

Doctoral Dissertation (Shinshu University)

**Impacts of invasive smallmouth bass and salmonids on native fish
in Japanese freshwater aquatic ecosystems**

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Abstract

Aquatic invasive species have spread globally and are a primary driver of native species decline and biodiversity loss in aquatic systems. In Japan, invasive freshwater fish have been introduced through a variety of pathways including aquaculture, aquarium trade, and sport fishing. These invasive fish are regarded as major social problem with many of the species listed in the 100 worst alien invasive species of Japan. The negative effects of these species on native fish are thought to include direct predation, resource competition and hybridization. Globally, the effects of invasive fish on native species have been studied nearly exclusively in experimental settings and an understanding of how these complex relationships play out in natural settings is lacking. Therefore, I designed four studies, all in natural settings, to elucidate the interspecific interactions between invasive and native fish species, and to determine the negative effects that native fish are experiencing.

In the following studies (1~4) I focused on the effects of smallmouth bass (*Micropterus dolomieu*) on native fish in rivers and lakes and the niche overlap of invasive trout (brown trout: *Salmo trutta* and brook trout: *Salvelinus fontinalis*) and native whitespotted charr (*Salvelinus leucomaenis*) in headwater streams located in central Japan. Smallmouth bass and salmonids are known to be some of the most destructive fish invaders and cause many issues related to predation, competition and hybridization with native fish throughout the world. In areas where smallmouth bass have invaded, native small-bodied fish species such as cyprinids often face decline due to predation. In addition, native piscivorous fish species also can face decline due to competition for fish prey resources. Similarly in areas where salmonids have been introduced, native salmonids generally decline, most often due to prey resource competition and hybridization. Although these generally trends are well studied, predominantly in laboratory setting, the specific mechanisms through which invasive smallmouth and salmonids displace native fish species are lacking, especially in complex natural habitat. Detailed examinations of these specific interactions are paramount and will provide concrete recommendations for management and conservation of native fish in systems where they coexist with these invaders.

Therefore, the goal of this study is to examine the detailed relationships between these invaders and native fish, while providing concrete recommendations on how management practices can enhance native fish survival.

(1) Microhabitat use and diets of smallmouth bass and native cyprinids were examined in a large river system where smallmouth bass were introduced in the early 2000s. In this system, smallmouth bass were found to have shifting effects on native cyprinids based on habitat, with direct predation in slow flowing habitat to prey resource (aquatic invertebrate) competition in faster flowing habitat.

(2) In order to understand how these native cyprinids were responding and possibly adapting to the novel predator, smallmouth bass, the response of Japanese dace (*Pseudaspius hakonensis*) to vicinity smallmouth bass was studied in the lake where the two species have coexisted for ca. 30 years. Dace were found to recognize smallmouth bass as a predation threat and change their behavior accordingly, demonstrated by decreased foraging, a shift from benthic to water column foraging, and increased vigilance.

(3) Smallmouth bass populations continue to increase and spread into a variety of Japanese aquatic systems. To examine favorable spawning conditions, considered to be a driving factor in the continued spread of smallmouth bass, I conducted a two-year daily nest survey and determined egg predators by experimental removal of guarding males in the same lake as study (2). Spawning was found to be highly successful with nearly all nests proceeding to fry dispersal, gobies were the only egg predator observed, and the number of eggs predated was minimal.

(4) In headwater stream systems invasive salmonids are causing problems thought to stem from prey resource competition and direct predation of native species. The interspecific interactions between native whitespotted charr and two invasive salmonids (brown trout and brook trout) were examined in headwater streams. Brook trout and whitespotted charr have nearly identical foraging, diet and habitat niches, while brown trout are distinct and likely have terrestrial ecosystem impacts due to predation on terrestrial prey.

Throughout these examinations of a host of invasive and native species interactions, the effects of aquatic invasive species in the studied region were generalized to be negative but shifted based on habitat and species assemblage as follows: (1) smallmouth bass are not only direct predators of native cyprinids but also compete for prey resources in faster flowing habitat; (2) Japanese dace that have coexisted with these smallmouth bass utilized effective predator avoidance behaviors in response to the novel predator; (3) smallmouth bass may likely continue to increase and spread throughout Japan if favorable spawning habitat is present and native egg predators are lacking; (4) Similar issues were also found in headwater streams as invasive salmonids were competing with native charr for prey resources (primarily aquatic invertebrates) and habitat, while brown trout were found to directly predate on

whitespotted charr, and likely also have terrestrial ecosystem impacts due to high rates of terrestrial insect predation.

Overall, this study is novel in that it examines interspecific interactions of invasive and native fish species exclusively in natural settings, and provides important insights for management of these species. Going forward managers should consider a variety of conservation techniques such as the introduction of underwater cover structure to provide complex habitat where native cyprinids can escape from smallmouth bass, especially in systems where a complete removal of the invasive species is unlikely, or where the invasive species is predicted to expand its' range due to climate change. In areas where native fish are showing behavioral adaptation to the novel predator, the genetic basis of these adaptations should be studied to possibly enhance these traits in native fish populations coexisting with invasive predators. In areas where the invasion range is still limited, targeted removal of invasive fish species and re-introduction of native species should also be undertaken.

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1. Introduction

1-1. Background

Invasive species have been introduced globally and have spread widely causing the decline and loss of native biodiversity in a host of ecosystems (Tran et al. 2015). These species have been introduced through a variety of anthropogenic processes including pet and aquarium trade, shipping, and illegal introductions. In aquatic systems, invasive freshwater fish species have been introduced through aquaculture, pet release, as well as stocking for recreational angling opportunities. In terms of recreational angling, largemouth (*Micropterus salmoides*) and smallmouth bass (*Micropterus dolomieu*) have been stocked, legally and illegally, in all continents besides Antarctica, creating a large global market for bass fishing (Jackson 2002).

In Japan, smallmouth bass were first introduced in 1925, and then again illegally in the 1990's which led to the rapid spread of this species in lakes and rivers throughout the country (Iguchi et al. 2004). Smallmouth bass are considered highly detrimental to native fish, due to their piscivorous diet and aggressive foraging, and are listed in the 100 worst invasive species in Japan (Ecological Society of Japan 2002). Therefore, in many Japanese lakes and rivers smallmouth bass were actively culled in an attempt to fully remove the species. However, due to the large scale of the invasion and the size of many of the invaded systems, a complete removal is typically impractical. At the same time, recreational angling for smallmouth bass has grown in popularity and certain lakes are experiencing a significant increase in revenue from these anglers (Peterson and Kitano 2022).

1-2. Review of Previous Studies

Smallmouth bass are originally native to the Eastern United States and Canada, but have been introduced throughout the world, as early as the 1800s, as a sport-fishing target (Carey et al 2011). These fish are known to be one of the hardest fighting freshwater species when caught by rod and reel and have consequently become enormously popular for sport-fishing (Jackson 2002). Although they may be fun to catch, smallmouth bass often cause the decline of native fish species due to direct predation. Smallmouth bass are known to be piscivorous, meaning that they prey on fish, and because of this smaller bodied prey fish often face obvious decline after smallmouth bass introductions (Zanden et al. 2004). In many areas, managers are faced with the difficult task of balancing angler satisfaction and native fish conservation (Jackson 2002). This can range from a complete acceptance and even enhancement of smallmouth

where angling is set as the leading priority, or the opposite of mechanical removal and culling of smallmouth where native fish conservation is paramount such as in areas where the impacted native species is endangered (Jackson 2002, Rahel and Taniguchi 2019).

In Japan, there was much concern when smallmouth bass first began spreading in the 1990s mainly in Nagano Prefecture, Central Japan (Iguchi et al. 2004). Due to the concern of declining native or historically introduced fish species that were important to angling and commercial inland fisheries, there was also much research at this time on the impacts of smallmouth bass on native fish (reviewed by Katano and Matsuzaki 2012). Through these studies smallmouth bass were found to have negative effects on native cyprinids through a combination of direct predation and also decreased growth rates of the cyprinids while in sympatry with smallmouth bass, likely due to energy spent on predator avoidance (Katano and Aonuma 2001, 2002). These studies provided the basis for much of the extermination efforts of smallmouth bass in Nagano Prefecture in the early 2000s.

When I began my research of smallmouth bass in 2018, the general understanding both of the public and fisheries managers was that smallmouth bass were causing the decline of native fish in a variety of aquatic systems due to their direct predation of other fish. These declines of native fish were first seen in lakes but soon after in rivers as well, as smallmouth bass expanded their range into many of the large river systems in Nagano Prefecture. In Nagano, smallmouth bass are thought to have first been introduced to Lake Nojiri and Lake Kizaki in the 1990s (Iguchi and Yodo 2004) and have subsequently spread into many river systems including the Chikuma and Sai Rivers (Peterson et al 2019). Smallmouth bass were first recorded in the Chikuma River in the early 2000s and rapidly spread and increased in number especially in the middle Chikuma River that flows from Ueda city to Nagano city. Native fish conservation is especially focused on in this area of the Chikuma River as there is a historical commercial fishery for native cyprinids (Peterson et al. 2019). In this area, native and historically introduced cyprinid species are thought to be declining in recent years due to a variety of factors including habitat loss and predation from smallmouth bass (Peterson et al 2019).

Brown trout and brook trout, like smallmouth bass, have been introduced globally due to their popularity of angling and also as a food source. In areas where they are invasive there are often impacts on native fish primarily due to competition and hybridization (Buoro et al. 2016). Behavior changes and foraging shifts of whitespotted charr in response to prey availability and invasive salmonids has been studied extensively in an experimental stream setting in Hokkaido (Nakano et al. 1994, 1999). However, how these relationships play out

between the globally rare three species assemblage of brown trout, brook trout and whitespotted charr remain unstudied and the impacts on whitespotted charr conservation warrant detailed study. In Japan, whitespotted charr are typically found in sympatry with other native salmonids such as masu salmon (*Oncorhynchus masou*) or dolly varden (*Salvelinus curilus*), 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 (Hoxmeier and Dieterman 2015, Dieterman and Mitro 2019, 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 associated 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 whitespotted 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.

Therefore, I decided to focus on these interactions between invasive fish species, smallmouth bass, brown trout and brook trout, and native fish, but to examine them fully in their natural habitat. Lab experiments had shown that direct predation negatively affects native cyprinids (Katano and Aonuma 2001, 2002), but detailed examination of if this simple predator-prey interactions were the same in a more complex natural habitat setting was lacking. I hypothesized that these interactions are likely not just simple predation, but may shift based on habitat type and fish species assemblage. I found that in a small river setting, smallmouth bass not only predate on native cyprinids but also compete with them for aquatic invertebrate prey resources through drift foraging (Peterson and Kitano 2019). I also found that favorable smallmouth bass spawning habitat was allowing these invaders to successfully reproduce in a large river system (Peterson et al. 2019). Although I was beginning to understand how smallmouth bass and native cyprinids were interacting in natural settings the balance between predation and competition was still unclear.

1-3. Aims and Purpose of this Study

A detailed examination of these inter-specific interactions was conducted in the following four studies (1-4).

(1) To understand how predator-prey interactions play out in a natural river setting, microhabitat use and diet overlap of smallmouth bass and native cyprinids were studied in the Chikuma River, in an area where smallmouth bass invaded in the early 2000s (Chapter 2)

(2) Behavior change and adaptation are critical for native species in order to survive under threat from a novel invasive predator. Therefore, behavior changes were studied in a population of Japanese dace which have coexisted with smallmouth bass in Lake Nojiri for 30 years. Foraging and predator avoidance behaviors were analyzed to examine how dace were avoiding predation from smallmouth bass (Chapter 3).

(3) A key factor driving the continued spread of smallmouth bass in Japan is thought to be favorable spawning conditions and a possible lack of native egg predators. To test these hypothesis, smallmouth bass spawning habitat, male guarding behavior, and egg predation when guarding males were removed was studied for two seasons in Lake Nojiri where smallmouth bass are the predominate fish species (Chapter 4).

(4) Invasive salmonids, brown trout and brook trout are thought to be causing the decline of native whitespotted charr in Japan, due to competition, predation and hybridization. In order to examine the competition and predation aspects of this issue, microhabitat, foraging and diet niche overlaps were studied between these three species in headwater tributary streams in the Kamikouchi area (Chapter 5).

For both smallmouth bass and the invasive salmonids, the current study aims to use habitat and diet niche modeling, as well as detailed underwater behavioral observation to determine the extent of habitat, foraging, and diet niche overlap between the invasive and native fish species, as well as the mechanisms by which the invaders displace native species, and the response or possible adaptations that native species are utilizing to coexist with the invaders. Overall, the goal of this study to examine the relationships between invasive and native aquatic species and determine the effects that these invaders have. A detailed examination of these relationships will provide managers with a better understanding of the mechanisms by which native species are negatively affected and eventually decline due to these invasive fish. This will in turn provide the opportunity for targeted management of native fish species, especially in areas where a complete removal of the invasive species is

unlikely, such as enhancement of underwater cover where small bodied native fish can escape from invasive predators.

1-4. Published Manuscript Specifics

Chapter 2 has been published as: Peterson, M. I., Kitano, S. (2021) Habitat dependent predation-competition interaction shifts of invasive smallmouth bass (*Micropterus dolomieu*) and resident cyprinids in the Chikuma River, Nagano Japan. *Environmental Biology of Fishes*, 104, 155-169. DOI: 10.1007/s10641-021-01067-x

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Chapter 3 has been published as: Peterson, M. I., Kitano, S. (2021) Changes in foraging and predator avoidance behavior of Japanese dace (*Pseudaspius hakonensis*) to predation risk by invasive smallmouth bass (*Micropterus dolomieu*) in a Japanese lake. *Environmental Biology of Fishes*, 104, 1381-1389. DOI: 10.1007/s10641-021-01166-9

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Chapter 4 has been published as: Peterson, M. I., Kitano, S. (2022) Spawning season and nest guarding behavior of invasive smallmouth bass (*Micropterus dolomieu*) in a Japanese Lake. *Ecological Research*, 37, 598-608. DOI: 10.1111/1440-1703.12316

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2. Habitat dependent predation-competition interaction shifts of invasive smallmouth bass (*Micropterus dolomieu*) and resident cyprinids in the Chikuma River, Nagano Japan

2-1. Introduction

In addition to direct predation, habitat and dietary overlap between invasive and native species are key factors in determining the effects and extent of an invasion (Tran et al. 2015). Smallmouth bass, *Micropterus dolomieu*, are a freshwater fish native to the United States that were introduced into Japan in 1925 (Jackson 2002). The original population did not spread, but a likely illegal re-introduction in Nagano prefecture, central Japan in the early 1990's led to the rapid increase and spread of smallmouth bass throughout Japan (Yodo and Iguchi 2003a, Iguchi et al. 2004). They are now common in many lakes and reservoirs as well as rivers (Peterson and Kitano 2019). While some managers at certain lakes have embraced smallmouth bass as they provide sport fishing opportunities and increased revenue for rural areas (Rahel and Taniguchi 2019), the majority of managers and local citizens are concerned by the possible negative impacts of smallmouth bass predation on native or historically introduced species such as Japanese dace, *Pseudaspius hakonensis*, ayu, *Plecoglossus altivelis altivelis*, various salmonid species and Japanese smelt, *Hypomesus nipponensis* (Katano and Aonuma 2002, Rahel and Taniguchi 2019).

The spread of smallmouth bass into river systems has caused concern as many inland rivers have active traditional commercial fisheries (Katano and Matsuzaki 2012). In areas where ocean fish were not readily available, commercial fisheries for river fish such as ayu, Japanese dace and pale chub, *Opsariichthys platypus*, were traditionally present (Nakamura 1952, Koyama and Nakamura 1955). These fisheries are in decline due to a variety of factors including fish habitat loss and decreasing stock numbers, introduction of exotic predators such as smallmouth bass, largemouth bass (*Micropterus salmoides*), snakehead (*Channa argus*) and channel catfish (*Ictalurus punctatus*), as well as an ageing fishing population and availability of fresh ocean fish (Matsuzaki and Kadoya 2015). Predation of these species by smallmouth bass could have negative effects as many studies have demonstrated that increasing smallmouth bass populations can cause the decline of native cyprinid and minnow species (Jackson and Mandrak 2002, Weidel et al. 2007, Sharma et al. 2009). In native river smallmouth bass populations in the United States, diets are known to shift from plankton and

aquatic invertebrates in juveniles, to fish and crayfish in adults (Doan 1940, Olson and Young 2003). In Japan, many smallmouth bass populations have been thought to mainly prey on fish (Yodo and Iguchi 2003b). However, recent studies in rivers have shown that in some populations, invertebrates such as mayflies and caddisflies make up a large part of the diet even in adults (Yodo and Iguchi 2004, Peterson and Kitano 2019). These invertebrates are also important prey for native river fish such as chub and dace (Nakagawa et al. 2011, Peterson and Kitano 2019). Therefore, increased invertebrate predation by smallmouth bass likely results in feeding and habitat competition in addition to predation between these species (Peterson and Kitano 2019). However, the balance of competition and predation between smallmouth bass and cyprinids lacks examination, especially in how this balance shifts between habitat types.

The present study aims to determine habitat use and diet of smallmouth bass, Japanese dace and pale chub in the Chikuma River, central Honshu, Japan. Although other cyprinid species are also prevalent in this river section, chub and dace were chosen as they are abundant throughout the study area and have the greatest economic importance as there is an active commercial fishery. An understanding of the balance between predation and competition in different habitats of these species will be beneficial in determining effects of the smallmouth bass invasion and possible mitigation techniques.

2-2. Materials and Methods

2-2-1. Study Area

This study was conducted on a 21km stretch (elevation: 338–387 m) of the middle Chikuma River, Nagano Prefecture of central Honshu in Japan (Fig. 2-1). This section of the Chikuma River flows through Sakaki, Chikuma and Nagano City and is characterized by gentle flowing runs and pools with intermittent fast flowing riffles. Water temperature was approximately 20°C during the study period in both years. This section of the river has relatively high fish diversity with populations of Japanese dace, pale chub, smallmouth bass, Japanese barbel (*Hemibarbus labeo*), ayu, carp (*Cyprinus carpio*) and Amur catfish (*Silurus asotus*) as well as the recently described Tanigawa catfish (*Silurus tomodai*) (Yamashita et al. 2004, Hibino and Tabata 2018, Peterson et al. 2020) among others.

Fish were collected during Sep. 18-21 in 2018 and Sep. 26-29 in 2019. Each year, fish were collected at eight locations using cast nets (15 mm mesh in runs and pools and 18 mm

mesh in riffles) in accordance with the basic survey manual by the Japanese Ministry of Land, Infrastructure, Transport and Tourism (2016). At each location, nets were cast approximately ten times each in one run and pool habitat respectively. Riffles were also sampled by ten casts at the Akasaka and Kamuriki sites both years. In 2018, additional riffles were sampled at the other locations for diet analysis, but this data was not used in habitat analysis. Riffles were categorized as areas with fast flow and turbulent surface, runs as areas with fast flow and non-turbulent surface, and pools as areas with slow flow (see Hawkins et al. 1993 for habitat classification). At eight locations, baseline habitat data was also collected at ten points in each habitat (riffle, run and pool) type respectively. Although habitat types were determined visually according to Hawkins et al. 1993, baseline habitat data corroborated these categorizations (Supplementary Material 2-1). At each cast site (center of open net) habitat data of flow velocity (measured using flow meter CR7: Cosmo-Riken Ltd., at 60 % of total depth), water depth and dominant and subdominant substrate type was collected. Substrate size was classified into six categories: 1, silt or sand (< 2 mm); 2, gravel (2–16 mm); 3, pebble (17–64 mm); 4, cobble (65–256 mm); 5, boulder (> 256 mm) and 6, flat bedrock (Mark et al. 1985). Each captured fish larger than 50 mm was measured (standard length: SL) and species was determined. The main survey was conducted in September when the three study species have finished spawning and are most likely to be feeding in order to accumulate nutrients for the coming low temperature season (Peterson et al. 2020, Satoshi Kitano unpublished data). However, to examine seasonal fish distribution trends, riffle, run and pool habitat was also sampled at the Akasaka and Kamuriki sites during May (18-19 in 2018, 14-15 in 2019), June (12-13 in 2018, 10-11 in 2019) and July (19-20 in 2018, 11-12 in 2019) each year using the same habitats as in September. These additional surveys used a simplified method in which ten casts were used in each habitat but habitat data for each cast was not taken.

2-2-2. Data Analysis

Habitat variables were analyzed for the three study species: dace, chub and smallmouth bass. Mean habitat flow velocity, water depth and dominant substrate size were compared between species using ANOVA, and post-hoc Tukey's HSD tests were used to find posteriori differences ($\alpha = 0.05$) between species means. Fish density by species was calculated for each habitat at each location by adding the total biomass of a certain species caught at that habitat and dividing by the total surveyed area. Fish weight was calculated from individual

SL using specific equations for each species (Kitano unpublished) (Supplementary Material 2). The total surveyed area was calculated by multiplying the area covered by an open cast net (12.56m²) by the number of casts in each habitat. To visualize the spatial and temporal differences in species distribution and abundance, non-metric multidimensional scaling (NMDS) was performed using species density at each study site habitat by the function *metaMDS* in the R package *vegan* 2.5-6.

To categorize and compare habitat use between the three species, a habitat index value of the flow velocity divided by water depth was also calculated (Dolling 1968, Jowett 1993). High values indicate riffle-like habitat (shallow and fast) while low values indicate pool-like habitat (deep and slow) with run habitat falling between the two. Factors associated with habitat use were analyzed using a general linear model (GLM) with Gamma error structure. Fish species, fish size and density of each species at each location were set as fixed effect variables and habitat index value as a response variable. Smallmouth bass were set as the reference category for species. An explanatory variable was considered to have a statistically significant effect when the estimated coefficient of the variable did not include zero in a 95% confidence interval.

