Resource partitioning as a factor limiting gene flow in hybridizing populations of Dolly Varden char (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*)

John Hagen and Eric B. Taylor

Abstract: Dolly Varden char (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) hybridize in areas of secondary contact in northwestern North America but maintain their genetic integrity in spite of gene flow. We examined juvenile stream ecology and adult reproductive ecology of these species in sympatry to test for specializations to alternative niches that may act as a basis for natural selection against hybrids. Juvenile Dolly Varden occupied deeper, faster water and foraged more during daytime in the drift, whereas bull trout used shallower, slower water and made more night-time foraging attempts towards the substrate. The species showed extensive diet overlap (Schoener's index = 0.7–0.9), and we found evidence for density compensation between species among sites. Our results suggest no obvious basis for selection against hybrids at the juvenile stream-rearing life-history stage. Bull trout, however, are adfluvial, whereas Dolly Varden are permanent stream residents. Bull trout are also much larger at maturity (50–80 cm vs. 12–15 cm) and build redds in faster, deeper water using larger substrate sizes. Consequently, hybrid genotypes may be selected against in these alternative life-history niches or during reproduction owing to their intermediate size at maturity and size-dependent spawning habitat use and mate choice.

Résumé : La Dolly Varden (*Salvelinus malma*) et l'Omble à tête plate (*Salvelinus confluentus*) s'hybrident dans les régions de contact secondaire dans le nord-ouest de l'Amérique du Nord, mais ils maintiennent leur intégrité génétique malgré le flux de gènes. Nous avons étudié l'écologie des juvéniles dans les cours d'eau et de l'écologie de la reproduction des adultes de ces espèces dans des situations de sympatrie pour vérifier l'existence de spécialisations à des niches de rechange qui pourraient servir de base à une sélection naturelle contre les hybrides. Les juvéniles de la Dolly Varden vivent en eau plus profonde et plus rapide et font plus de recherche de nourriture pendant le jour, alors que l'Omble à tête plate occupe des eaux moins profondes et moins rapides et fait plus de tentatives de recherche de nourriture sur le substrat la nuit. Les deux espèces ont des régimes alimentaires qui se chevauchent considérablement (indice de Schoener = 0,7–0,9) et il y a des indices de l'existence d'une compensation de densité entre les espèces parmi les sites. Nos résultats n'identifient aucune base évidente pour postuler l'existence d'une sélection négative des hydrides au cours de la partie du cycle biologique correspondant au développement des juvéniles en eau courante. L'Omble à tête plate, cependant, fait des migrations vers les eaux stagnantes (c'est une espèce « adfluviale »), alors que la Dolly Varden réside en permanence dans les eaux courantes. Les ombles sont aussi beaucoup plus grands à maturité (50–80 cm contre 12–15 cm) et construisent leurs gravières en eau plus profonde et plus rapide, utilisant des substrats plus grossiers. Conséquemment, il peut y avoir une sélection négative des génotypes hybrides dans ces niches de rechange de développement juvénile ou alors durant la reproduction; les hybrides possèdent, en effet, une taille intermédiaire à maturité et leur utilisation des habitats de fraie et leur choix de partenaire sont des comportements reliés à la taille.

[Traduit par la Rédaction]

Introduction

Hybrid zones can be broadly defined as geographic areas where mating occurs between genetically distinct populations to produce individuals of mixed genetic ancestry (Arnold 1997; Jiggins and Mallet 2000). Although once considered rare in most animals, hybrid zones have long been recognized in various groups of fishes (Arnold 1997) and are viewed as excellent "natural laboratories" that provide opportunities to understand processes that maintain genetic

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distinction in the face of gene flow and perhaps the processes that lead to the evolution of reproductive isolation (Barton and Hewitt 1989; Jiggins and Mallet 2000).

Natural hybridization and introgression have been reported in a number of co-existing salmonid species in the Northern Hemisphere (e.g., reviewed by Verspoor and Hammer 1991). In most cases, the parental species appear to maintain their integrity in the face of hybridization and some gene flow (e.g., Verspoor and Hammer 1991; Baxter et al. 1997), but in some cases, hybrid swarms may develop (e.g., Forbes and Allendorf 1991). However, there have been no detailed studies of the potential genetic or ecological factors that might limit gene flow in salmonid hybrid zones. One hypothesis that could explain the maintenance of parental genomes in spite of gene flow is environment-dependent natural (or sexual) selection against hybrids in nature (Arnold 1997). Under this model, parental species are adapted to alternative niches and hybrids between species suffer reduced fitness if they are phenotypically intermediate to the parental species in traits important to exploitation of the parental species' niches (Arnold and Hodges 1995).

Two species of char, Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*), hybridize in several watersheds along a broad zone of secondary contact in northwestern North America (Taylor et al. 2001). In one area, Thutade Lake of the upper Finlay (Peace) River drainage, the two species interact in what appears to be a "bimodal" hybrid zone (sensu Jiggins and Mallet 2000) where most genotypes are either parental or backcrosses with relatively few F_1 hybrids (Redenbach 2000). Given that intrinsic (i.e., genetic) mechanisms of selection against hybrids appear to be absent in these species owing to high viability of laboratory crosses (Haas and McPhail 1991) and to the production of backcross genotypes in nature (Baxter et al. 1997; Redenbach 2000), hybrid zones between Dolly Varden and bull trout may represent a case where extrinsic (ecological) selection against hybrids may constrain gene flow between the species.

