Phylogeography of a salmonid fish, white-spotted charr (Salvelinus leucomaenis), in a historically non-glaciated region in the northwestern North Pacific

ECOLOGICAL RESEARCH

Species-specific foraging behavior and diets of stream salmonids: an implication for negative impacts on native charr by nonnative trout in Japanese mountain streams

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	spotted charr, and have ecosystem-level impacts due to predation on terrestrial prey.

Original Article

Species-specific foraging behavior and diets of stream salmonids: an implication for negative impacts on native charr by nonnative trout in Japanese mountain streams

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Abstract

Salmonids have been introduced globally as a food source and recreational fishing target. In Japan, brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) were introduced in the 19th century and have since spread. In many headwater streams, native white-spotted charr (*Salvelinus leucomaenis*) are thought to be experiencing negative impacts from ~~brown and brook trout~~these species. The current study examined foraging behavior, microhabitat use, and diet overlap of these three species in Kamiko~~u~~chi, Nagano Prefecture: one of Japan's premier mountain ~~protected~~ areas. In Kamiko~~u~~chi, many spring-fed headwater streams are currently dominated by these invasive salmonids and white-spotted charr have declined drastically over the last half century. Underwater video analysis revealed that while total foraging rates and foraging modes were similar between the three species, brook trout and white-spotted charr ~~primarily~~ foraged benthically ~~but~~ more frequently than brown trout ~~utilized drift and benthic foraging~~. Microhabitat water depth and flow velocity were similar between species, and fish size had a positive effect on water depth and flow velocity in all three species. Diet analysis indicated that brook trout and white-spotted charr diets were nearly identical, ~~(Schoener Index of Overlap: >92%),~~ comprised primary of aquatic invertebrates, while brown trout preyed on a mix of terrestrial and aquatic invertebrates, as well as amphibians and fish. These results indicate that in Kamiko~~u~~chi, the decline of white-spotted charr is likely most influenced by direct competition with brook trout for prey resources. ~~.~~However, brown trout likely also predate on juvenile white-spotted charr, while also possibly causing a foraging niche shift of white-spotted charr, and ~~and~~ have ecosystem-level impacts due to predation on terrestrial ~~invertebrates and amphibians~~prey. ~~.~~

Keywords: brook trout, brown trout, white-spotted charr, competition, predation

Introduction

Aquatic invasive species have spread globally, causing problems associated with predation, competition, and hybridization with native species (Almela et al., 2021). In terms of freshwater fish species, salmonids have been widely introduced in nearly all continents as a food source and recreational angling target (Buoro et al., 2016). Within the salmonid family, which has a wide species diversity of both anadromous and landlocked forms, rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta)*, and brook trout (*Salvelinus fontinalis*) are commonly introduced (Buoro et al., 2016). Rainbow trout and brown trout are ranked in the global 100 worst alien invasive species by the International Union for Conservation of Nature (IUCN), due to their negative impacts on native fish (Lowe et al., 2000). In areas such as the United States, where brown trout were introduced from Europe in the 1800s and rainbow trout and brook trout were introduced domestically, these species have become dominant and have caused the severe decline of native species such as cutthroat trout (*Oncorhynchus clarkii*) and bull trout (*Salvelinus confluentus*) (Al-Chokhachy & Sepulveda, 2019; Bell et al. 2021; Kruegar & May, 1991). However, due to the angling popularity of these introduced species, in many areas stocking continues and these species make up the base of the recreational salmonid fishery in the United States (Halverson, 2008; Swink, 1983). Although largescale stocking continues, in certain areas with remnant populations of native species, these invasive salmonids have been removed and targeted re-introduction of native species has shown success (Budy et al., 2021; Quist & Hubert, 2004).

In Japan, rainbow trout, brown trout and brook trout were introduced in the 1800s (Kitano, 2004). Although rainbow trout have not spread wildly (except for certain areas in Hokkaido and Nagano), likely due to juvenile-survival limitations (Fausch et al., 2001), brown trout are currently spreading throughout the country (Hasegawa, 2020). Therefore, rainbow trout and brown trout are also listed in the 100 worst invasive species in Japan (Ecological Society of Japan, 2002). Brook trout have also not spread widely, but are found in a few particular spring-fed streams (Kitano, 2004). The spread of invasive salmonids in Japan is concerning as native salmonids such as white-spotted charr (*Salvelinus leucomaenis*) and masu salmon (*Oncorhynchus masou*) will likely occur negative impacts based on direct predation and resource competition. Hybridization between white-spotted charr and brook trout, as well as rare hybridization between white-spotted and brown trout, have been found in Japan (Kitano et al. 2009;~~,~~ Kitano et al., 2014) and appear to be an increasing concern as these invasive salmonids continue to spread, especially in Hokkaido. In Honshu Island of the Japanese archipelago, white-spotted charr are a headwater species typically found at high elevations in cold water. In many areas, white-spotted charr populations are currently under threat from rising water temperatures and habitat degradation and fragmentation (Dunham et al., 2008; Takami et al., 1997). White-spotted charr habitat use and diet has been studied in detail throughout their distribution range in Japan. On Honshu they are typically found exclusively in high altitude headwater streams while in Hokkaido, where water temperatures are colder, they can be found in a range of habitats (Morita, 2019; Yamamoto et al., 2004). White-spotted charr distribution often overlaps with other native salmonids such as masu salmon and southern Asian D~~d~~olly V~~v~~arden (*Salvelinus curilus*), and habitat and diet niche partitioning has been studied in great detail, especially in Hokkaido (Miyasaka et al., 2003). In headwater stream habitat, white-spotted charr commonly forage on a variety of aquatic insects such as caddisflies (Trichoptera), mayflies (Ephemeroptera~~Ephemeratpera~~) and stoneflies (Plecoptera~~Plectoptera~~) (Iguchi et al., 2004). Terrestrial insects such as camel crickets and grasshoppers have also been shown to be an important part of white-spotted charr diets in certain settings (Miyasaka et al., 2003; Sato et al., 2011). Foraging modes have been described as typically benthic or drift foraging with occasional surface foraging (Nakano & Furukawa-Tanaka, 1994).