To evaluate habitat selection based on flow velocity and water depth, the Manly-Chesson index α (Manly 1974, Chesson 1978, 1983) was calculated.

$$\alpha = \frac{r_i/P_i}{\sum_{i=1}^n r_i/P_i}$$

Where r_i = the proportion of a habitat range in the total habitat use of each species, P_i = the proportion of a habitat range in the total available habitat and n = the number of habitat ranges in the environment. The values of α range from 0 (complete avoidance) to 1 (complete preference). If $\alpha = 1/n$, the fish is using habitat randomly and use of each habitat range is used in proportion to abundance in the environment. $\alpha > 1/n$ indicates habitat preference while $\alpha < 1/n$ indicates habitat avoidance (Jarv et al. 2011).

2-2-3. Diet Analysis

In 2018, fish (smb n=96, dace n=66 and chub n=92) were collected at each location for diet determination. Collected fish were euthanized using an anesthesia overdose of FA 100 (DS Pharma Animal Health Co., Ltd.), placed in formalin solution (10%) in the field and

transported to the laboratory (Nagano Environmental Conservation Research Institute Iizuna Office). At the laboratory, stomachs were removed from each fish and the wet weight of total stomach contents was recorded. For smallmouth bass, whole stomachs were removed, and in chub and dace, anterior gut segments (up to the first bend) were removed. Stomach contents were examined under microscope (Nikon 2782811 Tokyo, Japan) and classified into a variety of algae, invertebrate, and fish categories. Each prey category was recorded as the percentage of the total stomach content wet weight (%WT) and invertebrate and fish prey size was also measured. Percentage wet weight of each prey category was determined by evenly spreading the entire stomach content on a gridded petri dish and determining the ratio of surface area covered by each prey type. Percent occurrence (%OC) was calculated as the ratio of predator individuals with each prey category present and the total number of predator individuals examined. The alimentary index (IA) was also calculated for each prey category i by multiplying %WT $_i$ and %OC $_i$ to take into account the weight of different prey types and expressed as percentage (Lauzanne 1975, Mabragana and Giberto 2007).

The Schoener Index of Overlap, also known as the Percent Similarity Index (PSI) (Schoener 1974), was used to estimate diet overlap by species and habitat (Carbrol et al. 2002, Hilgers et al. 2018, Falke et al. 2020). PSI is calculated as

$$PSI = [1 - 0.5 \sum_{i=1}^n |P_{ik} - P_{jk}|] \times 100$$

where P is the proportion of biomass (wet weight) of the k th prey category consumed by predator species i and j . Diet overlap values (PSI) $\geq 60\%$ were considered biologically significant, and lowest available taxonomic groupings were used to calculate the PSI (Wallace and Ramsay 1983, Hill et al. 2015).

2-3. Results

2-3-1. Distribution and Density

In September 2018, smallmouth bass (n=281) were found at 6/8 sites and in September 2019 (n=42) at 6/8 sites. In 2018, dace (n=172) were found at 8/8 sites and in 2019 (n=117) at 8/8 sites. In 2018, chub (n=246) were found at 8/8 sites and in 2019 (n=257) at 8/8 sites (Fig. 2-1). In 2018, mean fish density per location throughout the study area was $9.76 \pm 2.31SE$ g/m²

and in 2019 it was $2.61 \pm 0.57\text{SE g/m}^2$. In 2018, within the three study species, smallmouth bass ($2.84 \pm 1.07\text{SE g/m}^2$) had the highest mean density throughout the study area followed by chub ($1.82 \pm 0.39\text{SE g/m}^2$) and dace ($1.12 \pm 0.30\text{SE g/m}^2$). In 2019, chub ($0.93 \pm 0.22 \text{g/m}^2$) had the highest mean density throughout the study area followed by smallmouth bass ($0.70 \pm 0.30\text{SE g/m}^2$) and dace ($0.36 \pm 0.10\text{SE g/m}^2$). NMDS indicated that smallmouth bass spatial distribution was closer to chub and dace in 2018 than in 2019, and that they were found in more downstream habitat than dace as chub as well (2D stress: 0.128) (Fig. 2-2). In addition to the three study species, in 2018 six other species, Japanese barbel, topmouth gudgeon (*Pseudorasbora parva*), ayu, tamoroko (*Gnathopogon elongatus elongatus*), funa (*Carassius sp.*) and carp were captured and in 2019 seven, Japanese barbel, topmouth gudgeon, ayu, freshwater goby (*Rhinogobius sp.*), higai (*Sarcocheilichthys sp.*), tamoroko and largemouth bass (*Micropterus salmoides*) were captured. Of these additional species, Japanese barbel were most prevalent, being found at nearly every site in both years, followed by ayu which were found at three sites in 2018 and two in 2019 (Table 2-I).

Additional simplified surveys conducted at the Kamuriki and Akasaka location each year showed that dace, chub and smallmouth bass were prevalent at both locations from May through September (Table 2-II). In 2018, smallmouth bass were found in high densities in run and riffle habitat in May, July and September especially at the Kamuriki site. However, in 2019 smallmouth bass at the Kamuriki site had highest densities in pool habitat in all months.

2-3-2. Size Distribution

In both years, captured individuals of all three species were predominantly in the 50-125mm size (standard length) class range (Fig. 2-3). Within this range, in both years all three species were most common in the 50-75mm size class. Larger (150mm+) smallmouth bass were captured both years although the frequency of these larger fish was low. In 2018, the large amount of smallmouth bass captured in run and riffle habitat were in the 50-125mm size range while larger individuals were mostly captured in pool habitat. In 2019 smallmouth bass were scarcely captured in run and riffle habitat. In both chub and dace larger individuals were more commonly found in run and riffle habitat compared to smaller individuals in pool habitat.

2-3-3. Habitat Use

In both years, dace, chub and smallmouth bass were found throughout riffles, runs and pools in the study section. In 2018, smallmouth bass were found in areas with the highest average flow velocity, followed by chub and dace. Smallmouth bass were found in areas with the deepest average water depth, followed by chub and dace. In 2019, dace had the highest average habitat flow velocity, followed by chub and smallmouth bass. Smallmouth bass were found in areas with the deepest average water depth, followed by chub and dace (Table 2-III). An index value (flow velocity divided by water depth) was used as an indicator of habitat type. High values indicate riffle-like habitat (shallow and fast) while low values indicate pool-like habitat (deep and slow). In 2018, smallmouth bass had the highest mean index value, followed by chub and dace. In 2019, dace had the highest mean index value, followed by chub and smallmouth bass (Table 2-III). In both years, Tukey HST tests indicated that mean index values for chub and dace did not significantly differ. However, smallmouth bass mean index values were significantly different than chub and dace, although they were higher in 2018 and lower in 2019. Smallmouth bass mean habitat index values and density were compared between 2018 and 2019. When smallmouth bass density was high in 2018, smallmouth bass had a significantly higher mean habitat index value ($p < 0.01$) compared to 2019 (Fig. 2-4). Smallmouth bass size (standard length) showed significant negative correlation with the habitat index value in 2018 but was not significant in 2019. Dace size was not significantly correlated with the habitat index value in 2018 but showed significant positive correlation in 2019. Chub size showed significant positive correlation with the habitat index value in both years (Fig. 2-5). Baseline habitat mean substrate values were $3.16 \pm 0.20SE$ in riffle, $3.66 \pm 0.09SE$ in run and $2.80 \pm 0.11SE$ in pool habitat. In 2018, smallmouth bass were found in areas with largest mean substrate, followed by chub and dace. In 2019, smallmouth bass also had the largest mean substrate value, followed by chub and dace (Table 2-III). All three species were found in areas with significantly larger substrate in 2018 compared to 2019, with smallmouth bass showing the most significance (dace $p = 0.01$, chub $p < 0.001$, smallmouth bass $p < 0.0001$).

Factors affecting habitat use were varied among species and years as shown by GLM modeling (Table 2-IV). In 2018, species density had the only significant effect on habitat index values. This effect was positive and did not significantly differ between the three fish species. In 2019, species density had no significant effect on habitat index values. However, species chub and dace both had a significant positive effect on habitat index value compared

to smallmouth bass. Fish size (SL) also had a significant positive effect on habitat index values.

2-3-4. Habitat Selectivity

In terms of habitat flow velocity, in 2018 the Manly-Chesson index indicated that smallmouth bass had a preference for slow flowing habitat (0-15cm/s) and relatively fast flowing areas (90-105 cm/s), dace had a preference for slow flowing habitat (15-30 cm/s) and chub habitat use was fairly random. In 2019, the only strong preference indicated was for smallmouth bass in slow flowing habitat (0-15 m/s) (Fig 2-6a). With regard to habitat water depth, in 2018 smallmouth bass showed a preference for habitat with a water depth of 135-150 cm and 165-180 cm, and in 2019 a similar trend was found for smallmouth in habitat with water depth of 105-135 cm as well as for dace 120-135 cm (Fig. 2-6b).

2-3-5. Stomach Contents

In 2018, smallmouth bass (n=96, 15 empty stomachs), dace (n=66, 28 empty stomachs) and chub (n=92, 6 empty stomachs) stomach contents were analyzed. In riffle and run habitat, dace, chub and smallmouth bass all had the highest %IA for ephemeroptera larva. Diatom, diptera and algae prey were also found in small amounts in chub and dace, while fish prey was found in small amounts for riffle smallmouth bass and was more prevalent in run smallmouth bass. In pool habitat, dace had the highest %IA for diatom prey, chub for ephemeroptera, while smallmouth bass had the highest %IA for fish prey (Table 2-V). Within smallmouth bass, size was also correlated with diet as larger fish consumed a significantly higher %AI of fish ($r= 0.73, P < 0.001$). Although much of the prey fish were unidentifiable due to advanced digestion, 13 individual prey fish were identified. These identified preys were all cyprinids, although species (likely dace, chub or barbel) was unclear. Percent similarity index (PSI) was calculated in each habitat between the three species. In riffles, chub, dace and smallmouth bass all showed biologically significant ($\geq 60\%$) diet similarity, with dace/chub being highest at 86.5%, chub/smallmouth bass at 70.3% and dace/smallmouth bass at 69.5%. In runs, chub and dace diets had significant similarity (84.7%) however, smallmouth bass diet was not significantly similar to either chub or dace. In pools, chub and dace diets also had significant similarity (83.2%) while smallmouth bass diet was not significantly similar to either chub or dace (Table 2-VI).

2-4. Discussion

This study produced comparative habitat use results for dace, chub and smallmouth bass for two years. Although there were similarities between the years as chub and dace were found in overlapping habitat that was significantly different from smallmouth bass habitat, a major difference was that in 2018 smallmouth bass were found in much faster flowing areas (Table 2-III). This trend was also found in the seasonal surveys, especially at the Kamuriki site and the result of smallmouth bass utilizing riffle or run habitat with high flow contradicts much literature on smallmouth bass river habitat use. In general, smallmouth bass are typically found in slower flowing areas of rivers such as pools (Dauwalter and Fisher 2008, Ettinger-Dietzel et al. 2015 Peterson and Kitano 2019). In this study, in 2019, smallmouth bass were found in much more typical slow flowing habitat. The other main difference between 2018 and 2019 was fish density. Overall density in all three species was significantly higher in 2018 than 2019, especially in smallmouth bass (Fig. 2-4). Therefore, we suspect that when fish density is high, interspecific and intraspecific competition occurs for habitat use and prey (Schlosser 1984, Dong and DeAngelis 1998). This was supported by the 2018 GLM results, as in areas where species density is high, all three species are found in habitat with higher index values (fast and shallow). In 2019, chub and dace were found in habitat with higher index values compared to smallmouth bass. These species effects found in 2019 were likely masked by the overall high fish density in 2018. Therefore, when density is high, fish, especially smallmouth bass, may be likely to expand their habitat range into more riffle-like habitat. If smallmouth bass, especially larger individuals, prefer to prey on fish in slow flowing habitat (Orth and Newcomb 2002, Todd and Rabeni 1989, Dauwalter et al. 2007), intraspecific competition will occur as this type of habitat is limited in the middle Chikuma River (Peterson et al. 2020). When this competition occurs, larger fish will likely settle in their preferred habitat while smaller individuals are able to expand their habitat range into faster flowing areas and feed on aquatic invertebrates (Pert et al. 2002). Yearling and subadult smallmouth bass have also been shown to have wider suitable habitat criteria in terms of flow velocity than adults (Orth and Newcomb 2002, Brewer and Orth 2014), and therefore are likely well suited for moving into faster flowing habitat when density is high. This is what was likely observed in 2018, as smaller smallmouth bass were found in faster flowing areas in which they were hardly found in 2019 and smallmouth bass size was significantly negatively correlated with habitat index values.

In terms of diet, this study indicates that within the Middle Chikuma River system, smallmouth bass diet differs based on habitat during the studied time period (Table 2-III). This diet shift based on habitat was also found for chub and dace, and the interactions between smallmouth bass and the cyprinids appeared to shift from predation in slow flowing habitat to prey competition in faster flowing habitat. In slower flowing habitat (pools and runs) the PSI was not biologically similar between smallmouth bass and the cyprinids. Smallmouth bass captured in pools were larger in size and fed primarily on fish prey as well as aquatic invertebrates while chub and dace fed on aquatic invertebrates and diatoms. However, in riffles, all three species fed primarily on ephemeroptera larva and PSI were biologically similar between the three species (Table 2-V, 2-VI). Smallmouth bass predation of ephemeroptera larva in faster flowing areas is likely influenced by the interspecific habitat competition mentioned above. In this study, the ratio of fish prey in a smallmouth bass' diet increased with fish size. However, even within smaller smallmouth bass, fish prey made up a larger part of their diet in slower flowing areas. Therefore, as smaller fish move out of slower flow areas, they enter faster flow and likely feed on ephemeroptera larva as indicated by juvenile smallmouth bass habitat and diet analysis conducted by Easton and Orth (1992), Sabo and Orth (1995 and Sabo et al. (1996). We have previously found smaller smallmouth bass feeding on ephemeroptera larva (Peterson and Kitano 2019) and the current study supports this observation. A possible reason for these smaller fish shifting to an invertebrate diet is that it may be more energy efficient to drift feed for invertebrates rather than pursue fish prey in faster flowing habitats as shown by juvenile smallmouth bass net energy gain studies conducted by Sabo et al. (1996) and Sabo and Orth (1995).

It should be noted that overall, very few large smallmouth bass were captured in the current study, and most fish were in the 0-2 age-class range for both years (age is estimated using SL and previous scale reading) (Supplementary Material 2-3). Also, habitat and diet analysis were conducted only in September for both years and a detailed understanding of seasonal trends requires further study. Cast net survey habitat requirements also limited the area that could be effectively sampled as habitats with water depth deeper than two meters and areas with large substrate size (boulder etc.) could not be surveyed. The lack of larger individuals may be a result of continued culling efforts of large smallmouth bass in this river section over the last five years (Kitano unpublished). The current results indicate that continued culling would likely be beneficial in keeping the smallmouth bass density at a low level. High smallmouth bass density, as in 2018, appears to have a greater negative impact on cyprinids as not only are they preyed on by smallmouth bass in low flow habitat, but they

also face invertebrate prey competition with smaller smallmouth bass in faster flowing habitat. These faster flowing habitats are likely a refuge where cyprinids can typically avoid contact with smallmouth bass, as Gorman (1987) and Schlosser (1984) found high densities of young minnows in fast and shallow habitat where predator density was low. However, when smallmouth bass density is high, cyprinids may encounter and be negatively affected by smallmouth bass in a wider habitat range.

These results provide a basis for predicting the effects of smallmouth bass invasion in different systems. A strong negative effect caused by direct predation of other fish by smallmouth bass will likely be found in areas with little or no flow including lakes, and slow flowing river systems. This has already been confirmed in lakes systems in Japan as well as worldwide (Jackson 2002, Yodo and Iguchi 2003b, Loppnow et al. 2013). However, the effect of smallmouth bass may be less negative in faster flowing systems as direct predation will likely decrease. When direct predation decreases though, the indirect negative effect of prey competition will likely increase. This also indicates that when smallmouth bass density increases in a certain system, the negative effects on other fish will likely exponentially increase. When density is high, not only will larger smallmouth bass predate on other fish in slow flow habitat, but smaller smallmouth bass will compete for invertebrate prey with other fish in faster flowing areas in which smallmouth bass are not typically found. This means that for other fish, there will be no escape from some type of negative affect of smallmouth bass regardless of habitat type. This model is also dependent on the abundance of aquatic invertebrate prey, which is high in the middle Chikuma River, as indicated by numerous studies on ephemeroptera in this section of the river (Inoue et al. 2011, Hirabayashi et al. 2016, Saito and Tojo 2016a, Saito and Tojo 2016b). In systems where aquatic invertebrate prey is scarce, smallmouth bass may predate on other fish even when habitat flow is faster. This same model can be used within a single system as well, as the effect of smallmouth bass on other fish shifts from predation in slow flowing areas to prey competition in faster flow areas. However, a more detailed seasonal analysis is needed to model interactions between these species in other seasons such as during spring and early summer spawning activity. The shifting effect of invading smallmouth bass depending on habitat is important to consider when thinking about the outcomes of invasions in different systems. However, an important factor that has not been considered is the ability of different fish species to adapt to invading smallmouth bass. If certain native species are better able to adapt and escape from predation they will likely fare better in coexistence with smallmouth bass. If species are unable to

adapt, they will likely face decline, especially in areas with slower flow and high rates of smallmouth bass predation.

3. Changes in foraging and predator avoidance behavior of Japanese dace (*Pseudaspius hakonensis*) to predation risk by invasive smallmouth bass (*Micropterus dolomieu*) in a Japanese lake

3-1. Introduction

In Chapter 2, I examined the balance between predation and competition of smallmouth bass and cyprinids in the Chikuma River and to see how these interactions change based on habitat. I found that the negative effects of smallmouth bass on native cyprinids are complex and that they shift between predation and resource competition based on habitat. This answered some of the questions regarding why smallmouth bass were feeding on invertebrates rather than fish in certain areas. However, an important question that arose was how these native cyprinids were responding to smallmouth bass, especially in areas where they have coexisted for 20-30 years. Are certain native species better suited to adapting their behavior to avoid smallmouth bass predation, as found in Canada (Zanden et al 2004)? If so, what behavior changes are utilized to enhance survival? In the Chikuma River, it seems like smallmouth are preying on cyprinids less than they did when they first invaded 20 years ago (Kitano unpublished). There are likely many factors that drive this diet shift, including energy efficient drift foraging found in Chapter 2, but a behavioral adaptation in native cyprinids may be making it more difficult for smallmouth bass to predate on them as well. These are crucial questions in regard to native cyprinid conservation in areas where a complete removal of smallmouth bass is impractical. Therefore, to examine detailed behavioral changes of cyprinids under smallmouth bass predation threat, in Chapter 3 I constructed a study in Lake Nojiri, which provides a unique opportunity in that Japanese dace and smallmouth bass have coexisted for 30 years, using underwater video observation and behavioral analysis. These types of experiments have been conducted in lab settings extensively, but I decided to try and observe behavior changes of dace in the vicinity of smallmouth in a natural in-lake setting.

Lake Nojiri in Nagano Prefecture provides an interesting case study in smallmouth bass management in Japan, as it is likely where the 1990s re-introduction of smallmouth bass occurred, and after a period of targeted removal, the local fisheries cooperation has embraced smallmouth bass sport fishing and it is now one of the premier smallmouth bass sport fishing destinations in Japan (Yodo and Iguchi 2003, Peterson et al. 2021). The Nojiriko Fisherman's Cooperative Association (NFCA) obtained prefectural government permission in 2009 to

allow catch and release angling of smallmouth bass, which was an exceptional case under the current Invasive Alien Species Act in Japan (Nagano Prefecture). Currently, managers at Lake Nojiri are attempting to balance the positive effects from increased smallmouth bass sport fishing and the negative effects from smallmouth bass predation on other species such as Japanese smelt and Japanese dace. Smallmouth bass are not stocked and spawn naturally, while native and historically introduced species such as dace, smelt and kokanee salmon are stocked annually (Peterson et al. 2021). In Lake Nojiri, the Japanese dace population experienced significant decline after smallmouth bass establishment likely due to direct predation but has rebounded in recent years possibly in part due to the resurgence of aquatic vegetation which may provide cover from smallmouth bass predation (Nakamura et al. 2004, NFCA personal communication). The aquatic vegetation of Lake Nojiri was decimated after the introduction of invasive grass carp, *Ctenopharyngodon idella*, in the 1970s aimed to control algae blooms, but has recently recovered due to the continued culling of grass carp along with the fact that they are not able to spawn in lentic environments (Chilton and Muoneke 1992, NFCA personal communication).

Recent research on interactions between smallmouth bass and other fish species in Japan has indicated that smallmouth bass directly predate on other fish as well as compete for prey resources and available habitat (Yodo and Iguichi 2004, Peterson and Kitano 2019, Peterson and Kitano 2021). In lakes, direct predation is likely the major driving factor for the decline in other species, while in rivers the negative effects appear to be a balance of predation and competition based on habitat (Peterson and Kitano 2021). Although smallmouth bass are targeted for removal in many systems, a complete removal, especially in larger lakes and rivers is considered impractical (Rahel and Taniguchi 2019). This reality along with the increasing popularity of smallmouth bass sport fishing in Japan indicates that the spread of smallmouth bass will likely continue. If complete removal is unattainable, the response and adaptation of native species to invading smallmouth bass requires study to understand if and how these species will be able to coexist. Laboratory experiments have indicated that species such as ayu and Japanese dace exhibit reduced growth due to reduced foraging rates while in the same tank as smallmouth bass (Katano and Aonuma 2002). In predator-prey interactions, not limited to fish, prey species often have reduced foraging rates as well as an increase in avoidance or vigilant behavior (Draulans 1987, Brown et al. 2001, Creel et al. 2014). In fish species, especially sportfishing game-fish, these interactions are mostly studied in laboratory settings (Magnhagen 1988, Katano and Aonuma 2002, Tang et

al. 2017) and an understanding of how these interactions play out in natural environments remains unclear.

