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Potential negative impacts of introduced rainbow trout on endangered Sakhalin taimen through redd disturbance in an agricultural stream, eastern Hokkaido

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Abstract – Sakhalin taimen (*Hucho perryi*) populations have decreased in Hokkaido, northernmost Japan, primarily because of overexploitation and habitat degradation. Here we document another threat to this species, that of spawning redd superimposition by artificially introduced rainbow trout (*Oncorhynchus mykiss*). Sakhalin taimen and rainbow trout are the only spring-spawning salmonid species in Hokkaido. In 2006–2008, spawning activities of both these species were observed in a Hokkaido stream, and it was determined that their spawning periods overlapped during mid-late April. They also spawned at similar water velocities, depths and substrate compositions. Although female Sakhalin taimen were larger than female rainbow trout, their egg burial depths were nearly identical. During the observation period, rainbow trout redds were approximately five times more abundant than Sakhalin taimen redds, and about 30% of the observed Sakhalin taimen redds were superimposed by rainbow trout redds. The high degree of spatial and temporal overlap in spawning, the similar egg burial depths of both species, and the high proportion of superimposed redds suggest that the introduced rainbow trout impact the endangered Sakhalin taimen in Hokkaido, and possibly, in other areas where the two species occur together.

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Key words: alien species; *Hucho perryi*; invasive species; *Oncorhynchus mykiss*; redd count

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Introduction

In addition to the endangering effects of habitat degradation and overexploitation, invasive alien species can be a serious threat to the conservation of freshwater ecosystems (Rahel 2000; Kolar & Lodge 2001; Cambray 2003; Simon & Townsend 2003). In particular, many salmonid species have been introduced worldwide for recreational and commercial purposes with little regard for their effects on native species (Fausch 2007). In many cases, such introductions have been widely implicated in the decline of native biota, especially native salmonids through predation (Macchi et al. 1999; Wissinger et al. 2006), competition (Fausch & White 1981; Fausch 1988; Hasegawa & Maekawa 2006), hybridization (Verspoor 1988; Henderson et al. 2000; Koizumi et al. 2005), and destruction of spawning nests, i.e., redd superimposition (Witzel & MacCrimmon 1983; Essington et al. 1998; Taniguchi et al. 2000).

Rainbow trout (*Oncorhynchus mykiss*), whose native range stretches along in the Pacific coast of North America and the Kamchatka Peninsula, have been introduced in 97 countries worldwide for

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recreational fishing purposes and as a food resource (Fausch 2007; Crawford & Muir 2008). The species has succeeded in establishing new populations in many areas (e.g., Crowl et al. 1992; Fausch et al. 2001; Pascual et al. 2002; Cambray 2003; Simon & Townsend 2003; Kitano 2004; Baxter et al. 2007; Crawford & Muir 2008), possibly because of their diverse life history forms, [e.g., anadromous (steelhead), fluvial, adfluvial and resident (Van Velson 1974; Busby et al. 1996; Meka et al. 2003; RivaRossi et al. 2004)] and their variable spawning timing (from November to July; Busby et al. 1996). Rainbow trout have hybridized with other rare species, such as inland cutthroat trout (*Oncorhynchus clarki*) (Allendorf & Leary 1988; Weigel et al. 2003), and have replaced native brook trout (*Salvelinus fontinalis*) throughout the long reaches of some southern Appalachian streams (Larson & Moore 1985). As a result, rainbow trout belong to the '100 of the World's Worst Invasive Alien Species' list prepared by the International Union for Conservation of Nature (IUCN) (IUCN 2000).

Sakhalin taimen (*Hucho perryi*), recently reported to be synonymous with *Parahucho perryi* (Oleinik & Skurikhina 2008), is a salmonid species inhabiting the streams of northeast Asia, from the Primorye region of Siberia (south of the Amur River) to the Sakhalin Island, as well as from the southern Kurile Islands to the northernmost island of Japan, Hokkaido (Kimura 1966; Holčík et al. 1988). In Hokkaido Island, Sakhalin taimen were reportedly present in over 42 River Basins in the 1960s; however, their distribution rapidly diminished to 24 river systems by 1980 (Fukushima 2008), and to only 13 river systems by 2008 (Edo 2007; K. Nomoto, unpublished data). At present, Sakhalin taimen is in danger of extinction, mainly because of habitat degradation and overexploitation, and has been listed under 'Critically Endangered' in the IUCN red list (IUCN 2006).