In this study, we examined the potential contribution of ecological factors to the maintenance of genetic distinction between the species. In most naturally co-existing salmonids, different species typically demonstrate strong resource partitioning as stream-resident juveniles (e.g., Ross 1986; Taylor 1991). If ecological selection is important to maintaining parental genomes in the face of gene flow, then this predicts that the parental species should exhibit distinct resource utilization along one or more ecological axes. We evaluated this prediction by testing for habitat, food, and time resource partitioning between co-existing juvenile Dolly Varden and bull trout in a pristine watershed in north-central British Columbia. Thus, two objectives of our study were to document, for the first time, the extent of resource partitioning between sympatric juvenile Dolly Varden and bull trout and to evaluate the extent of density compensation in areas of local sympatry and allopatry. Density compensation refers to the idea that co-existing species are so similar in resource use that reduced density or absence of one species is compensated for by an increase in the density of the other species while total fish density remains relatively constant. Consequently, the absence of density compensation would suggest that the species have evolved major differences in resource use such that the presence or absence of one species has little or no effect on the density of the other species. Finally, the reproductive phase of an individuals' life history also requires resource acquisition, e.g., in terms of habitat use. Consequently, our third objective was to test for habitat partitioning between Dolly Varden and bull trout during spawning by comparing size at maturity and habitat dimensions at identified spawning sites.

Materials and methods

Study area and design

The Thutade Lake watershed is situated near the Pacific–Arctic continental divide in north-central British Columbia, at approximately 57.0°N latitude and 126.7°W longitude (Fig. 1). Thutade Lake is the source of the Finlay River, a tributary of the Peace River and ultimately the Mackenzie River, which flows into the Arctic Ocean. The fish community is relatively simple, being dominated numerically by Dolly Varden and bull trout, although other native salmonids such as rainbow trout (*Oncorhynchus mykiss*), kokanee (*Oncorhynchus nerka*), and mountain whitefish (*Prosopium williamsoni*) also occur in small numbers in the tributary streams (Bustard 1999). At most sites, therefore, the potentially confounding effects of other competitors were not an issue. Because of the remote location of the Thutade watershed and the lack of fishing pressure or human-mediated habitat disturbance, the fish populations are also thought to be at levels that are close to the environment's natural carrying capacity, which was important for the assessment of the effects of interspecific competition. Importantly, gene flow between Dolly Varden and bull trout and hybrid fertility in the watershed have been documented. Hybridization occurs but is infrequent; 0.61% of all char examined were classified as F_1 hybrids, 4.8% as resulting from backcross matings, and 0.15% as post-F_1 hybrids (Redenbach 2000).

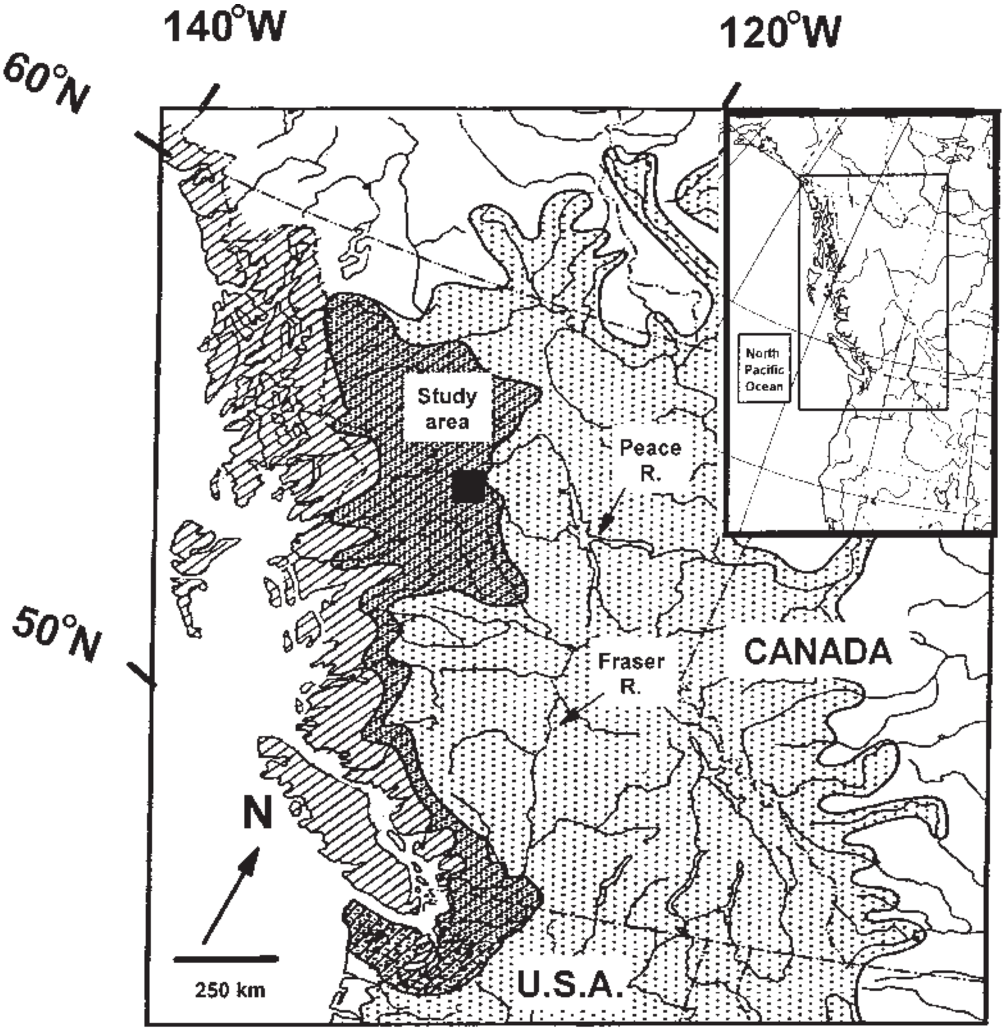
The life-history patterns of Dolly Varden and bull trout differ in the watershed and this influenced the design of our study. Dolly Varden reside in small streams for their entire life cycle. Bull trout spend up to 4 years in these same streams at the beginning of their lives but then migrate to Thutade Lake and become piscivorous. Consequently, we felt that it was important to consider both Thutade Lake and its tributary streams in our investigation of resource-use specializations. We sampled the lake environment to assess the apparent absence of Dolly Varden in this habitat and designed our sampling of the tributary streams around Schoener's (1974) three niche dimensions: habitat, food, and time, with the time dimension investigated by replicating habitat and food measures for day and night time periods. We assessed resource use along three habitat dimensions (water depth, mean current velocity, and hydraulic habitat type) and three food dimensions (foraging mode (benthic versus drift), mean prey item biomass, and taxonomic classification of prey).