In stream settings, larger individuals maintain favorable focal points and typically utilize drift foraging to prey on terrestrial and aquatic invertebrates while smaller subordinate individuals take up less favorable focal points and when drift prey is scarce, shift to benthic foraging (Nakano et al., 1991). In larger white-spotted charr individuals, especially in large river or lake habitats, fish prey can also make up a large part of diets (Takami & Nagasawa, 1996). Brown trout and brook trout habitat and foraging niches have also been studied in detail in the native and invasive ranges (Horka et al., 2017), although detailed studied are still lacking in Japan. Brown trout inhabit a wide range of habitats and have been found foraging on aquatic and terrestrial invertebrates in streams (Becer et al., 2011; Cochran-Biederman & Vondracek, 2017), while large individuals often prey on fish (Jensen et al., 2008), amphibians (Bylak, 2018), and in some cases even small birds and mammals (Milardi et al., 2016a; Milardi et al. 2016b). Brook trout inhabiting stream habitat also typically forage on aquatic and terrestrial invertebrates (Hubert and Rhodes, 1989; Tiberti et al., 2016) and have been shown to have overlapping foraging niches with brown trout when found in sympatry (Horka et al., 2017).

The Kamiko~~u~~chi area of Nagano Prefecture, in the Chubu Sangaku National Park, provides a stark example of just how damaging these invasive salmonids can be and a unique opportunity to study their interactions with native white-spotted charr. Brown trout and brook trout were introduced through larval stocking in 1925-1933, and have rapidly spread and established in the Azusa River which drains into the central Kamiko~~u~~chi area, and current dominate many of the small spring-fed tributaries. The Kamiko~~u~~chi area historically had abundant white-spotted charr populations. Despite all recreational angling being banned in 1975, white-spotted charr have nearly been complexly expatriated from many of the tributaries over the last 50 years (Azumi Village, 1998; Environment Agency, Government of Japan (EAGJ), 1982). Currently white-spotted charr remain abundant only in the very upper reaches of the Azusa watershed where brown trout and brook trout have yet to establish (Azumi Village, 1998). ~~These select small tributaries in the Kamikouchi area where brown trout, brook trout and white-spotted charr are found in sympatry provide a unique opportunity to examine this rare three species assemblage.~~ In Japan, white-spotted charr are typically found in sympatry with other native salmonids such as masu salmon or D~~d~~olly V~~v~~arden, and in certain areas with either brown trout or brook trout. In North American streams, it is rare to find brown trout and brook trout in sympatry as brook trout are typically found in more headwater habitat (Dieterman & Mitro, 2019; Hoxmeier & Dieterman, 2015; Mitro et al., 2019). ~~However, there is global concern that in areas where brown trout have been introduced, they are likely to expand their range into more headwater habitat due to rising water temperatures associate with climate change and in doing so displace native headwater fish species (Al-Chokhachy et al., 2016, Bell et al., 2021). The Kamikouchi area provides a setting where interactions between brown trout, brook trout, and native white-spotted charr can be studied. The results of this study will not only have implications for Japanese headwater streams where brown trout are likely to invade in the near~~

~~future, but also globally where brown trout establishment would put them in sympatry with other salmonids.~~

The current study aims to use detailed underwater observation and diet and habitat analysis to understand the species interactions between brown trout, brook trout, and white-spotted charr in small tributaries of the Kamiko~~u~~chi area. By examining foraging modes, aggressive behavior, diet composition and microhabitat use, direct impacts on white-spotted charr from each invasive species will be determined~~, and will contribute to future white-spotted charr restoration projects throughout Japan and also to global native salmonid management in areas where brown trout are invading~~.

Materials and Methods

Study Area

This study was conducted in six headwater streams (Table 1) in the Kamiko~~u~~chi area of Nagano Prefecture, Japan (36°14'55.84"N, 137°38'16.20"E, 1,500 m.a.s.l.) (Fig. 1). Underwater observation and electrofishing surveys were conducted periodically during June-September 2021 (see Table 1 for details). Kamiko~~u~~chi is one of Japan's most popular mountain recreation areas and is characterized by many short headwater streams that flow into the Azusa River which is surrounded by 3,000 m peaks (Fig. 2). These headwater streams are mostly spring-fed and have consistently cool water temperatures throughout the year. Of the six headwater streams selected, station 1 was a mountain stream with a steep gradient and large boulders, while the other five stations were all spring-fed streams with low gradient and fine substrate (Table 1, Fig. 2).