In Hokkaido, rainbow trout were first introduced in the 1920s for use in aquaculture. Their population was small initially, but the number of catchments inhabited by them has increased rapidly since the 1970s. By 1996, they were present in more than 70 catchments because of accidental or purposeful release into lotic and lentic environments (Takami & Aoyama 1999). Their invasion of Hokkaido is apparently continuing (Fausch et al. 2001). Before invasion by rainbow trout, Sakhalin taimen were the only spring-spawning salmonids in Hokkaido and had not been exposed to interspecific competition with other native, fall- and/or winter-spawning salmonids, e.g., masu salmon (*O. masou*), pink salmon (*O. gorbuscha*), chum salmon (*O. keta*) and white-spotted charr (*S. leucomaenis*). Thus, the present study was conducted to determine the impact of rainbow trout invasion on the Sakhalin taimen population in a tributary of the Furen River in eastern Hokkaido, where these two salmonid species coexist. The questions mainly addressed by us were: (i) when and where do Sakhalin taimen and rainbow trout spawn, and is there any overlap in their spawning times and/or locations, (ii) is the microhabitat use of the two species correlated with particular environmental variables and (iii) how many Sakhalin taimen redds are superimposed by rainbow trout?

Material and methods

Study area

The study area was located in an upstream tributary of the Furen River, which drains into the brackish waters of Lake Furen in eastern Hokkaido. We have not described the location of the studied stream in detail to protect the critically endangered Sakhalin taimen (Category 'Critically Endangered' in the IUCN red list) from fishing. In the Furen River, rainbow trout were first introduced because of an accidental release from a fish-breeding pond in the 1940s. A rapid expansion of their distribution occurred, particularly since the 1970s, when additional rainbow trout were frequently released into the river for sport fishing purposes. Although the Furen River is also inhabited by white-spotted charr, masu salmon, chum salmon and pink salmon, these species spawn in the autumn and/or winter unlike Sakhalin taimen whereas rainbow trout spawn in the spring similar to Sakhalin taimen.

This study was conducted in the springs of 2006, 2007 and 2008. The studied stream is sinuous with a low gradient (channel slope of about 0.65%), has a drainage area of 19.5 km^2, and is located at an average altitude of 70 m. The stream is 5–7 m wide and has a maximum depth of about 1 m. The surveyed reach was 6,380 m long and started at the stream mouth (lowermost of the surveyed reach). A preliminary survey revealed that there were no suitable salmonid spawning grounds upstream or downstream of the surveyed reach.

At a fixed station near the stream mouth, daily water temperature and water discharge were monitored throughout the spring observation periods. The daily water temperature was recorded at hourly intervals using a data logger (Optic StowAway; Onset Computer Corp., Bourne, MA, USA). Water discharge was measured using the U.S. Geological Survey Mid-Section method (Orth 1983).

Surveys of redds and redd superimposition

Redd construction and superimposition were surveyed almost every day throughout the overlapping spawning periods of Sakhalin taimen and rainbow trout.

After one of the species had finished spawning, the surveys were continued to be performed almost every week until the other species finished spawning. During each survey, we walked along the river bank of the study reach and searched for spawning females and their redds. When a female spawner was observed, its body size was estimated by visually marking two stones on the stream bed. The distance between the selected stones was considered to be equal to the total body length of the spawner. After the female had left the area, the distance between the stones was measured (Maekawa et al. 1994; Taniguchi et al. 2000). The location of newly constructed redds was recorded by a differential global positioning system (Mobile Mapper Pro; Thales Navigation Inc., SanDimas, CA, USA). The redd was marked with four pegs stuck to enclose the redd tail. The shape and size (total length, and tail length and width) of the redds were recorded on a plan view of the redd. During each survey, redd superimposition was detected by examining the plan view. In this study, superimposition was defined as a disturbance of more than 50% in the tail area by a later spawner (Essington et al. 1998).

Sakhalin taimen and rainbow trout occasionally construct false redds which do not have egg pockets. For potential true redd, which are identified by their pit shape (V-shaped or not) or sizes (>140 cm in total length and >75 cm in tail width), the redd superimposition were recorded. In addition, we excavated the potential true redd, which had not been superimposed, to examine the probability that those actually have egg pockets. Results of the examination showed that the potential true redd for Sakhalin taimen could be distinguished from its false redds with 93% accuracy, while rainbow trout redds could also be distinguished from its false redds with 78% accuracy. On the other hand, a potential false redd for Sakhalin taimen could be distinguished from its true redd with 100% accuracy, while those for rainbow trout could be distinguished with 95% accuracy.