The current study aims to directly observe Japanese dace response to smallmouth bass in a natural lake setting. Change in foraging rates, foraging modes, and predator avoidance behaviors when dace are in proximity to smallmouth bass are examined to visualize the effects of smallmouth bass on dace and the methods by which dace respond to smallmouth bass.

3-2. Materials and Methods

3-2-1. Study Area

Interactions between Japanese dace and smallmouth bass were studied in Lake Nojiri, Nagano Japan. Lake Nojiri is located at an altitude of 657m, with an area of 4.56km², and maximum depth of approximately 39m. In summer, water temperatures reach near 30 C° while in winter they decrease to 4 C°, although the lake rarely freezes in recent years. There are populations of gamefish such as Japanese smelt, smallmouth bass, largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), kokanee salmon (*Oncorhynchus nerka*), and common carp (*Cyprinus carpio*), as well as non-targeted species such as Japanese dace and freshwater gobies (*Rhinogobius* sp.) (Peterson et al. 2021). The present study focused on the interactions between dace and smallmouth bass in shallow areas near the shoreline. A 500m stretch of the southern shoreline was surveyed along with an additional site on the north-west shoreline (Fig. 3-1a). Fish were observed extensively on numerous occasions (n=38days) from 9 June, 2020 – 1 October, 2020, during daylight hours (5:00 – 17:00).

Fish were observed by underwater video recording. Cameras (GoPro Hero 7, 8) were placed in areas with dace by snorkeling the shoreline looking for dace schools. When schools were located, cameras were placed underwater right along the shore facing out into deeper water (Fig. 3-1b). After placing the camera, the snorkeler exited the water. At each site, video was recorded for 15 minutes, but the first 5 minutes were not used for analysis to allow fish to acclimate to the camera. Water visibility was typically 3 – 4m and fish observations were not made on days when water visibility was less than 3m. Dace less than 40mm TL (young of the year, which were not schooled and foraged sporadically) and smallmouth bass less than

75mm TL (not considered a direct predation threat: Katano and Aonuma 2001) were excluded from analysis.

3-2-2. Video Analysis

Each video file was analyzed to determine dace foraging rates and modes, habitat use, presence of predators, and dace schooling behavior. Foraging modes were set as benthic foraging, water column foraging and surface foraging. Foraging attempts were counted when a fish's mouth was observed to open and close. Dace individuals intercepted the water surface during surface foraging, contacted the substrate during benthic foraging, and all other foraging attempts were classified as water column foraging (modified from Fausch et al. 1997). Total foraging attempts of each type were counted for all individual dace visible in the camera frame that remained in the frame for more than 10 seconds. Foraging attempts were counted for each individual up to 60 seconds. If an individual left the camera frame prior to 60 seconds, the time (seconds) that the individual remained in-frame was recorded and used as an offset in the statistical modeling to account for varying observation times of each individual. Once an individual dace left the camera frame, all possible efforts (body size, color, distinguishing markers etc.) were made to not "double-count" the individual if it returned to the camera frame. Predators were considered to be in vicinity of an individual dace when they were visible in the same camera frame (approximately within 4m of each dace individual due to water visibility limitations) as that dace individual but not directly attacking. Predator size (total length TL) was estimated visually by comparing fish size to pre-measured lake bottom substrate. Total predator biomass was calculated using the TL of all predators (Peterson and Kitano 2021) present within the same camera frame as each dace individual. In addition to foraging attempts, number of total dace (within the same camera frame as each dace individual), distance of dace to nearest dace neighbor (estimated using pre-measured lake bottom rocks), and the ratio of the nearest 10 dace neighbors with the same body orientation were recorded for each individual dace. Body orientation was split into four categories: facing toward, away, right and left of the camera (simplified for in-lake use from Gautrais et al. 2012, Marras and Domenici 2013). Direct predator attacks were recorded when a predator accelerated swimming speed and lunged toward a dace individual causing the dace to disperse. Habitat data of water depth, dominant and subdominant substrate type and presence of cover within 50cm diameter were taken for each dace individual. Substrate

size was classified into the following six categories: 1, silt or sand (< 2 mm); 2, gravel (2–16 mm); 3, pebble (17–64 mm); 4, cobble (65–256 mm); 5, boulder (> 256 mm) and 6, flat bedrock (Mark et al. 1985). Cover consists of underwater structures such as undercut banks and rocks wider than 20cm which are wide enough for a fish to hide under, or overhead structures such as docks, logs or terrestrial vegetation within 1m from the water surface (Hasegawa and Maekawa 2008).

3-2-3. Data Analysis

In dace foraging and response variable analysis, each individual dace observation was treated as an individual data point. To examine the effects of a variety of factors on dace foraging and response variables, generalized linear mixed models (GLMM) were conducted for each dace response or foraging variable. Predator presence, predator biomass, dace size, number of dace, water depth, cover presence and days after start were set as fixed effect variables and total foraging, benthic foraging, water column foraging, nearest neighbor distance and neighbor orientation were set as response variables. For total foraging, benthic foraging and water column foraging, Poisson error structure was used with time if frame as an offset and video file number as a random effect variable to account for dace individuals in the same shoal likely affecting each other's behavior. Poisson error structure was also used for nearest neighbor distance, as distances were rounded to the nearest whole number (cm) to account for the limitations of underwater distance estimation, with video file number set as a random effect variable. Binomial error structure was used for neighbor orientation with video file set as a random effect variable. An explanatory variable was considered to have a statistically significant effect when the estimate coefficient of the variable did not include zero in a 95% confidence interval. Stepwise model selection was used to determine the best model (with the lowest Akaike's information criterion (AIC)) for each GLMM (Burnham and Anderson 2002). Fixed variables were checked for multicollinearity using the Pearson's correlation before inserting into the GLMM models. All analysis was conducted using R: version 4.1.0. (R core team 2021).

3-3. Results

Dace individuals (mean TL \pm SE: 8.3 ± 0.9 cm, range: 4.0 – 17.0cm) were observed with a vicinity predator (N = 193, Fig. 3-1c) and without a vicinity predator present (N=311, Fig. 3-1d) during the study period. A total of 59 predators, all smallmouth bass (mean size TL \pm SE: 19.5 ± 1.3 cm, range: 7.5 – 50.0cm), were observed (Fig. 3-2). Dace and predators were found along the 500m stretch of southern shoreline from 9 June, 2020 – 21 August, 2020. After 21 August the dace schools dispersed and could not be found along the southern shoreline. Therefore, the survey area was moved to a point on the north-west shoreline where dace were prevalent and observations were made at this point until 1 October. At the southern shoreline, dace were found in habitat with shallow water depth (mean \pm SE: 40.7 ± 0.9 cm) and a mix of gravel and pebble substrate (mean substrate score \pm SE: 2.5 ± 0.08). At the north-west shoreline point, dace were found in similar shallow habitat (mean \pm SE: 44.9 ± 1.8 cm) and either flat bedrock or sand substrate (mean substrate score \pm SE: 2.1 ± 0.2). Docks were the only type of cover observed where dace were present. Water temperature remained above 20C° for the entire study period and reached a maximum of 28C° on 25 August.

3-3-1. Foraging and Avoidance Behavior

Dace were observed using primarily water column foraging and benthic foraging, while surface foraging was rare (Fig. 3-3). Overall dace had a mean foraging rate of 15.51 ± 0.41 SE attempts/min, and remained in frame for a mean of 27.62 ± 0.31 SE seconds. When predators were absent, dace foraged significantly more frequently (mean 20.83 ± 0.41 SE attempts/min) than when predators were present (mean 6.84 ± 0.31 SE attempts/min) (t-test, $p < 0.001$). Direct predator attacks were rare as they were only observed on three occasions during the entire study period. In each of these attacks smallmouth bass were not successful in capturing dace. Factors affecting dace foraging and response variables were tested using GLMM analysis (Table 3-I). For all response and foraging variables, predator presence had a significant effect. For dace total foraging, predator presence, number of dace, days after start and predator biomass all had significant negative effects. For benthic foraging, predator presence and predator biomass had significant negative effects. For water column foraging, predator presence had a significant positive effect. For nearest neighbor distance, predator presence and water depth had significant negative effects. For neighbor orientation, predator presence had a significant positive effect (Table 3-I).

3-4. Discussion

This study produced comparative foraging and behavior results for Japanese dace in the presence and absence of predators. In Lake Nojiri, dace schools and smallmouth bass, the only observed predator, were found overlapping in shallow habitat along the shoreline throughout the study period. Dace continuously foraged during the study period, but foraging rates and foraging modes were significantly changed when smallmouth bass were nearby. Dace encountering vicinity smallmouth bass foraged significantly less and foraging modes shifted from predominantly benthic foraging in dace without smallmouth bass to water column foraging in dace with smallmouth bass (Table 3-I, Fig. 3-3). Dace avoidance behavior was also significantly influenced by the presence of smallmouth bass, as dace schooled tighter by reducing nearest neighbor distance and increasing neighbor alignment when smallmouth bass were in vicinity (Table 3-I). GLMM modeling indicated that the presence of a predator had highly significant effects on all dace foraging and response variables. Predator presence had a negative effect on total foraging and benthic foraging, while it had a positive effect on water column foraging. With regard to predator avoidance behavior, predator presence had a negative effect on nearest neighbor distance and a positive effect on neighbor orientation (Table 3-I). All of these results indicate that in response to smallmouth bass, dace forage less, shift their foraging mode and exhibit predator avoidance behavior. GLMM modeling also indicated that several other factors besides predator biomass have significant effects on dace behavior. Days after start had a significant negative effect on total foraging. During the study period, dace appeared to be foraging most frequently in early summer and foraging became less frequent as the study progressed regardless of smallmouth bass presence. The reasons for this are unclear but might be related to reduced prey availability and oxygen supply required for metabolic demands with increasing water temperatures as the study progressed (Peng et al. 2014). Water depth also had a significant negative effect on nearest neighbor distance. This indicates that dace in deeper water may be more vulnerable to predator attacks and therefore school tighter by reducing neighbor distance.

These findings indicate that in Lake Nojiri, dace likely recognize smallmouth bass as a predator and act accordingly to try to avoid direct predation. Although dace seem to be able to successfully avoid the majority of direct predation attempts, at least during daylight hours, the significant decrease in foraging may have negative impacts on dace growth and survival,

as found in many fish species under predation threat (Reinhart and Healey 1997, Allouche and Gaudin 2003, Angradi 2011). Katano and Aonuma (2002) found that in experimental ponds, prey species, goldfish and Japanese dace, had significantly reduced total foraging in the presence of smallmouth bass and goldfish had reduced growth rates while Japanese dace growth rates were not significantly reduced. However, they discuss that dace growth rates likely remained constant due to the direct predation of dace by smallmouth bass reducing dace density and consequently intraspecific prey competition. In the present study, direct predation of dace was not observed, and reduced dace foraging would likely cause reduced growth rates if dace density remained relatively constant. When smallmouth bass are not present, dace appear to prefer benthic foraging and forage aggressively. However, when smallmouth bass are nearby, dace shift to water column foraging and the foraging rate decreases. This shift to water column foraging is likely also a predator avoidance behavior, as by lifting their head and line of sight, dace are likely able to better see advancing smallmouth bass and may be more aware of their surroundings, as indicated in studies on other fish species (Krause and Godin 1996, Brandl and Bellwood 2015). Although dace diets are known to consist of a mix of aquatic invertebrates and algae (Peterson and Kitano 2019, Peterson and Kitano 2021), a detailed study on dace diet in Lake Nojiri would be beneficial in determining the energy tradeoffs between prey items foraged using different foraging modes. Increased foraging rates of fish utilizing cover is common (Reinhart and Healey 1997, Tugend et al. 2002), however, it is interesting that in this study, the presence of cover (docks) did not have significant effects on any of the dace foraging and response variables.

Previous studies have indicated that the interactions between cyprinids such as dace, and smallmouth bass shift between competition and predation depending on habitat types (Peterson and Kitano 2021). In lentic systems, such as Lake Nojiri, direct predation is likely the main interaction type between dace and smallmouth bass. It appears that due to the continued interaction of dace and smallmouth bass, and likely predation of dace in Lake Nojiri over the last 30 years, dace in Lake Nojiri have recognized smallmouth bass as a predator, likely through recognition of chemical, visual and/or dietary cues of smallmouth bass (Ferrari et al. 2010), and utilize avoidance behaviors, although overall foraging is severely reduced when smallmouth bass are in vicinity. Laboratory experiments comparing dace response to native fish predators along with invasive smallmouth bass would be beneficial in determining whether dace have acquired these avoidance behaviors specific to the novel smallmouth bass, and if responses would be different with native predators. An interesting point is that there are relatively few native predator fish in Central Japan, and the

native predators that are common, such as Japanese eel (*Anguilla japonica*) and Amur catfish (*Silurus asotus*) typically forage nocturnally (Katano et al. 2003, Wakiya and Mochioka 2021) and their predatory behavior is likely quite different than smallmouth bass. These types of reduced foraging and increased vigilance have been shown in a wide variety of organisms (Krause and Godin 1996, Turney and Godin 2014), including similar fish species in laboratory settings (Katano and Aonuma 2002) but confirmation of these behaviors in natural lake settings, especially in areas where smallmouth bass have been introduced are lacking. This study is an exciting step in further understanding the interactions between these invasive predators and native prey species.

4. Spawning season and nest guarding behavior of invasive smallmouth bass (*Micropterus dolomieu*) in a Japanese Lake

4-1. Introduction

Chapter 2 and 3 showed that the effects of smallmouth bass on native cyprinids shift based on habitat, and that in a Japanese dace population that has coexisted with smallmouth bass for 30 years, dace individuals recognize smallmouth bass as a predation threat and change their behavior accordingly. These were important findings as they indicated that the negative effects of smallmouth bass are not limited to simple predation of native fish, and that to a certain extent native fish are able to change their behavior to avoid direct smallmouth bass predation. Although the mechanisms by which smallmouth bass impact native fish were becoming clearer, the factors driving the highly successful invasion of smallmouth bass were still unclear. Two factors that are essential in any fish establishment are the presence of suitable spawning habitat, and successful spawning. In a previous study I found that in the Chikuma River, smallmouth bass spawning was successful in specialized habitat and that there appeared to be a lack of native egg predators (Peterson et al. 2020). However, the evidence for a lack of egg predators was circumstantial, and there was a need to experimentally test the presence of these egg predators, especially as Japanese dace and carp have been shown to predate on smallmouth bass eggs in Japan (Iguchi and Yodo 2004). Therefore, I set up a study in Lake Nojiri to closely monitor the smallmouth bass spawning season, using daily nest checks, and to experimentally remove guarding males and analyze egg predation using underwater video recording.

Smallmouth bass spawning is well studied in the native range (Cooke et al. 2003; Ridgway et al. 1989; Winemiller et al. 1982). Males construct circular nests on the bottom substrate using their fins to clean rocks and make a depression. After eggs are deposited by the female and fertilized by the male, the male will then remain on the nest as a guard until eggs have hatched and fry have dispersed (Dauwalter et al. 2007). This process can take several weeks and the timing is largely water temperature dependent with spawning typically starting once water temperatures reach 15 C° (Graham & Orth 1986; McNeill 1995). In the native range, males ward off a variety of egg predators, most commonly sunfishes (*Lepomis* spp.) such as bluegill (*Lepomis macrochirus*) and pumpkinseed (*Lepomis gibbosus*) (Gravel & Cooke 2009) and invasive crayfish (*Orconectes rusticus*) (Baldrige & Lodge 2013; Morse et al. 2013). In the Great Lakes, invasive round gobies (*Neogobius melanostomus*) have

become a major egg predator and are causing issues with maintaining stable smallmouth bass reproduction (Steinhart et al. 2004). These round gobies are aggressive nest predators and where they are found in high densities, that have been shown to consume all the eggs in a nest in approximately 15 min (Steinhart et al. 2004; Tufts et al. 2019). Due to the potential for egg predation, in the native range, sport fishing is often not allowed or highly regulated during the spawning season (Suski & Philipp 2004; Tufts et al. 2019). Multiple studies have shown that regulating fishing during the spawning season is highly beneficial in increasing egg survival and maintaining stable natural reproduction (Gwinn & Allen 2010; Long et al. 2015).

In Japan, natural spawning of smallmouth bass has been confirmed in a variety of lakes and rivers (Iguchi, Yodo, & Matsubara 2001; Iguchi, Yodo, & Matsubara 2004; Peterson, Kitano, & Ida 2020). Spawning is generally successful where suitable habitat is present, and there seems to be a lack of native eggs predators (Peterson, Kitano, & Ida 2020), although Iguchi & Yodo (2004) proposed that Japanese dace could be effective egg predators. Iguchi, Yodo, & Matsubara (2001) surveyed smallmouth bass spawning site selection in Lake Nojiri and Lake Aoki. They examined three sites at Lake Nojiri on 13 and 14 June, 2001 and found abundant smallmouth bass nests with a relatively high prevalence of eggs or fry (51% of nests). At two sites, where dock cover was present, the majority of nests were found under or near the docks, while at a third site lacking dock cover, nests were found offshore in water depths with mean 121cm. Spawning bed diameter also increased with area of cover indicating that larger nests were constructed near cover. They concluded that artificial structures such as docks are likely beneficial for smallmouth bass spawning in Lake Nojiri and aided in the establishment of the invasive population. They also propose eradication methods using artificial spawning nests placed near cover to remove eggs, and also discuss that native fish predators such as common carp (*Cyprinus carpio*) and Japanese dace are likely effective nest predators when guarding male smallmouth are removed.

The current study aims to build on previous observations (Iguchi et al. 2001) by determine the spawning season at a daily level, habitat use of spawning smallmouth bass in Lake Nojiri and provide a detailed examination of male guarding behavior and egg predation when males are removed. Previous studies in Japan have typically only surveyed individual nests a few times during the spawning season and primarily focus on physical characteristics of suitable spawning habitat (Iguchi et al 2001, Peterson and Kitano 2020). The extensive daily nest observations in the current study will provide a much more detailed understanding of spawning season progression and egg development. The use of underwater time-lapses to

analyze male guarding behavior and egg predation is also novel in Japan, and builds on Iguchi and Yodo's 2004 observation of native egg predators. A lack of native egg predators may be beneficial in the spread of smallmouth bass throughout Japan along with the driving force of continued illegal stocking of smallmouth bass, and determination of egg predation potential is also needed to effectively manage smallmouth bass populations in Japan where stocking is not an option.

4-2. Materials and Methods

Smallmouth bass spawning was surveyed for two seasons at Lake Nojiri (Lat: 36° 48' 59.99" N, Long: 138° 11' 60.00" E), Nagano Prefecture Japan, from 2020 to 2021 (Figure 4-1).

There are populations of gamefish such as Japanese smelt, smallmouth bass, largemouth bass, bluegill, kokanee salmon, and common carp as well as non-game species such as Japanese dace and freshwater gobies (*Rhinogobius kurodai*) (Kitano et al. 2010; Peterson et al. 2021).

This survey focused on a 100 m stretch of shoreline along the southwestern shore. This section of shoreline is a designated swimming area and has concrete docks extending 40 m from shore. Water depth reaches approximately 4 m at the ends of the docks (Figure 4-2).

This stretch of shoreline is one of the few areas in Lake Nojiri where recreational shore-fishing is not allowed throughout the year. Smallmouth bass nests were located by walking along the docks and snorkeling in the swimming area. When nests were located, they were marked on a map and were subsequently visited daily (once a day during daylight) during the spawning season. Nest stage (no eggs, eggs, alevin, black fry or disappeared fry) was visually confirmed by snorkeling each day and nests were observed daily until fry dispersed. For each nest, physical characteristics of nest length, width and depth, dominant and subdominant substrate size, presence of cover and water depth were measured underwater and guarding male size (total length: TL cm) was estimated using nest length measurements. Substrate size was classified into six categories based on Mark et al. (1985): 1, silt or sand (< 2 mm); 2, gravel (2-16 mm); 3, pebble (17-64 mm); 4, cobble (65-256 mm); 5, boulder (> 256 mm) and 6, flat bedrock. Cover consists of underwater structures such as banks and rocks wider than 20 cm, wide enough for a fish to hide under, or overhead structures such as docks and terrestrial vegetation within 1 m from the water surface (Hasegawa & Maekawa 2008).

Surface water temperature was approximately 15 °C when eggs were first observed in May for both years and warmed to near 25°C when fry dispersed in June and July. Water temperature rose quicker in 2020 compared to 2021 (Fig. 4-3).

Male guarding behavior and nest predators were observed using underwater camera (GoPro Hero 8 & 9) time-lapse videos (at approximately two nests per day). For time-lapse s observing male guarding behavior (2020 and 2021), a camera was placed on the bottom substrate near the nest and was set on time-lapse mode (0.5 s/frame) and run for 30-45 min. In 2021, to observe nest predators, male smallmouth bass were angled from their nests and placed in a live-well near shore. Time-lapse s (0.5 s/frame) were then run at the nest, in the absence of the guarding male for 30-45 min, after which the male was released back to the nest.

Male guarding time-lapse videos were analyzed and the time (s) each male was on the nest and off the nest was recorded, along with total clockwise and anticlockwise rotations, nest predator lunges, guarding radius and distance from bottom substrate. Nest predator time-lapse videos were analyzed and data on predator species, predator number, predator size (estimated from pre-recorded nest size measurements), time until arrival of first predator after guarding male removal, and number of eggs predated (by visually observing the opening and closing of a gobies mouth, and the subsequent disappearance of a smallmouth bass egg) was taken.