Underwater Observation

At each stream~~ reach~~, fish behavior and microhabitat use were examined using underwater snorkel observation. Study reaches at stations 1, 2 and 4, were the downstream (from confluence with the main Azusa River) 300m, and at stations 3, 5 and 6, the entire stream was surveyed. Researchers entered each stream reach from downstream and slowly snorkeled upstream through the entire study reach observing ~~each ~~all individual fish (> 80mm Total Length: TL, estimated from pre-measured stream-bottom substrate and rounded to the first nearest integer to account for limitations of underwater observation) for at least one minute. Five-minute underwater video recordings (Go Pro Hero 7, 8 ,9) were taken for each individual fish after allowing the fish to adjust to the snorkeler's presence for three minutes, to determine foraging and agnostic behavior as well as microhabitat use (Fig. 3). After video recording, a marker was placed on the bottom substrate at the location of each individual fish at the end of filming, and microhabitat data was taken. At each fish marker, water depth, focal point water depth (distance from the bottom substrate), flow velocity (60% of water depth), focal point flow velocity (flow velocity of each individual's focal point) and dominant and subdominant substate types were recorded. Substratesize class was estimated following Bain et al. (1985), with modification for prevalent algae cover, and separated into five categories: 1: algae, 2: silt or sand (< 2 mm), 3: gravel (2–16 mm), 4: pebble (17–64 mm), 5: cobble (65–256 mm), and 6: boulder (>256 mm). Mean substrate score was calculated for each individual microhabitat. Fish density of each reach was calculated by measuring the reach length, wetted channel width, and water depth to calculate the surface area.

Video Analysis

Video files were analyzed to determine foraging and agonistic behavior of ~~each~~ all fish observed in each study reach. Foraging modes were set as surface, drift and benthic. A fish's mouth breached the surface during surface foraging, touched the bottom substrate during benthic foraging, and all other foraging was considered drift (Fausch et al., 1997). All foraging attempts were counted for fish individuals that remained in the camera frame for at least 30 seconds. Foraging attempts were counted up to 60 seconds, and if an individual left the camera frame prior to 60 seconds, the time in frame was recorded and used as an offset in the statistical modeling. Agnostic behavior was categorized as either aggressive or defensive and the ~~total length (~~TL~~)~~ of both individuals involved in the interaction was recorded. Due to the prevalence of hybridization between white-spotted charr and brook trout and the difficulty of visually distinguishing white-spotted charr from hybrids (Iguchi et al. 2001), all fish that visually appeared as white-spotted charr were categorized as white-spotted charr and fish that appeared as brook trout were categorized as brook trout.

Fish Collection

Fish were collected by electro-fishing (Model LR-24, Smith-Root Inc., Vancouver, Washington) at four of the streams, throughout the same reaches as the underwater observation (details in Table 1) to determine species assemblage, size and diet. At each reach, fish were kept alive in mesh bags and buckets, sedated with anesthesia FA 100 (DS Pharma Animal Health Co., Ltd.) and stomachs were pumped (Strange & Kennedy, 1981) in all individuals greater than 50mm TL. The stomach contents of each individual were placed in labeled mesh bags (<1mm mesh, Eiken Chemical Co., Ltd.), preserved in 99.5% ethanol, and transported on ice to the lab for analysis. Total length and fork length (FL) were also measured for each individual and fish were allowed to recover for 30 minutes and then released at the site of capture. Hybridization was dealt with as above in video analysis.

Diet Analysis

Each mesh bag containing an individual fish's stomach contents were emptied into a petri dish and the total ~~wet~~ weight of the contents was recorded. Stomach contents were examined under a microscope (Model SMZ, Nikon Instruments Inc., Tokyo, Japan) on a gridded petri dish and prey were classified into a variety of categories down to the family level. Each prey category was recorded as a percentage of the entire stomach content ~~wet~~ weight (%WT) and prey size was also recorded. Percent ~~wet~~ weight was determined by evenly spreading the stomach contents and visually determining the ratio of the total surface area occupied by each prey category. Percent occurrence (%OC); the ratio of fish individuals with each prey category present and the total number of fish examined was also calculated, and the alimentary index (%AI) was calculated to take into account the differing weights of each prey type by multiplying the %WT and %OC of each prey category and expressed as a percentage. To compare diet similarity between the three study species the Schoener Index of Overlap or Percent Similarity Index (PSI) (Schoener, 1974) was used and is calculated as

$$\mathrm{PSI} = [1 - 0.5\textstyle\sum_{i=1}^{n}|Pik - Pjk|]\ x\ 100$$

where P is the proportion of ~~wet~~ weight of the kth prey category consumed by predator species *i*~~I~~ and j. PSI values greater than 60% are considered to be biologically significant (Wallace and Ramsay, 1983).

When stomachs contained fish or amphibians that were unidentifiable due to digestion, DNA barcoding was applied using the Cytochrome Oxidase Subunit 1 (COI) molecular marker. The components were separated macroscopically into fish, frogs or salamanders and weighed. Samples for DNA analyses were washed with water and stored separately in an bottle containing 95% ethanol at 4°C prior to DNA extraction. Genomic DNA was extracted from the muscle or vertebrae tissue by Qiagen D~~d~~Neasy Blood and Tissue Kit (Qiagen, Inc., Hilden, Germany) following the manufacturer's protocol. A fragment of the COI gene was amplified using universal primers LCO1490 and HCO2198 (Folmer et al., 1994), which have been commonly used in DNA barcoding studies of vertebrates (e.g. Becker et al., 2015; Xia et al., 2012). Amplifications were performed with 30 cycles and 55°C annealing temperature, with AmpliTaq Gold 360 Master Mix (Thermo Fisher Scientific, Inc.) Amplified DNA was purified using ExoSAP-IT (Thermo Fisher Scientific, Inc.) and sequenced directly using the BigDye Terminator v3.1 Ready Reaction Cycle Sequencing Kit (Thermo Fisher Scientific, Inc.) with an automated DNA sequencer ABI PRISM 3730-XL DNA Analyzer (Applied Biosystems™). Sequences generated in this study have been deposited in DNA Data Bank of Japan (DDBJ accession numbers: LC760029-LC760032, LC761623-LC761626). ~~AB***–AB***).~~