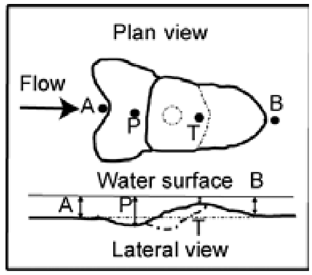

Fig. 1. Standard shape of redd. Water depth was measured at A (upstream of redd), P (pot), T (tail) and B (downstream of redd), and the depth of each redd was represented as the means of these four values. Water velocities were measured 5 cm above the stream bed at A and T and the velocity at each redd was represented as the means of the two values.

Measurement of water velocity, water depth and pebble size on each redd

At each redd, water velocity was measured 5 cm above the stream bed at two positions (A and T in Fig. 1) using a portable current metre (CR-7WP; Cosmo Riken Inc., Kashihara, Japan; as described by Tanida et al. 1985). Water depth was measured at four positions (A, P, T and B in Fig. 1). In addition, substrate composition was determined by placing a 50 cm square metal grid (mesh size: 7 cm × 7 cm) on the stream bed adjacent to both sides of the redd tail and by classifying the diameters of 100 pebbles at the grid intersections into eleven categories (<2 mm, 2–2.8 mm, 2.8–4 mm, 4–5.6 mm, 5.6–8 mm, 8–11.3 mm, 11.3–16 mm, 16–22.6 mm, 22.6–32 mm, 32–45.3 mm, 45.3–64 mm) using a template (US SAH-97; Wildco, Buffalo, NY, USA) according to methods described by Potyondy & Bunte (2002). In the present study, streambed substrate compositions were expressed as D50 (median diameter), which correspond to the 50th percentiles on the cumulative curve. These microhabitat measurements were conducted within seven days after a redd was first discovered.

Species identification of each redd

During the time of redd construction, the species present was immediately identified *in situ*. In the absence of spawner, the species of each redd was identified based on egg size, DNA sequencing or cultivation of eggs collected from one egg pocket per redd. When the accumulated daily water temperature reaches about 200 °C, the eggs are reported to be eyed and relatively tolerant to physical disturbance (Kawamula et al. 1996). Therefore, at that time, generally late May, all redds whose spawning species were unknown were excavated, and a few eyed eggs were collected from the upper area of each egg pocket for species identification. Based on species identification from DNA sequences (detailed in the next section) and on spawner observations undertaken in 2006, the diameter of eggs deposited by two salmonids were found to be 5.0–6.8 mm in Sakhalin taimen and 3.8–5.5 mm in rainbow trout. Therefore, in the 2007 and 2008 surveys, we considered eggs larger than 5.5 mm as those of Sakhalin taimen and those smaller than 5.0 mm as those of rainbow trout. Species identification of medium-sized eggs from overlapping region (i.e., from 5.0 mm to 5.5 mm in diameter) was performed by egg cultivation as follows. Ten to twenty eyed eggs were collected from each egg pocket and reared in a mesh cage placed in flowing water drawn from a natural stream. Each eyed egg hatched to a fry after 50–60 days of cultivation. Sakhalin taimen and rainbow trout fry were discriminated from each other based on parr marks and body morphology.

DNA analysis

Prior to performing species identification by DNA analysis, new primers were developed by analyzing the control region of mitochondrial DNA extracted from eyed eggs of eight Sakhalin taimen and eight rainbow trout redds. A comparison of DNA sequences between the two fish species found relatively abundant substitutions in a 214-bp region, and the side sequences were used for developing new primers: OHCR-F1, GTATAATATTACATATTATGTATTTACCC and OHCR-R1, TGGTCGGTTCTTACTACATTAAG (Fig. 2). One and three haplotypes were observed from Sakhalin taimen and rainbow trout, respectively, and these sequence data were the same as the published data (Sakhalin taimen: position 161–373 in AY862364; Rainbow trout: position 3806–4019 in AY032631, position 15748–15961 in DQ288271, position 114–327 in AF044150). For species identification by DNA analysis, one eyed egg was collected from each redd and DNA was extracted from it; this DNA was amplified by PCR using the aforementioned primers. PCR was performed in 20 μl reaction volumes, which included 10× PCR buffer (Applied Biosystems, Foster city, CA, USA), 0.2 mM each dNTP, 3.0 mM $MgCl_2$, 0.5 μM of each primer, 0.3 units of *Taq* DNA polymerase (Applied Biosystems) and 1 μl of the DNA extract. The reaction conditions were 94 °C for 10 min followed by 40 cycles of 30 s at 94 °C, 30 s at 55 °C and 50 s at 72 °C, and a final extension for 10 min at 72 °C. PCR products were directly sequenced in forward and reverse directions using a BigDye Terminator kit (Applied Biosystems) and an ABI 3100 automatic sequencer (Applied Biosystems).

