

Evaluating a pattern of ecological character displacement: charr jaw morphology and diet diverge in sympatry versus allopatry across catchments in Hokkaido, Japan

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Similar species that overlap in sympatry may diverge in characters related to resource use as a result of evolution or phenotypic plasticity. Dolly Varden charr (*Salvelinus malma*) and whitespotted charr (*S. leucomaenis*) overlap along streams in Hokkaido, Japan, and compete by interference for invertebrate drift-foraging positions. Previous research has shown that as drift declines during summer, Dolly Varden shift foraging modes to capture benthic prey, a behaviour facilitated by their subterminal jaw morphology. We compare body and jaw morphology of Dolly Varden in sympatry vs. allopatry in two locations to test for character displacement. Statistical analysis showed significant divergence in characters related to foraging, which was correlated with variation in individual charr diets. Dolly Varden in sympatry had shorter heads and lower jaws than in allopatry, and even within sites charr with these characteristics fed less on drifting terrestrial invertebrates but more on benthic aquatic invertebrates. Those in allopatry had longer heads and lower jaws, and fed more on terrestrial invertebrates. The close proximity of sites in one stream suggests that Dolly Varden may display phenotypic plasticity similar to other charr, allowing rapid responses in morphology to the presence of competitors. These morphological shifts probably help them maintain positive fitness when competing with whitespotted charr in Hokkaido streams.

ADDITIONAL KEYWORDS: Dolly Varden charr – interspecific competition – morphometrics – phenotypic plasticity – resource polymorphism – *Salvelinus* – whitespotted charr.

A common pattern in assemblages of animal taxa is that similar species diverge in morphology or behaviour where their ranges overlap and they occur in sympatry (Schluter, 2000; Pfennig & Pfennig, 2012). Brown & Wilson (1956) termed this phenomenon character displacement and proposed interspecific competition

as the primary mechanism. Support for this theory has waxed and waned over the last 60 years (Stuart & Losos, 2013), as criteria for testing the theory were made explicit (Schluter & McPhail, 1992) and more rigorous studies were conducted in response. Evidence gathered from field research includes strong support for the role of resource competition in driving rapid evolution of adaptive divergence (Schluter, 1994; Grant & Grant, 2006; Stuart *et al.*, 2014), but also clear cases where phenotypic plasticity

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induced by resource competition results in character shifts within the lifetimes of individuals (Pfennig & Murphy, 2002; Pfennig *et al.*, 2006). To create a clear logic for investigating these questions, Stuart *et al.* (2017) proposed a two-step process, to first document the pattern of character displacement and then investigate the particular processes that create the pattern. They argue that more careful study is needed, which requires unlinking character displacement from simple resource competition.

Character displacement when closely related species come into secondary contact is considered to be the final stage in an evolutionary cycle of adaptive radiation (Lack, 1947; Schluter, 2000; Snorrason & Skúlason, 2004). This pattern is prevalent in fishes, especially those that occur in recently deglaciated lakes (Robinson & Wilson, 1994). Salmonids, and charrs (genus *Salvelinus*) in particular, are adept at rapid changes in morphology when they encounter the 'ecological opportunity' afforded by unused habitats and food resources available in these lakes (e.g. Smith & Skúlason, 1996; Piggott *et al.*, 2018). For example, two to four morphs of Arctic charr (*S. alpinus* L.) that occupy different benthic, pelagic and littoral habitats and forage on different prey have been reported from lakes in Iceland (Skúlason & Smith, 1995; Skúlason *et al.*, 1999; Snorrason & Skúlason, 2004), Norway (Amundsen *et al.*, 2008; Klemetsen, 2010), and Scotland (Adams *et al.*, 1998, 2008). Brook charr (*S. fontinalis* Mitchell; Bertrand *et al.*, 2008) and lake charr (*S. namaycush* Walbaum; Chavarie *et al.*, 2015, 2017) have diversified into two to five benthic and pelagic or deep- and shallow-water morphs in North American glacial lakes. Dolly Varden charr (*S. malma* Walbaum) isolated in the Lake Kronotskoe watershed on the Kamchatka Peninsula about 12 000–14 000 years ago produced seven distinct morphs that use littoral, pelagic and benthic habitats, forage on different prey, and spawn in different locations or at different times (Esin *et al.*, 2018; Markevich *et al.*, 2018). For cases that have been studied in detail, the mechanisms producing the morphs are a mixture of genetic variation and adaptive phenotypic plasticity in response to their environment, and often involve complex effects on developmental pathways (Klemetsen, 2010; Küttner *et al.*, 2014; Skúlason *et al.*, 2019).

Fewer examples of morphological divergence in fishes have been reported from flowing waters (Robinson & Wilson, 1994; Whiteley, 2007), perhaps because temporal fluctuations in water flow and habitat homogenize phenotypic responses in comparison with more stable benthic and pelagic habitats in lakes (Wimberger, 1994; Senay *et al.*, 2015). McLaughlin *et al.* (1994, 1999) and McLaughlin (2001) reported that newly emerged brook charr adopted discrete foraging strategies in stream margin

habitats, as either sit-and-wait predators of benthic crustaceans or active predators of insect prey at or near the surface. They found no difference in three morphological characteristics related to swimming performance, but argued that these behavioural differences are an important prerequisite for developing resource polymorphisms (Skúlason & Smith, 1995). Nevertheless, a variety of fish species captured in habitats with fast vs. slow current velocity (e.g. pools vs. riffles in streams), including brook charr, show morphological differences in size and shape of the body and caudal fin that relate to swimming performance (McLaughlin & Grant, 1994; Samways *et al.*, 2015; Senay *et al.*, 2015).

A model system for investigating character displacement in stream fishes concerns the congeneric charrs of Hokkaido Island, Japan, for which there is a wealth of supporting phylogeographical and ecological information. Based on analysis of mitochondrial DNA, Dolly Varden charr apparently colonized the island during the Pleistocene from a source in the adjacent Sea of Okhotsk or Sea of Japan (Yamamoto *et al.*, 2014), followed later by its congener, the whitespotted charr (*S. leucomaenis* Pallas; Yamamoto *et al.*, 2004). Dolly Varden now occur in upstream reaches throughout the island, and whitespotted charr downstream, with relatively narrow zones of sympatry where they overlap (Ishigaki, 1984; Fausch *et al.*, 1994). The zone of overlap is related to temperature, and increases in elevation westward across the island with a warmer climate. Density compensation occurs in individual pools in these sympatric segments (Fausch *et al.*, 1994), where the two species compete for positions in interspecific dominance hierarchies (Nakano & Furukawa-Tanaka, 1994). Positions near the heads of pools, from which salmonids make forays to capture drifting invertebrates, provide the most net energy (Fausch, 1984) and are vigorously contested (Nakano, 1995a, b). However, as this shared invertebrate drift resource declines throughout summer, individual Dolly Varden shift from sit-and-wait foraging to ranging widely and picking benthic invertebrates from the streambed (Nakano & Furukawa-Tanaka, 1994), a behaviour facilitated by their subterminal mouth compared to whitespotted charr (Fig. 1). A replicated field experiment coupled with a 4-year field comparative study confirmed that declines in drifting prey caused the foraging mode shift (Fausch *et al.*, 1997; Nakano *et al.*, 1999a).

During intensive field research in one catchment to measure these ecological mechanisms, the senior author noticed differences in the jaw morphology of Dolly Varden in the allopatric segment upstream compared to the sympatric zone downstream. He and several co-authors subsequently gathered charr from another pair of streams with allopatry and

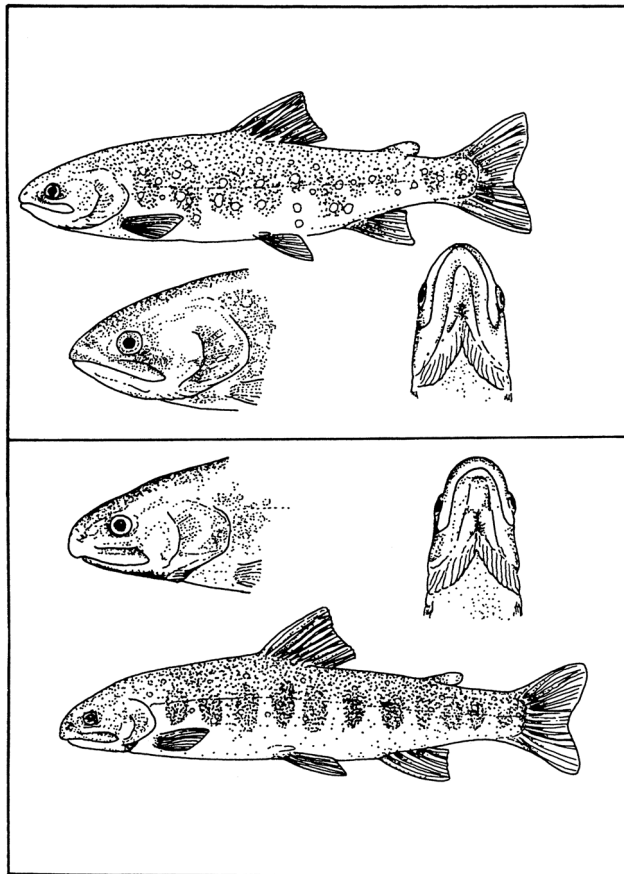


Figure 1. Body and mouth morphology of whitespotted charr (top) and Dolly Varden charr (bottom). Drawings by S. Nakano (from [Fausch 2015](#); used with permission).

sympatry in another region of Hokkaido. They also sampled charr diets and drifting and benthic prey at all locations. Here, we first evaluate the pattern of character displacement, and then weigh the evidence for the mechanisms that caused it based on standard criteria ([Schluter & McPhail, 1992](#); [Schluter, 2000](#); [Stuart & Losos, 2013](#)). We present not only the first report of divergence in morphological characters of a stream salmonid where they are sympatric with a congener, but also show the functional significance of this divergence for diets of individual fish, a key link in understanding the consequences of character displacement for resource acquisition, and hence fitness.