The obtained COI sequences were assembled and edited in MEGA 5.0 (Tamura et al. 2011). After ambiguous nucleotides in the first and last 100bp of the sequences were removed, the sequences (*ca.* 400-600bp) were blasted in GenBank using NCBI software version 2.2.28+ (Camacho et al 2009). The sequence was accepted as correct species identification when it showed a higher similarity of over 98% with the regionally listed fish and amphibians (EAGJ, 1982, EBHAV, 1998).

Data Analysis

In fish behavior and microhabitat use analysis, each individual fish observation was considered as an individual data point. To determine the effects of a variety of factors on foraging and microhabitat use, Generalized Linear Mixed Models (GLMM) were used. Fish species, fish size (TL), days after start (days after the first survey date), and density of invasive salmonids were set as fixed effect variables and surface foraging count, drift foraging count, benthic foraging count, total foraging count, water depth, focal point water depth, flow velocity, focal point flow velocity, substrate size, interspecific aggressive behavior count, intraspecific aggressive behavior count, interspecific avoidance behavior count, and intraspecific avoidance behavior count were set as response variables. Poisson error structure was used for all response variables with time in frame set as an offset, video file as a random effect variable and brook trout set as the reference category for species as they were most prevalent in the study area. For the aggressive and avoidance behavior models, the ratio of conspecific individuals visible in each video file was calculated and added as a random effect variable to take into account the differing species interaction potentials of each area. ~~Measurements of fish size and water depth were rounded to the nearest whole number (cm) to account for limitations of underwater observation. An explanatory variable was considered significant when the estimate coefficient did not include zero in a 95% confidence interval.~~ Model selection was determined step-wise using the model with the lowest Akaike's information criterion (AIC) for each GLMM (Burnham & Anderson, 2002). Variables were checked for multicollinearity using the Pearson's correlation prior to inserting into each model and highly correlated variables were removed.~~.~~ All analysis were conducted in R software: version 4.1.2. (R core team, 2021).

Results

Study Area and Species Assemblage

~~Six headwater tributary streams were surveyed by snorkeling and electrofishing from June-September 2021 (see Table 1 for survey details).~~ Brook trout and brown trout were found in each of the six study streams while white-spotted charr were rare, and only found in two streams (Table 1). Out of the six streams, brook trout has the highest density in four streams while brown trout had the highest density in two (Table1). No other fish species were observed in any of the study streams.

Microhabitat

The three study species, observed by snorkeling (brook n= 141, brown n= 130, white-spotted charr n= 20), were found in overlapping habitat where present in sympatry within the study area. Although overall mean habitat water depth, flow velocity, and substrate size values have slight differences between species (Table 2), GLMM analysis, with brook trout set as the reference category, showed no significant species effects for water depth, flow velocity and substrate size

(Table 3). However, GLMM analysis indicated that fish size had a positive effect on flow velocity and ~~,~~ water depth ~~and substrate size~~, while white-spotted charr had a positive effect on focal point flow velocity and fish size has a negative effect. For focal point water depth, brown trout and fish size had positive effects while white-spotted charr and invasive salmonid density had negative effects (Table 3).

Foraging

Total foraging rates were similar between the three species, approximately 1.8 attempts·min^{-1}. All three species foraged primary using drift foraging with brown trout exhibiting the highest rate and brook trout and white-spotted charr having similar rates (Fig. 4). Brook trout and white-spotted charr also foraged benthically at a higher rate than brown trout. Brown trout exhibited occasional surface foraging while brook trout and white-spotted charr did not. GLMM analysis showed brown trout had a positive effect while fish size had a negative effect on drift foraging. For benthic foraging, brown trout had a negative effect and no significant effects were found for surface foraging (Table 3).

Aggression

Inter and intra-specific aggressive and defensive behavior was infrequent (approximately 0.2 aggressive behaviors·min^{-1}), however brown trout were primarily aggressive toward other species while brook trout were aggressive conspecifically. In general, throughout the three species, aggressive and defensive behavior followed a size gradient as the aggressor was larger in size in almost all interactions. GLMM analysis indicated that brown trout, white-spotted charr and fish size had positive effects on interspecific aggression and brown trout has a negative effect on intraspecific aggression while the effect of fish size was positive. Brown trout and fish size had negative effects on interspecific avoidance (Table 3).

Diet

The three study species, collected by electrofishing (brook n= 193, brown n= 74, white-spotted charr n= 36), were found to prey on a variety of aquatic and terrestrial prey items with terrestrial Hemipetra and aquatic Trichoptera being the most prevalent prey items in all three species.