Measurement of egg burial depth

Egg burial depth has been considered as a criterion that indicates the robustness or vulnerability of the redds to superimposition and is correlated with spawner body size (DeVries 1997). Of all the redds excavated for species identification, 53 redds, including those of eight Sakhalin taimen and 14 rainbow trout females, whose total length had been visually measured using the above-mentioned method, were excavated from the bottom of the egg pocket. Following the methods used by previous authors (e.g., Fukushima 1994; Elliott 1995; Edo et al. 2000), redds at the eyed egg stage were excavated, and all eggs were carefully collected from each egg pocket area. The eggs were placed in a hatchbox (Whitlock-Vibert; as described by Garrett & Bennett 1996) and the vertical distance between the surface of the stream bed and the bottom of the egg pocket was measured. Subsequently, with the exception of a few eggs collected for species identification, the eggs in the hatchbox were immediately returned to their natal pocket, which was then reburied to its original depth without adding silt and sand.

Statistic

Two assumptions for parametic test, normality and homogeneity of variance were checked by Shapiro-Wilk normality test and F-test, respectively, and then a two-way ANOVA was performed to verify differences in median pebble size among redd locations along the stream or between the two species. Microhabitat uses were compared between Sakhalin taimen and rainbow trout using a complete-randomization test described by Manly (2006) with 10,000 iterations. In the randomization test, the number of variables (N) in the smaller groups was used to randomly select the values of an equal sample size from the larger group. The R computer package (R Development Core Team 2009; http://www.R-project.org/) for Windows was used for statistical testing.

Results

The number of rainbow trout redds was about five times greater than that of Sakhalin taimen redds in all three years (Table 2). As shown in Fig. 3, spawning in

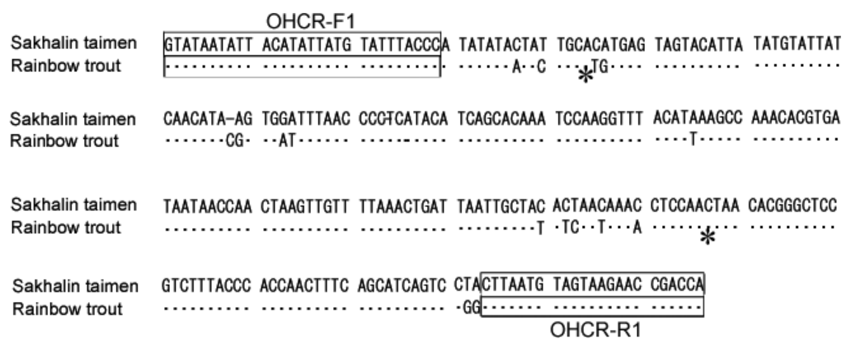

Fig. 2. Nucleotide sequences of a part of the control region (265–266 bp) from Sakhalin taimen and rainbow trout DNA amplified using primers OHCR-F1 and OHCR-R1. A eighteen-bp difference (17 substitutions and one deletion) was observed between the two species. Intraspecific variation was detected at two sites (*) in rainbow trout.