CRITERIA FOR TESTING CHARACTER DISPLACEMENT

[Stuart *et al.* \(2017\)](#): table A1) described nine ecological and evolutionary processes proposed by others to account for the patterns of morphological divergence

Table 1. Six criteria used to weigh the evidence for character displacement and the mechanisms causing it, adapted from [Schluter & McPhail \(1992\)](#), [Schluter \(2000\)](#) and [Stuart & Losos \(2013\)](#)

Criterion	Supporting evidence
1. There is independent evidence that similar phenotypes compete for limited resources	Nakano & Furukawa-Tanaka (1994) , Fausch <i>et al.</i> (1994, 1997) , Nakano <i>et al.</i> (1999a) , Taniguchi & Nakano (2000) , Watz <i>et al.</i> (2019)
2. Ecological shifts in resource use should match changes in morphology or other phenotypic traits	Nakano & Furukawa-Tanaka (1994) , Fausch <i>et al.</i> (1997) , Nakano <i>et al.</i> (1999a) , this study
3. Differences are due to evolution, and not simply species sorting	Fausch <i>et al.</i> (1994) , Morita (2019)
4. Environmental differences between sites of sympatry and allopatry must be controlled	This study
5. Chance should be ruled out as an explanation for the pattern	This study
6. Phenotypic differences between populations and species have a genetic basis	No data available

Supporting evidence for each criterion from previous research on congeneric charrs in Hokkaido is shown, as well as those tested in this study.

termed character displacement. These mechanisms range from sexual selection to interspecific competition for resources, agonistic behaviour, phenotypic plasticity and chance. [Schluter & McPhail \(1992\)](#) compiled the six most important criteria for distinguishing among these mechanisms ([Table 1](#)) and these have been used to test the strength of evidence within and across studies ([Schluter, 2000](#); [Dayan & Simberloff, 2005](#); [Stuart & Losos, 2013](#)).

Previous research provides evidence to address three of these six criteria for Hokkaido charr ([Table 1](#)). There is independent evidence that fish of similar phenotypes compete for limited resources (Criterion 1). As described above, Dolly Varden and whitespotted charr set up interspecific dominance hierarchies in stream pools where they compete by interference for drift foraging positions, and sequential removals of the highest-ranking individual resulted in rapid shifts of the entire dominance hierarchy into the positions vacated (K. D. Fausch *et al.*, unpubl. data). The two species also show density compensation across pools,

an indirect measure of interspecific competition (Wiens, 1989). In a laboratory study of age-0 charr at two temperatures typical of allopatric segments in Hokkaido streams (6°C vs. 12°C; upstream and downstream of the sympatric zone), whitespotted charr dominated Dolly Varden by aggressive interference, held more favourable upstream positions, foraged more, grew faster and survived better at the higher temperature, whereas at the lower temperature Dolly Varden were about equal competitors, grew similarly and survived much better than whitespotted charr (Taniguchi & Nakano, 2000). This difference was confirmed in a replicated field experiment on growth in cold vs. warm tributaries (c. 7.4°C vs. 10–14°C; Watz *et al.*, 2019), and provides a mechanism to explain their longitudinal distribution in Hokkaido streams.

There is a clear functional link between the displaced trait and the partitioned resource (Criterion 2), and we test this prediction more fully in this study. Dolly Varden have more subterminal mouths and are more adept at picking benthic insects from the substrate than whitespotted charr (Fig. 1). When drifting prey were depleted with nets set upstream from replicate pools, Dolly Varden switched to benthic feeding within 1 h, and switched back to drift feeding within 20 min when drift was restored, whereas few whitespotted charr shifted foraging modes (Fausch *et al.*, 1997). The switch occurred at a specific threshold of drifting prey, and measurements of charr displaying drift vs. benthic feeding throughout a 1.5-km segment of the stream over four summers revealed identical behaviours and the same switching point (Nakano *et al.*, 1999a).

Differences in phenotypes represent evolutionary shifts, and not simply species sorting (Criterion 3). In speciose taxa, patterns of character displacement may occur because some competitors with similar morphology die out, leading to trait overdispersion in sympatric congeneric species (Schluter, 2000). However, Schluter (2000) proposed that this criterion is satisfied if the differences between sympatry and allopatry are intraspecific, which we test here. In addition, only two other salmonids occur in Hokkaido, namely masu salmon (*Oncorhynchus masou* Brevoort) and Sakhalin taimen (*Parahucho perryi* Brevoort), and both are distributed in lower-elevation streams compared with Dolly Varden (Fausch *et al.*, 1994; Fukushima *et al.*, 2011; Morita *et al.*, 2016). Masu salmon overlap with Dolly Varden in eastern Hokkaido where these charr also inhabit low-elevation streams (Ishigaki, 1984; Morita, 2019), but the salmon are anadromous and most emigrate after 1 year. Therefore, competition from the more distantly related masu salmon is likely to be weaker than from congeneric whitespotted charr, and to occur for a shorter period.

Criterion 4 is that environmental differences between sites of sympatry and allopatry must be controlled. Schluter (2000) proposed that it is never possible to rule out all environmental factors that might differ between sites and cause a pattern of character displacement, but that the most obvious alternative agents such as resource availability and habitat characteristics should be tested. This is the approach we take here. Criterion 5 is that chance should be ruled out as an explanation for the pattern. Species in sympatry at one location and in allopatry at two others are expected to show greater divergence in sympatry one time in four ($P = 0.25$) if population means are assigned randomly to locations (Schluter, 2000). Hence, based on this simple model this same pattern is expected to occur at two independent sets of sites with a probability of $P = 0.0625$. Here we analyse the pattern in replicate pairs of sites in different regions and draining to different seas to assess the evidence for its repeatability across Hokkaido.

Finally, Criterion 6 is that phenotypic differences between populations and species have a genetic basis. We have no data to test this criterion, but it will be a key for evaluating the importance of evolution and phenotypic plasticity in explaining any pattern of morphological differences in these charr. This will require further detailed study, because plasticity often involves a complicated interplay among competitors, their environment and the genome that affects development and leads to morphological divergence (e.g. Pfennig *et al.*, 2010; Küttner *et al.*, 2014; Esin *et al.*, 2018; Skúlason *et al.*, 2019).

MATERIAL AND METHODS

STUDY LOCATIONS

Dolly Varden charr were sampled from two pairs of sites in streams in central and eastern Hokkaido Island, Japan (Fig. 2). Each pair had Dolly Varden populations in allopatry vs. sympatry with whitespotted charr. One pair was in Poroshiri Stream (42°41'N, 142°41'E) a tributary of the Niikappu River in the Hidaka Mountains of south-central Hokkaido, which drains south to the Pacific Ocean. Dolly Varden were captured in allopatry in a 400-m-long headwater site and in sympatry in a 1350-m site that began 650 m downstream. The second pair of sites was in the headwaters of the Hidarsookippukaomanai River (800 m of allopatry, HSK hereafter, 43°44'N, 144°53'E) and in Ichani Stream (600 m of sympatry, 43°41'N, 145°5'E) in eastern Hokkaido, streams in adjacent catchments which drain east to the Nemuro Strait bordering the Sea of Okhotsk.

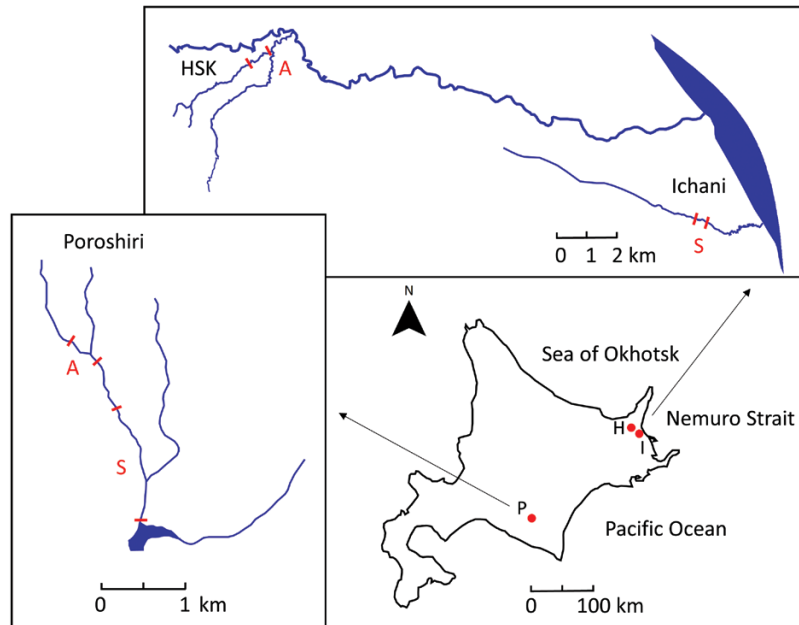


Figure 2. Location of the two pairs of study sites in Hokkaido Island, Japan where Dolly Varden charr were collected in sympatry (S) and allopatry (A). The pairs were in Poroshiri Stream (P), and Ichani Stream (I) and Hidarisookippukaomanai River (H or HSK).

Within each pair, physical characteristics were generally similar and the charr made up most of the fish assemblage. The sites in Poroshiri were of higher elevation and gradient whereas the sites in Ichani–HSK were in streams of moderate to low elevation and gradient (Supporting Information, Table S1). Sites had similar wetted width (c. 4–5 m) and discharge (c. 0.15–0.30 m³ s⁻¹), and had pools of similar area (c. 6–7.5 m²) and maximum depth (c. 0.50–0.60 m). They had primarily gravel and cobble substrate and low average summer water temperatures suitable for both charr (c. 8–9 °C). Juvenile masu salmon occurred in Ichani Stream, but at lower abundance compared to charr, and were rare in HSK. River sculpin (*Cottus nozawae* Snyder) occurred in Poroshiri Stream, but were rare (Nakano *et al.*, 1999a), and ninespine stickleback (*Pungitius pungitius* L.) inhabited Ichani Stream.

No barrier prevented fish movement between the sites in Poroshiri Stream. Dolly Varden of all age classes (age 1–4, based on otoliths) were captured at sites in sympatry, allopatry and the boundary between (Fausch *et al.*, 1994), and age-0 and age-1 charr were observed throughout by snorkelling (Nakano *et al.*, 1999a), indicating that these fish spawned locally throughout the stream. Poroshiri Stream drains into a reservoir of the Niikappu River (Fig. 2) inhabited by whitespotted charr, but only two of the large silvery fish were encountered in the stream while snorkelling (K. Fausch and S. Kitano, pers. observ.). In contrast, all Dolly Varden were stream residents.

SAMPLING FISH, DIETS AND PREY

Charr were captured by electrofishing (Model 12 backpack electrofisher, Smith-Root Inc., Vancouver, WA, USA) in all streams, and also by cast net and angling in Poroshiri Stream (Fausch *et al.*, 1994; Nakano *et al.*, 1999a). Fish were captured from throughout the 400–1350-m-long sites in study streams during late June to early August (Supporting Information, Table S1). At each study site, a representative sample of approximately 80–215 charr of ages 1 and older were preserved in 10% buffered formalin for analysis of morphometrics and diet. All fish were collected under appropriate permits from the Hokkaido government to S. Nakano, and euthanized via an overdose of tricaine methanesulfonate (MS-222) according to an Animal Care and Use protocol issued by Colorado State University to K. Fausch.