4-2-1. Data Analysis

Correlations between guarding male size and nest (length, width and depth) and habitat (water depth) variables were tested using the Pearson's correlation. To examine the effects of a variety of factors on male guarding behavior and nest predators, generalized linear mixed models (GLMM) were used. For male guarding behavior models, nest progression (days after first egg), nest length, nest width, nest depth and water depth were set as fixed effect variables and ratio of time the male smallmouth bass is on the nest, total rotations, guarding radius (linear distance from center of the nest and center of the guarding male) and male distance from bottom were set as response variables. Binomial error structure was used for the ratio of time the male smallmouth bass is on the nest, and Poisson error structure was used for total rotations, guarding radius and male distance from bottom, with video time set as an offset and file ID set as a random effect variable. For nest predator models, nest progression, nest length, nest width, nest depth and water depth were set as fixed effect variables, and predator number and number of eggs predated were set as response variable. For both models Poisson error was used with video time set as an offset to account for differing time-lapse times and file ID set as a random effect variable. An explanatory variable

is statistically significant when the estimate coefficient does not include zero in a 95% confidence interval. Best models were determined using stepwise model with lowest Akaike's information criterion for each GLMM (Burnham and Anderson 2002).

Multicollinearity was checked in fixed variables using the Pearson's correlation prior running the GLMM models. Mean days for each nest stage were tested using Student's t-tests. All analysis was conducted using R: version 4.1.0. (R core team 2021).

4-3. Results

4-3-1. Spawning Season

In 2020, nests were first observed on 19 May and fry dispersal was complete on 14 July. There were two peaks to the spawning season with the majority ($n = 22$) of nests during 22 May - 9 June, and the second peak of nests ($n = 14$) were found during 22 June - 14 July. In 2021, a similar two-peaked spawning season was observed with most nests ($n = 30$) found during 12 May - 14 June and the second peak of nests ($n = 10$) during 21 June - 1 July (Figure 4). Nests that received eggs (2020: $n = 21$; 2021: $n = 27$) progress from the egg stage to fry dispersal (Figure 4-5) unless eggs were predated and could no longer be found (2020: $n = 1$; 2021: $n = 2$). In both years nests constructed during the first peak were more likely to receive eggs than in the second peak (2020: first peak: 90.1%, second peak: 28.6%; 2021: first peak: 76.6%, second peak: 30.0%). The rate at which nests progressed from the egg stage to fry dispersal was significantly faster in 2020 compared to 2021 (Fig. 4-6) (t-test $p < 0.001$).

4-3-2. Nest and Male Characteristics

Nests were found throughout the swimming area in both years. Nearly all nests were found underneath docks ($n = 71$ out of 76) in water depths ranging from 100 - 350 cm. Nest physical characteristics were similar between years and are shown in Table 1. Guarding males were found on every nest and ranged in size 20 - 50 cm. Male size was significantly correlated with nest physical characteristics in both years as larger males made longer, wider and deeper nests in deeper water depths (Figure 4-7).

4-3-3. Male Guarding Behavior

Observations by time-lapse s ($n = 20$) showed that males were found to be on the nest nearly the entire time (91.9%), rotating within the nest (0.48 ± 0.80 rotations/min; rotation radius:

56.9 ± 9.1 cm) and suspended from the bottom substrate (10.3 ± 3.5 cm) in both years (Figure 4-8). GLMM models revealed that nest progression had a significant negative effect on total rotations. Water depth had a significant positive effect on guarding radius, while nest progression had a positive effect on male distance from bottom. No significant effects were found for ratio of time on nest (Table 4-2).

4-3-4. Nest Predators

In 2021, guarding males were angled from nests (n = 20) (Figure 4-9a) to experimentally determine nest predation using time-lapse videos. Gobies were the only nest predator observed and they appeared at all nests in the egg stage (n = 12), most nests in the alevin stage (n = 4 out of 5), and no nests in the fry stage (n = 3) (Figure 4-9b). A total of 68 gobies were observed (mean total length: 36.2 ± 1.7 mm) with a mean of 4.5 ± 1.3 gobies/nest/30min. These gobies predated on mean 3.2 ± 1.0 eggs/nest/30 min and took mean 817.7 ± 149.4 seconds to first arrive at nests. GLMM models were run to analyze variables influencing the number of gobies present and the number of eggs predated. Nest progression significantly affected both response variables with a negative effect on number of gobies present and number of eggs predated. As nests progressed, fewer gobies were present and fewer eggs were predated. Nest length had a positive effect on predator number and number of eggs predated, while water depth had a negative effect on predator number and number of eggs predated (Table 4-3).

4-4. Discussion

This two-year study produced a detailed overview of the smallmouth bass spawning season in Lake Nojiri and the factors driving its success. Male smallmouth bass began nest construction once water temperatures reached 14 °C, and eggs progressed to fry dispersal in approximately two weeks, although eggs progressed faster in 2020 compared to 2021 (Figure 4-6), likely due to rapid water temperature increase in 2020 (Figure. 4-3). In both years the main spawning season was in late May through early June, with a smaller second peak of nests in late June through early July (Figure 4-4). Nests were found primarily under dock cover and male size was significantly correlated with nest physical characteristics in both years as larger males constructed longer, wider and deeper nests (Figure 4-7). This correlation of male size and nest characteristics is typical and has been found in the native

range as well as in Japan (Suski & Philipp 2004; Peterson et al. 2020). The use of dock cover is also consistent with previous observations in Lake Nojiri (Iguchi et al. 2001). Of the nests that received eggs, nearly all progressed successfully to fry dispersal (Table 4-1). This nest success rate of near 100% is much higher than what is typically found in the native range and previously in Japan (Iguchi & Yodo 2004; Lukas & Orth 1995; Peterson et al. 2019; Steinhart & Lunn 2011; Tufts et al. 2019). It was interesting that in both years, nests were much more likely to receive eggs during the first spawning peak, compared to the second one.

Guarding males were found on all nests and guarding behavior time-lapse s revealed that nest progression was that main factor driving guarding behavior. As nest progressed, males rotated less and were suspended off the bottom substrate (Table 4-2). This indicates that males guard more vigilantly when nests are in the egg stage and vigilance gradually reduces as eggs progress to alevin and fry. This is likely since nests appear to be most susceptible to egg predation during the egg stage as shown in previous studies (Baldrige & Lodge 2013; Steinhart et al. 2004). It is important to note that in the current study, time-lapse s used 0.5 s frame intervals and therefore detailed male behavior such as fin fanning which is used to aerate eggs (Scott & Crossman 1973) was not able to be observed. Male rotational behavior observed in the time-lapse s may be a product of fanning as well which also is thought to decrease with nest progression (Scott & Crossman 1973). However, Winemiller and Taylor (1982) separated male fanning behavior from rotational behavior and found that males rotate most during the early stages of the spawning season. Susceptibility of eggs during the egg stage was confirmed in the current study by the nest-predator time-lapse s where males were experimentally removed, as nests in the egg stage were predated most and as nests progressed, fewer gobies arrived and fewer eggs were predated (Table 4-3). Gobies were the only egg predators observed, and they arrived on all nests in the egg stage.

However, only a handful of gobies visited each nest and the total number of eggs predated was low. The guarding behavior of male smallmouth bass in the current study is likely different than that in areas with prominent egg predators. For example, when predators such as crayfish, sunfish, or Japanese dace are present, smallmouth bass males have been shown to aggressively attack these predators while guarding the nest (Baldrige & Lodge 2013; Gravel & Cooke 2009; Iguchi, Yodo, & Matsubara 2004). In the current study, no predators including gobies were observed approaching the nest when guarding males were present, and therefore male attacks on potential predators were not observed at all. The fact that only gobies arrived at the nests was surprising as Lake Nojiri also has populations of

dace and carp which have been shown to predate on smallmouth bass eggs (Iguchi & Yodo 2004).

Iguchi & Yodo (2004) found that dace were highly effective in predated smallmouth bass eggs even when males were guarding nests, in a lake in Nagano Prefecture 40 km from Lake Nojiri where smallmouth bass had recently invaded at the time. Dace schools were observed in the nest-predation time-lapse s in the current study, however the dace showed no interest in smallmouth bass eggs and did not approach the nests. These contrasting results may be a product of smallmouth bass and dace coexisting in Lake Nojiri for the last 30 years. Dace in Lake Nojiri have recently been shown to recognize smallmouth bass as a predation threat and utilize predator avoidance behavior (Peterson & Kitano 2021b). This threat recognition of smallmouth bass may make dace less inclined to predate on smallmouth bass eggs, especially when males are guarding the nests. The mean arrival time of gobies, about 13 minutes, was also quite slow compared to nest predators in other areas (Baldrige & Lodge 2013; Tufts et al. 2019), and likely enough time for a male smallmouth bass to be angled and released back to the nest before predators arrived (Stein & Philipp 2014; Steinhart et al. 2004).

In the native range, sunfishes, crayfish and round gobies have been shown to be highly destructive smallmouth bass egg predators. These predators arrive quickly after guarding male removal and can predate on a large portion of the eggs in just a couple minutes (Baldrige & Lodge 2013; Gravel & Cooke 2009; Tufts et al. 2019). Where nest-predator densities are high, guarding males are often busy warding off predators that arrive at the nest even when the male is present. In the current study no predators were observed at nests when the male guard was present, and even when males were removed only minimal eggs were predated by gobies. It's important to note that the gobies present in Lake Nojiri, as well as in most of Japan are much smaller in size (current study mean total length: 36.2 mm) than the round gobies (total length: 51-161 mm, from Gutowsky & Fox 2011) that predate on smallmouth bass eggs in North America (Gutowsky & Fox 2011; Kornis et al. 2012). This lack of destructive nest predators in Lake Nojiri is likely a driving factor behind the stable smallmouth bass population despite no stocking and no angling restrictions during the spawning season. It's important to note that the current study area in Lake Nojiri is likely ideal for successful spawning as it is one of the few areas where shore fishing is not allowed year-around, and also has an abundance of dock cover extending into deep water. The success rates for nests progressing to fry dispersal may be lower in other areas of the lake with less dock cover and greater angling pressure. This is corroborated by observations in

2001 in areas where fishing is allowed which showed lower rates of nests with eggs (51%) in Lake Nojiri and Lake Aoki compared to our results (58 and 68%) (Iguchi, Yodo, & Matsubara 2001). In the current study male guarding behavior and nest predator experiments were also only conducted during daytime and sample size is limited. A more extensive nest predator examination in multiple areas of the lake including areas where fishing is allowed and also areas without prevalent dock cover including nighttime surveys would be beneficial in further understanding interactions between native fish and smallmouth bass. It would also be beneficial to conduct nest surveys in areas where other potential predators such as bluegill and carp are found in higher densities compared to the current study area.

Although the current study was conducted only at Lake Nojiri, the results of highly successful smallmouth bass spawning and a lack of nest predators may correspond to other Japanese lakes as well, and might be a corroborating factor along with the primary driver of continued illegal stocking, in the continued spread of these invasive smallmouth bass. Stocking of smallmouth bass is illegal throughout Japan, and in many areas catch and release angling is also illegal. Despite these regulations, smallmouth bass continue to spread to new locations and there appear to be stable populations in many lakes and rivers (Peterson & Kitano 2019; Peterson & Kitano 2021a).

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

5-1. Introduction

Chapters 2-4 produced a detailed examination of smallmouth bass in Nagano Prefecture, providing important insights into the effects on native species, the response of these native species, and the favorable factors driving the continued establishment and spread of smallmouth bass in Japan. However, it turns out that smallmouth bass are not the only aquatic invasive species that are causing problems in Nagano Prefecture. There is another group of fish, the salmonids, especially brown trout (*Salmo trutta*), and brook trout (*Salvelinus fontinalis*), which have been imported from Europe and North America, that are established in headwater streams, and have likely caused the decline of native headwater species such as whitespotted charr (*Salvelinus leucomaenis*).

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). 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 clarkia* sp.) (Kruegar and May 1991, Al-Chokhachy and Sepulveda 2019). 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 (Swink 1983, Halverson 2008). 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 (Quist and Hubert 2004, Budy et al. 2021).

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), likely due to spawning limitations (Fausch et al. 2001), brown trout are currently spreading throughout the country (Hasegawa 2020). Brook trout have also not spread widely, but are found in a few select areas with spring-fed streams (Kitano 2004). The spread of invasive salmonids in Japan is concerning as native salmonids such as whitespotted charr (*Salvelinus*

leucomaenis) and masu salmon (*Oncorhynchus masou*) will likely occur negative impacts based on direct predation and resource competition. Hybridization between whitespotted charr and brook trout has also been found in Japan (Kitano et al. 2014) and appears to be a growing concern as brook trout continue to spread, especially in Hokkaido. In Honshu, whitespotted charr are a headwater species typically found at high elevations in cold water. In many areas, whitespotted charr populations are currently under threat from rising water temperatures and habitat degradation and fragmentation (Takami et al. 1997, Dunham et al. 2008). Whitespotted 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 (Yamamoto et al. 2004, Morita 2019). Whitespotted charr distribution often overlaps with other native salmonids such as masu salmon and southern asain dolly varden (*Salvelinus curilus*), and habitat and diet niche partitioning has been studied in great detail (Miyasaka et al. 2003). In headwater stream habitat, whitespotted charr commonly forage on a variety of aquatic insects such as trichoptera, ephemeroptera and plecoptera (Iguchi et al. 2004). Terrestrial insects such as camel crickets and grasshoppers have also been shown to be an important part of whitespotted 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 and Furukawa-Tanaka 1994). In larger whitespotted charr individuals, especially in large river or lake habitats, fish prey can also make up a large part of diets (Takami and 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 studies 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 and 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 Kamikouchi area of Nagano Prefecture, in the Chubu Sangaku National Park, provides a stark example of just how damaging these invasive salmonids can be. 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 through the center of the Kamikouchi area, and many of the small spring-fed tributaries. The Kamikouchi area historically had abundant whitespotted charr populations. Despite all recreational angling being banned in 1975, whitespotted charr have nearly been completely expatriated from many of the tributaries over the last 50 years (Kitano Unpublished). Currently whitespotted charr remain abundant only in the very upper reaches of the Azusa watershed where brown trout and brook trout have yet to establish.

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 whitespotted charr in small tributaries of the Kamikouchi area. By examining foraging modes, aggressive behavior, diet composition and microhabitat use, direct impacts on whitespotted charr from each invasive species will be determined, and will contribute to future whitespotted charr restoration projects throughout Japan.

5-2. Materials and Methods

5-2-1. Study Area

This study was conducted in six headwater streams (Table 5-1) in the Kamikouchi area of Nagano Prefecture, Japan (36°14'55.84"N, 137°38'16.20"E, 1,500 m.a.s.l.) (Fig. 5-1). Underwater observation and electrofishing surveys were conducted periodically during June-September 2021 (see Table 5-1 for details). Kamikouchi 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. 5-2). These headwater streams are mostly spring-fed and have consistently cool water temperatures throughout the year. The streams historically had populations of native whitespotted charr but currently are dominated by invasive brown and brook trout. Whitespotted charr are still prevalent in the higher altitude areas where the invasive salmonids have yet to establish (Environment Agency, Government of Japan (EAGJ) 1982).

5-2-2. Underwater Observation

At each stream reach, fish behavior and microhabitat use were examined using underwater snorkel observation using a mask, snorkel and dry suit. Researchers entered each stream reach from downstream and slowly snorkeled upstream observing each individual fish (>

80mm TL) 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. 5-3). After video recording, a marker was placed on the bottom substrate at the location of each individual fish, and microhabitat data was taken. At each fish marker, water depth, focal point water depth, flow velocity, focal point flow velocity and dominant and subdominant substrate types were recorded. Substrate size 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.

5-2-3. Video Analysis

Video files were analyzed to determine foraging and agnostic behavior of each fish. 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 whitespotted charr and brook trout and the difficulty of visually distinguishing whitespotted charr from hybrids (Iguchi et al. 2001), all fish that visually appeared as whitespotted charr were categorized as whitespotted charr and fish that appeared as brook trout were categorized as brook trout.

5-2-4. Fish Collection

Fish were collected by electro-fishing (Model LR-24, Smith-Root Inc., Vancouver, Washington) at four of the streams (details in Table 5-1) 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 and 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 99.8% 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.

5-2-5. 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), and days after start 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. 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 and Anderson 2002). Variables were checked for multicollinearity using the Person's correlation prior to inserting into each model. All analysis were conducted in R software: version 4.1.2. (R core team 2021).

5-2-6. 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. 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

$$PSI = [1 - 0.5 \sum_{i=1}^n |P_{ik} - P_{jk}|] \times 100$$

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

5-3. Results

5-3-1. Study Area and Species Assemblage

Six headwater tributary streams were surveyed by snorkeling and electrofishing from June-September 2021 (see Table 5-1 for survey details). Brook trout and brown trout were found in each of the six streams while whitespotted charr were rare, and only found in two streams (Table 5-1). Out of the six streams, brook trout has the highest density in four streams while brown trout had the highest density in two (Table 5-1).

5-3-2. Microhabitat

The three study species, observed by snorkeling (brook $n= 141$, brown $n= 130$, whitespotted charr $n= 20$), were found in overlapping habitat throughout the study area. Although overall mean habitat water depth, flow velocity, and substrate size values have slight differences between species (Table 5-2), GLMM analysis, with brook trout set as the reference category, showed no significant species effects for water depth, flow velocity and substrate size (Table 5-3). However, GLMM analysis indicated that fish size had a positive effect on flow velocity, water depth and substrate size, while whitespotted 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. Days after start had positive effect on substrate size (Table 5-3).

5-3-3. Foraging

Total foraging rates were similar between the three species, approximately $1.8 \text{ attempts} \cdot \text{min}^{-1}$. All three species foraged primarily using drift foraging with brown trout exhibiting the highest rate and brook trout and whitespotted charr having similar rates (Fig. 5-4). Brook trout and whitespotted charr also foraged benthically at a higher rate than brown trout. Brown trout exhibited occasional surface foraging while brook trout and whitespotted 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 5-3).

5-3-4. Aggression

Inter and intra-specific aggressive and defensive behavior was infrequent (approximately $0.2 \text{ aggressive behaviors} \cdot \text{min}^{-1}$), 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, whitespotted 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 while fish size also had a negative effect on intraspecific avoidance (Table 5-3).

5-3-5. Diet

The three study species, collected by electrofishing (brook $n=193$, brown $n=74$, whitespotted charr $n=36$), were found to prey on a variety of aquatic and terrestrial prey items with terrestrial hemiptera and aquatic trichopteran being the most prevalent prey items in all three species. Brown trout preyed most on terrestrial hemiptera followed by aquatic trichopteran, while brook trout and whitespotted charr preyed most on aquatic trichopteran followed by terrestrial hemiptera (Table 5-4). 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: $n=4$,

whitespotted 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 5-5). The Percent Similarity Index (PSI) showed that brook trout and whitespotted charr diets were nearly identical with high biological significance while brown trout diets were not significantly similar to either brook trout or whitespotted charr (Table 5-6).

5-4. Discussion

This study produced an overview of salmonid distribution in Kamikouchi headwater streams and showed clear niche overlap between native and invasive species. The lack of native whitespotted 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 5-3), foraging mode (Fig. 5-4) and diet analysis (Table 5-4, 5-6) clearly showed that brook trout and whitespotted charr had nearly identical foraging niches while brown trout were distinct. Brook trout and whitespotted 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 whitespotted 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 whitespotted 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 (Hoxmeier and Dieterman 2015, Dieterman and Mitro 2019, Mitro et al. 2019).

With these three species occupying similar habitat niches in Kamikouchi headwater streams, the possibility of foraging niche shifts due to pressure from the other species is likely. Whitespotted 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 (Nakano et al. 1999, Fausch et al. 1997) or a dominant individual pushes them out of their preferred focal point (Nakano et al. 1999,

Fausch et al. 2020). This niche shift in whitespotted charr has also been shown in relation to introduction of brown trout and rainbow trout (Hasegawa and Maekawa 2006), with these invasive salmonids pushing whitespotted 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 whitespotted charr and brook trout for drift foraging focal points. 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 (Fausch and White 1986, Dieterman and Mitro 2019). 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 and Wlizabeth 1992). With the nearly identical foraging and habitat niches and brook trout and whitespotted charr in the current study, brown trout likely have similar impacts on whitespotted 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 5-1) while only whitespotted charr are severely reduced. The specific mechanisms by which brook trout outcompete whitespotted charr are unclear, and the impact of hybridization also requires further study. Habitat characteristics and stream type likely also influence the persistence of whitespotted charr and warrant study, as the Zenroku stream, which is the only non-spring fed stream in the study area, had the highest densities of white-potted charr.

In terms of fish species distribution and density, the lack of native whitespotted charr and prevalence of invasive salmonids was strikingly evident and highlights the drastic decline of whitespotted charr in this area over the last 100 years. Of the six streams surveyed only one (Zenroku) had prevalent whitespotted charr while the other streams had either no whitespotted charr or very few individuals. This lack of whitespotted 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. Ideally, to further understand the negative impacts of invasive salmonids on whitespotted charr, streams with differing species densities (i.e. Recently invaded state: whitespotted charr are predominant with few invasive salmonids. Invaded state: similar densities of whitespotted charr and invasive salmonids) would provide a clearer picture of how the negative impacts of these invaders directly causes the decline of whitespotted charr. Unfortunately, in the Kamikouchi area, this is no longer possible as in many of the small headwater streams, whitespotted charr populations have experienced drastic decline over the last century. 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 Kamikouchi, brook trout directly compete with whitespotted charr for prey resources. Brown trout also compete with whitespotted 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 whitespotted charr while also significantly altering the headwater stream ecosystems found in the Kamikouchi area. Although whitespotted charr have nearly been wiped out in the study area, 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 whitespotted charr. The effectiveness of such practices in restoring native headwater stream biodiversity should be examined in future studies.