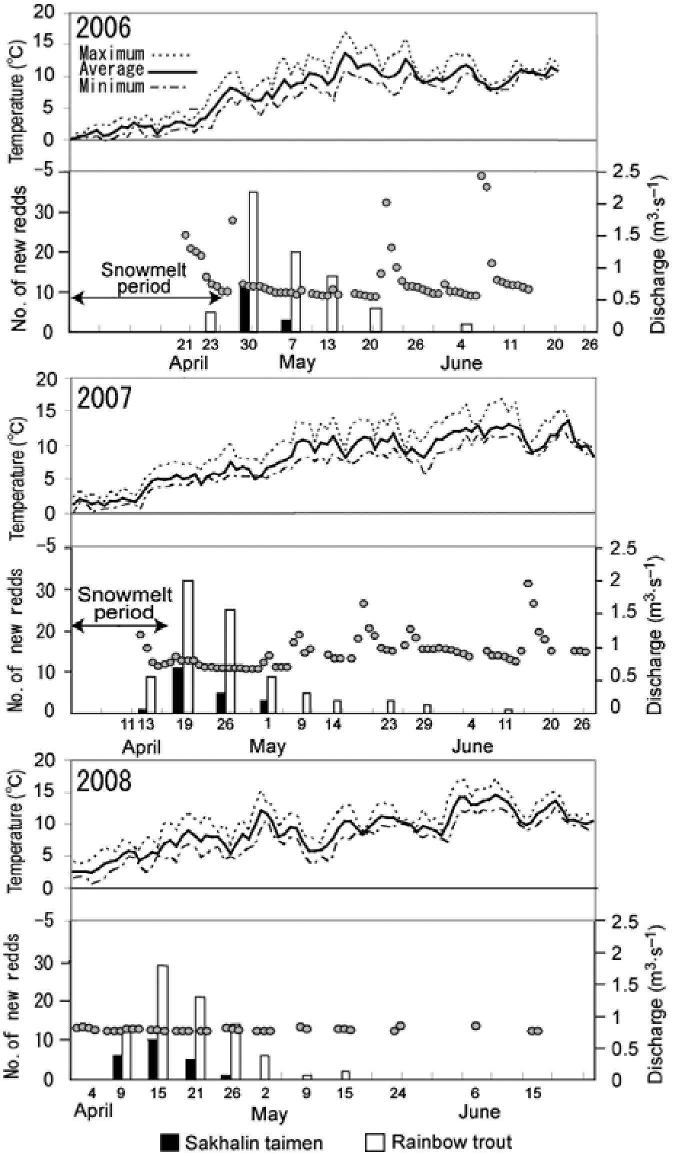

Fig. 3. Temporal fluctuation in water temperature and discharge as well as in the number of newly constructed redds during survey intervals of approximately one week in three spawning years. Bars represent redd counts and dots represent water discharge. Arrows indicate snowmelt periods an accumulation of snow observed at the riverside of the stream.

both species did not begin until the maximum water temperature exceeded 5 °C and as the average water temperature exceeded 4 °C following formation of freshets due to snowmelt in 2006 and 2007, but spawning began in the absence of a snowmelt-related freshet in 2008. The construction of new redds peaked shortly after the beginning of spawning and lasted until early May in Sakhalin taimen, but continued until early or middle June in rainbow trout.

With the exception of the uppermost reach, which was dominated by rainbow trout, redds of both species were widely distributed along the surveyed reach in all the years (Fig. 4), indicating no interspecific separation of the stream reach for redd construction. Thus, microhabitat uses were compared between the two species based on pebble size, water velocity and water depth data for each redd. The water depths and velocity at redd site of Sakhalin taimen and rainbow trout were very similar, with no statistically significant difference (Table 1). Data from 17 Sakhalin taimen and 20 rainbow trout redds were collected at intervals of 500 m along the stream, and the median pebble size in each interval ranged from 9.8 ± 95% CL 1.2 mm to 12.61 ± 95% CL 0.6 mm. Substrate composition at redd site of the two salmonids was not significantly different either among redd locations (Two-way ANOVA: $F = 1.79$, $P = 0.23$) along the stream or between the two species ($F = 0.08$, $P = 0.78$). These results suggest that the microhabitat uses for spawning are not significantly different between the two species. D50 values at the redd sites were not significantly different between two species (Table 1).

Although Sakhalin taimen redds were more frequently superimposed by rainbow trout, Fisher's exact test did not detect a significant difference, suggesting that both species almost randomly disturbed the

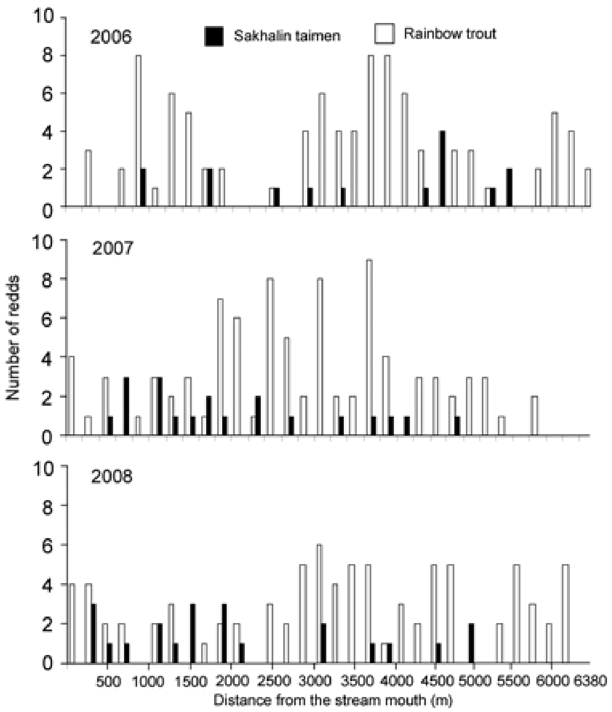

Fig. 4. Distribution of Sakhalin taimen and rainbow trout redds along the 6,380 m reach at intervals of 200 m during three spawning years.