Drifting and benthic invertebrates are potential prey resources for charr, and were sampled at all sites. Drift was sampled once in Ichani and HSK during the same period fish were sampled, and two to three times in Poroshiri Stream during late June to mid-August each of four summers during 1991–1994 ($N = 10$ periods; Nakano *et al.*, 1999a). During each sampling, drift nets (20 × 20 cm opening, 60 cm long, 400- μ m mesh) were set for 20 min across the inlet of a representative pool at each study site. In Ichani and HSK, nets were set at four locations three times between 0900 and 1700 h, and in Poroshiri Stream nets were set at 6 to 10 locations two to three times

during the same period. At each location, drift nets were deployed to sample the entire water column, to ensure capturing invertebrates drifting throughout the water column and on the water surface. The depth of water filtered was measured at the mouth of each net that was not fully submerged, and water velocity was measured in the centre of the flow for each net with a portable current meter (Tanida *et al.*, 1985). Nets of similar mesh size but smaller openings (10 × 10-cm openings, 20 cm long, 475- μ m mesh, set for 30 min) were used in Poroshiri Stream in 1991 and 1992 (Nakano *et al.*, 1999a).

Benthos was collected during each sampling at five locations distributed throughout the pools where drift was sampled. Samples were collected with a 100- μ m mesh Surber sampler (25 × 25-cm quadrat). Both drift and benthic samples were preserved in 5% buffered formalin. Unfortunately, data for the benthic samples and the taxonomic composition of the drift samples in Poroshiri-allopatry could not be found among the files left when the senior author passed away (see Fausch, 2000).

MEASURING FISH MORPHOMETRICS, FISH DIETS AND PREY RESOURCES

All morphometric measurements and analysis of fish diets and prey resources were conducted during 1995–1999. We restricted all analyses to age-1 and older fish, which for all sites during the seasons sampled were those ≥ 85 mm standard length (SL). Morphometric measurements were made using a digital calipers (nearest 0.01 mm) on the left side of the fish. Nine morphometric characters defined in Hubbs & Lagler (1970) were measured, including: SL, head length, snout length, upper jaw length, lower jaw length (length of mandible), eye diameter, pectoral fin length, body depth and caudal peduncle depth. Two additional characters were measured: lower jaw width (transverse distance between the most posterior points of the mandibles) and body width (width at the widest point, exclusive of fins). Sex and maturity of gonads were determined by dissection.

Methods for analysing fish diets, drift and benthos followed those reported in Nakano *et al.* (1999a). Briefly, samples of all three types were sieved once (475- μ m mesh) to equalize the minimum size of invertebrates. Terrestrial invertebrates were identified to Order (larvae and adults were separated) and aquatic invertebrates to Family. Biomass for each of 27–37 categories in the study streams was measured as damp mass (nearest 0.01 g) after blotting for ~10 s (repeated measurements varied $\leq 6\%$; Nakano *et al.*, 1999a). Damp mass was converted to dry mass using regressions through the origin calculated from random

subsamples dried at 60 °C for 24 h ($N = 11\text{--}30$, $r^2 = 0.76\text{--}0.99$, $P < 0.001$ for the 37 categories; S. Nakano & Y. Kawaguchi, unpubl. data).

Biomass of drifting invertebrates (mg m^{-3}) was estimated by dividing the total estimated dry weight from each net by the water volume sieved. Total biomass of drift passing the cross-section at the inlet of the pool sampled (mg s^{-1}) was calculated by multiplying the mean drift biomass by stream discharge ($\text{m}^3 \text{s}^{-1}$). This measure is the most meaningful given fluctuations in streamflow, because higher streamflow may carry less biomass of drift per unit volume but more total biomass. Benthic food resources were expressed simply as benthos biomass (g m^{-2}) because the wetted width of the study streams and hence stream area changed little with discharge.

PREDICTIONS AND STATISTICAL ANALYSIS

PREDICTIONS

Based on the previous research described above, we hypothesized that competition for prey resources between the congeneric charrs in sympatry would favour divergence in head and body shape of Dolly Varden charr compared to allopatry. Moreover, observations by the senior author of charr in Poroshiri Stream indicated that lower jaw length and mouth position (terminal or subterminal) could be key morphometric characteristics that change. Jaw length also varies between males and females in charrs (Morton, 1965). Males develop longer lower jaws, termed a kype, as a secondary sex characteristic during the breeding season (October–November; Kitano, 1996). The kype is a different type of bone that develops rapidly in the weeks before spawning (Witten & Hall, 2002) and most is resorbed rapidly when competition for breeding ceases (Haugland *et al.*, 2011). Only a small amount is converted into permanent dentary bone (Witten & Hall, 2003), contributing to sexual dimorphism in jaw length. We sampled charr only during the non-breeding season (June–August) when the kype is absent, and accounted for sex in our analysis.

Based on these considerations, we made four predictions regarding body morphology of Dolly Varden charr (DV):

1. Body shape, and especially head shape, of DV are different in sympatry vs. allopatry
2. DV of a given length in sympatry have shorter snouts and lower jaws, and more subterminal mouths, than those in allopatry
3. Female DV of a given length have shorter snouts and lower jaws than males

4. These differences in morphology are similar in the two pairs of allopatric and sympatric sites from across Hokkaido.

We also made seven specific predictions relating shifts in use of trophic resources to size-adjusted differences in morphology (i.e. the functional significance, addressing Criterion 2; [Table 1](#)) for Dolly Varden in sympatry vs. allopatry. We first predicted that:

1. DV in sympatry consume more benthic prey than those in allopatry
2. DV in allopatry consume more terrestrial prey than those in sympatry

We then predicted that in both sympatry and allopatry:

3. DV with shorter heads and lower jaws consume more benthic prey
4. DV with more subterminal mouths consume more benthic prey
5. DV with longer heads and lower jaws consume more terrestrial prey
6. DV with more terminal mouths consume more terrestrial prey
7. Larger DV consume more terrestrial invertebrates, because they dominate favourable upstream positions in pools which offer greater access to this drifting prey resource

MULTIVARIATE ANALYSIS

For each pair of sites, we conducted a separate multivariate analysis to assess whether Dolly Varden have a different body shape in sympatry vs. allopatry, and what morphometric characters contribute to that difference. All morphometric characters were first transformed using natural logarithms and standardized (mean = 0; SD = 1). Characters were then adjusted for body length by calculating the residuals of each from the allometric regression on SL, which removes the effect of body size from the analysis ([Thorpe, 1976](#)). The allometric relationships were calculated separately for males and females in sympatry and allopatry, and the slopes and intercepts of the four groups were averaged to estimate an unbiased common regression, after the methods of [Thorpe \(1976, 1983\)](#) and [Reist \(1986\)](#).

Overall differences in body shape of Dolly Varden in sympatry vs. allopatry were first tested using multivariate analysis of variance (MANOVA) on the residuals of the 10 morphometric characters described above (all except SL). Homogeneity of variance was assessed using Bartlett's test for each individual character (with Bonferroni correction across tests) because Box's M test is known to be inaccurate ([Field *et al.*, 2012](#)). Characters contributing to differences in

body shape were assessed using principal components analysis (PCA) on the covariance matrix of these same residuals of the 10 characters. The MANOVA was conducted using the *manova* function in R v.3.5.3 ([R Core Team, 2019](#)), and PCA was conducted using the *prcomp* function.

UNIVARIATE ANALYSES

To evaluate differences in key morphometric characters more fully, we conducted univariate analyses of four characters that consistently had the highest loadings on the first two principal components across the two pairs of sites: lower jaw length, upper jaw length, snout length and eye diameter (see Results). We conducted the same analysis on mouth position, the distance between the most anterior tips of the upper jaw and lower jaw ([Morita & Suzuki, 1999](#)), which we estimated from the upper and lower jaw lengths and lower jaw width using geometry (see Supporting Information, [Fig. S1](#)). Fish with greater distance values have more subterminal mouths.

We used general linear models (*lm* function in R) to test the prediction that each character differed between sympatry and allopatry, and between the sexes, after accounting for length. We transformed all variates using natural logarithms and fitted allometric relationships of each character as a function of ln SL, sympatry vs. allopatry (hereafter 'patry'), sex, and all two-way interactions among these covariates. Given the clear hypotheses, few covariates and large sample sizes, we considered this a confirmatory analysis and drew inference from the full model ([Burnham & Anderson, 2002](#)).

RELATING DIETS TO MORPHOLOGY

For the analysis to test the seven predictions relating diet to morphology, terrestrial prey included all terrestrial invertebrates in fish diets. The prey that Dolly Varden are most likely to capture from the benthos are those that rarely occur in the drift (i.e. are found only in catastrophic drift). We determined these prey taxa based on information for this functional trait of invertebrates reported by [Poff *et al.* \(2006\)](#), who categorized occurrence in the drift (rare, common or abundant) for 311 genera in 75 families of aquatic insects in North America, many of which also occur in Japan. We compared this against three categories developed independently by Y. Miyake based on his research (e.g. [Miyake *et al.*, 2003](#)) and experience, describing the likelihood that Dolly Varden captured each prey taxon: (1) primarily from the drift, (2) from either drift or benthos depending on the invertebrate species or environmental conditions, or (3) primarily

from the benthos. We found that the two sources agreed.

Two additional prey categories strongly selected by benthic foraging Dolly Varden were also included as benthic prey. Fish observed by snorkelling in Poroshiri-sympatry to have switched to benthic foraging averaged $\leq 8\%$ terrestrial prey in their diets, and these fish had high selectivity for chironomid larvae (0.65 based on Manly's Index, >6 times that expected at random; see Nakano *et al.*, 1999a: fig. 5). We classified Dolly Varden in Ichani and HSK that had $\leq 8\%$ terrestrial prey in their diets as benthic foragers also, and found that those in HSK also had high selectivity for chironomids (0.54, >3 times expected at random) whereas those in Ichani strongly selected amphipods (0.46, >3 times expected at random). Therefore, these taxa were also included among the prey most likely to have been captured directly from the benthos. Most chironomids were also probably captured from the benthos in Ichani Stream, because most Dolly Varden (72%) were benthic foragers, chironomids made up a higher proportion of benthic than drift samples by weight (25% vs. 8%), and both benthic and drift foragers selected this prey approximately at random (0.16–0.20). Amphipods were found in drift and benthic samples only in Ichani Stream, but are reported to be primarily benthic in Japan (Tomikawa & Morino, 2012). They were found in only one fish stomach in HSK and never in those in Poroshiri.

In contrast to the confirmatory analysis of morphometrics, we considered the analysis of the functional significance of morphology for Dolly Varden diet to be exploratory, and so used a formal model-selection framework (Burnham & Anderson, 2002). All analyses of diets were confined to summer low-flow periods in July and August when drifting prey become limiting for charr (Nakano *et al.*, 1999a). We used general linear models to relate two diet response variables to explanatory variables, after first assessing normality and homoscedasticity. To meet assumptions, total biomass (mg) was log transformed ($\log X + 1$), and the proportion of total biomass was logit transformed (cf. Warton & Hui, 2011) after first constraining proportions to 0.025–0.975 to minimize effects of extreme values.

