morita et al. 2004; Hasegawa and Maekawa 2009).

Stocking of brook trout in Japan is not as popular and expansion is less evident when compared to rainbow or brown trout (Kitano 2004); yet brook trout originated from eastern North America could be one of concerned invasive species in headwater drainages because of their short life cycle, wider habitat preference, and tendency to overpopulate small streams (Scott and Crossman 1973). In western North America, introduced brook trout have displaced native cutthroat trout (*Oncorhynchus clarki*)

through interspecific competition after the widespread establishment of reproducing populations in headwater streams and lakes (Griffith 1988; Dunham et al. 2002; Benjamin et al. 2007). Moreover, hybridization between native bull trout (*Salvelinus confluentus*) and introduced brook trout occurs over a wide geographic area in the western North America (Kanda et al. 2002). For example, Leary et al (1993) described a rapid and almost complete displacement of native bull trout by introduced brook trout in Montana streams, in which initial phases were characterized by frequent hybridization. Although this is not introgressive hybridization forming hybrid swarms mainly due to low fertility of F₁ hybrids, wasted reproductive potential can promote displacement of bull trout by brook trout since hybridization tends to occur predominantly between female bull trout and male brook trout (Leary et al. 1993, 1995; Kanda et al. 2002). Also in Japanese headwater streams, introduced brook trout and white-spotted charr hybrids have been documented based on appearance in streams in Honshu, central Japan (Suzuki and Kato 1966; EAGJ 1982). Little is known about mechanism of hybridization between these two species due to lacking of genetic analysis, but we should pay attention to interspecific hybridization when brook trout invaded into native charr habitats in Japan.

In the present study, we focus on the invasion of brook trout and their potential impacts on native white-spotted charr in upper reaches of the Sorachi River, Hokkaido where brook trout have already been documented (Kondo et al. 2000). We predicted that interspecific hybrids between brook trout and white-spotted charr should be present within this river and utilized genetic analyses to verify putative hybrids identified by external appearance.

Materials and methods

106 *Study area.* The study was conducted during 24–27 June, 2003 at three tributaries of
107 upper Sorachi River in the Sorachi district in central Hokkaido, Japan (Fig. 1).
108 Twenty-two study sites (reach length: ca. 50–100 m) were established over the three
109 streams to investigate fish distribution and abundance. Streams at the study sites were
110 generally small (first to third order), with low to moderate channel gradient (Table 1).
111 The upper Sorachi River is inhabited by white-spotted charr, Dolly Varden, brook trout,
112 rainbow trout, Sakhalin taimen, crucian carp (*Carassius auratus langsdorffii*), Siberian
113 stone loach (*Noemacheilus barbatula toni*), sculpin (*Cottus nozawae*) and brook
114 lamprey (*Lethenteron reissneri*).

115 *Field surveys.* We made one pass electrofishing to estimate the relative
116 abundance of fish using an electrofishing unit (Model 12 Backpack Electrofisher,
117 Smith-Root Inc.). The captured fish were identified to species based on appearance
118 (Nakabo 2000) and standard lengths were measured. Individuals with ambiguous wavy
119 lines on the dorsal fin were marked as putative hybrids between brook trout and
120 white-spotted charr (Suzuki and Fukuda, 1973; Fig. 2). For *Salvelinus* spp. which were
121 well known for interspecific hybridization (e.g., Suzuki and Fukuda 1974; Leary et al.
122 1993), fin clips (less than several square millimeters) were preserved in 99 % ethanol
for subsequent DNA analyses.

123 We recorded physical environmental variables (water temperature, reach length,
124 channel width, a maximum depth, and dominant substrate) by measuring at the center of
125 each reach. The channel gradient, expressed as percent change in relative height to
126 reach length, was estimated on 1:25,000 topography maps (Published by Geographical
127 survey institute, government of Japan) by measuring stream length at 10m-incremental
128 changes in elevation.

129 *Genetic analyses.* Total genomic DNA of fish were isolated from fin tissue by
130 Proteinase K/SDS digestion at 55 °C, and followed by phenol-chloroform extractions.
131 Samples were precipitated in 2.5 volumes of EtOH and 0.1 volume of 2 M NaCl.