Table 1. Water depth, velocity and pebble size at Sakhalin taimen and rainbow trout redds in the study stream. Values in parentheses are mean ± 95% confidence limit. Randomization tests were performed for each value between the two species.

Species	Water depth (cm)	Velocity (cm·second^{-1})	Pebble size D50 (mm)
Sakhalin taimen	11.0–43.3 (23.9 ± 2.2) *N* = 52	31.8–85.3 (54.0 ± 4.5) *N* = 50	8.21–12.66 (10.67 ± 0.54) *N* = 17
Rainbow trout	10.5–41.5 (25.5 ± 2.0) *N* = 61	16.4–77.7 (55.1 ± 3.6) *N* = 61	8.30–14.07 (10.73 ± 0.60) *N* = 22
Randomization test (*P*)	>0.05	>0.05	>0.05

existing redd locations; thus, not showing any species-specific superimposition (Table 2). This test was conducted each year separately and no species-specific superimposition was detected in any year (Table 2).

Figure 5 shows the correlation between the total body length (x) and egg pocket depth (y) of eight Sakhalin taimen and 14 rainbow trout females. Among conspecific females, the egg pocket depth correlated significantly with the total body length in case of Sakhalin taimen and rainbow trout, with larger females excavating deeper egg pockets. However, while the slopes of the linear regression lines were not significantly different between the two species (ANCOVA: $F = 0.25$, d.f. = 1, $P = 0.62$), the y-intercept of the line was significantly higher in rainbow trout than in Sakhalin taimen ($F = 1.63$, d.f. = 1, $P < 0.001$). This may be a reflection of the similarity in egg pocket depths in Sakhalin taimen (13–25 cm; mean: 17.4 ± 95% CL 3.3 cm, $N = 8$) and rainbow trout (11–23 cm; mean: 17 ± 95% CL 2.4 cm, $N = 14$), even though the total body length of females was much larger in Sakhalin taimen (58–117 cm; mean: 74.6 ± 95% CL 9.5 cm, $N = 14$) than in the rainbow trout (26–60 cm; mean: 40.5 ± 95% CL 2.9 cm, $N = 36$). Furthermore, excavation of 53 redds over three years indicated that the egg pocket depths were nearly equal in Sakhalin taimen (13–32 cm; mean: 18.7 ± 95% CL 2.9 cm, $N = 15$) and rainbow trout (11–25 cm; mean: 17.0 ± 95% CL 1.1 cm, $N = 38$) with no significant difference between the two species (Randomization test: $P > 0.05$).

Discussion

During the three years of observation, rainbow trout redds were about five times more abundant than Sakhalin taimen redds, and about 30% of the observed Sakhalin taimen redds were superimposed by rainbow trout. These results suggest that superimposition by the artificially introduced rainbow trout species is impacting the indigenous Sakhalin taimen species, which are rare and require urgent conservation in Hokkaido.

During the embryo stage of salmonids, redd superimposition seems to be a major mortality factor (Hayes 1987; Van den Berghe & Gross 1989). For instance, in New Zealand streams, introduced rainbow trout has been reported to superimpose 94% of the brown trout (*Salmo trutta*) redds, and occasionally, brown trout egg survival rates are reduced to less than 1% (Hayes 1987). As a result of such intensive superimposition by rainbow trout, one brown trout population has been extirpated (Scott & Irvine 2000). Thus, rainbow trout have been reported to be a cause of serious damage to eggs deposited by an early spawner species through redd superimposition in oceanic streams where none of the indigenous salmonids inhabit. On the other hand, in other regions including the northern hemisphere, where numerous salmonids are native, such high levels of redd superimposition by rainbow trout on native salmonids have not been reported. Among the limited literature available on redd superimposition by rainbow trout, Taniguchi et al. (2000) reported that introduced rainbow trout superimposed redds constructed by two indigenous autumn spawners (13% of Dolly varden charr redds and 3% of white-spotted charr redds) in a southern Hokkaido stream. Over the three years of our study, as many as 28% of Sakhalin taimen redds were superimposed by introduced rainbow trout, while less than 6% of their redds were superimposed by

Table 2. Number of redds superimposed by conspecific or other salmonid species in 2006, 2007 and 2008. Fisher's exact tests (FET) were performed for each year's 2 × 2 contingency tables (data enclosed in squares).