Brown trout preyed most on terrestrial Hemiptera followed by aquatic Trichoptera, while brook trout and white-spotted charr preyed most on aquatic Trichoptera followed by terrestrial Hemiptera (Table 4). The ratio of "others" was also higher in brown trout as numerous large individuals were found to be preying on amphibians. Three individuals (brown: n=2, brook: n=1) were found preying on fish (prey ID: brook: n=4, white-spotted charr: n=1, brown: n=1), while five brown trout were found preying on amphibians (prey ID: salamander: *Onychodactylus japonicus*: n=1, toad: *Bufo japonicus formosus*: n=4) (Table 5). The Percent Similarity Index (PSI) showed that brook trout and white-spotted charr diets were nearly identical with high biological significance while brown trout diets were not significantly similar to either brook trout or white-spotted charr (Table 6).

Discussion

This study produced an overview of salmonid distribution in Kamikochi's small, predominantly spring-fed, headwater tributaries ~~Kamikouchi headwater streams~~ and showed clear niche overlap between native and invasive species. The lack of native white-spotted charr and abundance of invasive brown and brook trout was glaringly evident. While habitat use analysis indicated that all three species utilize similar habitat in the small headwater streams (Table 3), foraging mode (Fig. 4) and diet analysis (Table 4, 6) clearly showed that brook trout and white-spotted charr had nearly identical foraging niches while brown trout were distinct. Brook trout and white-spotted charr primarily foraged in drift and also benthically, with diets composed largely of aquatic Trichoptera while brown trout foraged primarily in drift and diets were composed largely of terrestrial Hemiptera. Diets composed of Trichoptera and Hemipetra are consistent with previous studies on white-spotted charr (Iguichi et al., 2004) and brook trout (Tiberti et al., 2016) residing in small streams. These results indicated that while the three species inhabit similar habitat in these small headwater streams, they occupy slightly different foraging niches with brook trout and white-spotted charr being similar and distinct from brown trout. The habitat niche overlap of all three species in the current study area is likely influenced by the small scale of the tributaries and the lack of potential habitat for habitat partitioning. In larger-scale streams where brown trout and brook trout are found sympatrically, habitats are often partitioned with brook trout in headwater areas with cooler water temperatures and faster flow velocity (Dieterman & Mitro, 2019; Hoxmeier & Dieterman, 2015; Mitro et al., 2019).

With these three species occupying similar habitat niches in Kamiko~~u~~chi headwater streams, the possibility of foraging niche shifts due to pressure from the other species is likely. White-spotted charr have been shown to have flexible foraging niches that can shift from predominantly drift foraging for terrestrial prey, to benthic foraging for aquatic invertebrates when prey resources change (Fausch et al., 1997; Nakano et al., 1999~~a~~) or a dominant individual pushes them out of their preferred focal point (Fausch et al., 2020; Nakano et al., 1999~~a~~). This niche shift in white-spotted charr has also been shown in relation to introduction of brown trout and rainbow trout (Hasegawa & Maekawa, 2006), with these invasive salmonids pushing white-spotted charr individuals out of their preferred foraging position. The high rate of brown trout drift foraging for terrestrial prey in the current study may be a product of large brown trout individuals outcompeting white-spotted charr and brook trout for drift foraging focal points. This is corroborated by the GLMM analysis that showed white-spotted charr and invasive salmonid density having a negative effect on focal point water depth. This means that in areas where invasive salmonid density is high, focal points of white-spotted charr become closer to the bottom substrate. Numerous studies in North America have shown that brook trout are negatively affected by the presence of brown trout, due to the combined effects of direct predation, interspecific competition and induced behavior changes (Dieterman & Mitro, 2019; Fausch & White, 1986). Brook trout are displaced from preferred foraging and resting positions, exhibit reduced aggressive and foraging behavior, which result in weight loss and disease susceptibility (DeWald & Wlizbach, 1992).

With the nearly identical foraging and habitat niches ~~and~~ of brook trout and white-spotted charr in the current study, brown trout likely have similar impacts on white-spotted charr as they do on brook trout in North America. However, it is interesting that in the study area, brook trout and brown trout are found at similar densities (Table 1) while only white-spotted charr are severely reduced. The specific mechanisms by which brook trout outcompete white-spotted charr are unclear, and the impact of hybridization as well as reproductive interference from brook trout and brown trout using redds where white-spotted charr have already spawned also require~~s~~ further study. Habitat characteristics and stream type likely also influence the persistence of white-spotted charr and warrant study, as the Zenroku stream, which is the only non-spring fed stream in the study area, had the highest density~~ies~~ of white-spotted charr. The prevalence of white-spotted charr and brook trout, and the lack of brown trout in the Zenroku stream is likely influenced by the mountain stream type which has high flow during spring snowmelt. Brown trout invasion success in Japan has been shown to be negatively influenced by flood disturbance (Kawai et al. 2013) and therefore, in the current study area, the high densities of brown trout in spring-fed streams with relatively stable flow levels is likely a product of their stream type preference. ~~These results of brown trout primarily drift foraging for terrestrial invertebrates while brook trout and white-spotted charr forage benthically for aquatic invertebrates also offer important insights into the potential interactions of brown trout globally, which are likely to expand their habit range into more headwater areas due to climate change (Al-Chokhachy et al., 2016, Bell et al., 2021). If brown trout are dominant over native salmonids they will likely take up favorable focal points and forage on the most energy rich prey items, often terrestrial invertebrates in summer (Eros et al., 2012; Nakano et al., 1999b; Sweka & Hartman, 2008)~~