We hypothesized that diet is most affected by four covariates: sympatry vs. allopatry, lower jaw length, mouth position and relative length. Lower jaw length was chosen as a measure of head length for this analysis, because all three measures of jaw and snout length found to be important in the multivariate analysis were highly correlated among themselves and with head length ($r = 0.83$ – 1.00 , median $r = 0.98$, $N = 48$ for the eight site \times sex combinations).

Lower jaw length and mouth position increase with fish length, so for both measures we used the residual of the distance from the regression on SL as the covariate

for analysis. For lower jaw length we used the residual from the allometric relationship, whereas regressions of mouth position on SL were linear so we calculated the residuals from this relationship. In both cases, we used an unbiased common regression calculated by averaging over the four sex \times patry groups, as for the morphometric analysis (see above).

We also reasoned that diet could be affected by the relative length of fish, which affects their dominance rank (Nakano, 1995a, b). For example, larger, more dominant fish hold positions near the heads of pools and are likely to have greater access to drifting terrestrial insects. Relative length for individual fish was defined as their SL divided by the 90th percentile SL among all fish at a given site (Saunders & Fausch, 2012, 2018). Individuals at the 90th percentile or larger were assigned a relative length of 1.0. None of the four covariates used to predict diet, including the residuals of lower jaw length and mouth position, were highly correlated (absolute values of $r = 0.05$ – 0.55 , median $r = 0.09$).

The set of candidate models for this analysis consisted of all subsets of the four covariates, and three two-way interactions we considered to be most likely. First, Dolly Varden with more subterminal mouths might capture more benthos in sympatry where competition is stronger than in allopatry where all fish have more access to terrestrial insects (patry \times mouth position). Second, Dolly Varden with shorter, blunter heads (as measured by lower jaw length) might also capture more benthos in sympatry than allopatry for this same reason (patry \times lower jaw length). Third, larger Dolly Varden might capture more terrestrial insects than small Dolly Varden in allopatry, in comparison to sympatry where all Dolly Varden are expected to have little access to terrestrial insects (patry \times relative length).

Given the exploratory nature of this analysis, we also elected to use the most conservative approach to model selection to ensure the most robust evidence for any effects detected. This involves 'full' model averaging over all models fit, rather than conditional averaging over only those in which each effect occurs. Conditional model averaging tends to inflate weak effects because they occur only in a few models, where their effects are overestimated (Anderson, 2008). Simulations showed that full model averaging and calculation of the unconditional variance based on all models ensures that confidence intervals cover the true value at the level selected whereas 95% confidence intervals based on conditional model averaging covered the true value less than half the time (Lukacs *et al.*, 2010). We also report the variable importance for each covariate and interaction, defined as the sum of the model weights for all models in which each covariate appears (range: 0–1). Fitting and averaging all possible subsets of the covariates and interactions was done using the *MuMIn* package (Barton, 2018) in program R.

Data on stream salmonid diets are known to be highly variable (e.g. Nakano *et al.*, 1999b; Giller & Greenberg, 2015; Saunders & Fausch, 2018) because diets depend on many factors that entrain benthic and terrestrial insects into the flow, prey visibility (which varies with flow and time of day), fish positions in dominance hierarchies, and many other factors. Given this variability, and the conservative method of model selection we used, we developed a hierarchy of metrics to assess the strength of evidence for each prediction tested. We considered the evidence for an effect to be very strong in cases where the effect was in the direction predicted, the 95% confidence interval (95% CI) of the slope parameter did not overlap zero and variable importance values were ≥ 0.98 . Cases where the effect was in the direction predicted but the 95% CI overlapped zero were designated as providing strong evidence if variable importance was 0.80–0.97 and modest evidence if variable importance was 0.35–0.79. Finally, cases with lower variable importance, where the effect was close to zero or in the opposite direction predicted, were considered to offer no evidence in support of an effect.

RESULTS

A total of 561 Dolly Varden charr were measured for morphometric analysis (Supporting Information, Table S2). Both sexes were well represented at each site, and they ranged from *c.* 120 to 130 mm in mean SL among sites (total range: 85–204 mm SL). A large proportion of the fish were analysed for diet (68% in Poroshiri, 86% in Ichani–HSK; $N = 416$ total), and these spanned the entire range of lengths and had similar mean length as the entire sample from each site.

MULTIVARIATE ANALYSIS

Bartlett's test revealed significant heterogeneity in variances of morphometric characters when the sexes were analysed together for each pair of sites, so separate MANOVAs were conducted by sex. For these, only one of the 10 characters was found to have heterogenous variance for only one of the four groups (snout length for females in Ichani–HSK; $P < 0.005$), so the overall assumption of homogeneous variance was considered met. The MANOVAs revealed highly significant differences in the set of morphometric characters between sympatry and allopatry for each sex at each pair of sites ($P < 3.0 \times 10^{-12}$ for all), indicating strong differences in body shape.

PCA revealed that the first two principal components for each pair of sites accounted for substantial variation in the multivariate space defined by the 10 traditional morphometric characters (62% for Poroshiri, 73% for Ichani–HSK; Table 2). Three characters related to the head and jaws (lower and upper jaw lengths, snout length) and eye diameter had among the highest loadings on the first principal component for both pairs of sites, and eye diameter had high loadings on both the first and the second principal components. As a result, we focused on these four morphometric characteristics and mouth position in univariate analyses.

Plots of the data with centroids and 95% confidence ellipses show that Dolly Varden of each sex in sympatry had shorter jaws and snouts and smaller eyes than their counterparts in allopatry, and in Poroshiri Stream they also had deeper and wider bodies and wider lower jaws (Fig. 3; Table 2). In both pairs of sites, female Dolly Varden had shorter jaws and snouts and smaller eyes than males, when compared within sympatry and allopatry.

Table 2. Loadings of the 10 original morphometric characters on the first two principal components for the two pairs of sites, based on PCA

Character	Poroshiri		Ichani–HSK	
	PC1	PC2	PC1	PC2
Lower jaw length	-0.4013	0.2861	-0.5085	0.1476
Upper jaw length	-0.3802	0.2252	-0.4763	0.1046
Snout length	-0.5426	-0.1218	-0.3167	0.2986
Head length	-0.2301	0.1988	-0.2349	0.1214
Lower jaw width	-0.3080	-0.3924	-0.2727	0.1710
Body depth	-0.1384	-0.3372	-0.0714	0.1940
Body width	-0.2261	-0.6331	-0.0140	0.2105
Eye diameter	-0.3238	0.3232	-0.3862	-0.8630
Pectoral fin length	-0.2190	0.1824	-0.3458	0.0405
Caudal peduncle depth	-0.1706	-0.0824	-0.1039	0.0754
Cumulative proportion of variance explained	38%	62%	61%	73%

Cumulative proportion of the variance explained is shown for each pair. Loadings with absolute value >0.30 are in bold type.

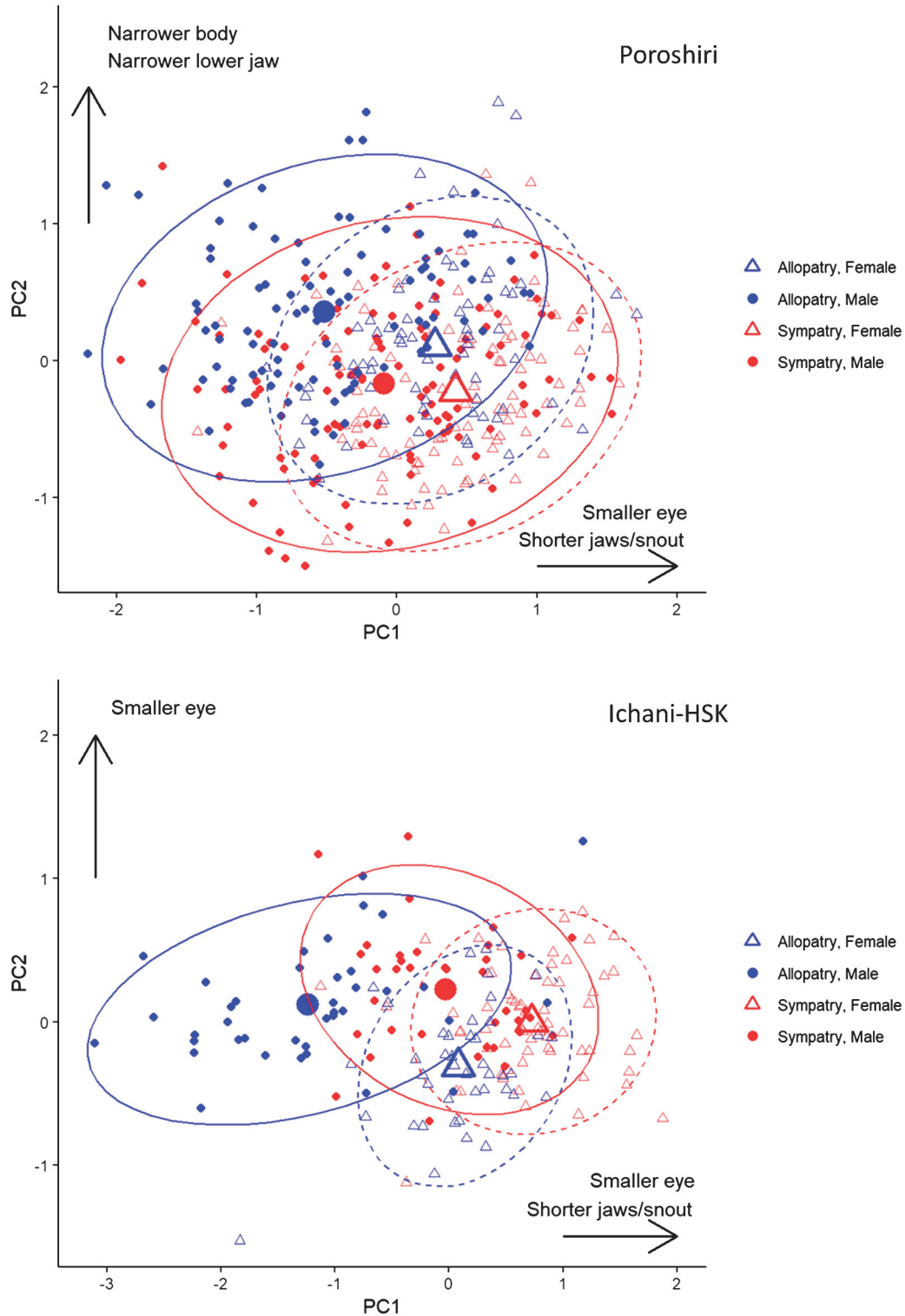


Figure 3. Principal components analysis for 10 morphometric characteristics of Dolly Varden charr in Poroshiri Stream (top) and Ichani Stream and HSK River (bottom). Labels along each axis show characters with the highest loadings (see Table 2). Centroids and 95% confidence ellipses are shown for each sex in allopatry and sympatry.