6. Discussion and Conclusions

6.1 General Discussion

The current study produced a detailed in-situ examination of smallmouth bass, and brook trout and brown trout in Nagano Prefecture. In chapters 2-4, invasive smallmouth bass populations were studied in the Chikuma River and in Lake Nojiri. The impacts of smallmouth bass on native fish were found to shift from predation to competition based on habitat type within a single river system. This was a novel result in that no studies had previously examined this predation-competition interaction balance at a microhabitat scale. Japanese dace were also shown to recognize smallmouth bass as a predation threat and they changed their behavior by shifting from predominantly benthic foraging to water column foraging, and exhibited predator avoidance behaviors such as schooling when in the vicinity of smallmouth bass. This finding was also novel as prey fish response to smallmouth bass had not been studied in natural in-lake settings. In general, smallmouth bass are thought to have negative impacts on native fish, especially smaller cyprinids, through direct predation (Yodo and Iguchi 2004). Although I did find instances of direct predation, especially in slow flowing pool habitat in the Chikuma River, it was surprising that smallmouth bass were competing for aquatic invertebrate prey resources with native cyprinids in faster flowing river habitat.

There are likely two main factors that are driving this reduced predation. One is that like we found in the Chikuma River, when smallmouth bass densities are high, many fish, especially smaller individuals move into faster flowing habitat. In slow flowing or lake habitat smallmouth bass are known to be fish predators (Crane and Einhouse 2016), and therefore when they move into faster flowing habitat they likely encounter difficulty chasing and predating on other fish. Like we found in the Nogu River (Peterson and Kitano 2019), these smallmouth bass in fast flowing habitat then utilize drift foraging to predate on aquatic invertebrates, and in doing so compete with native cyprinids for prey resources. This shift from fish to invertebrate prey based on microhabitat flow velocity is a novel finding for smallmouth bass. However, it is likely based on energy tradeoffs, as it would be inefficient to try and predate on fish in fast flowing habitat, and drift foraging for invertebrates is likely much more cost efficient (Sabo and Orth 1995, Sabo et al. 1996).

The second factor driving smallmouth bass diet shifts may be that native cyprinids are adapting to the novel predators and are better able to avoid direct predation. This would be a

rapid behavioral change in populations that have been in contact with smallmouth bass for 20-30 years since their introduction. There is circumstantial evidence that in the Chikuma River, smallmouth bass are predating less on cyprinids, regardless of habitat type, than they did 20 years ago soon after the first introduction (Kitano Satoshi unpublished). However, study of the mechanisms by which cyprinids recognize smallmouth bass as a predation threat, and change their behavior to avoid predation was lacking especially in non-laboratory settings. Chapter 3 provides the first in-situ observation of Japanese dace behavior change in response to smallmouth bass, and clearly indicates that dace effectively change their behavior to reduce predation risk. Although this study was conducted only at one lake, it provides hope for native cyprinid populations in areas where a complete removal and smallmouth bass is unlikely. The majority of the previous studies have focused on the detrimental effects of smallmouth bass predation on native fish (Jackson 2002, Zanden et al. 2004), and studies on other effects such as prey competition and behavior adaptations are lacking. Going forward, studies on other cyprinid populations, under predation threat from smallmouth or largemouth bass, in a variety of habitat types and times since invasive predator introduction, would be highly beneficial in determining the behavioral adaptability of cyprinids in response to these predators, and the extent to which populations of each cyprinid species can persist and coexist with the predators.

Chapters 2 and 3 showed the negative impacts of smallmouth bass and the mechanisms by which a native cyprinid reduces predation risk. However, the factors driving the continued spread and establishment of new smallmouth bass populations throughout Japan required study. My previous work in the Chikuma River (Peterson et al. 2020) indicated that spawning success was high and that native egg predators may be lacking. I tested this in Lake Nojiri (chapter 4) using detailed daily nest observations for two spawning seasons, and experimentally removing guarding males from nests to test egg predator presence. Spawning success was found to be much higher than in the native range, egg predators were scarce and egg predation was minimal even when male guards were removed from the nests. This confirmed our suspicions from the Chikuma River that native egg predators are scarce, and if this trend holds true in other lake and river systems, it is likely a driving factor of the continued spread and establishment of smallmouth bass in Japan, along with the primary driving factor of continued illegal introductions. However, this lack of native egg predators may be unique to Lake Nojiri, and study in other invaded areas is needed.

In addition to my work on smallmouth bass I was also interested how another group of invasive fish, the salmonids, were affecting native headwater fish species. Therefore, I examined the interactions between two invasive salmonid species (brook trout and brown trout) and native whitespotted charr were examined in headwater tributary streams in the Kamikouchi area in chapter 5. These interspecific interactions were similar to smallmouth bass and cyprinids in that prey resource competition and direct predation were found. However, the prevalence of competition and predation was different between the two invasive species. Brook trout were found to have nearly identical microhabitat, foraging, and diet niche overlaps with whitespotted charr and little direct predation. In contrast, brown trout were in similar habitat as brook trout and whitespotted charr, but their diet was distinct with high rates of predation on terrestrial invertebrates as well as aquatic invertebrates. Large brown trout individuals were also found to be directly predating on fish and amphibians. Therefore, the decline of whitespotted charr in Kamikouchi over the last century is likely due to the combined effects of these two invasive salmonids, competition with brook trout, and predation from brown trout. An unexplored factor in the current study that requires examination is hybridization of brook trout and whitespotted charr. In Kamikouchi, brook trout – whitespotted charr hybrids are common, and this hybridization likely also contributes to the decline of native whitespotted charr. These results of brown trout primarily drift foraging for terrestrial invertebrates while brook trout and whitespotted 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 (Nakano et al. 1999, Sweka and Hartman 2008, Eros et al. 2012), which will in turn lead to decreased growth and survival of the native species.

6-2. Conclusions

Throughout this study I've had the fantastic opportunity to study a host of different aquatic invasive species and the impacts that they have on native species. In general, I've found these impacts to be negative and the continued spread of these invasive species causes concern for native species conservation. However, the impacts are not limited to simple predation, as they are complicated and vary based on habitat type and species assemblage of

each unique aquatic system. Going forward, management of aquatic invasive species should focus on targeted removal when possible, but also detailed study of native species behavioral adaptations, and management practices that can enhance native species survival are essential.

In terms of smallmouth bass, in lakes where a complete removal is unlikely, habitat enhancements should be made to facilitate native fish survival. Shoreline cover such as submerged terrestrial vegetation and aquatic vegetation will likely provide hiding spots for native fish to escape from smallmouth bass predation and lead to higher survival rates. Also, in lakes where native fish are exhibiting adaptations to avoid smallmouth bass predation, the genetic basis of these adaptations should be studied to understand how these traits are passed down and to conserve these traits in the population going forward. Stocking of native fish species that are better adapted to coexisting with smallmouth bass may also be a viable option in lakes where the native fish have been decimated following introduction of the invasive predator. For invasive salmonids, examining the extent of the effects of hybridization is also highly important as this may also be a leading cause of native species decline. I am planning to continue my work on the interactions between brown trout, brook trout and whitespotted charr in headwater streams and plan to experimentally remove the invasive salmonids and re-introduce native charr in select spring-fed streams. My next goal is to understand the ecosystem effects of invasive species removal and native species re-introductions.

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9. Figures

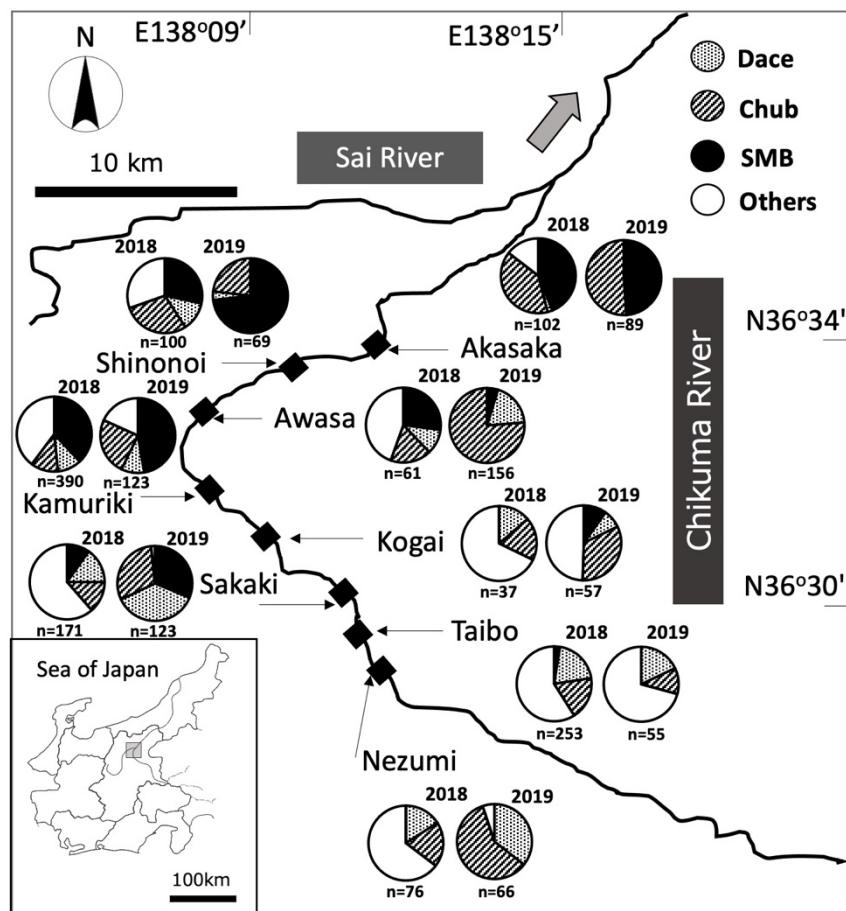


Fig. 2-1

Map of the Chikuma River including a detailed map of the study area and fish species composition (smallmouth bass (SMB), Dace, Chub and Others) at eight survey sites (Nezumi, Taibou, Sakaki, Kougai, Kamuriki, Awasa, Shinonoi and Akasaka)

*Modified from Peterson and Kitano 2021a

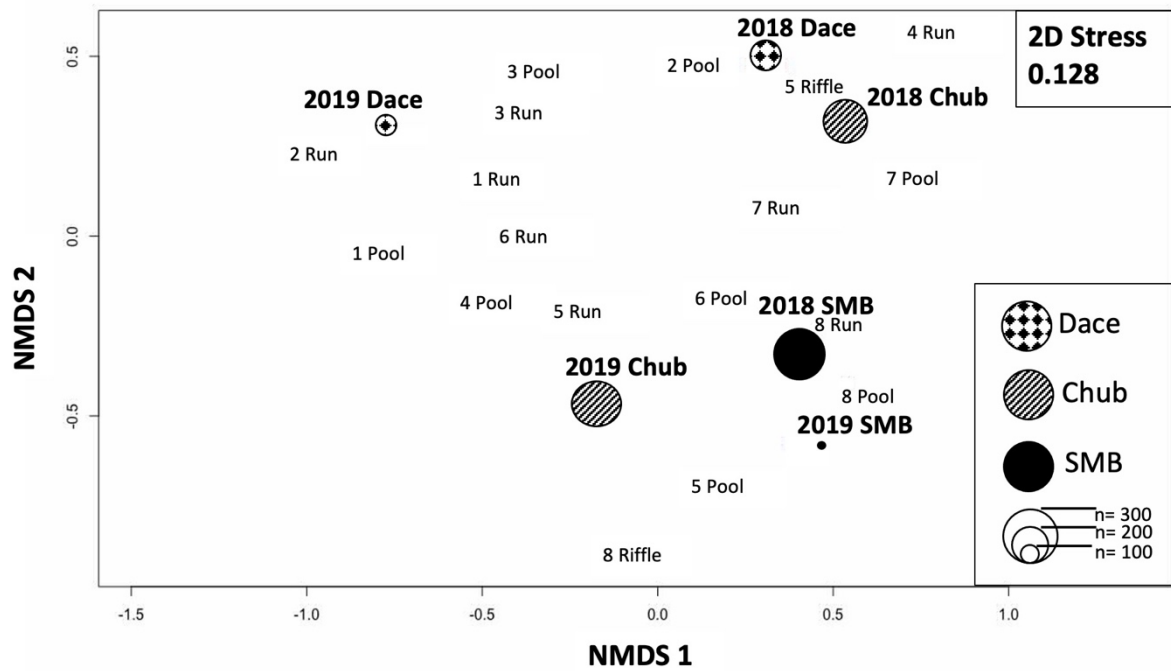


Fig. 2-2

Non-metric Multidimensional Scaling (NMDS) plot of Dace, Chub and smallmouth bass (SMB) densities across survey site habitats for 2018 and 2019. Survey sites are numbered from upstream to downstream (1: Nezumi, 2: Taibou, 3: Sakaki, 4: Kougai, 5: Kamuriki, 6: Awasa, 7: Shinonoi and 8: Akasaka). Sample size of each species for each year is indicated by circle size

*Modified from Peterson and Kitano 2021a

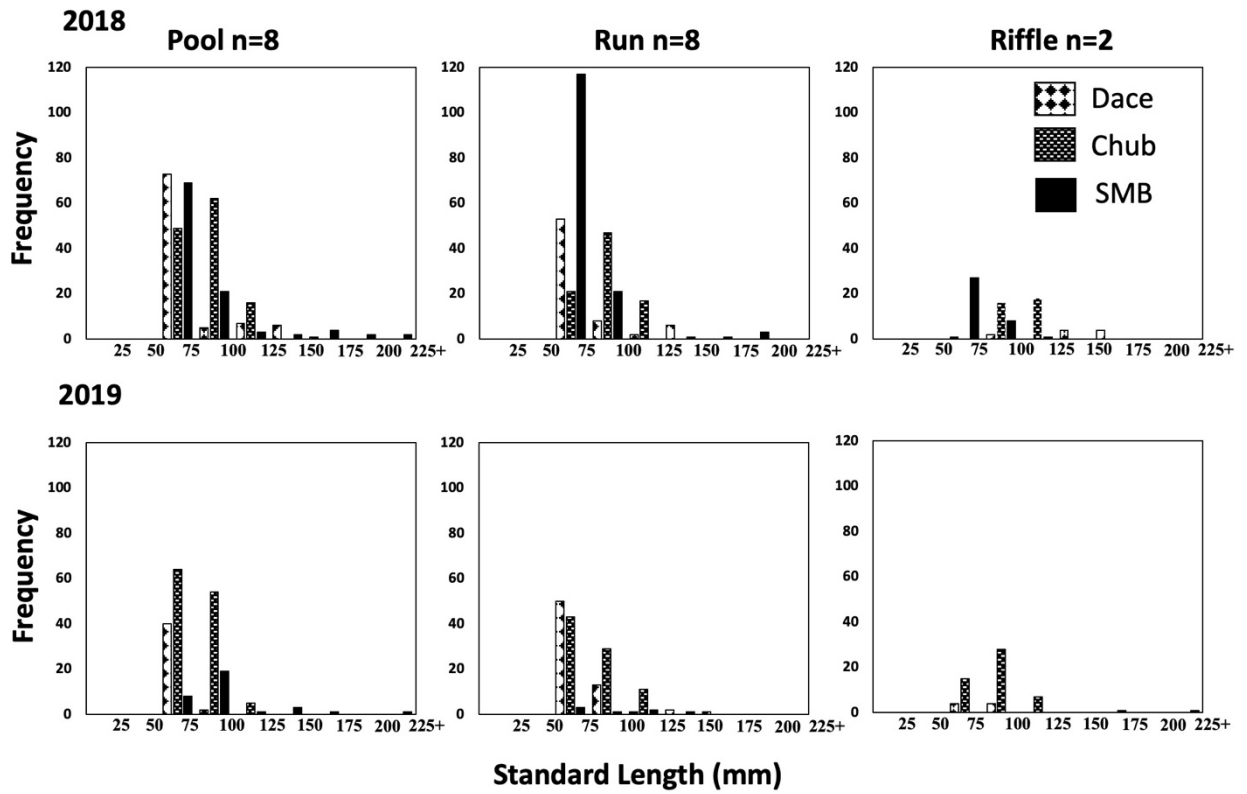


Fig. 2-3
 Standard length distribution of Dace, Chub and smallmouth bass (SMB) in pools, runs and riffles throughout the study area in 2018 and 2019

*Modified from Peterson and Kitano 2021a

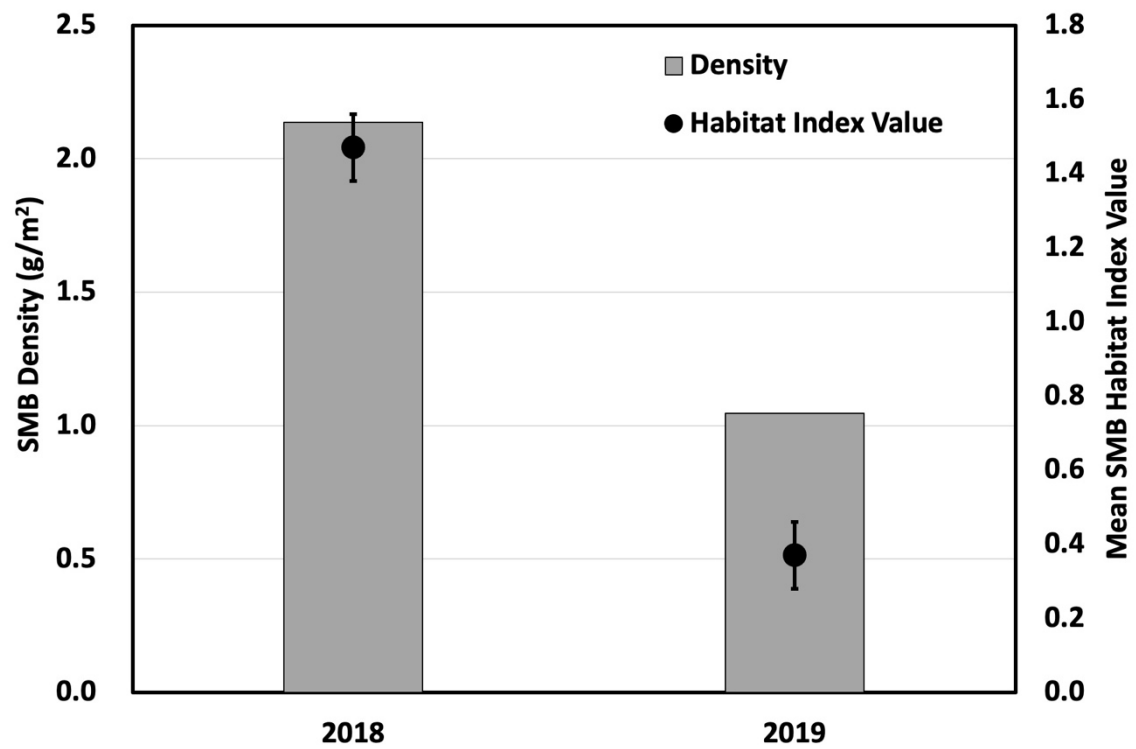
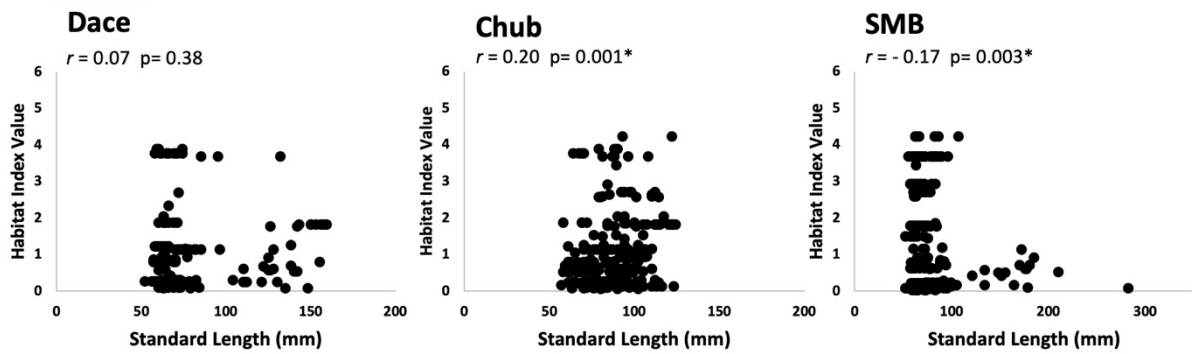


Fig. 2-4

Total smallmouth bass (SMB) density throughout the study area indicated by bars and mean (\pm SE) smallmouth bass habitat index value (velocity divided by water depth) indicated by dots, in 2018 and 2019

*Modified from Peterson and Kitano 2021a

2018



2019

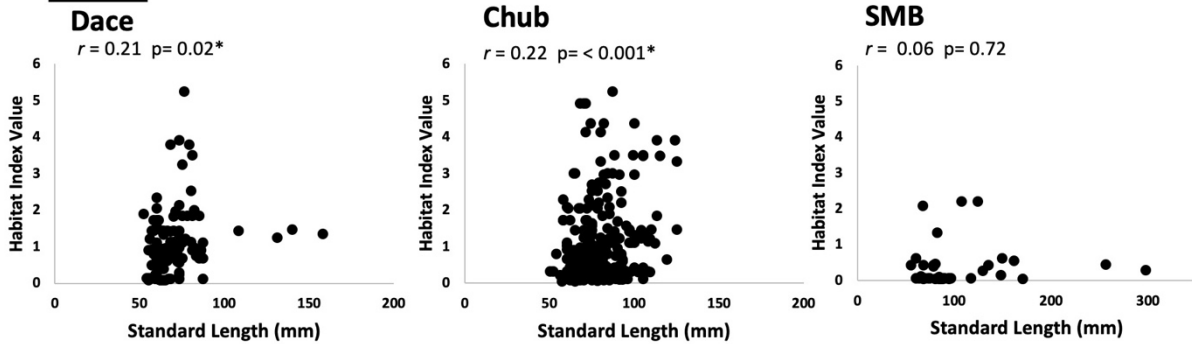


Fig. 2-5

Correlation of fish size (standard length) and habitat index values (velocity divided by water depth) of Dace, Chub and smallmouth bass (SMB) for 2018 and 2019. Correlation coefficient r and p -value were calculated using Pearson's correlation