Life history

To confirm the suspected migratory life-history differences between the species, we surveyed Thutade Lake between June 16 and June 21, 1998. We captured fish with small-mesh, monofilament tangle nets set both on the bottom and suspended from the surface at two different locations on the lake. From June 16 to June 18, we fished an open-water, deeper location adjacent to a large shoal, and from June 19 to June 21, we fished a shallower, more complex shoreline near the lake outlet. All fish captured were anaesthetized, identified, measured, and released. We sampled char stomach contents using the pulsed gastric lavage method (Foster 1977) and preserved them in a solution of 10% formalin for later measurements.

To assess species differences in reproductive ecology, body size data and tissue samples for molecular genetic analysis (Redenbach

Fig. 1. Distributions of Dolly Varden, *Salvelinus malma* (hatched), and bull trout, *S. confluentus* (stippled), and areas of overlap (dark hatching and stippling) in western Canada. The study site is the Thutade Lake watershed (black square), located at approximately 57.0°N latitude and 126.7°W longitude at the source of the Finlay River (upper Peace River). The inset shows the general study area in relation to western North America.



2000) were collected during the bull trout spawning migration by angling at locations along the Attichika River and along its tributary Kemess Creek. The Attichika River accounts for most (about 60%) total adult bull trout abundance in the Thutade watershed (500–600 spawners annually, Bustard 1999). Adult Dolly Varden from throughout the watershed were also sampled, but because they were difficult to distinguish from immature char, we sacrificed a sample to determine maturity status of individuals by dissection. Spawning timing was investigated in the Kemess Creek watershed for both species in all years of a population-monitoring program undertaken from 1995 to 1998 (e.g., Bustard 1999). All known spawning areas in the watershed were surveyed on a weekly basis by foot over the spawning period for both species combined (early August until early October of each year). For each species, the numbers of spawners present in stream reaches were recorded, as was the cumulative total number of redds (spawning sites excavated into the stream substrate) on each survey date. A subsample of redds from the total number identified from three independent reaches for both species (Table 1) was compared in terms of hydraulic and substrate characteristics after spawning had been completed. Mean stream depth and current velocity were recorded as the average of two measures, each over undisturbed substrate on opposite sides of the midpoint of the redd. To minimize disturbance to incubating char embryos, particularly for bull trout where the substrate was imbedded, we limited our redd substrate size comparisons to estimates of surface substrate size. For each redd, we visually estimated a single substrate particle of "median-like" size, i.e., 50% of the remaining pieces were smaller than the selected pieces and 50% were larger. The maximum diameter of each selected piece was measured and constituted the D_{50} of substrate size for each redd. The D_{50} measure was not intended to represent the average substrate size within the total redd volume (which would require disturbing embryos) but rather an approximate quantification of differences in surface substrate size between species that appeared obvious by eye. Notwithstanding the approximate nature of substrate size measure, our data were consistent with substrate sizes of redds recorded for other populations of bull trout (e.g., Baxter and McPhail 1996) and with more detailed measures of interspecific differences in mean particle sizes for smaller numbers of redds (D. Bustard, Bustard and Associates, Smithers, B.C., personal communication). Spawning locations were recorded within 400-m marked sections of the surveyed reaches and also in smaller or seepage-fed tributaries located along these marked sections.