UNIVARIATE ANALYSES

Dolly Varden charr of each sex in sympatry with whitespotted charr had shorter lower and upper jaws and snout lengths at a given body length than those in allopatry, at both pairs of sites (Fig. 4; Supporting Information, Table S3). Female charr had shorter lower and upper jaws and snouts than males at both pairs of sites. These effects were often manifested as significant interactions ($P \leq 0.05$) among covariates (Table 3), such as the greater effect of allopatry vs. sympatry for

larger vs. smaller fish ($\ln SL \times \text{patry}$) and for males vs. females ($\text{patry} \times \text{sex}$). For example, estimated effect sizes for males across a range of SL from 105 to 150 mm (the 25th to 90th percentiles of length) showed that lower jaw length was about 5–10% shorter in sympatry than in allopatry in Poroshiri Stream and 11–16% shorter in Ichani–HSK (Fig. 5; Table S3). For females, lower jaw length was about 1–2.5% shorter in sympatry than in allopatry in Poroshiri and 8% shorter in Ichani–HSK. The only exception to this pattern was

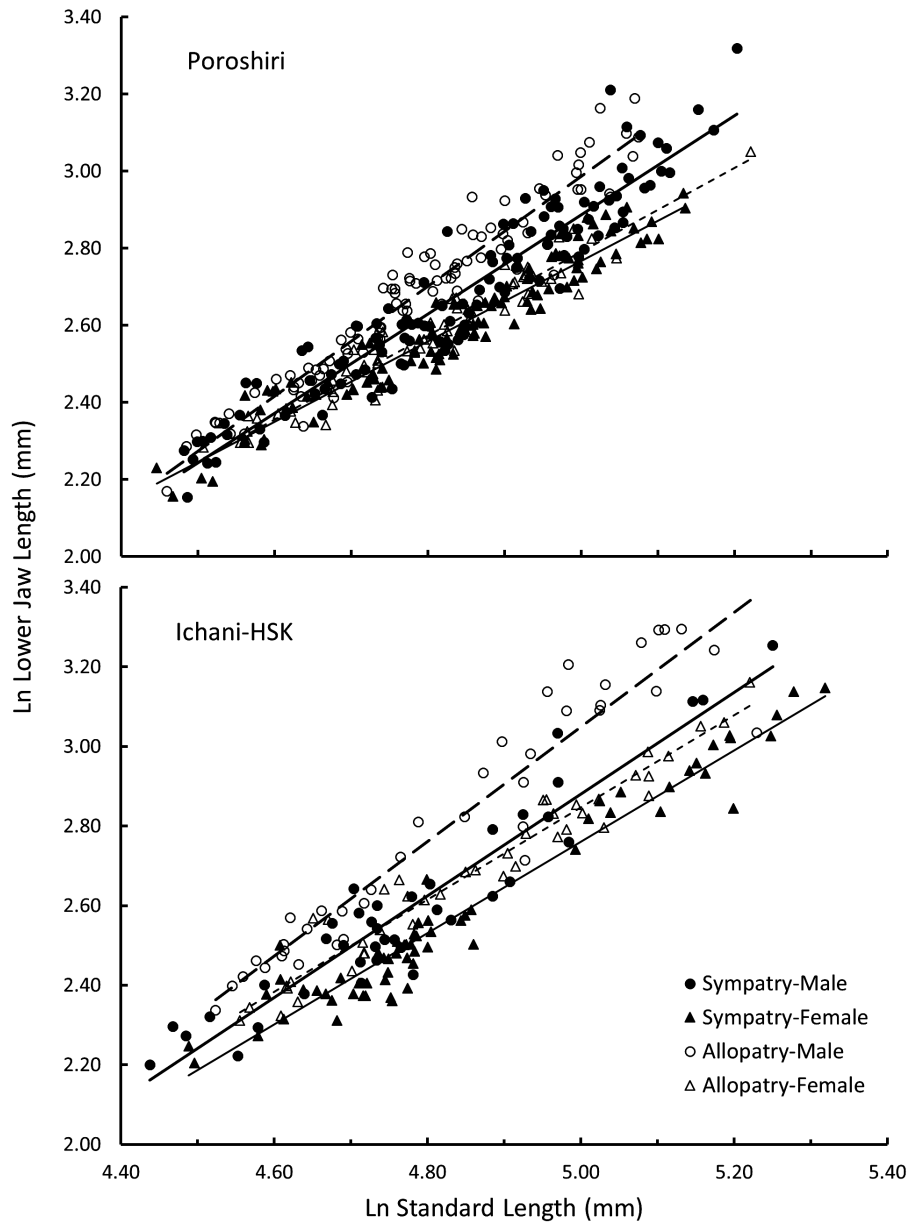


Figure 4. Allometric relationships of lower jaw length as a function of standard length for Dolly Varden charr in Poroshiri Stream (top) and Ichani Stream–HSK River (bottom). Relationships predicted from the linear model (see Table 3) are shown for each sex in allopatry (dashed lines) and sympatry (solid lines). Lines for males are above those for females in each case.

Table 3. Results of univariate analyses of allometric relationships for four morphometric characters of Dolly Varden in the two pairs of study sites

Character	Model fit (adjusted R^2)	Model significance	d.f.	Ln SL	Patry	Sex	Ln SL × Patry	Ln SL × Sex	Patry × Sex
Poroshiri Stream									
Lower jaw length	0.92	<0.0001	370	<0.0001	0.01	<0.0001	0.01	<0.0001	<0.0001
Upper jaw length	0.93	<0.0001	370	<0.0001	0.06	<0.0001	0.06	<0.0001	<0.0001
Snout length	0.79	<0.0001	369	<0.0001	0.06	0.04	<0.05	0.02	0.13
Mouth position	0.17	<0.0001	370	0.03	0.06	0.57	0.04	0.54	0.70
Eye diameter	0.78	<0.0001	369	<0.0001	0.93	0.01	0.80	0.01	0.05
Ichani Stream–HSK River									
Lower jaw length	0.93	<0.0001	177	<0.0001	0.30	0.003	0.18	0.0004	0.01
Upper jaw length	0.94	<0.0001	177	<0.0001	0.12	0.005	0.06	0.0006	0.003
Snout length	0.90	<0.0001	177	<0.0001	0.21	0.06	0.23	0.02	0.0007
Mouth position	0.31	<0.0001	177	<0.0001	0.49	0.33	0.53	0.35	0.61
Eye diameter	0.86	<0.0001	176	<0.0001	<0.0001	0.72	<0.0001	0.81	0.18

Results of tests of effects shown in bold are significant ($P \leq 0.05$). Missing values reduced sample size by one for snout length and eye diameter in three cases. SL, standard length; d.f., degrees of freedom.

that snout length of females in Ichani (sympatry) was slightly longer than in HSK (allopatry), opposite to that predicted. Eye diameter was smaller in sympatry than in allopatry for both sexes at both pairs of sites. This effect was greater for larger than for smaller fish, and in Poroshiri was greater for males than for females.

Mouth position was more subterminal for Dolly Varden in sympatry than in allopatry, although the effect was small in the Ichani–HSK stream pair (Supporting Information, Table S3). Mouth position of larger fish was greater (more subterminal) in sympatry than in allopatry for both sexes in Poroshiri Stream ($P = 0.04$; $\ln \text{SL} \times \text{patry}$; Table 3; Fig. 6), but only length had a significant effect in Ichani–HSK. In both pairs there was a substantial amount of individual variation in mouth position.

PREY RESOURCES

The data available on drifting and benthic invertebrates show that biomass was similar between sympatry and allopatry within each pair of sites, but different between pairs (Table 4). There was no evidence that drift abundance was different in the allopatric vs. sympatric site of Poroshiri Stream across the four to six summer baseflow periods during 1991–1994 (t -test, $t = 0.52$, $P = 0.62$), and the estimate at baseflow for Ichani was only 14% less than that for HSK and within about 1 SE. Similarly, the estimate of biomass for benthic invertebrates was only 5% more for Ichani than for HSK. No estimate of benthos biomass was available for the allopatric section of Poroshiri (see Methods), so no comparison was possible for this stream.

The composition of benthic and drifting invertebrate taxa varied across sites and streams, but the percentage of terrestrial vs. benthic taxa in the drift was similar. Terrestrial invertebrates made up 21–41% of prey in drift samples from the three sites for which data were available (Supporting Information, Table S4). Dominant benthic invertebrates in the drift were chironomids in Poroshiri (sympatry), amphipods in Ichani, and caddisflies (Trichoptera) in HSK. In benthic samples, mayflies (Ephemeroptera) made up 24–42% of the biomass and were the dominant taxon in Poroshiri. As in the drift, amphipods dominated the benthos in Ichani and caddisflies in HSK. Chironomids made up 5–25% of the benthic biomass across streams.

RELATING DIET TO MORPHOLOGY

Analyses of Dolly Varden diets as a function of morphology offer strong or very strong support for the effects of sympatry vs. allopatry on diets, and modest to very strong support for the effects of head and jaw functional morphology on diets over and above these ecological effects (Fig. 7; Supporting Information, Table S5). For example, Dolly Varden in sympatry consumed a higher proportion of biomass (Fig. 8) and higher total biomass of benthic prey than those in allopatry in each pair of sites, for which there was strong (Poroshiri) or very strong (Ichani–HSK) evidence. Likewise, there was the same pattern of strong or very strong evidence in each pair of sites that Dolly Varden in allopatry consumed a higher proportion and total biomass of terrestrial prey than those in sympatry.