Species spawned	No. of redds	No. of total superimposed redds (%)	Superimposed by			FET (*P*)
			Unidentified	Sakhalin taimen	Rainbow trout	
2006						
Sakhalin taimen	15	5 (33.3)	0	0	5	0.55
Rainbow trout	93	26 (28)	1	5	20	
Unidentified	11	7 (63.6)	1	2	4	
2007						
Sakhalin taimen	20	8 (40)	0	2	6	1
Rainbow trout	89	23 (25.8)	2	5	16	
Unidentified	14	10 (71.4)	3	1	6	
2008						
Sakhalin taimen	22	6 (27.3)	0	1	5	1
Rainbow trout	85	19 (22.4)	3	4	12	
Unidentified	15	10 (66.6)	4	1	5	

FET, Fisher's exact tests.

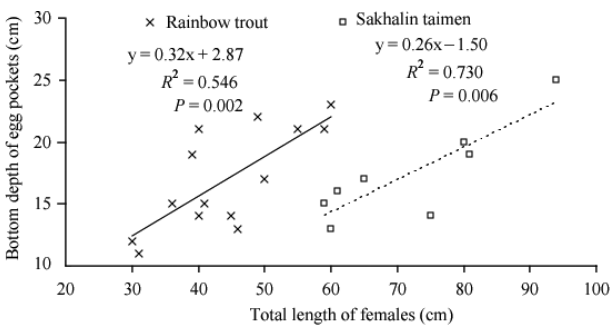

Fig. 5. Correlations between the egg pocket depth and total body length in female Sakhalin taimen and rainbow trout.

conspecific Sakhalin taimen (Table 2). Moreover, the rate of superimposition by conspecific Sakhalin taimen is similar to that (about 10%) observed in another stream in central Hokkaido (Edo et al. 2000).

Redd superimposition by rainbow trout on Sakhalin taimen is considered to be due to four main reasons. First is the spawning time of rainbow trout, i.e., large numbers of rainbow trout spawn just after the main spawning period of Sakhalin taimen, a period when the uneyed embryos are vulnerable to physical shock. Second is the similarity in microhabitats used by the two species, i.e., similar microhabitats used by the two species can result in reuse of Sakhalin taimen redd sites by rainbow trout. Third is the higher abundance of rainbow trout spawner in an area where suitable spawning ground is limited. Fourth is the similarity in the depth of the egg pockets in the two species. These four reasons are discussed below in detail.

In the three observed spawning periods, spawning timing considerably overlapped between the Sakhalin taimen and rainbow trout. Such overlaps seem to enhance interspecific competition through occurrences such as redd superimposition between the two species. In many salmonid species, reproductive timing is considered to be affected by genetic adaptation (e.g., Siitonen & Gall 1989; Quinn et al. 2000) and phenological flexibility (Jonsson 1991). Although we have no information about the strain or population of the rainbow trout that had been introduced into the study area, there are rainbow trout populations in Hokkaido that spawn between January and April (Taniguchi et al. 2000) and others that spawn between April and June (Aoyama et al. 1999; present study). The overlap in the spawning timing between the two species can be partly attributed to the reproductive timing of the original rainbow trout strains that spawn in the same season even in other regions. Nevertheless, it may also be attributed to phenological flexibility, i.e., adaptation to environmental conditions such as water temperature, discharge and the fluctuation (Jonsson 1991). In this study area, most streams are frozen until mid-March and freshets may suddenly occur in late March or early April due to the melting of snow and ice. If a spawner lays its eggs too early, the survival rate of the embryos may decline as a result of egg loss caused by a freshet, while if the eggs are laid too late, the resulting fry may be at a territorial disadvantage among earlier emerging fry (Einum & Fleming 2000; Carlson et al. 2004). In this study, spawning in both species started just after a strong freshet was produced due to snowmelt in both 2006 and 2007 (Fig. 3). Such a relationship between the initiation of spawning and water fluctuation has also been reported in other spring-spawning populations of Sakhalin taimen (Fukushima 1994) as well as in golden trout *O. mykiss aguabonita* (Knapp & Vredenberg 1996), redband trout *O. mykiss gairdneri* (Muhlfeld 2002) and in adfluvial rainbow trout (Holecek & Walters 2007).