Resource use in tributary streams

Resource-use measurements in the tributary stream environment, for both species concurrently, were made during July and August 1998. The study reach was a 2.0-km section of North Kemess Creek, a third-order tributary of the Attichika River (which in turn flows into Thutade Lake) and an area of known, strict sympatry. We used snorkel surveys to locate and observe individual fish of each species. If the snorkeler deemed the fish undisturbed, he observed it for 5 min and recorded the number of surface, drift, and benthic foraging attempts as well as the species identification. The fish was then captured using two large aquarium nets and measured, its adipose fin was clipped for genetic analysis, and the fish was then preserved in a solution of 10% formalin for laboratory gut content analysis. At the focal point (nose-point location) for each fish, we measured stream depth and mean current velocity and also noted hydraulic habitat type (riffle or pool, main channel or side channel). In habitats too shallow for snorkel observations, we used a battery-powered electrofisher and short pulses of electricity to capture individual fish. Depth, velocity, and hydraulic habitat type were recorded and the fish was sampled as above. The location of focal points through electrofishing is obviously less precise than through diver surveys, but the resulting relationships, even though less precise, would provide more accurate overall conclusions by including all available habitats.

We replicated underwater observations at nighttime using a diver's light shining through a sanded translucent red filter that minimized disturbance to fish (Heggenes et al. 1993) and sampled fish by electrofishing at night using high-powered lighting. Young-of-the-year char (those less than 50 mm based on size frequency data; Bustard 1999) were not included in the study because it was difficult to distinguish Dolly Varden from bull trout based on physical appearance alone and because areas of sympatry do not correspond with those for older juveniles (Redenbach 2000).

We conducted stomach content analysis on the entire preserved sample. Intact prey items removed from the fish stomachs were identified, counted, and measured by using a dissecting microscope connected through a digitizing tablet to a computer. We also measured mouth width and gill raker spacing because Wankowski (1979) has suggested that these are morphological constraints that salmonids can manipulate that influence the size range of prey. We obtained the total biomass of intact prey items for each stomach by weighing the gut contents after they had been dried in a dessicator, and regular measurements indicated that the weights had stabilized (typically after 2 days).

Density compensation

Density compensation between the species was investigated for all 4 years (1995–1998) of the population-monitoring surveys (Bustard 1999). Fieldwork was conducted during August across 22 sites for which multiple years of data exist. We sampled fish populations by the two-pass removal method (Seber and LeCren 1967) using electrofishing equipment at sites completely enclosed by stopnets. Sites included the entire wetted channel unless flow characteristics did not allow this, in which case an area enclosed along the stream margin was used. All fish captured were anaesthetized, identified to species, measured, weighed, and then released. At sites for which aging and molecular genetic analyses were planned, scales and tissue samples were also taken.

Statistical analyses

In strict sympatry of the tributary stream environment, resource-use traits for both species were generally positively related to body size and lognormally distributed, which is typical for allometric data (Harvey 1982). We adjusted for the effect of body size differences by collecting samples of both species from across an overlapping size range. We removed the affect of body size differences statistically using analysis of covariance (ANCOVA) on log-transformed data, which we could apply to resource-use traits with continuous distributions (habitat traits water depth and mean current velocity; food traits drift foraging rate, benthic foraging rate, and mean prey item biomass; and time traits total foraging rate and total gut content biomass). We also used ANCOVA to compare mouth width and gill raker spacing for the species. Hydraulic habitat type data (riffle–pool, main channel – side channel) were in discrete categories, so we tested for differences between species using contingency table χ^2 analysis. Patterns of diet utilization consist of several non-independent variables, which often take extreme distributions that complicate their statistical analysis (e.g., Sevenster and Bouton 1998). Consequently, we limited our interspecific comparisons of average diet proportions of each prey category to using the Schoener (1970) index of proportional overlap, as recommended by Wallace (1981). Adjustment of the significance levels for individual tests, which we conducted using the sequential Bonferroni adjustment, took place for three tables (Rice 1989) of statistical tests corresponding to the niche dimensions habitat, food, and time. The overall Type I error rate for the stream resource-use study was then no greater than 15%, within the 10–15% guidelines for exploratory surveys suggested by Chandler (1995).

We tested for interspecific differences in mean of adult body size using Mann–Whitney *U* tests owing to the small sample sizes for Dolly Varden and hybrid adults. Differences in spawning site depth, spawning site current velocity, and spawning site substrate size were assessed using two-sample *t* tests. Significance levels were adjusted for multiple comparisons using the sequential Bonferroni adjustment.

For our density compensation analysis, we considered the proper replicate for comparison to be the average for each stream reach of all site densities averaged over all of the years. This was because the individual sites and years were not independent, potentially belonging to the same population. We calculated the regression of the average reach densities of each species on that of the other species using the ordinary least squares technique with untransformed data, which enabled us to compare the resulting slope with null hypotheses slopes of zero (i.e., density of one species is independent of density of the other) and –1 (i.e., complete density compensation) using *t* tests. Mean total densities of sympatric and allopatric reaches were compared using the two-sample *t* test.