In terms of fish species distribution and density, the lack of native white-spotted charr and prevalence of invasive salmonids was strikingly evident and highlights the drastic decline of white-spotted charr in this area over the last 100 years (Azumi Village, 1998). Of the six streams surveyed only one (Zenroku) had prevalent white-spotted charr while the other streams had either no white-spotted charr or very few individuals. This lack of white-spotted charr limited the sample size for this species compared to brook and brown trout in this study and required combining of the survey dates and stream locations in the foraging mode and diet analysis. Also, as white-spotted charr were not found in four of the streams, the three species could not be observed in sympatry in many parts of the study area. Ideally, to further understand the negative impacts of invasive salmonids on white-spotted charr, streams with differing species densities (i.e. Recently invaded state: white-spotted charr are predominant with few invasive salmonids. Invaded state: similar densities of white-spotted charr and invasive salmonids) would provide a clearer picture of how the negative impacts of these invaders directly causes the decline of white-spotted charr. Unfortunately, in the Kamiko~~u~~chi area, this is no longer possible as in many of the small headwater streams, white-spotted charr populations have experienced drastic decline over the last century (Azumi Village, 1998). It is also important to note that the current study was conducted only during summer (June-September) and habitat use likely differs especially in the fall when all three species spawn.

The current study indicates that in Kamiko~~u~~chi, brook trout directly compete with white-spotted charr for prey resources. Brown trout also compete with white-spotted charr for prey resources, although to a lesser extent than brook trout, and likely have ecosystem-level impacts due to high predation rates of terrestrial insects as well as amphibians and fish. The combined impacts (prey resource competition, direct predation, and hybridization) of these two invasive salmonid species have likely contributed to the drastic decline of white-spotted charr in tributary habitat, while also significantly altering the headwater stream ecosystems found in the Kamiko~~u~~chi area. Although white-spotted charr have nearly been wiped out in the studied tributary habitat, they can still be found in relatively high densities just a few kilometers upstream in the Azusa River where brook trout and brown trout have yet to invade. Conservation of these areas is paramount, and the prevention of further spread of the invasive salmonids should be highly prioritized. The small spring-fed streams in the current study, which are very short (from headwater to confluence with the Azusa River) (Table 1) also provide an opportunity for complete removal of the invasive salmonids and reintroduction of white-spotted charr. The effectiveness of such practices in restoring native headwater stream biodiversity should be examined in future studies.

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Availability of data and materials

All data collected and analyzed in this study are available upon request.

Declarations

Conflict of interest

The authors declare no conflict of interest.

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Figure Captions

Fig. 1 (a) Map of the study area (Kamiko~~u~~chi, Nagano, Japan) and (b) the six study streams. St.1 = Zenroku, St.2 = Nakagawa, St.3 = Kitano, St.4 = ~~Myojin~~Miyagawa, St.5 = Shimizu, St. 6 = ~~Baikamo~~Nameless Stream

Fig. 2 Photos of the study area. (a) Main flow of the Azusa River, (b) Zenroku (St.1), (c) Kitano (St.3), and (d) Nakagawa (St.2)

Fig. 3 Underwater photos taken from video recordings (GoPro Hero). (a) Brook and brown trout located in ~~Baikamo~~ the Nameless Stream (St.6), and (b) brown trout located in ~~Baikamo~~ the Nameless Stream (St.6)

Fig. 4 Mean foraging attempts/min for brook trout, brown trout, and white-spotted charr in the study area

Table 1: Descriptions of the six survey streams in the Kamikochi area. Stream length is the stream distance from headwaters to confluence with the Azusa River. Fish densities are calculated from a single pass snorkel survey. WSC = White-spotted charr.

(St. #) Stream	Lat/Long	Elevation (m)	Stream length (km)	Type	Gradient (m/km)	Snorkel Dates	Fish Collection Dates (2021)	Brook (Ind. m^{-3})	Brown (Ind. m^{-3})	WSC (Ind. m^{-3})
(1) Zenroku	36°15’11 N/ 137 °38’12E	1817-1514	1.70	Mountain	178.23	22 Jun, 16 Sep	10 Jun, 15 Jul, 16 Sep	0.07	0.01	0.03
(2) Nakagawa	36°15’2 N/ 137 °38’18E	1530-1508	0.86	Spring	25.58	6, 28 Jul, 16 Sep	15 Jul, 16 Sep	0.08	0.01	0.01
(3) Kitano	36°15’7 N/ 137 °38’29E	1524-1511	0.31	Spring	41.94	6, 28 Jul, 16 Sep	28 Jul, 16 Sep	0.17	0.15	NA
(4) ~~Myojin~~Miyagawa	36°15’13 N/ 137 °40’57E	1535-1529	0.91	Spring	6.59	22 Jun	16 Sep	0.20	0.07	NA
(5) Shimizu	36°14’58 N/ 137 °38’20E	1519-1506	0.34	Spring	38.26	6 Jul, 16 Sep	NA	0.05	0.17	NA
(6) ~~Baikamo~~ Nameless Stream	36°15’10 N/ 137 °38’14E	1520-1511	0.41	Spring	21.95	22 Jun, 16Sep	NA	0.02	0.03	NA

Table 2: Means ± SE for microhabitat characteristics of each species. WSC = White-spotted charr.