In three years' survey, Sakhalin taimen and rainbow trout in this study stream began spawning when the daily average temperature exceeded 4 °C, which is similar to spawning water temperatures reported for Columbia River rainbow trout (Holecek & Walters 2007) and northern Hokkaido River Sakhalin taimen (Fukushima 1994), in spite of differences of fluctuation patterns in stream temperatures among three years, probably suggesting that the initiation of spawning in the two species reflected increasing water temperature in each year. Before and during peak discharge due to snowmelt, the temperature of the stream was probably too low for the two species to start spawning activities in the study stream, as mentioned in other rainbow trout populations (Holecek & Walters 2007; Muhlfeld et al. 2009). The relationships between spawning timing and an environmental cue may be a common characteristic of spring-spawning salmonids inhabiting streams where freshets occur due to spring snowmelt. In other words, the temporal overlap in spawning of both species is occurred probably because of their similar response to changes of the environmental cues such as water discharge and temperature.

In the present study, Sakhalin taimen and rainbow trout selected very similar microhabitats (water depth, velocity and substrate composition) for their redd sites. The characteristics of microhabitat variables associated with the redds observed in this study were similar to other published values for adfluvial rainbow trout (Holecek & Walters 2007) and resident redband trout (Muhlfeld 2002). Of the physical variables assessed, substrate composition is considered to be a critical factor in spawning and embryo survival (Chapman 1988). As a result of geological and human influences, sediment particles that are appropriately sized for salmonid spawning were not abundant in the reach studied here. The stream bed was dominated by silt and sand with few pebbles because the stream originates in a volcanic stratum where there are few pebbles. In addition, intensive grazing by live stock within the catchment has led to a decrease in the spawning ground area due to sedimentation of silt and sand because of soil washout from the adjacent grazing land (K. Nomoto, unpublished data). This limitation of available habitat suitable for spawning and the large numbers of sympatric rainbow trout spawners may contributed to the increased rate of redd superimposition, because redd superimposition seems to attribute to the limited availability of spawning habitat (e.g., McNeil 1964, 1966; Hayes 1987; Ligon et al. 1995; Fukushima et al. 1998).

A critical question in this study is whether eggs deposited by an early spawner Sakhalin taimen die, after the redd has been superimposed by a later spawning, rainbow trout. To answer this question, egg burial depth was used as the criterion to determine the loss of Sakhalin taimen eggs due to redd superimposition by rainbow trout. Steen & Quinn (1999) reviewed published and unpublished data from different regions and compared regression lines (egg burial depth vs. female body length) among five salmonid species including Sakhalin taimen (data collected in northern Hokkaido rivers; Fukushima 1994). Their review indicated that Sakhalin taimen bury eggs at a relatively shallow depth for their body size compared with other salmonids such as rainbow trout, brown trout, Atlantic salmon (*S. salar*) and sockeye salmon (*O. nerka*). In the present study, Sakhalin taimen also buried eggs at a relatively shallow depth for their body size compared with the sympatric-spawning rainbow trout. Although egg burial depth is also reported to vary with changes in physical factors, such as water velocity and substrate composition (DeVries 1997), the egg burial depths of the two species observed here were within the ranges reported by previous researchers (for Sakhalin taimen, Edo et al. 2000; for rainbow trout, Crisp & Carling 1989). Their data were collected in a central Hokkaido river or British rivers, suggesting that the vertical overlap of egg burial depth observed here is probably attributable to a species-specific characteristics rather than related to local circumstances. Thus, if Sakhalin taimen redds in other streams are superimposed by rainbow trout redds, Sakhalin taimen eggs in those streams would also be affected as observed here.

However, we should take into consideration that the data presented here were derived from only one stream for three years. Additional observations in other regions or streams for longer periods will help determine the impacts of redd superimposition by rainbow trout on Sakhalin taimen. Such impacts may change according to changes in other factors such as biological (rainbow trout strain, body size and abundance, etc.) and environmental (stream substrate composition, spawning ground availability and water regime, etc.) conditions. Thus, further studies in other regions and streams are needed.

This study has several management implications for the conservation of endangered Sakhalin taimen. To reduce the deleterious impacts on Sakhalin taimen, we suggest that rainbow trout should not be released, neither purposefully nor accidentally, into catchments inhabited by Sakhalin taimen. Furthermore, removal of rainbow trout using efficient and reasonable methods such as electrofishing (as described by Peterson et al. 2008), may be needed especially in streams where Sakhalin taimen abundance is declining.

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