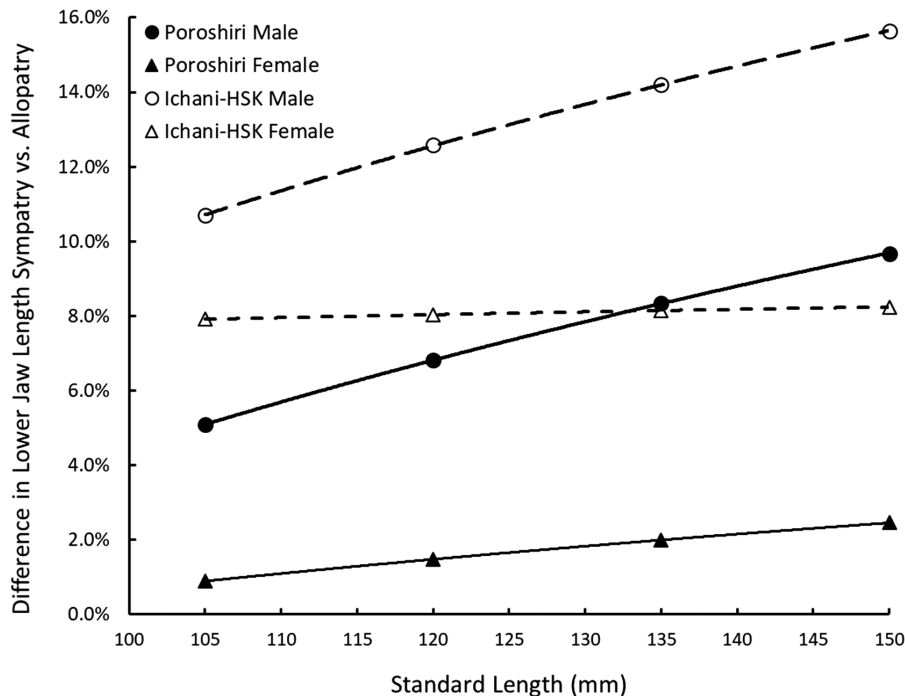


Figure 5. Effect sizes for the percentage difference in lower jaw length for Dolly Varden charr of each sex in sympatry relative to allopatry, at the 25th, 50th, 75th and 90th percentiles of standard length.

There was evidence from at least one pair of sites that jaw and head characteristics had effects on diet in addition to that owing to sympatry or allopatry. For Ichani–HSK streams, there was strong or very strong evidence that Dolly Varden with shorter lower jaws (and, therefore, smaller mouths and blunter heads) foraged more on benthos (proportion or total biomass) and those with longer lower jaws foraged more on terrestrial insects, and modest or strong evidence for most of these effects in Poroshiri Stream (Figs 7, 9). There was modest evidence from Poroshiri Stream that Dolly Varden with more subterminal mouths (larger residual of mouth position) captured a higher proportion of prey biomass from the benthos, and that those with more terminal mouths captured a higher proportion and total biomass of terrestrial prey. Finally, in three of four cases there was very strong evidence that larger Dolly Varden, which are assumed to be more dominant, captured a higher proportion and total biomass of terrestrial prey than smaller fish (Figs 7, 8 for Poroshiri).

DISCUSSION

The results confirmed our four predictions regarding body morphology, and show a clear divergence in morphology of Dolly Varden charr across two pairs

of allopatric vs. sympatric sites in Hokkaido. This is the first step in describing a pattern of character displacement and the processes that explain it (Stuart *et al.*, 2017). MANOVA detected highly significant differences in body shape for each sex in each pair of sites based on a set of 10 morphometric characters, and PCA revealed that three measures of head and jaw length, and eye diameter, were most highly correlated with the first two principal axes. Univariate analyses of these four characters confirmed that Dolly Varden in sympatry with whitespotted charr had shorter snouts and lower jaws, and smaller eye diameters, than Dolly Varden in allopatry. Effect sizes were greater for males than for females and greater for large vs. small fish. Dolly Varden in sympatry also had more subterminal mouths than those in allopatry, although this effect was not significant for Ichani–HSK. Nevertheless, the shorter jaws in sympatry in Ichani Stream also result in a smaller mouth opening, adapting these Dolly Varden for feeding on benthic prey, which are smaller than terrestrial prey (Nakano *et al.*, 1999b).

The shifts in morphology we found in sympatry are similar to those reported for Dolly Varden and other charr in recently deglaciated lakes that diverged in response to opportunities to exploit benthic prey. Differences among the seven morphs of Dolly Varden in a Kamchatkan lake were also primarily due to head and jaw length, mouth position, and eye diameter

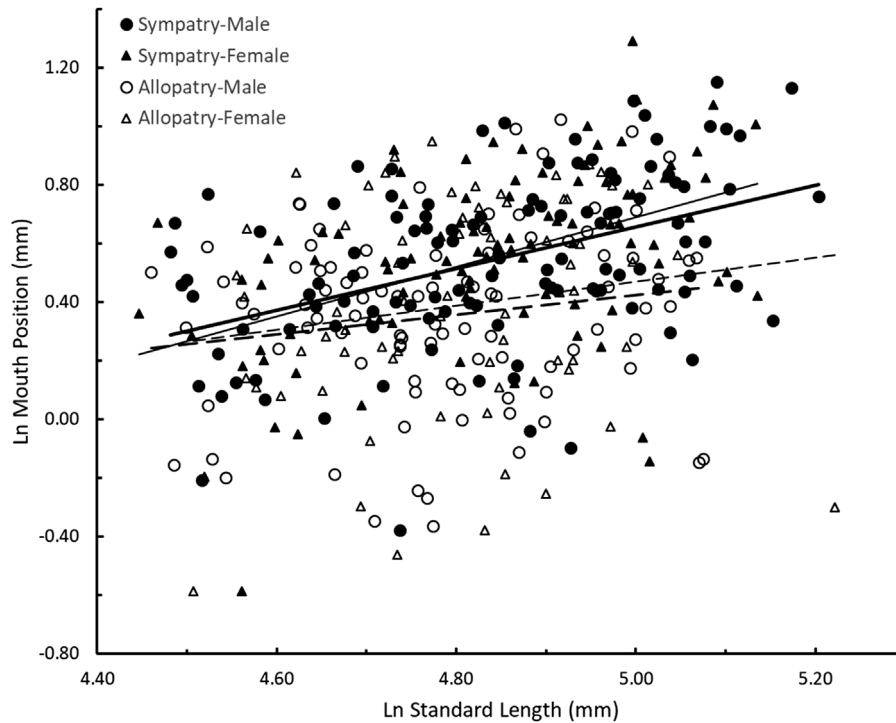


Figure 6. Allometric relationships of mouth position as a function of standard length for Dolly Varden charr of each sex in the sympatric and allopatric sites of Poroshiri Stream. Relationships predicted from the linear model (Table 3) are shown for sympatry (solid lines) and allopatry (dashed lines), and for males (thick line or long dashes) and females (thin line or short dashes).

Table 4. Biomass of benthic invertebrates and total abundance of drifting invertebrates at the four study sites during summer low-flow sampling periods (July and August)

Stream and reach	Benthos biomass (g m^{-2})		Drift abundance (mg s^{-1})	
	Mean	Range	Mean	Range
Poroshiri – sympatry	1.15* (0.12)	0.92–1.66 ($N = 6$)	0.180* (0.043)	0.053–0.318 ($N = 6$)
Poroshiri – allopatry	NA	–	0.141* (0.066)	0.051–0.331 ($N = 4$)
Ichani	3.80 (0.40)	–	0.051 (0.007)	–
HSK	3.63 (0.51)	–	0.059 (0.008)	–

Means (SE) and ranges for Poroshiri Stream are for four to six sampling periods during 1991–1994. For Ichani–HSK, the means (SE) are based on subsamples (Surber samplers or drift nets) for the one sampling period. NA, data were not available (see text).

*One sample during high flow after a rainstorm in early August 1992 was not included.

(Esin *et al.*, 2018), with shallow-water benthic morphs having blunt heads and subterminal mouths (Markevich *et al.*, 2018). Similar blunt head shape and subterminal mouth position have been reported for benthic morphs of Arctic charr (e.g. Skúlason *et al.*, 1989, 1999). Although eye diameter often contributes strongly to differences in shape among morphs of Dolly Varden (Esin *et al.*, 2018) and Arctic charr (Sandlund *et al.*, 1992) in lakes, those that forage in deep water where light is limited often have larger eyes regardless of whether they select benthic or pelagic prey. It

is plausible that in clear streams where light is not limited larger eyes allow more efficient feeding on drifting terrestrial insects, which may account for the larger eyes of Dolly Varden in allopatry.

EVALUATING CRITERIA FOR CHARACTER DISPLACEMENT

The next step, aimed at investigating the processes that can explain this pattern of divergence, is to address the six criteria developed to distinguish among various

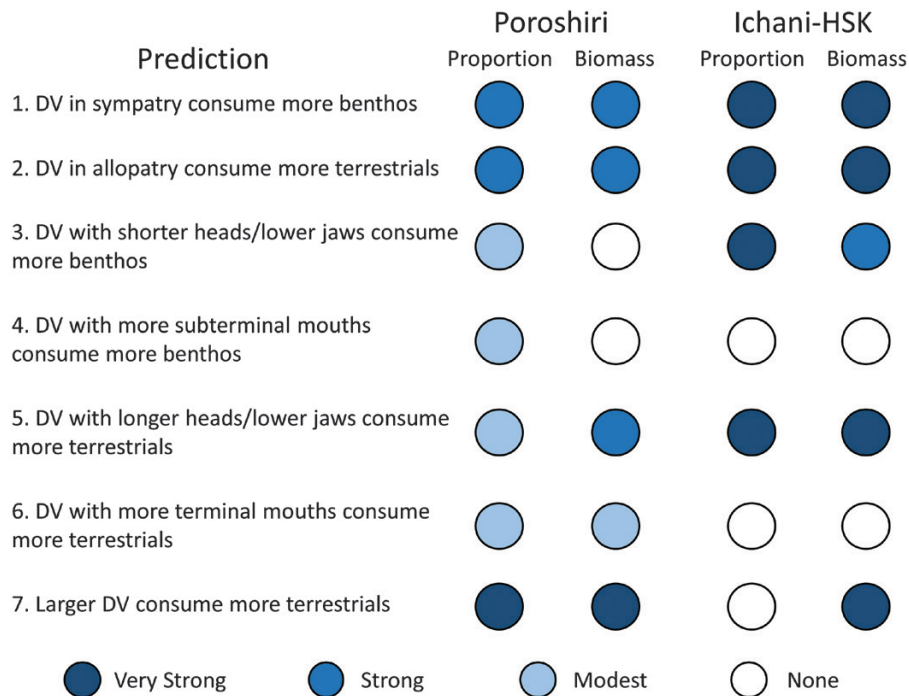


Figure 7. Results for tests of seven predictions regarding the functional significance of morphological shifts for diets of Dolly Varden (DV) in two pairs of sites, and the strength of supporting evidence based on linear models (see text for criteria). Response variables were proportion of biomass and total biomass of benthic or terrestrial prey in the diet.

mechanisms for character displacement (Table 1; Schluter, 2000; Stuart & Losos, 2013). Previous studies by ourselves and others have provided sufficient evidence to address two of the criteria (see above, Criteria for testing character displacement). In summary, comparative field studies and experiments in both field and laboratory have shown clearly that similar phenotypes compete for limited resources (Criterion 1; Nakano & Furukawa-Tanaka, 1994; Fausch *et al.*, 1997; Taniguchi & Nakano, 2000; Watz *et al.*, 2019), a criterion satisfied in only 17% of previous studies reviewed by Stuart & Losos (2013). Likewise, species sorting among guild members (Criterion 3) can be ruled out because we found intraspecific differences in Dolly Varden between sympatry and allopatry (Schluter, 2000). In addition, although competition with masu salmon may have had some additional unknown effect on Dolly Varden in Ichani Stream, the salmon are more distantly related than the congeneric whitespotted charr, were at lower abundance, and most emigrate after 1 year, so any effect was probably weak.