Species	Fish Size (cm TL)	Water Depth (cm)	Flow Velocity ($cm \cdot s^{-1}$)	Substrate (Size Class)
Brook n= 141	15.7 ± 0.45	56.4 ± 2.20	25.5 ± 1.14	3.2 ± 0.10
Brown n= 130	20.3 ± 0.58	65.7 ± 2.13	31.3 ± 1.46	3.2 ± 0.12
WSC n= 20	19.5 ± 1.10	49.0 ± 3.46	34.1 ± 3.47	3.7 ± 0.19

Table 3: Generalized linear mixed model (GLMM) results for factors affecting microhabitat, foraging and aggressive response variables. Only explanatory variables with significant effects are shown. ΔAIC values are between the chosen (best) model and the next best model. Abbreviations: FP = Focal point, Inter = Interspecific, Intra = Intraspecific, WSC = White-spotted charr, Days = days after start.

Response Variable	Effect Variable	Coefficient Estimate	Standard Error	Z-Value	Pr (>|z|)	ΔAIC
Flow Velocity	Fish Size	0.01	0.01	3.48	0.001	1.62
FP Velocity	WSC	0.24	0.09	2.54	0.01	1.98
	Fish Size	-0.01	<0.01	-2.07	0.04	
Water Depth	Fish Size	0.01	<0.01	6.29	<0.001	3.87
FP Water Depth	Brown	0.48	0.06	7.03	<0.001	2.51
	WSC	-0.35	0.16	-2.20	0.03	
	Fish Size	0.03	<0.01	6.19	<0.001	
	Invasive Density	-2.00	0.82	-2.45	0.01	
Benthic Foraging	Brown	-2.21	0.38	-5.75	<0.001	2.19
Drift Foraging	Brown	0.34	0.10	3.43	0.001	2.37
	Fish Size	-0.02	0.01	-2.09	0.04	
Inter-aggression	Brown	2.08	0.78	2.66	0.01	1.75
	WSC	2.62	0.98	2.56	0.01	
	Fish Size	0.12	0.04	3.27	0.001	
Intra-aggression	Brown	-1.65	0.58	-2.86	0.004	1.99
	Fish Size	0.13	0.04	3.34	<0.001	
Inter-avoidance	Brown	-1.67	0.77	-2.18	0.03	2.12
	Fish Size	-0.14	0.06	-2.42	0.02	

Table 4: Stomach content % weight (% WT) and % alimentary index (% AI) of brown trout, brook trout and white-spotted charr (WSC).

Prey taxa	Brook		Brown		WSC	
	%WT	%AI	%WT	%AI	%WT	%AI
Aquatic						
Trichoptera	60.4	84.1	22.1	34.6	60.7	80.9
Ephemeroptera	6.2	1.4	1.6	0.4	2.7	0.4
Plecoptera	4.4	1.4	2.9	1.0	6.5	3.1
Fish	1.0	<0.1	11.4	1.0	<0.1	<0.1
Terrestrial						
Coleoptera	2.1	0.1	NA	NA	3.1	0.4
Hemiptera	17.8	11.5	29.9	52.4	18.0	13.4
Lepidoptera	6.4	1.1	5.9	1.7	2.1	0.1
Hymenoptera	2.9	0.2	9.8	2.3	5.9	1.3
Others	2.2	0.2	20.3	6.5	1.6	0.2

Table 5: Fish and amphibian prey species found in brook and brown trout stomachs, identified by DNA barcoding

Predator Species	Size (mm FL)	Location	Date	Prey Species (weight mg)
Brook	215	Nakagawa	16 Jul	Brook (261)
Brown	206	Nakagawa	16 Jul	Brook (432)
Brown	270	Kitano	28 Jul	Brook (355), Brook (263), White-spotted charr (1252), Brown (2994)
Brown	178	Nakagawa	17 Sep	*Onychodactylus japonicus* (386)
Brown	275	Nakagawa	16 Jul	*Bufo japonicus formosus* (340)
Brown	215	Zenroku	16 Jul	*Bufo japonicus formosus* (283)
Brown	403	Zenroku	16 Jul	*Bufo japonicus formosus* (896)
Brown	225	Zenroku	16 Jul	*Bufo japonicus formosus* (1493)

Table 6: Schoener Index of Overlap values of diet overlap between brown trout, brook trout and white-spotted charr (WSC). Diet overlap is considered biologically significant if PSI ≥ 60% and is indicated by bold lettering.