*Modified from Peterson and Kitano 2021a

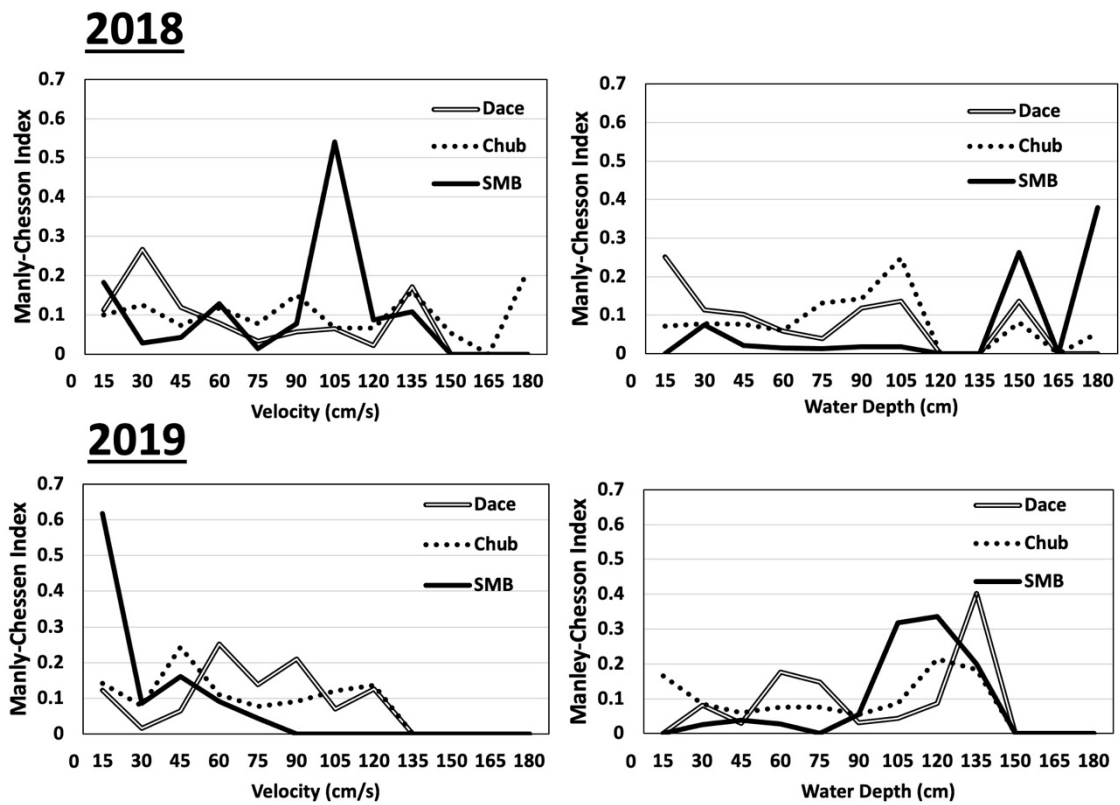


Fig. 2-6

Manly-Chesson habitat selectivity index values of velocity and water depth for dace, chub and smallmouth bass throughout the study area in 2018 and 2019

*Modified from Peterson and Kitano 2021a

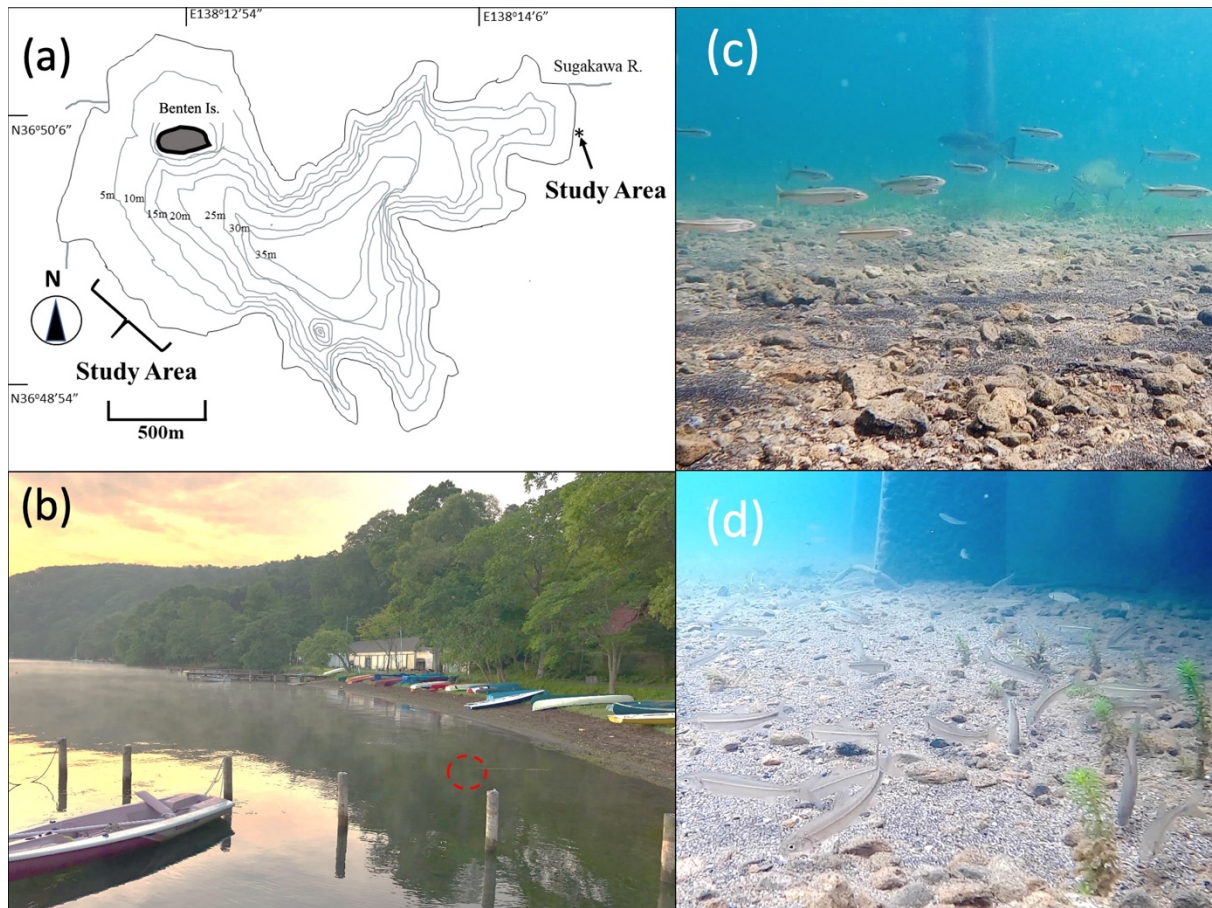


Fig. 3-1

(a) Map of the study area located in Lake Nojiri, Nagano Prefecture, Japan, and (b) a typical location where interactions between Japanese dace and smallmouth bass were filmed (camera location indicated by red circle). Images taken from underwater video observations: (c) Japanese dace schooled and foraging in the water column with two smallmouth bass in vicinity. (d) Japanese dace feeding benthically with no smallmouth bass in vicinity

*Modified from Peterson and Kitano 2021b

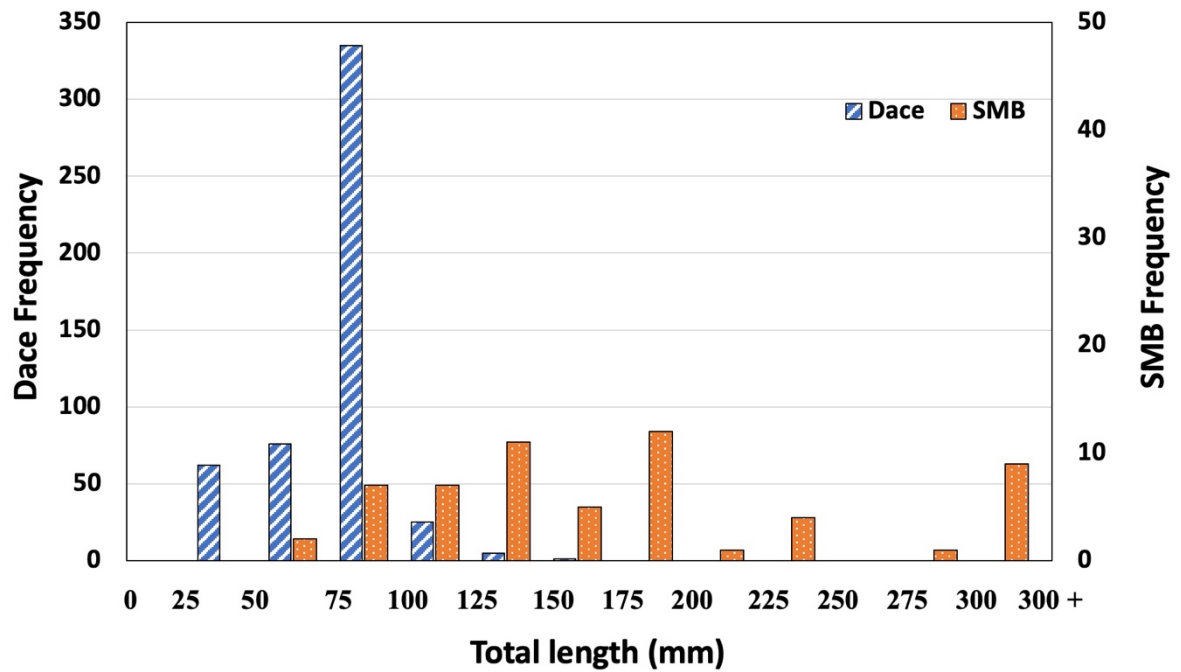


Fig. 3-2

Total length distribution of Japanese dace and smallmouth bass (SMB) (secondary y-axis) observed during the study period

*Modified from Peterson and Kitano 2021b

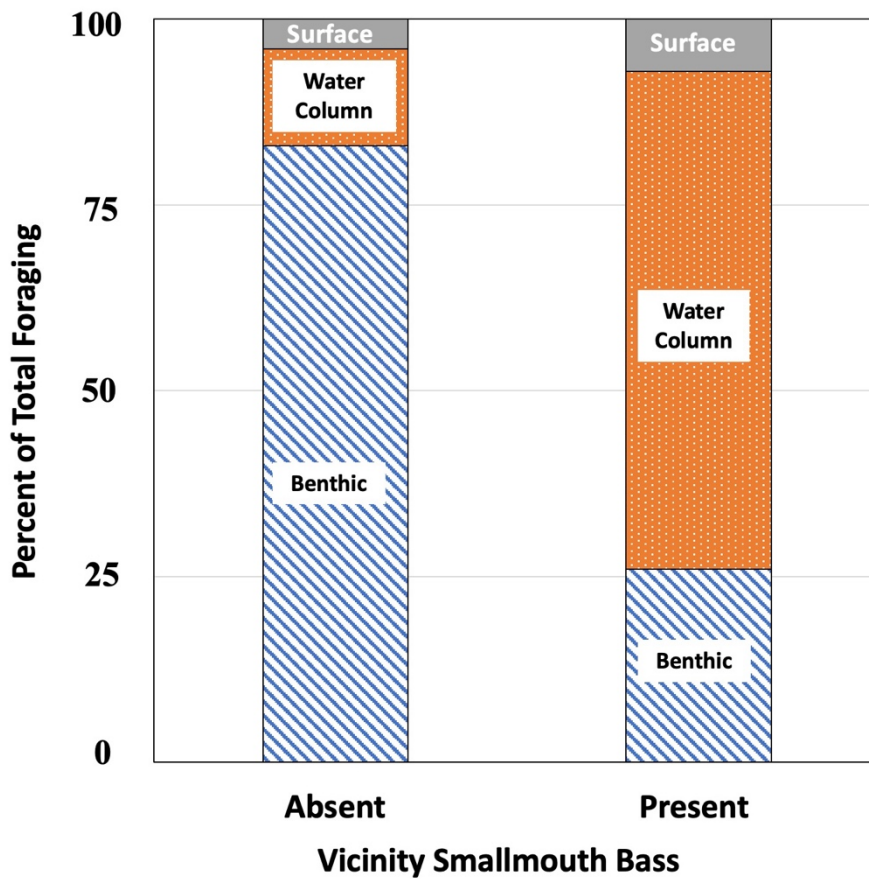


Fig. 3-3

Percentage of each Japanese dace foraging type (surface, water column and benthic foraging) when vicinity smallmouth bass were absent and present

*Modified from Peterson and Kitano 2021b

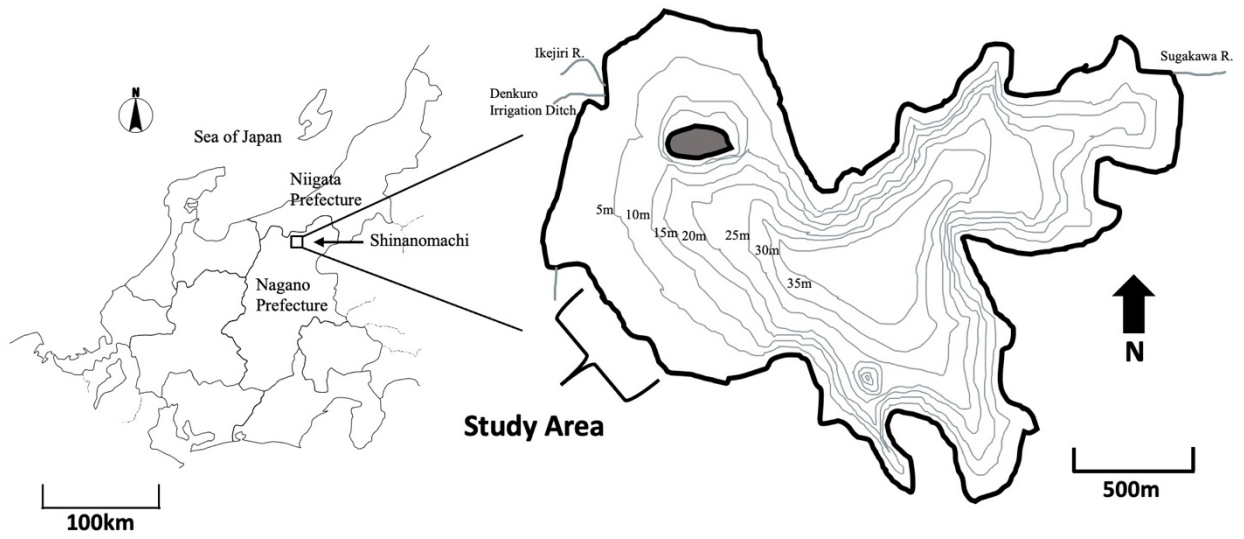


Fig. 4-1

Map of Lake Nojiiri and the study area

*Modified from Peterson and Kitano 2022

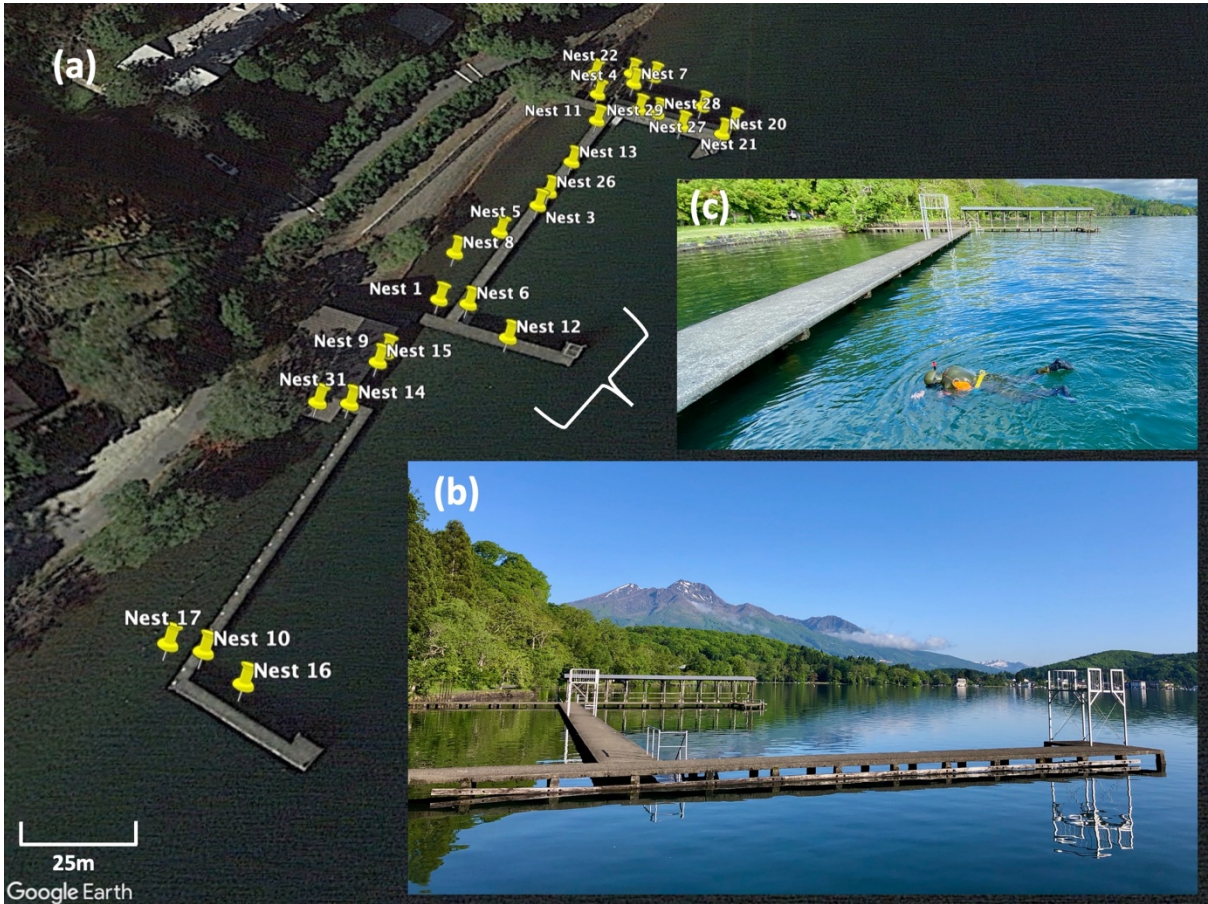


Fig. 4-2

- (a) Google Earth image of the study area with yellow pins marking nests examined in 2020.
- (b) Author snorkeling during daily nest observation and (c) photo of the study area during the spawning season

*Modified from Peterson and Kitano 2022

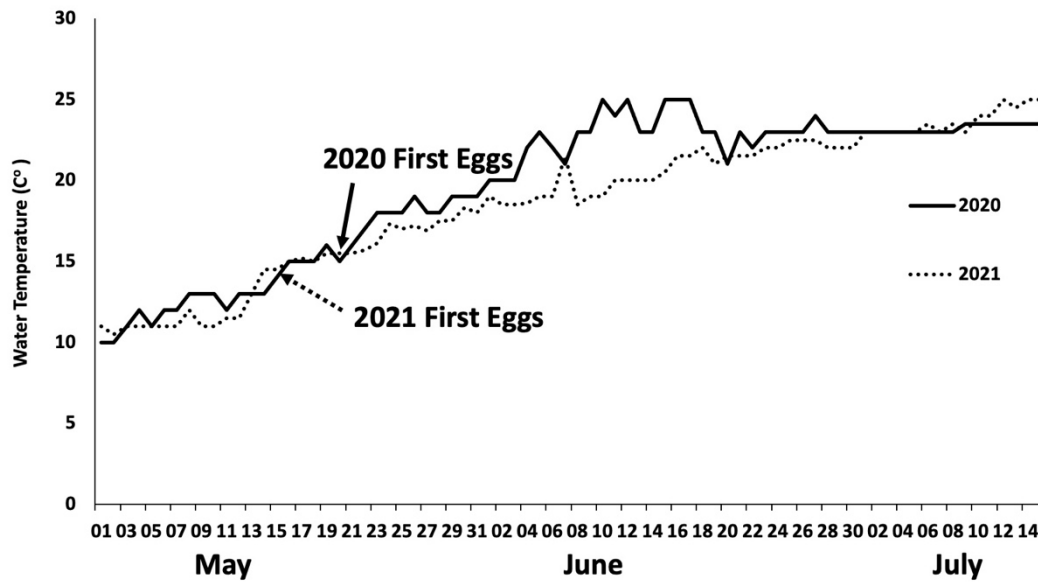


Fig. 4-3

Daily maximum surface water temperatures at Lake Nojiri during the smallmouth bass spawning season in 2020 and 2021