Results

Life history

Sixteen char, ranging in length from 199 mm to 850 mm, were captured in Thutade Lake from approximately 80 h of tangle-netting effort in both near-shore and open-water habitats. Through molecular analysis, all char were identified as bull trout, consistent with the hypothesis that bull trout undergo a niche shift to the lake that Dolly Varden do not. Of the 12 bull trout for which sex could be determined, eight were female and four were male. Piscivory was also indicated for bull trout in the lake; two mountain whitefish of 132 mm and 123 mm were recovered from the stomach of a 410-mm bull trout, and kokanee salmon (*Oncorhynchus nerka*) of 197 mm and 184 mm were recovered from bull trout of 800 mm and 600 mm, respectively. All other char stomachs were empty, although the stomachs of three bull trout of fork length <230 mm were not sampled.

The two species were different in their reproductive biology (Table 1). The most obvious distinction was a large difference in body size at maturity ($U_{0.05,3,12} = 408$, $P < 0.001$). Mature Dolly Varden (six females and six males) ranged from 113 to 235 mm fork length, averaging 148 mm (N = 12, standard error (SE) = 11 mm), whereas mature bull trout (15 males and 19 females) ranged in size from 440 to 890 mm and averaged 741 mm (N = 34, SE = 16 mm).

Table 1. Interspecific comparisons of reproductive biology of Dolly Varden and bull trout in sympatry.

	Dolly Varden		Bull trout		
Life-history attribute	Mean	SE	Mean	SE	
Adult fork length (mm)	148 (*n* = 12)	10.9	740 (*n* = 34)	15.7	**<0.001**
Spawning site depth (mm)	9.3 (*n* = 30)	0.9	34.7 (*n* = 28)	2.2	**<0.001**
Spawning site current velocity (cm·s^{-1})	20.7 (*n* = 30)	1.5	36.8 (*n* = 28)	2.5	**<0.001**
Spawning site substrate material D_{50}* (cm)	1.6 (*n* = 30)	0.1	4.9 (*n* = 28)	0.3	**<0.001**
Spawning period**	03 Sept. to 08 Oct.	22 Aug. to 12 Sept.	na		

Note: Means were compared with the two-sample *t* test or the Mann–Whitney *U* test (fork length). *, defined in methods; **, from incidental observations of spawning behaviour in addition to surveys of spawning areas; na, not applicable.

Three of the larger adult migrants were diagnosed using molecular markers as Dolly Varden – bull trout hybrids and they were intermediate in body size at maturity and averaged 403 mm (SE = 33 mm). These differences were statistically significant in both directions (Dolly Varden versus hybrid, $U_{0.05,3,12} = 36$, $P = 0.005$; bull trout versus hybrid, $U_{0.05,3,12} = 101$, $P = 0.001$). No precocious, stream-resident bull trout have ever been identified during snorkel surveys of spawning areas or during juvenile population assessment, which has typically taken place immediately before the onset of spawning.

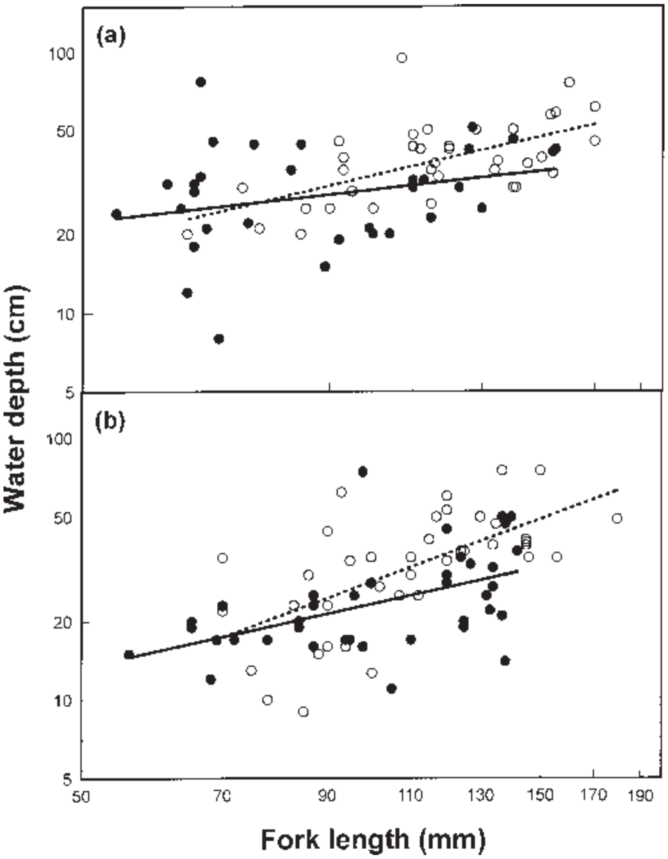
Spawning sites for the two species differed significantly in terms of their physical characteristics (Table 1). Bull trout spawned in deeper water than did Dolly Varden (34.7 cm versus 9.3 cm; $t_{0.05(2),56} = 10.80$, $P < 0.001$) and at sites with higher current velocities (36.8 cm·s^{-1} versus 20.7 cm·s^{-1}; $t_{0.05(2),56} = 5.51$, $P < 0.001$). The bull trout sites were also found in much larger bed material (D_{50} = 4.9 cm versus 1.6 cm: $t_{0.05(2),56} = 12.59$, $P < 0.001$).