We used three microsatellite loci (SFO-12, SSA-197, MST-85), which are expected to be diagnostic between brook trout and white-spotted charr, to determine nuclear DNA ancestry of putative hybrids (Angers et al. 1995; O'Relly et al. 1996; Presa and Guyomard 1996; Angers and Bernatchez 1998). Microsatellite amplification was performed on a thermal cycler (Perkin-Elmer Inc.) in a 10 µl reaction containing 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris-HCl (pH 8.3), 0.2 mM dNTP, 0.5µM of each primer and 0.25 units of *Taq* DNA polymerase. Microsatellites were analyzed on an ABI 310 (Applied Biosystems) automated sequencer. Scoring of allele sizes was performed using Genescan version 2.1 and Genotyper version 2.0 (Applies Biosystems), with reference to the internal standard. We included a reference white-spotted charr sample with known allelic sizes on all runs.

PCR-RFLP of NADH dehydrogenase 1 region (ND1: ca. 2,000 bp) of mitochondrial DNA were used for determining maternal ancestry of putative hybrids (Cronin et al. 1993). The ND1 region was chosen because it is expected to have a less intraspecific variation when compared to other mtDNA regions (see Kanda and Allendorf 2001). Amplifications were in 20-µL reaction mixtures containing 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris-HCl (pH 8.3), 0.2 mM dNTP, 0.5 units of *Taq* DNA polymerase. The PCR profile consisted of 95 °C, 9 min followed by 30 cycles of denaturation (94 °C, 0.5 min), annealing (55 °C, 0.5 min) and extension (72 °C, 2 min). Amplified segments were initially screened for variation with 8 different enzymes: *Hinf*I, *Hpa*II, *Hae*III, *Xba*I, *Taq*I, *Alu*I, *Afa*I (*Rsa*I), and *Dde*I. Digests were performed in 12-µL of PCR product and 2–3 units of restriction enzymes. Digested fragments were separated with 6 % polyacrylamide gels and ethidium bromide staining then visualized by ultraviolet transillumination. Based on the results of an initial screening of baseline samples of *Salvelinus leucomaenis* and *S. fontinalis*, *Taq*I was selected as the restriction enzyme with highest interspecific resolution. *Taq*I digested ND1 into six segments (657, 525, 510, 208, 90, and 21 bp) for brook trout (DDBJ: AF154850) and six segments (ca.

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

The software NewHybrids (Anderson and Thompson 2002) was used to estimate the posterior probabilities that each individual belongs to one of six genotypic classes: two parental (P_0 , P_1), first generation hybrids (F_1), second generation hybrids (F_2), backcrosses of F_1 with the first parental (B_0), backcrosses of F_1 with the second parental (B_1). Software parameters were set as follows: without individual or allele frequency prior information and independent of “Jeffreys-like” or “Uniform” priors for both mixing proportions and allele frequencies (posterior probabilities were not affected by these priors). Posterior distributions were evaluated after discarding an initial “burnin” of 25,000 sweeps and 10^5 iterations of the Monte Carlo Markov Chain. Individuals were assigned to the class with the highest posterior probability. Individuals with probability under 0.9 were not assigned.

Results

Fish distribution and abundance. Eight fish species and 12 putative interspecific hybrid individuals were caught in this study (Table 1). Salmonid fish were major taxa occurring in 19 study sites, and native white-spotted charr was the predominant salmonid fish occurring in 13 (59 %) of 22 sites. Dolly Varden was caught in only one headwater site. Nonnative trout were common, brook trout occurred in nine (41 %) sites and rainbow trout in six (27 %) sites. Five (23 %) study sites were comprised only of nonnative salmonids, and in eight (36 %) sites nonnative salmonids were sympatric with native charr. The sites with highest occurrence of brook trout were steeper in gradient ($F_{1,11} = 7.81$, $P = 0.02$ by ANOVA for arcsin-square-root-transformed gradient) than those of rainbow trout. The density of white-spotted charr was not significantly correlated with that of brook trout (Pearson’s $r = -0.31$, $P > 0.05$) nor with that of

rainbow trout ($r = -0.029$, $P > 0.05$) among 17 sites (F1–3, F5, N1–4, N6–11, T1, T3–4) where either of these salmonid fish (SL > 6 cm) was caught. Putative hybrids between white-spotted charr and brook trout were detected in two sites where these two species co-occurred or inhabited closely. Abundant hybrids were found on the site F5 with the highest salmonid density. The benthic fish abundance differed greatly among streams, rather than among the study sites. For example, stream loaches were abundant in the Furebetsu and Nishitappu streams, but uncommon in the Nunobe stream. Similarly, sculpin commonly occurred in the Nunobe and Nishitappu streams but not in the Furebetsu. Negative effects of invasive nonnative trout on the benthic fish were not clear.