*Modified from Peterson and Kitano 2022

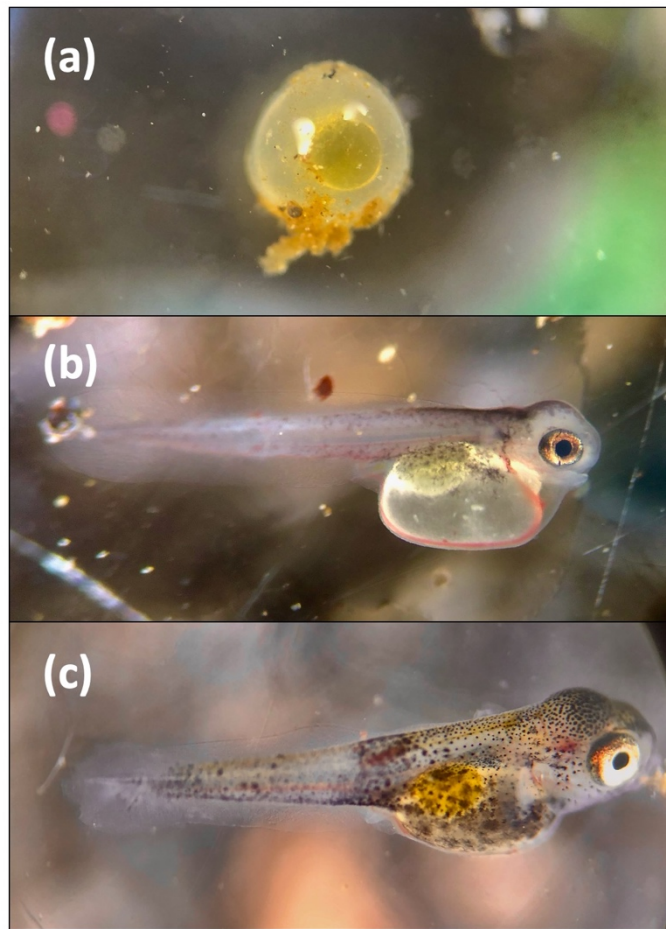


Fig. 4-5

Development of smallmouth eggs in Lake Nojiri: egg stage (a), alevin stage (b) and fry stage (c)

*Modified from Peterson and Kitano 2022

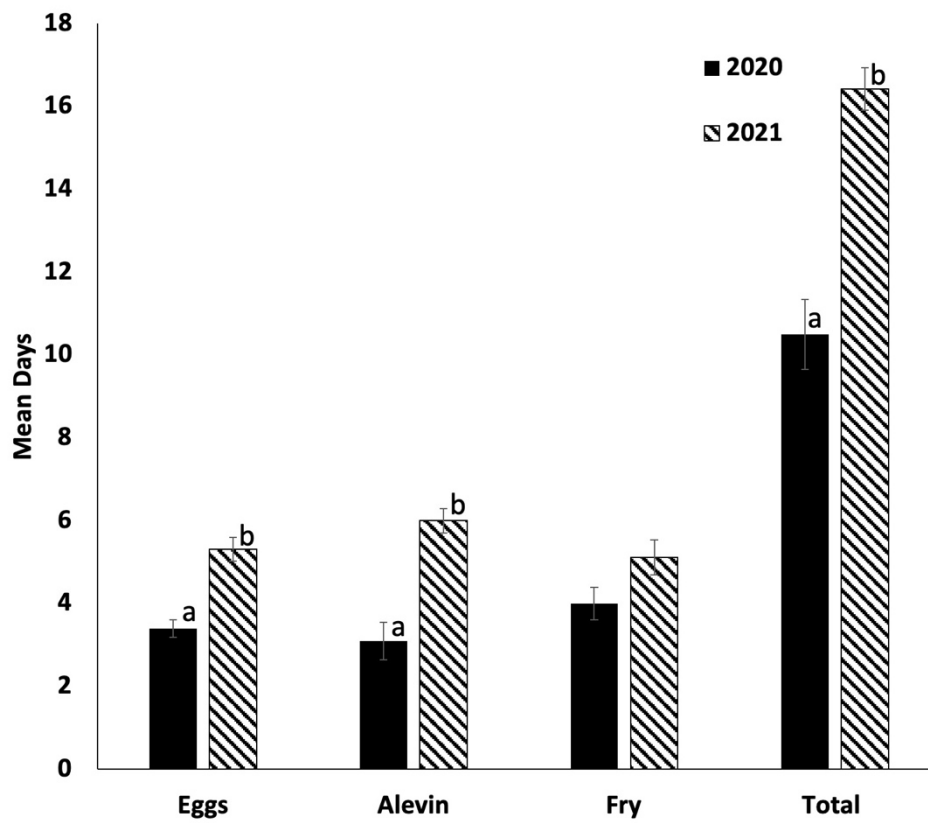


Fig. 4-6

Mean days at each stage for smallmouth bass nests in Lake Nojiri, 2020 and 2021.

Significance indicated by lowercase letters using t-tests

*Modified from Peterson and Kitano 2022

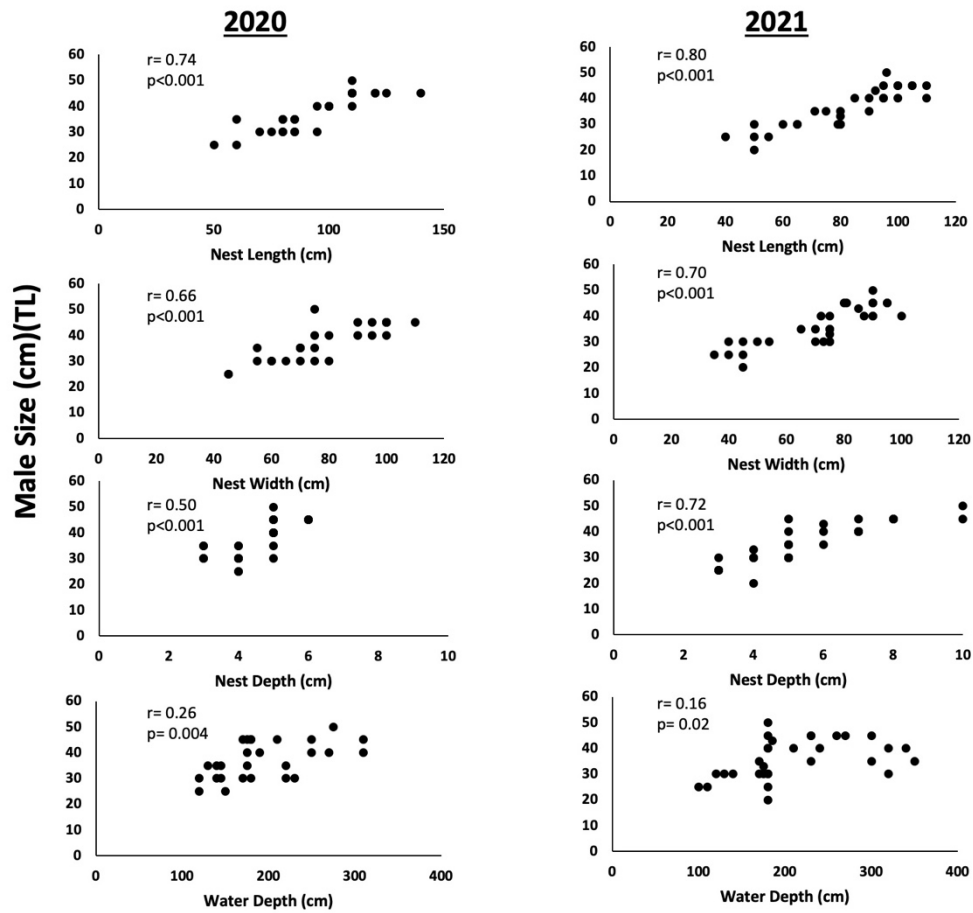


Fig. 4-7

Guarding male size correlations with nest characteristics (nest length: (a) 2020 and (b) 2021, nest width: (c) 2020 and (d) 2021, nest depth: (e) 2020 and (f) 2021 and water depth: (g) 2020 and (h) 2021). R and p-values shown using the Pearson's test

*Modified from Peterson and Kitano 2022

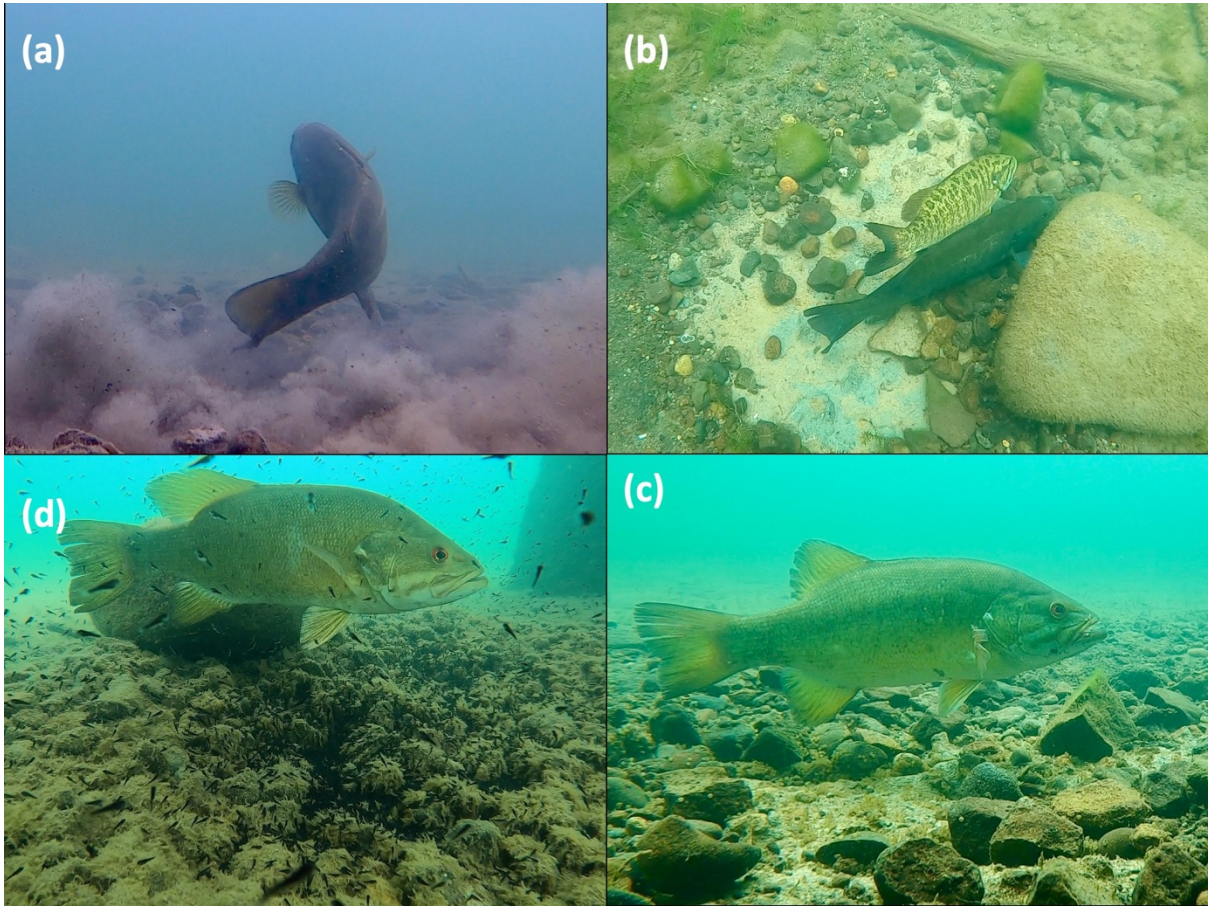


Fig. 4-8

Underwater photographs of nesting smallmouth bass in Lake Nojiri. (a) Male excavating nest with caudal fin, (b) female (lighter coloration) laying eggs with male present, (c) male guarding nest with eggs and (d) male guarding nest with fry

*Modified from Peterson and Kitano 2022

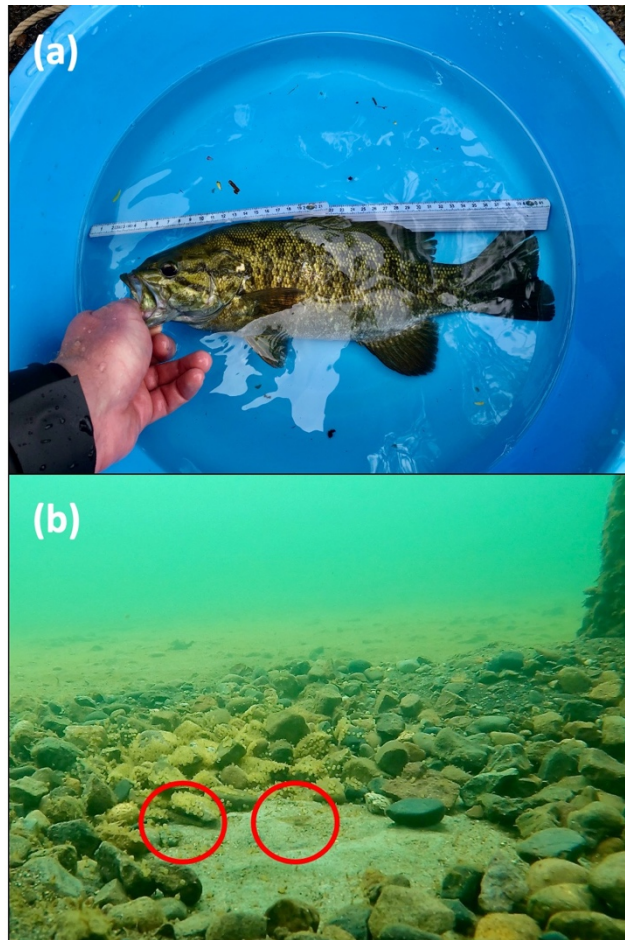


Fig. 4-9

Photographs from nest predator time lapses. (a) Male angled from nest and (b) two gobies (*Rhynogobius kurodai*), indicated by red circles, preying on smallmouth bass eggs in a nest where the male has been removed

*Modified from Peterson and Kitano 2022

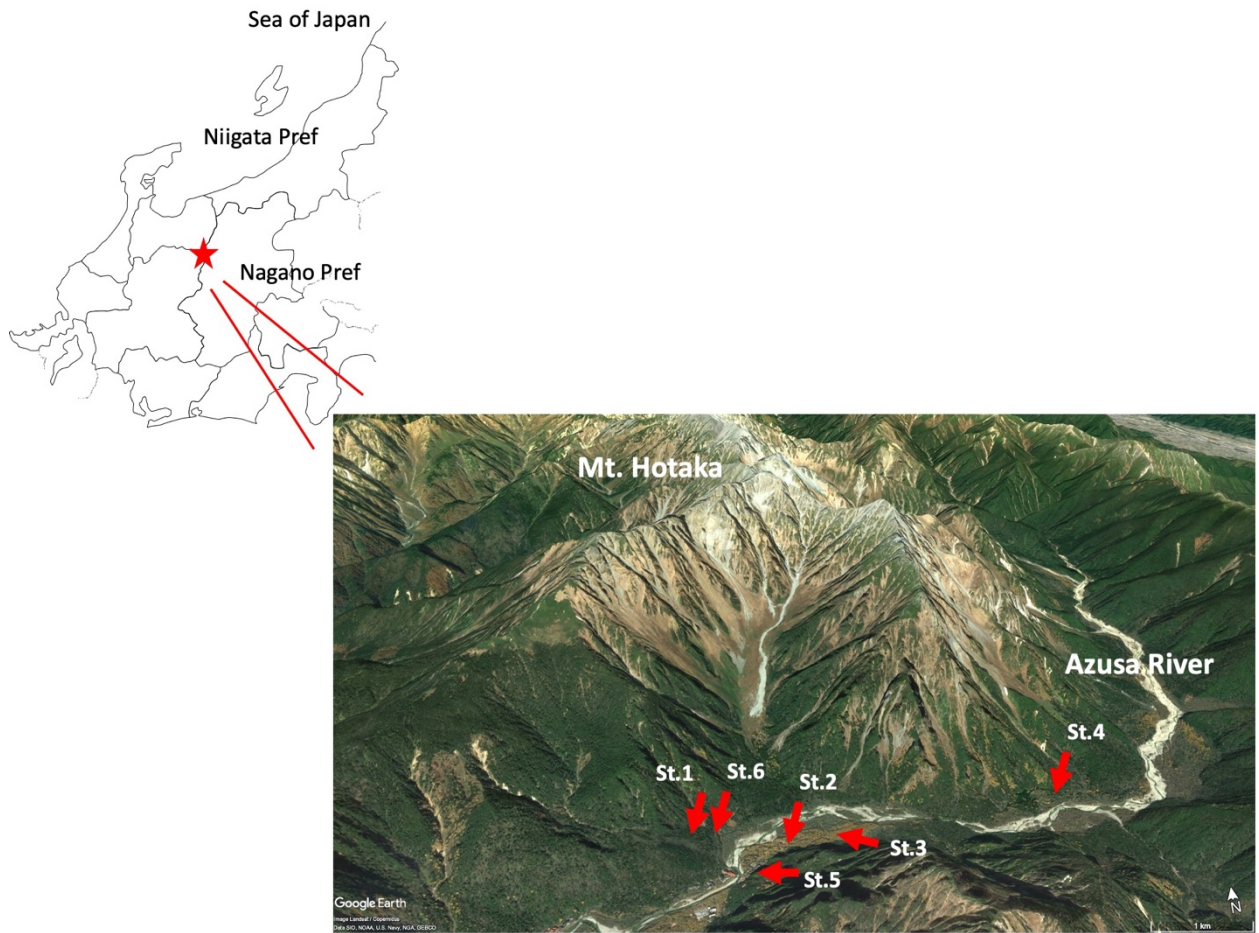


Fig. 5-1

Map of the study area (Kamikouchi, Nagano, Japan) and the six study streams. St.1 = Zenroku, St.2 = Nakagawa, St.3 = Kitano, St.4 = Myojin, St.5 = Shimizu, St. 6 = Baikamo

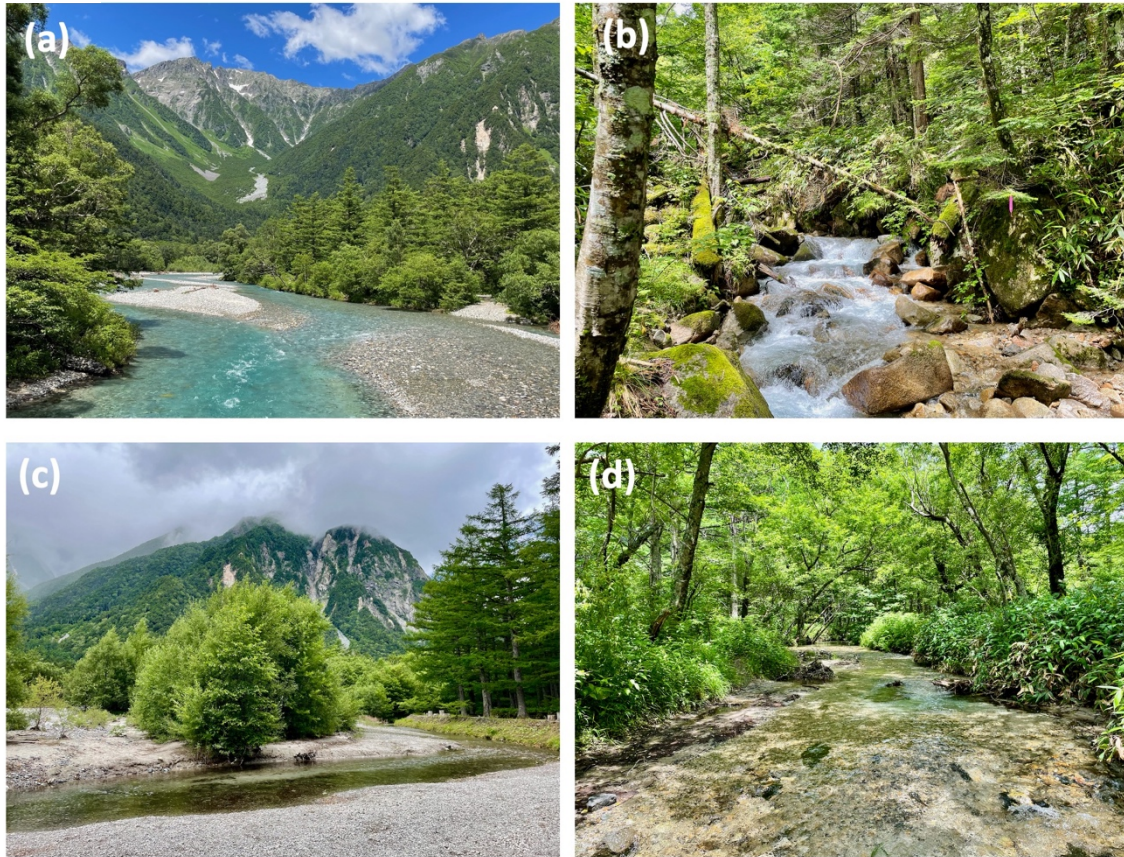


Fig. 5-2

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



Fig. 5-3

Underwater photos taken from video recordings (GoPro Hero). (a) Brook and brown trout located in Baikamo (St.6), and (b) brown trout located in Baikamo (St.6)

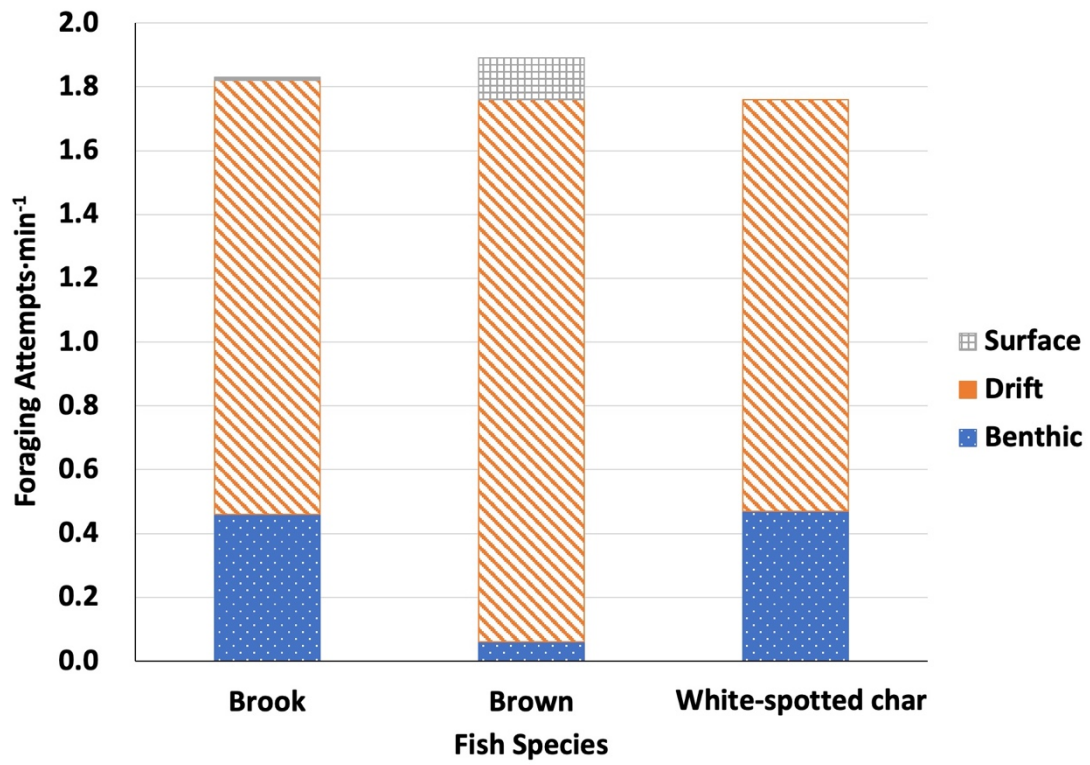


Fig. 5-4

Mean foraging attempts/min for brook trout, brown trout, and whitespotted charr in the study area

10. Tables

Table 2-I

Densities (g/m²) of all captured fish species: dace, chub, smallmouth bass (SMB), Japanese barbel (Barbel), topmouth gudgeon (Gud), ayu, tamoroko (Tam) funa, carp, freshwater goby (Goby), higai and largemouth bass (LMB), in surveyed habitat types at each location in 2018 and 2019. Modified from Peterson and Kitano 2021a.