Bull trout spawned earlier; the maximum range of spawning activity observed was from August 22 to September 12 compared with September 3 to October 8 for Dolly Varden (Table 1). The spatial distributions of spawning sites also showed little overlap. Within the North Kemess watershed, virtually all Dolly Varden spawning was limited to the reach comprised of the top 4.0 km of North Kemess Creek and the small, mostly seepage fed tributaries that enter this stream section. No bull trout were observed to enter or spawn in the seepage tributaries that are the most important spawning habitats for Dolly Varden, and in most years, very few (<10) spawned in the section of the main stem of Kemess Creek used by Dolly Varden.

Habitat use in tributary streams

Co-existing Dolly Varden and juvenile bull trout in the tributary streams could be distinguished statistically in terms of their resource use, but the differences were subtle. The comparison of focal point water depths (Fig. 2) between Dolly Varden and bull trout was not significant for the daytime observation period ($F = 1.99$, $P = 0.16$). The nighttime difference was greater; Dolly Varden were found in deeper water ($F = 6.91$, $P = 0.010$, adjusted $\alpha = 0.013$). The interspecific comparison of daytime mean focal point current velocity revealed different relationship slopes for the two species (Fig. 3*a*). Dolly Varden showed a greater difference relative to bull trout between small and large body size categories (slopes; $F = 6.32$, $P = 0.014$, adjusted $\alpha = 0.015$). Both Dolly Varden (DV) and bull trout (BT) were found at locations of reduced current velocity at night, and the interspecific comparison (Fig. 3*b*) revealed no significant difference ($F = 2.83$, $P = 0.097$). All fish observed were closely associated with the stream bottom. The two species used the hydraulic habitat categories (pool, riffle, side-channel pool, and side-channel riffle) in similar proportions, both during the day ($\chi^2 = 5.76$, $P > 0.10$, BT $N = 32$, DV $N = 37$) and night ($\chi^2 = 3.10$, $P > 0.25$, BT $N = 40$, DV $N = 39$) observation periods.

Fig. 2. Interspecific comparisons stream depth at focal point versus fork length (log scale) during (*a*) daytime ($F = 1.99$, $P = 0.16$) and (*b*) nighttime ($F = 6.91$, $P = 0.010$, adjusted $\alpha = 0.013$) for sympatric bull trout (solid circles and solid line; daytime $N = 32$, nighttime $N = 40$) and Dolly Varden (open circles and broken line; daytime $N = 37$, nighttime $N = 39$). See Appendix 1 for relationship equations.



Foraging and diets in tributary streams

Dolly Varden and juvenile bull trout were more distinguishable with respect to the food resource dimensions. During the day, foraging from the drift was more important than

Fig. 3. Interspecific comparisons of mean current velocity at focal point versus fork length (log scale) during (*a*) daytime (slopes: *F* = 6.32, *P* = 0.014) and (*b*) nighttime (slopes: *F* = 2.83, *P* = 0.097) for sympatric bull trout (solid circles and solid line; daytime *N* = 32, nighttime *N* = 40) and Dolly Varden (open circles and broken line; daytime *N* = 37, nighttime *N* = 39). See Appendix 1 for relationship equations.

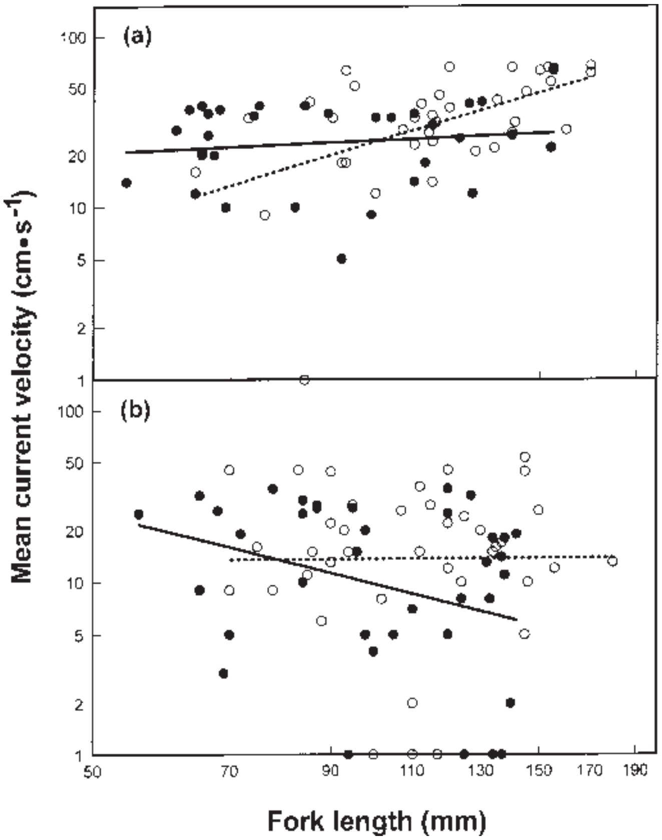
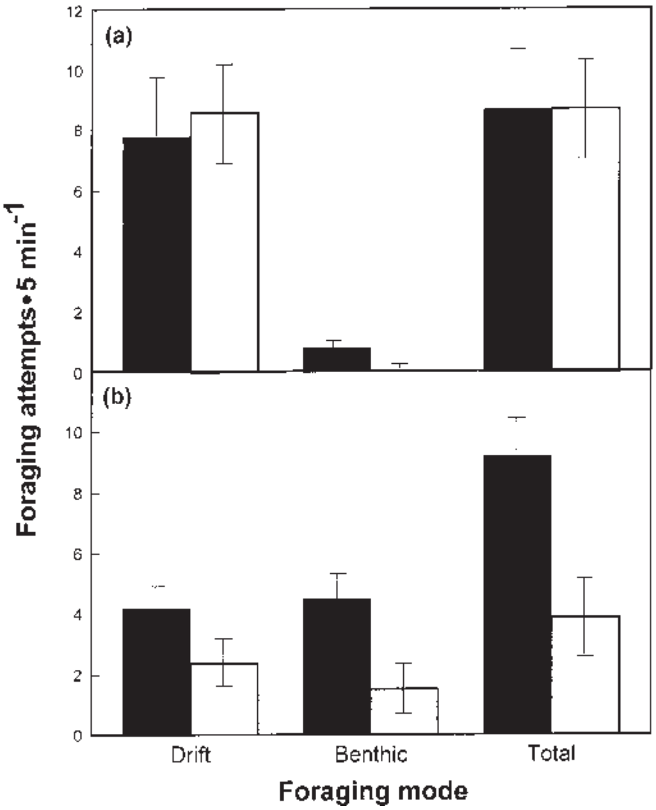