Species	Brook	Brown
Brook	---	---
Brown	52.8	---
WSC	**92.2**	52.1

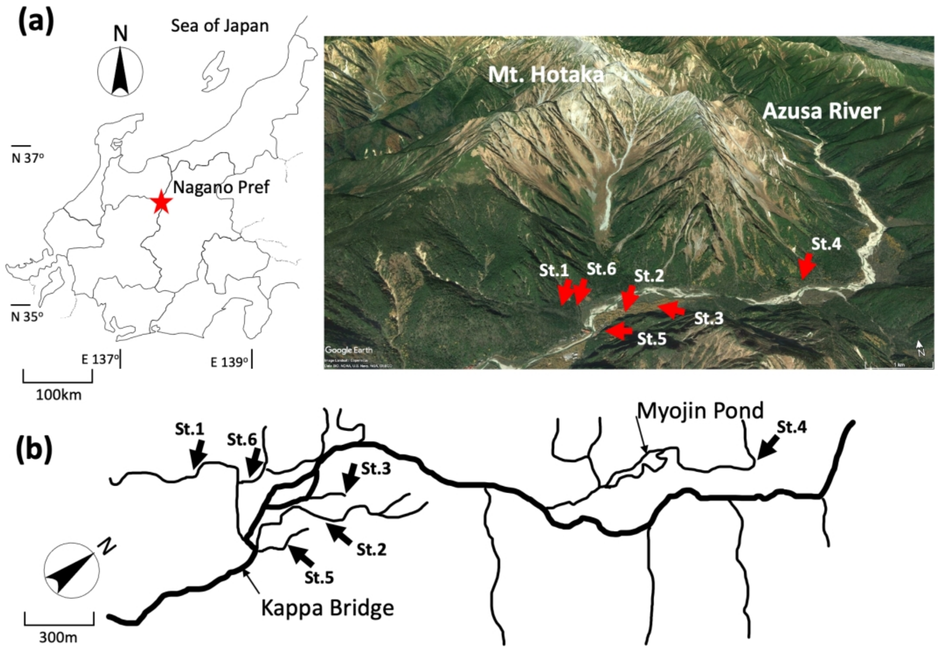

Figure 1

254x190mm (200 x 200 DPI)

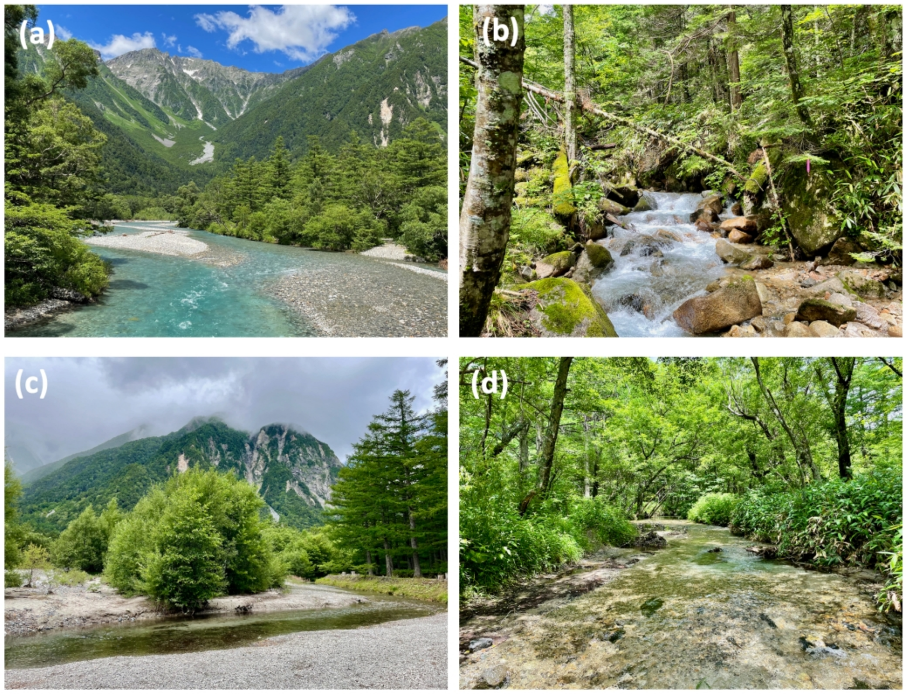

Figure 2

254x190mm (200 x 200 DPI)

Figure 3

254x190mm (200 x 200 DPI)

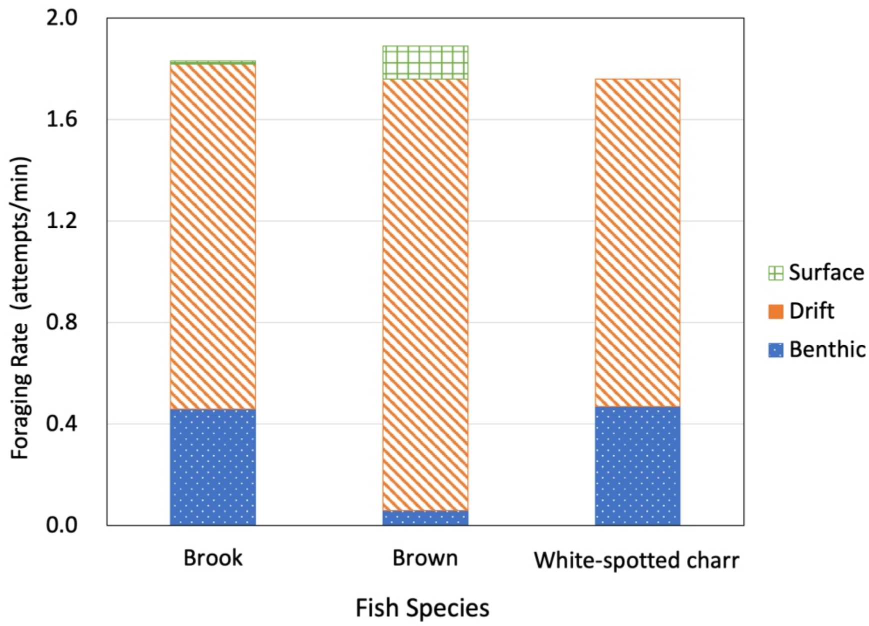

Figure 4

254x190mm (200 x 200 DPI)

GTOC: This study examines habitat, foraging, and diet niche overlaps of invasive salmonids (brown trout and brook trout) and native white-spotted charr in headwater streams. All three species are found in similar habitat, while brook trout and white-spotted charr have nearly identical foraging and diet niches. The combined effects of brown trout and brook trout have likely lead to the decline of white-spotted charr over the last century.

254x190mm (200 x 200 DPI)