Sites	Habitat	2018					2019				
		Dace	Chub	SMB	Barbel	Others	Dace	Chub	SMB	Barbel	Others
Nezumi	Run	0.62	1.12				0.37	0.45		0.05	
	Pool	0.34	0.10		3.54	0.16 (Gud), 0.03 (Tam)	0.69	1.21		0.08	0.14 (Gud), 0.03 (Goby)
Taibou	Run	0.25	0.06	0.02			0.94	0.55		0.33	2.98 (Ayu)
	Pool	1.19	3.08	0.42	6.66	0.12 (Gud), 0.36 (Funa)	0.19	0.30		0.03	3.32 (Ayu)
Sakaki	Run	1.96	1.71	0.29	1.27	3.30 (Ayu)	0.67	0.50	0.06	0.98	0.04 (Higai)
	Pool	0.52	0.18	0.96	7.30	0.04 (Ayu)	0.24	0.07	0.57	5.23	
Kougai	Run	0.04	0.08		2.87	0.02 (Gud)				0.03	
	Pool	0.54	0.69		0.19		0.29	1.02	0.25	1.00	
Kamuriki	Riffle	6.10	8.81	3.85	19.72	3.30 (Ayu)	0.40	0.73		1.02	0.41 (Ayu)
	Run	1.20	1.17	11.95	1.71		0.90	1.65		0.43	0.06 (Tam)
	Pool	1.06	0.56	8.43	0.57		0.14	1.19	6.87		0.72 (LMB)
Awasa	Run	0.46	1.92	1.86	0.13		0.59	1.12	0.17	3.00	
	Pool	1.96	2.06	4.23	9.89		0.09	1.61	0.00	1.26	
Shinonoi	Run	1.51	3.54	2.78	4.86	0.93 (Ayu)	0.09	0.56	0.46	2.26	
	Pool	1.84	4.18	4.82	1.20	1.17 (Carp)			1.32		
Akasaka	Riffle		1.11	0.82	0.49		0.06	2.65	4.03		
	Run	0.38	1.44	3.03	2.10			0.42	0.66		
	Pool		4.45	3.94				1.88			

Table 2-II

Densities (g/m²) of dace, chub and smallmouth bass (SMB) captured in monthly surveys at the Kamuriki and Akasaka locations in riffle, run and pool habitat types. Modified from Peterson and Kitano 2021a.

Month, Year	Species	Kamuriki			Akasaka		
		Riffle	Run	Pool	Riffle	Run	Pool
May, 2018	Dace	2.40	0.19	0.97		0.23	
	Chub					0.44	
	SMB	3.26	5.62	1.82			
June, 2018	Dace	0.27	2.52	0.78	2.40	1.40	0.89
	Chub	0.29	1.80	0.06	0.17	0.45	2.75
	SMB		0.89	2.87		0.51	2.76
July, 2018	Dace	10.38	0.26	0.44	0.81	0.51	1.28
	Chub	1.38	0.13	0.02	1.64	0.43	2.21
	SMB	4.22	11.48	2.32	1.11	1.32	10.95
Sept, 2018	Dace	6.10	1.20	1.06		0.38	
	Chub	8.81	1.17	0.56	0.11	1.44	4.45
	SMB	3.85	11.95	8.43	0.82	3.03	3.94
May, 2019	Dace					0.69	
	Chub			0.32	0.18	0.71	0.38
	SMB		0.69	28.60		5.40	
June, 2019	Dace				0.04	0.55	0.37
	Chub	0.38			1.36	0.32	0.53
	SMB	2.41	3.83	9.63			
July, 2019	Dace		0.75	0.13	0.04	0.23	1.54
	Chub	0.47	4.56	0.39	0.56	1.03	3.50
	SMB	0.89	2.16	7.17	0.28	0.02	1.04
Sept, 2019	Dace	0.40	0.90	0.14	0.06		
	Chub	0.73	1.65	1.19	2.65	0.42	1.88
	SMB			6.87	34.03	0.66	

Table 2-III

Mean habitat variables \pm SE of smallmouth bass (SMB), dace and chub captured in 2018 and 2019. Different letters represent significant differences among species (post-hoc Tukey HST $p < 0.05$). Modified from Peterson and Kitano 2021a.

*Habitat Index Value: Flow velocity divided by the water depth

Year	Species	n	Mean Velocity (cm/s)	Mean Water Depth (cm)	Mean Substrate Size Class	Mean Habitat Index Value *
2018	Dace	172	35.46 \pm 2.11 ^b	52.76 \pm 2.07 ^b	2.93 \pm 0.08 ^b	1.02 \pm 0.08 ^b
	Chub	248	48.44 \pm 2.29 ^a	62.30 \pm 1.56 ^c	2.85 \pm 0.06 ^b	1.02 \pm 0.06 ^b
	SMB	281	51.99 \pm 2.27 ^a	68.12 \pm 3.00 ^a	3.86 \pm 0.03 ^a	1.56 \pm 0.09 ^a
2019	Dace	117	53.37 \pm 2.37 ^b	62.04 \pm 2.37 ^b	2.61 \pm 0.01 ^b	1.09 \pm 0.08 ^b
	Chub	281	44.46 \pm 1.64 ^c	64.80 \pm 1.88 ^b	2.41 \pm 0.07 ^{ab}	1.02 \pm 0.07 ^b
	SMB	42	19.52 \pm 2.82 ^a	87.26 \pm 5.00 ^a	2.87 \pm 0.16 ^a	0.37 \pm 0.09 ^a

Table 2-IV

General linear model (GLM) results of the factors affecting habitat index values (flow velocity divided by the water depth) for 2018 and 2019. Species reference category: smallmouth bass. An explanatory variable effect is significant when its 95% confidence interval (CI) excludes zero. Significant results are indicated by * and highlighted in boldface. Modified from Peterson and Kitano 2021a.

Year	Factors	Coefficient Estimate	SE	P	Lower CI	Upper CI
2018	(Intercept)	-0.451	0.245	0.066	-0.920	0.037
	Species Density *	0.123	0.027	<0.001	0.067	0.175
	Species (dace)	-0.321	0.184	0.082	-0.061	0.682
	Species (chub)	-0.220	0.155	0.158	-0.116	0.536
	Fish Size	-0.0002	0.002	0.889	-0.004	0.003
2019	(Intercept)	-2.128	0.279	1.62e-13	-2.675	-1.560
	Species Density	0.028	0.089	0.753	-0.148	0.203
	Species (dace) *	1.380	0.211	<0.001	0.984	1.754
	Species (chub) *	1.198	0.182	<0.001	0.819	1.552
	Fish Size *	0.012	0.002	<0.001	0.006	0.017

Table 2-V

Stomach content % wet weight (% WT) and % alimentary index (% IA) of dace, chub and smallmouth captured in riffle, run and pool habitats respectively. Modified from Peterson and Kitano 2021a.

Habitat	Species	Total n (#of empty stomachs)	Size (SLmm) Mean \pm SE (min ~ max)	Indices	Prey item					
					Diatom	Algae	Diptera	Ephemeroptera	Trichoptera	Fish
Riffle	Dace	16 (2)	94.0 \pm 9.1	% WT	2.5	0	5.5	90.8	0	0
			(56 ~ 153)	% IA	0.4	0	0.9	98.7	0	0
	Chub	38 (4)	98.2 \pm 2.5	%WT	10.5	7.7	0.4	77.8	0	0
			(72 ~ 125)	% IA	2.8	1.7	< 0.1	95.5	0	0
	SMB	33 (2)	67.8 \pm 1.8	%WT	0	0	0.8	69.5	0.7	31.8
			(52 ~ 100)	% IA	0	0	< 0.1	96.3	< 0.1	3.6
Run	Dace	24 (10)	85.0 \pm 5.7	%WT	8.3	7.6	2.4	74.1	0	0
			(59 ~ 139)	% IA	3.2	1.0	0.3	95.5	0	0
	Chub	22 (0)	95.1 \pm 2.7	%WT	12.8	22.2	0	65.0	0	0
			(69 ~ 116)	% IA	5.1	7.1	0	87.8	0	0
	SMB	28 (1)	69.8 \pm 5.3	%WT	0	0	0.03	21.3	0.01	78.0
			(55 ~ 167)	% IA	0	0	< 0.1	58.5	< 0.1	41.5
Pool	Dace	26 (16)	96.0 \pm 6.2	%WT	39.4	14.1	21.1	21.8	0	0
			(60 ~ 152)	% IA	57.2	4.1	7.0	31.7	0	0
	Chub	31 (2)	90.9 \pm 2.6	%WT	39.7	14.4	0.9	39.4	0	0
			(63 ~ 121)	% IA	46.3	4.9	< 0.1	48.7	0	0
	SMB	32 (12)	83.4 \pm 7.5	%WT	0	0	0.1	16.6	0	81.4
			(53 ~ 270)	% IA	0	0	< 0.1	35.5	0	64.5

Table 2-VI

Percent Similarity Index values of diet overlap between dace, chub and smallmouth bass in riffle, run and pool habitat. Diet overlap is considered biologically significant when $PSI \geq 60\%$ and is indicated by bold lettering. Modified from Peterson and Kitano 2021a.

Habitat	Species	Dace	Chub
Riffle	Dace	----	----
	Chub	86.5	----
	SMB	69.5	70.3
Run	Dace	----	----
	Chub	84.7	----
	SMB	25.4	21.6
Pool	Dace	----	----
	Chub	83.2	----
	SMB	23.9	20.5

Table 3-I

Generalized linear mixed model (GLMM) results for factors affecting Japanese dace response variables. Only explanatory variables with significant effects are shown. Stepwise model selection was used to determine the best model for each GLMM and only the best models are shown. An explanatory variable effect is significant when its 95% confidence interval excludes zero. Modified from Peterson and Kitano 2021b.

Response Variable	Effect Variable	Coefficient Estimate	Standard Error	Z-Value	Pr (> z)
Total Foraging	Predator Presence	-1.04	0.07	-14.11	<0.001
	Number of Dace	-2.46e-3	1.07e-3	-2.30	0.02
	Days After Start	-4.04e-3	1.31e-3	-3.09	0.002
	Predator Biomass	-2.39e-4	6.15e-6	-3.88	<0.001
Benthic Foraging	Predator Presence	-1.48	0.17	8.59	<0.001
	Predator Biomass	-3.85e-4	1.47e-4	-2.62	0.008
Water Column Foraging	Predator Presence	0.57	0.12	4.85	<0.001
Nearest Neighbor Distance	Predator Presence	-0.63	0.02	-34.75	<0.001
	Water Depth	-0.01	5.94e-4	-18.00	<0.001
Neighbor Orientation	Predator Presence	1.86	0.11	16.78	<0.001

Table 4-I

Description of Lake Nojiri smallmouth bass nests in 2020 and 2021. Modified from Peterson and Kitano 2022.

	2020	2021
Male Size (cm)	37.0 ± 1.4	35.8 ± 1.4
Nest Length (cm)	93.3 ± 4.2	81.6 ± 3.5
Nest Width (cm)	78.0 ± 3.5	71.8 ± 3.4
Nest Depth (cm)	4.6 ± 0.2	5.6 ± 0.3
Dominant Substrate (cm)	3.0 ± 0.01	3.0 ± 0.03
Water Depth (cm)	193.7 ± 10.6	210.2 ± 12.3
% Nests with Eggs	58.3	67.5
% Eggs progressed to fry	95.2	92.6
% Cover Use	92.6	90.3

Table 4-II

Generalized linear mixed model (GLMM) results for factors affecting male smallmouth bass (SMB) nest guarding response variables. Only explanatory variables with significant effects are shown. Stepwise model selection was used to determine the best model for each GLMM and only the best models are shown. An explanatory variable effect is significant when its 95% confidence interval excludes zero. Modified from Peterson and Kitano 2022.

Response Variable	Effect Variable	Coefficient Estimate	Standard Error	Z-Value	Pr (> z)
Total Rotations	Nest Progression	-0.11	0.03	-3.90	<0.001
Guarding Radius	Water Depth	0.01	0.001	3.00	0.003
Distance from Bottom	Nest Progression	0.12	0.02	5.17	<0.001

Table 4-III

Generalized linear mixed model (GLMM) results for factors affecting nest predation response variables. Only explanatory variables with significant effects are shown. Stepwise model selection was used to determine the best model for each GLMM and only the best models are shown. An explanatory variable effect is significant when its 95% confidence interval excludes zero. Modified from Peterson and Kitano 2022.

	Effect Variable	Coefficient Estimate	Standard Error	Z-Value	Pr (> z)
Predator Number	Nest Progression	-0.17	0.06	-2.86	<0.004
	Nest Length	0.08	0.02	3.97	<0.001
	Water Depth	-0.02	0.01	-3.50	<0.001
Number of Eggs Predated	Nest Progression	-0.28	0.09	-2.86	<0.004
	Nest Length	0.08	0.03	3.23	0.001
	Water Depth	-0.02	0.01	-2.82	0.005

Table 5-I

Descriptions of the six survey streams in the Kamikouchi area. Distance is the stream distance from headwaters to confluence with the Azusa River. Fish densities calculated from a single pass snorkel survey.

Stream	Lat/Long	Elevation (m)	Reach length (km)	Source	Snorkel Dates	Fish Collection Dates	Brook (Ind. m ⁻³)	Brown (Ind. m ⁻³)	WSC (Ind. m ⁻³)
Zenroku	36°15'11N/ 137°38'12E	1817- 1514	1.70	Snowmelt	22 Jun, 16 Sep	10 Jun, 15 Jul, 16 Sep	0.07	0.01	0.03
Nakagawa	36°15'2N/ 137°38'18E	1530- 1508	0.86	Spring	6, 28 Jul, 16 Sep	15 Jul, 16 Sep	0.08	0.01	0.01
Kitano	36°15'7N/ 137°38'29E	1524- 1511	0.31	Spring	6, 28 Jul, 16 Sep	28 Jul, 16 Sep	0.17	0.15	NA
Myojin	36°15'13N/ 137°40'57E	1535- 1529	0.91	Spring	22 Jun	16 Sep	0.20	0.07	NA
Shimizu	36°14'58N/ 137°38'20E	1519- 1506	0.34	Spring	6 Jul, 16 Sep	NA	0.05	0.17	NA
Baikamo	36°15'10N/ 137°38'14E	1520- 1511	0.41	Spring	22 Jun, 16Sep	NA	0.02	0.03	NA

Table 5-II

Means \pm SE for microhabitat characteristics of each species. WSC = Whitespotted charr.

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

Table 5-III

Generalized linear mixed model (GLMM) results for factors affecting foraging, microhabitat and aggressive response variables. Only explanatory variables with significant effects are shown. Stepwise model selection was used to determine the best model for each GLMM and only the best models are shown. An explanatory variable effect is significant when its 95% confidence interval excludes zero. Species reference category is set as brook trout.

Abbreviations: FP = Focal point, Inter = Interspecific, Intra = Intraspecific, WSC = Whitespotted charr, Days = days after start.

Response Variable	Effect Variable	Coefficient Estimate	Standard Error	Z-Value	Pr ($> z $)
Flow Velocity	Fish Size	0.01	0.02	3.45	0.001
FP Velocity	WSC	0.23	0.09	2.44	0.01
	Fish Size	-0.01	<0.01	-2.16	0.03
Water Depth	Fish Size	0.01	<0.01	6.39	<0.001
FP Water Depth	Brown	0.47	0.06	7.90	<0.001
	Fish Size	0.03	<0.01	6.42	<0.001
Substrate Size	Fish Size	0.01	0.01	3.37	0.001
	Days	0.01	<0.01	2.19	0.03
Benthic Foraging	Brown	-2.22	0.38	-5.78	<0.001
Drift Foraging	Brown	0.34	0.10	3.41	0.001
	Fish Size	-0.02	0.01	-2.60	0.009
Inter-aggression	Brown	2.05	0.78	2.64	0.008
	WSC	2.40	0.95	2.52	0.01
	Fish Size	0.12	0.04	3.23	0.001
Intra-aggression	Brown	-1.64	0.57	-2.87	0.004
	Fish Size	0.12	0.04	3.09	0.002
Inter-avoidance	Brown	-1.73	0.77	-2.24	0.03
	Fish Size	-0.11	0.05	-2.25	0.02
Intra-avoidance	Fish Size	-0.08	0.04	-2.01	0.04

Table 5-IV

Stomach content % wet weight (% WT) and % alimentary index (% AI) of brown trout, brook trout and whitespotted charr (WSC).

Prey taxa	Brook		Brown		WSC	
	%WT	%AI	%WT	%AI	%WT	%AI
<i>Aquatic</i>						
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
<i>Terrestrial</i>						
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-V

Fish and amphibian prey species found in brook and brown trout stomachs.

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	<i>Onychodactylus japonicus</i> (386)
Brown	275	Nakagawa	16 Jul	<i>Bufo japonicus formosus</i> (340)
Brown	215	Zenroku	16 Jul	<i>Bufo japonicus formosus</i> (283)
Brown	403	Zenroku	16 Jul	<i>Bufo japonicus formosus</i> (896)
Brown	225	Zenroku	16 Jul	<i>Bufo japonicus formosus</i> (1493)

Table 5-VI

Schoener Index of Overlap values of diet overlap between brown trout, brook trout and whitespotted char (WSC). Diet overlap is considered biologically significant if $PSI \geq 60\%$ and is indicated by bold lettering.

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

11. Supplementary Material

Supplementary Material 2-1

Mean habitat variables \pm SE (range: min \sim max) (velocity, water depth, substrate size class and habitat index value) of baseline riffle, run and pool habitat respectively, sampled in both years. Modified from Peterson and Kitano 2021a.

	Mean Velocity (cm/s)	Mean Water Depth (cm)	Mean Substrate Size Class	Mean Habitat Index Value*
Riffle	81.3 \pm 5.4 (14.4 \sim 141.2)	35.5 \pm 3.5 (12 \sim 97)	3.16 \pm 0.20 (2 \sim 5)	2.72 \pm 0.22 (0.44 \sim 5.26)
Run	61.6 \pm 2.6 (5.9 \sim 195.1)	48.6 \pm 1.8 (10 \sim 121)	3.66 \pm 0.09 (0.5 \sim 5)	1.44 \pm 0.06 (0.12 \sim 3.90)
Pool	28.3 \pm 1.6 (4.6 \sim 91.5)	73.4 \pm 2.0 (25 \sim 170)	2.80 \pm 0.11 (0.5 \sim 5)	0.42 \pm 0.03 (0.04 \sim 1.59)

*Habitat Index Value: flow velocity divided by the water depth

Supplementary Material 2-2

Equations ($Y = a * x^b$) to estimate wet weight (Y: gram) from standard length (x: cm) for each fish species. Modified from Peterson and Kitano 2021a.

Species	Sample Year	a	b	n	r ²
Dace	2016	0.0176	2.808	200	0.983
Chub	2016	0.0117	3.159	251	0.988
SMB	2016	0.0227	3.068	601	0.992

Supplementary Material 2-3

Smallmouth bass age class sizes (SL) determined through scale reading. Fish were collected in the middle Chikuma River during September 2017. Modified from Peterson and Kitano 2021a.

Age	n	Mean SL (mm) \pm SE	Range: min ~ max
0	28	63.1 \pm 1.7	47 ~ 78
1	126	86.0 \pm 0.9	62 ~ 118
2	35	114.5 \pm 2.3	98 ~ 150
3	35	160.6 \pm 2.2	129 ~ 194
4	8	179.5 \pm 5.2	164 ~ 208
5	3	250.7 \pm 1.2	249 ~ 253
6	1	274 \pm NA	NA
7	1	301 \pm NA	NA

NA: Not available due to small sample size