Fig. 4. Observations of foraging mode during (*a*) daytime and (*b*) nighttime for sympatric bull trout (solid bars; daytime *N* = 18, nighttime *N* = 26) and Dolly Varden (open bars; daytime *N* = 24, nighttime *N* = 24). See Appendix 1 for relationship equations. Figures show adjusted means, but interspecific comparisons were made by the analysis of covariance technique (see Results). Error bars are ± standard errors of adjusted means.



benthic foraging for both species (Fig. 4), but the relative importance of benthic foraging increased at night. Interspecific comparison, however, revealed that benthic foraging was more important for bull trout than for Dolly Varden, during both daytime ($F = 7.82$, $P = 0.008$, adjusted $\alpha = 0.01$) and nighttime ($F = 6.72$, $P = 0.010$, adjusted $\alpha = 0.013$) observation periods. Dolly Varden had a slightly higher drift foraging rate by day ($F = 3.65$, $P = 0.062$). Conversely, at night, the bull trout drift foraging rate was higher. This difference was pronounced only for larger individuals, resulting in a difference in the relationship slopes that was not significant ($F = 5.01$, $P = 0.031$, adjusted $\alpha = 0.015$), however, after the correction for the multiple tests. Surface foraging was not important to either species during the period of the study, and thus this category was dropped from the analysis.

Daytime overall foraging rates (Fig. 4) for the two species were very similar ($F = 0.12$, $P = 0.73$), but the nighttime rates were significantly different ($F = 8.77$, $P = 0.0048$, adjusted $\alpha = 0.008$). Dolly Varden expressed a nighttime reduction in overall foraging rate relative to bull trout. The difference in diel foraging patterns, however, was not accompanied by a similar difference in stomach fullness indices between the species (day, $F = 1.31$, $P = 0.26$; night, $F = 0.10$, $P = 0.76$).

Stomach content analysis also revealed interspecific differences, chiefly that juvenile bull trout consumed larger prey items (Fig. 5) than did Dolly Varden ($F = 5.89$, $P = 0.015$, adjusted $\alpha = 0.015$). For the daytime stomach samples, the taxonomic composition of the average Dolly Varden diet was different from that of the bull trout (Fig. 6), having much higher proportions of winged insects. Adult dipterans and hymenopterans made up 33.6% and 7.5%, respectively, of the Dolly Varden diet, compared with 11.3% and 0.6%, respectively, of the bull trout diet. The value of the Schoener (1970) index of proportional overlap between species, however, was 0.71, which implies significant overlap by exceeding the commonly used "biological significance" level of 0.60 (Wallace 1981). Average diet proportions at night were more similar still, with the value of the Schoener index being 0.89 (data not shown).

Juvenile bull trout had relatively larger mouths than Dolly Varden (slopes, $F = 4.8$, $P = 0.03$) and wider gill-raker spacing (slopes, $F = 7.14$, $P = 0.009$), consistent with the finding of relatively larger prey in bull trout diets. Even though the slope differences precluded testing differences in these measures using the analysis of covariance, the separation between them was clear (Fig. 7).

Density compensation

We investigated the effects of the two species' densities

Fig. 5. Interspecific comparison of average prey item biomass versus fork length (log scale) (F = 5.89, P = 0.015, adjusted α = 0.015) for sympatric bull trout (solid circles and solid line; N = 49) and Dolly Varden (open circles and broken line; N = 39). See Appendix 1 for relationship equations.