A key criterion tested here, Criterion 2, is that shifts in resource use match changes in morphology, so that there is a clear functional link between them. Dolly Varden in sympatry clearly captured more prey from the benthos and fewer terrestrial prey from the drift than those in allopatry, which matches the functional significance of their shorter heads and jaws, more subterminal mouths,

and perhaps also smaller eyes. However, it is difficult to ascribe these differences in diet to a unique mechanism, because Dolly Varden can also undergo a behavioural shift to benthic feeding within 1 h when drifting prey decline and resources become limiting (Fausch *et al.*, 1997). This decline is generally a result of interspecific and intraspecific competitors depleting drift upstream, but drift also declines naturally through the summer so this shift occurs each year as the season progresses (Nakano *et al.*, 1999a).

Despite this well-studied shift in foraging behaviour, however, our results showed that additional variation was explained by differences in lower jaw length and mouth position of individual charr within these sites, even after accounting for differences owing to sympatry vs. allopatry. Evidence from one or both pairs of sites supported the predictions that Dolly Varden with shorter heads and more subterminal mouths foraged more on benthic prey, and that those with longer heads and more terminal mouths foraged more on terrestrial prey. These results indicate that the two mechanisms of character displacement, behavioural and morphological, work in concert to enhance energy intake by Dolly Varden under the pressures of intraspecific and interspecific competition in sympatry when drifting prey resources are limiting in late summer. Moreover, the magnitudes of these character differences (e.g. 1–16% for lower jaw length) are similar

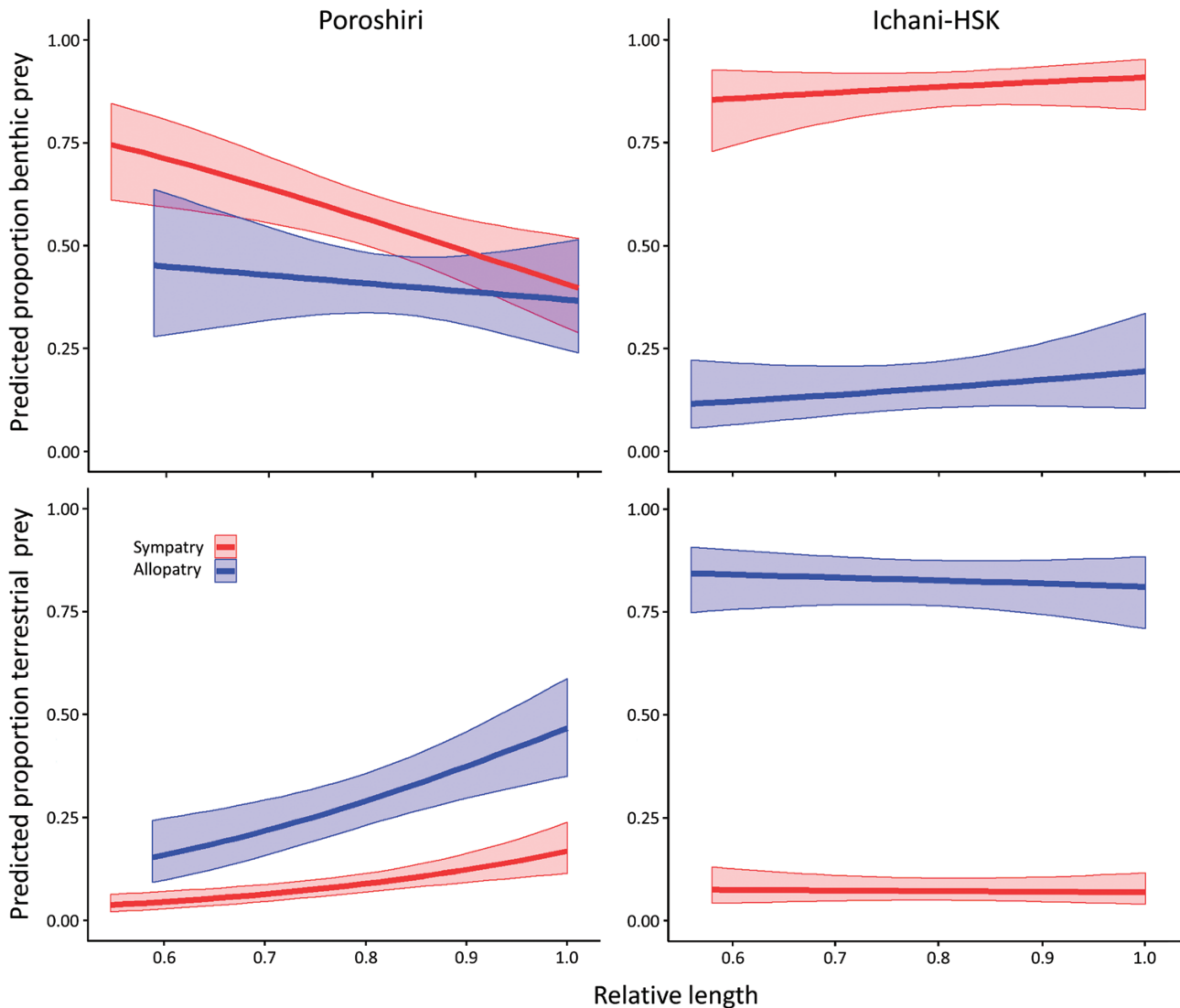


Figure 8. Predicted proportion of biomass of benthic prey (top) and terrestrial prey (bottom) in diets of Dolly Varden in sympatry and allopatry as a function of fish relative length in two pairs of sites in Hokkaido streams. The 95% confidence limits are shown for each predicted relationship.

to those found among morphs of other fishes for which differences in foraging efficiency have been documented (Ehlinger & Wilson, 1988; Wimberger, 1994).

The pattern of divergence we detected is not consistent with other explanations, including species sorting (Criterion 3, see above), or fundamental differences in characteristics of the environment or prey availability at sympatric vs. allopatric sites (Criterion 4). For example, physical habitat characteristics such as elevation, stream gradient and substrate were generally similar within each pair of sites, and other key characteristics were similar across all sites, including stream width and discharge, summer water temperature, and area and depth of pools. The available data on invertebrate prey showed that drift abundance

and biomass of benthos were also similar within pairs of sites, and the proportion of terrestrial vs. benthic invertebrate prey in drift samples was generally similar across sites even though the composition of specific taxa varied. Although Schluter (2000) reported that it is never possible to rule out all environmental factors that might explain a pattern of divergence, we found no evidence that differences in habitat or prey availability could explain the morphological divergence we found. For example, we expected that the greater riparian vegetation and higher air temperatures at the lower-elevation sympatric site in Poroshiri Stream would result in input of more terrestrial invertebrates (Baxter *et al.*, 2005; Saunders & Fausch, 2012, 2018), yet Dolly Varden head and mouth morphology shifted

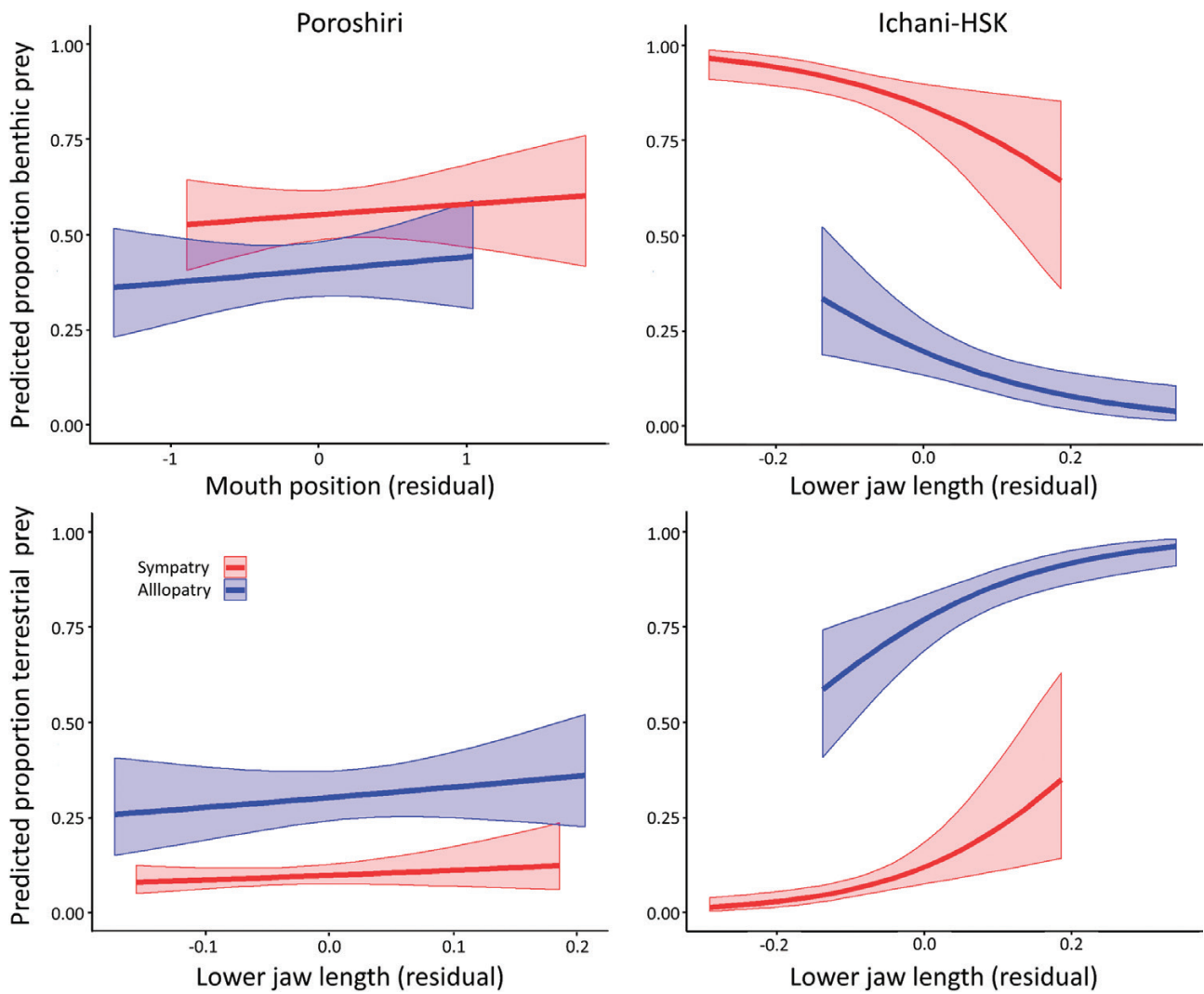


Figure 9. Predicted proportion of biomass of benthic prey (top) and terrestrial prey (bottom) in diets of Dolly Varden in sympatry and allopatry as a function of the residual of mouth position (upper left) and the residual of lower jaw length (other three panels) in two pairs of sites in Hokkaido streams. The 95% confidence limits are shown for each predicted relationship.

to adapt them to foraging on benthic prey at this site, which is opposite that expected if such differences in prey taxa drove morphological divergence.