Body length distribution of white-spotted charr and brook trout was bimodal with fry (age-0: 4–6 cm in standard length, SL) and older fish (age > 0: SL > 8 cm), indicating self-reproducing populations (Fig. 3). Body lengths of rainbow trout and putative hybrids also varied among individuals, although age-0 cohorts were not clearly identified for them.

DNA analyses of charr. Genetic variation at microsatellite loci was assessed in 63 individuals with >10 cm SL identified by appearance as white-spotted charr, brook trout, and putative hybrids. All three microsatellite loci were polymorphic and variable in both white-spotted charr and brook trout with certain differences in allele size distributions [Table 2, Electronic Supplemental Material (EMS) Table S1].

Of these individuals, most were assigned to pure white-spotted charr ($n = 23$), pure brook trout ($n = 29$) or F_1 hybrid category ($n = 7$) with a posterior probability higher than 0.9 (Table 2). The remaining 4 individuals (#119, #120, #121 and #127) could not be assigned to a particular class and may present backcrosses or later generation hybrids (Table 3). Among these, only individual #120 (14.7 cm SL) obtained relatively high support $P = 0.72$ for parental brook trout. One small putative hybrid #117 (12.7 cm SL) was assigned to pure white-spotted charr. These may partly due to

phenotypic variability along developmental stage.

Of the seven individuals (#1, #118, #122–126) assigned to the F₁ category with high probability ($P \geq 0.9$) by NewHybrids, white-spotted charr was identified as the maternal parent in all cases, i.e., the first generation hybrids were from mating between male brook trout and female white-spotted charr. Variable genotypes of microsatellite markers also indicate that they were not derived from single clutch, though these hybrids were from almost single study site.

Discussion

We found a broad zone of brook trout and rainbow trout invasion in the upper Sorachi River in central Hokkaido. Moreover, we report the first instance of interspecific hybridization between introduced brook trout and native white-spotted charr in Hokkaido. Nonnative trout invasions and following interspecific interactions potentially have negatively impacts on native salmonid species.

Nonnative brook and rainbow trout were most likely introduced into the Sorachi River area during the 1950's to 1990's for aquaculture. Based on a questionnaire to a local angler's shop, aquaculture escapees of brook trout had successfully established self-reproducing populations in tributaries of Nishitappu Stream as early as the 1980's (M. Yamamoto, personal communication). Rainbow trout might be occasionally stocked by private anglers since we found some had deformed fins, a common occurrence on aquaculture fish. Water temperature, especially maximum summer temperature, is probably the chief factor determining the success in establishment of nonnative trout (e.g., Dunham et al. 2002; Benjamin et al. 2007). The temperatures recorded in this study (10–15 °C) are consequently conducive to survival of nonnative brook and rainbow trout.

Although negative relationship was not clearly observed between nonnative trout and native white-spotted charr abundance, the absence of native salmonids in some study sites might result from strong ecological interactions between natives and nonnatives. The potential impacts of nonnative salmonines on native species are widely reported (e.g., Allendorf and Leary 1988; Dunham et al. 2002). Because nonnative salmonids are ecologically very similar to native salmonids, there is a strong potential for common resource requirements (i.e., niche overlap) and for interspecific competition. Interspecific competition is the most widely recognized mechanism of displacement of native cutthroat trout by nonnative brook trout in western North America (Griffith 1988; Dunham et al. 2002). Furthermore, interspecific hybrids between native white-spotted charr and nonnative brook trout were detected, as we had predicted. However, such hybrids were not caught on all co-occur sites. Therefore, ecological factors such as high density, relatively narrow spawning space or time, may play a role in determining the occurrence of interspecific hybridization. Further studies are necessary to examine the relative importance of each effect of competition, predation, pathogen transmission, and hybridization, which may vary in time and space (Taylor et al. 1984).