Finally, the role of chance (Criterion 5) is also an unlikely explanation for the pattern we found across two pairs of sites. Schulter (2000) reported that two species occurring together at one site (sympatry) and separately at two others (allopatry) are expected to show greater divergence in sympatry one time in four ($P = 0.25$). Hence, the probability of the same pattern occurring by chance at two independent sets of sites is low ($P = 0.0625$).

We have no data to address whether the phenotypic differences we found have a strictly genetic basis (Criterion 6), but we suspect phenotypic plasticity

may play an important role. Salmonids, and charr in particular, are known for displaying phenotypic plasticity, especially when single species disperse into new environments, such as recently deglaciated lakes (Smith & Skúlason, 1996; Klemetsen, 2013). This plasticity is most often manifested as resource polymorphisms (Skúlason & Smith, 1995), where discrete intraspecific morphs use different resources, occupy different niches, and display differing degrees of reproductive isolation and genetic variation from slight to substantial (Skúlason *et al.*, 1996; Snorrason & Skúlason, 2004; Klemetsen, 2010). As described above, Arctic charr, brook charr and lake charr produced two to five morphs that segregate among littoral, benthic and pelagic habitats and forage on different prey

resources (e.g. Skúlason *et al.*, 1999; Adams *et al.*, 2008; Bertrand *et al.*, 2008; Chavarie *et al.*, 2015), and Dolly Varden diversified into seven distinct morphs filling different niches that are apparently reproductively isolated in a Kamchatkan lake and its watershed (Esin *et al.*, 2018; Markevich *et al.*, 2018). In Poroshiri Stream plasticity may play an important role in phenotypic divergence compared to genetic adaptation because any two locations sampled were separated by only 0.65–2.4 km. In contrast, Dolly Varden in another Hokkaido river showed significant genetic divergence among populations that are generally separated by more than 5 km (Koizumi *et al.* 2006).

The mechanisms underlying phenotypic plasticity that contribute to the generation of different morphs in charrs are now understood to result from a complex interplay among ecology, evolution and development (i.e. eco-evo-devo processes; Skúlason *et al.*, 2019). For example, variation in head and jaw shape of both Arctic charr and Dolly Varden emerges during embryonic development (Skúlason *et al.*, 1989; Kapralova *et al.*, 2015), correlates with variable timing of ossification of different cranial bones in the different morphs (termed heterochrony; Esin *et al.*, 2018), and is affected by diet (Parsons *et al.*, 2011). Thus, specific sets of gene signalling pathways are expressed at higher levels in progeny of benthic vs. pelagic morphs, resulting in development of the corresponding head shape (reviewed by Skúlason *et al.*, 2019). Moreover, these gene regulation processes can respond to environmental signals, stemming from the mechanics of foraging (Wainwright *et al.*, 1991; Wimberger, 1994) or differences in diet. For example, in Arctic charr, foraging on benthic prey promotes development of blunt head shapes and subterminal jaws, whereas foraging on pelagic prey promotes longer heads and terminal jaws (Parsons *et al.*, 2010, 2011). Finally, theory suggests that selection can act on this novel phenotypic diversity, favouring further evolution of plastic responses in more variable environments such as streams, and genetic assimilation and canalization of phenotypes in more structured and stable environments such as some lakes (West-Eberhard, 2005b; Gilbert *et al.*, 2015; Skúlason *et al.*, 2019). Moreover, behavioural plasticity can be particularly important in the early stages of divergence, acting as a precursor to morphological plasticity (West-Eberhard, 2005a; Skúlason *et al.*, 1999, 2019). If this mechanism is occurring in Hokkaido charr, then the behavioural shift to benthic feeding by juvenile Dolly Varden caused by the increased interspecific and intraspecific competition for drifting prey in sympatry may promote development of the blunter snouts and more subterminal jaws that our results revealed. This hypothesis bears testing.

Ideally, the morphology of both charr species could be compared to determine whether characters of Dolly Varden in allopatry had shifted in the direction of whitespotted charr, and whether whitespotted charr were different in sympatry vs. allopatry. Unfortunately, whitespotted charr were collected only in sympatry, and the data left by the senior author were incomplete (i.e. sex was not recorded for those from Poroshiri, and only males were measured in Ichani), but the data available match the first prediction. When both sexes were combined, the length of the upper and lower jaws and snouts of Dolly Varden in allopatry in Poroshiri approached those of whitespotted charr in sympatry, and even exceeded them at greater body lengths (Supporting Information; Fig. S2). Surprisingly, male Dolly Varden in allopatry in HSK had diverged even farther, with lower jaws, upper jaws and snouts that were significantly longer than those of male whitespotted charr in Ichani Stream (Fig. S3). In both pairs of sites, eye diameter of Dolly Varden in allopatry was larger than in sympatry, but smaller than that of whitespotted charr, although the difference was significant only for Dolly Varden in Ichani vs. HSK. Dolly Varden in sympatry and in allopatry had more subterminal mouths (greater mouth position) than whitespotted charr in both pairs of sites, but no differences could be detected among the groups owing to high variation relative to sample size.

AGENDA FOR RESEARCH

Our results, combined with studies conducted to date, suggest an agenda for research on character displacement in Hokkaido charrs. A necessary first step will be a broad field survey of the morphological and genetic differences of sets of Dolly Varden and whitespotted charr coexisting in sympatric populations and separated in adjacent allopatric populations. It is possible that any differences revealed evolved independently in each set of populations, so we expect that divergence may not be uniform across them (termed ‘non-repeatable character evolution’; Germain *et al.*, 2018), and that cases of character convergence could also occur. A second step is to conduct intensive field studies at one or more sites to determine whether differences in sympatry allow similar, or better, growth, survival and fecundity of charr than in allopatry (i.e. demonstrating a link to fitness). These three close correlates of population growth and fitness are the most tractable to measure for long-lived organisms such as fish (Germain *et al.*, 2018).

A third step is to conduct relatively long-term laboratory experiments in artificial streams that include key features unique to stream habitat, such as riffle-pool morphology, flow, and real or simulated

benthic and drifting prey (cf. Fausch, 1984; Taniguchi & Nakano, 2000). Germain *et al.* (2018) presented a new experimental protocol termed invisibility tests, a type of common-garden experiment to assess whether organisms from sympatric populations can sustain greater rates of population growth when they are rare and their competitor is abundant than can organisms from allopatric populations, owing to the character shifts. They acknowledge that it is not tractable to measure population growth rates for long-lived organisms like fish, and so measuring correlates such as behaviour, growth and survival are the only suitable options (for examples see Fausch, 1984; Taniguchi & Nakano, 2000). Such experiments on specific life stages of interest (e.g. age-1) coupled with a broad field survey and focused field studies are the most likely to provide useful evidence to test patterns and mechanisms leading to the character displacement we found.

Finally, detailed studies should be conducted to explore the role of phenotypic plasticity in shaping the character divergence of Dolly Varden in sympatry vs. allopatry. These could follow the model studies of Arctic charr, including laboratory experiments to measure differences in head and jaw shape and timing of bone ossification during development (Kapralova *et al.*, 2015), manipulating diets fed to different morphs (Parsons *et al.*, 2010, 2011) and measuring differences in gene expression (Ahi *et al.*, 2014; Guðbrandsson *et al.*, 2018). A key is to determine whether presence of the congeneric whitespotted charr and the environmental conditions they help to create (i.e. lower drift abundance) are sufficient to alter developmental pathways and create facultative character shifts within the lifetime of individual Dolly Varden (cf. Pfennig *et al.* 2006).

A fundamental question for the coexistence of Dolly Varden with whitespotted charr in streams of Hokkaido and other regions of the Far East where they overlap is whether the morphological and behavioural shifts we documented allow Dolly Varden to feed and grow sufficiently in sympatry to survive periods of scarce resources despite the presence of their congener. Our results indicate that, like other charr, and Dolly Varden in other locations, those in Hokkaido can diverge in morphology in response to interactions with other species and their environment to fill diverse niches and partition food resources, which should allow them to maintain positive fitness in dynamic stream environments. Future studies are warranted to measure the genetic and developmental bases for these changes, and understand the interplay among the eco-evo-devo processes that shape the response by Dolly Varden to their congeners and environment.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Table S1. Habitat characteristics of study sites where charr were collected (see Fig. 2). Standard errors of baseflow discharge measurements ($N = 4-5$) are shown for Poroshiri Stream.

Table S2. Numbers and standard lengths of Dolly Varden measured for morphometrics at four study sites in Hokkaido. M = male; F = female.

Table S3. Predicted values of five morphometric characters for Dolly Varden charr jaws and head, for males (M) and females (F) in allopatry (A) and sympatry (S) at the 25th, 50th, 75th and 90th percentile lengths. Results are reported at the precision measured (nearest 0.01 mm).

Table S4. Percentage composition by dry weight of benthic and drifting invertebrates in three study sites. Data for the sympatric site in Poroshiri Stream are means ($N = 6$) for baseflow periods during July and August of four summers, 1991–1994, and those for Ichani Stream and HSK River are for the baseflow period during summer 1996.

Table S5. Slope coefficients (above) and variable importance (below) for model-averaged linear models relating four characteristics of diet to four covariates and three interactions for Dolly Varden charr in two pairs of sites in Hokkaido streams. Coefficients with confidence intervals that do not overlap zero (*95% CI, **99% CI, ***99.9% CI), and variable importance values ≥ 0.80 , are in bold.

Figure S1. Ventral view of the head of a Dolly Varden charr showing morphometric measurements of the upper jaw length (UJL), lower jaw length (LJL), and lower jaw width (LJW). Drawing by S. Nakano (modified from Fausch, 2015; used with permission).

Figure S2. The Ln lower jaw length as a function of Ln standard length for charr of both sexes combined in Poroshiri Stream: whitespotted charr in sympatry (thin solid line), Dolly Varden in allopatry (dashed line), and Dolly Varden in sympatry (thick solid line).

Figure S3. The Ln lower jaw length as a function of Ln standard length for males of Dolly Varden in HSK River (allopatry; dashed line), whitespotted charr in Ichani Stream (sympatry; thin solid line), and Dolly Varden in Ichani Stream (sympatry; thick solid line).