The genetic analyses of three microsatellite loci and ND1 region RFLPs of mtDNA clearly showed interspecific hybridization between white-spotted charr and brook trout, although possibilities of misidentifying individual fish to each criterion were still remained to some extent based on a limited number of genetic marker (e.g., Allendorf et al. 2001). The hybrids were comprised of abundant F_1 , with the near absence of F_2 , when we confined to data with reliable identification. This composition may imply that the hybridization is not introgression. Similar interspecific hybridization has been documented between bull trout and introduced brook trout in North America which produce nearly sterile progeny (Leary et al. 1993, 1995; Kanda et al. 2002). This idea would be supported by the experimental data that survival rates of hybrid progenies between brook trout and white-spotted charr decreased with increased generation of

backcrosses (Suzuki and Fukuda 1974). More diagnostic nuclear loci may help with resolution for this hybridization.

Directional hybridization has been implicated in the population decline of endangered species (Leary et al. 1993). Because eggs are generally a crucially limited resource for population growth of a species than sperms, it is possible that the population of white-spotted charr suffers more detrimental effects from hybridization due to reduced egg availability. According to the maternally inherited mtDNA analysis, most F₁ hybrids had white-spotted charr mtDNA. This indicates that the detected hybridization between native white-spotted charr and introduced brook trout is unidirectional, with brook trout males mating with female white-spotted charr in the study streams. Directional hybridization can be caused by various pre- and post-mating factors (Taylor 2004). For post-mating factor of salmonid species, it is often observed that progeny of one direction of hybridization displays higher survival than the reciprocal cross (Suzuki and Fukuda 1974). However, since the rates of survival and growth of F₁ hybrids differ little irrespective of parent combination between brook trout and white-spotted charr (Suzuki and Fukuda 1971), post-mating factors would be less important for hybridization between white-spotted charr and brook trout. Pre-mating factors, such as differences in mating tactics, competitive ability, and/or reproductive timing between parental species, may be the strongest determinants of directional hybridization (Wirtz 1999; Taylor 2004). In cases where there are differences in size at maturity between species, sneaking tactics employed by smaller species have been proposed as an explanation for directional hybridization (Baxter et al. 1997; Taylor 2004). However, size range of adult fish was quite similar for white-spotted charr and brook trout in the study area, which also indicates that they have similar competitive potential for mate acquisition. The observed directional hybridization may be due to the asynchronous spawning patterns of these species. In a stream in Honshu, white-spotted charr spawn from late October to early November, whereas brook trout spawn from

November to December (Uehara and Yoshida 1984). The reproductively active period of males generally begins earlier than that of females in stream charr (e.g., Kitano 1996). The spawning period of brook trout males is more likely to overlap with white-spotted female charr than *vice versa*. Such hybridization processes have also been suggested in other species of salmonids (e.g., Rosenfield et al. 2000; Kitano et al. 2009).

Our results indicate the occurrence of hybridization between native white-spotted charr and nonnative brook trout, which may play a role in the displacement of native charr by nonnative trout in Hokkaido streams. Further ecological studies should attempt to reveal mechanisms and impacts of nonnative trout invasion so that managers can develop effective conservation strategies of native endangered species.

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

References

- Allendorf FW, Leary RF (1988) Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conserv Biol* 2:170–184
- Allendorf FW, Leary RF, Spruell P, Wenburg JK (2001) The problems with hybrids: setting conservation guidelines. *Trends Ecol Evol* 16:613–622
- Anderson EC, Thompson EA (2002) A model-based method for identifying species hybrids using multilocus genetic data. *Genetics* 160:1217–1229
- Angers B, Bernatchez L, Angers A, Degroiseillers L (1995) Specific microsatellite loci

312 for brook charr (*Salvelius fontinalis* Mitchell) 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

Fig. 1 Study area location in the Sorachi River with relative percent composition of salmonid species (pie chart). Site and species codes correspond to Table 1

Fig. 2 Appearances of *Salvelinus fontinalis* (top: 18.4 cm SL), a putative hybrid (middle: 25.3 cm SL), and *Salvelinus leucomaenis* (bottom: 15.3 cm SL)

Fig. 3 Size-frequency distribution of salmonid fishes. *OM* *Oncorhynchus mykiss*, *HB* putative hybrids between *Salvelinus leucomaenis* and *Salvelinus fontinalis*, *SF* *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	Gradient	Width	Depth	Substrate	WT	Salmonids density ^a	Fish species								
	(m)	(%)	(m)	(cm)		(°C)	(N·100m ⁻²)	<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