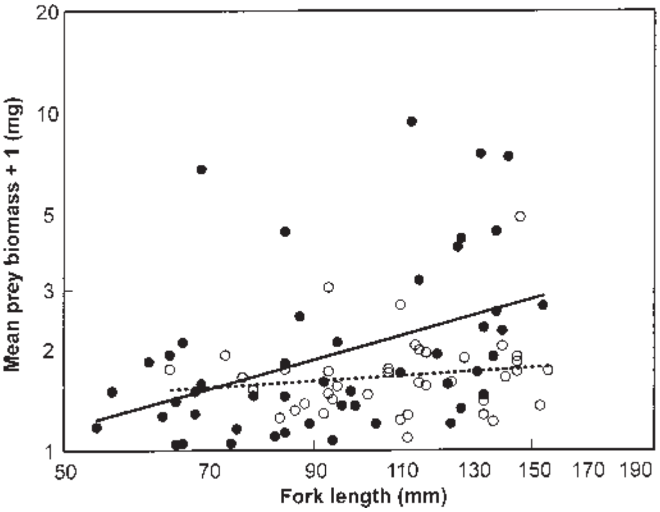
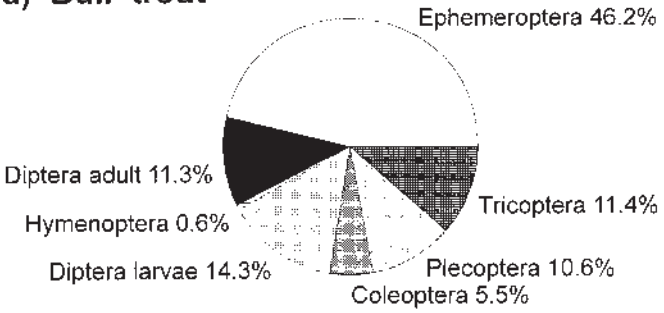
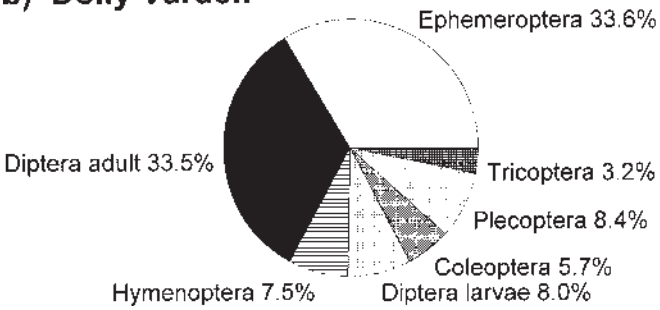


Fig. 6. Interspecific comparison of taxonomic composition of daytime stomach contents (Schoener index of proportional overlap = 0.71) for sympatric (a) bull trout (N = 24) and (b) Dolly Varden (N = 21).

a) Bull trout

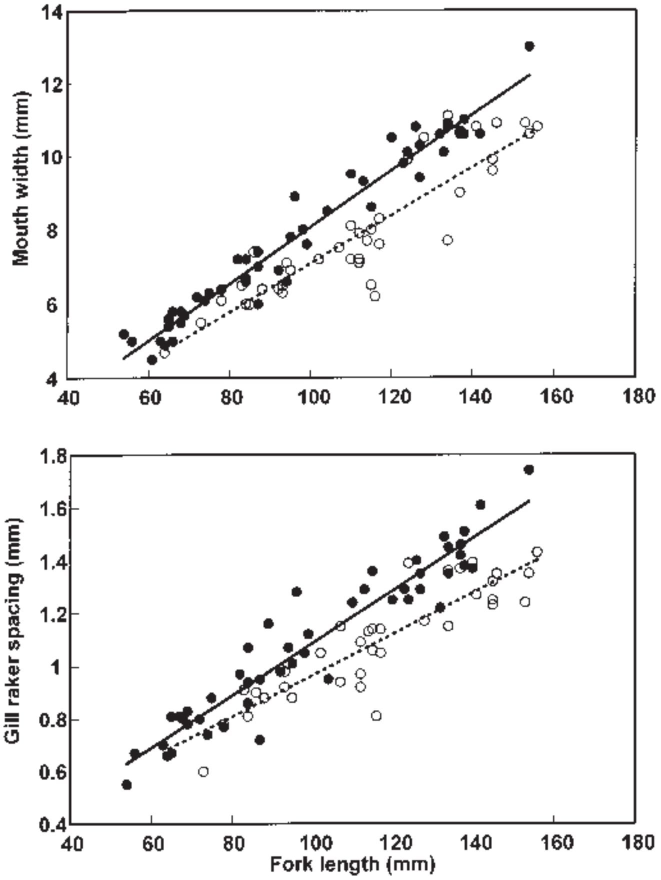


b) Dolly Varden



on each other in sympatry and local allopatry using data from seven reaches that we considered to be independent (Table 2, N = 21 sites total). All were sections of small- to medium-sized tributaries of either Thutade Lake or two larger rivers that drain into the lake (Table 2). Site areas ranged from 55.5 m^2 to 291.2 m^2, averaging 148.3 m^2 (SE = 15.7 m^2).

Fig. 7. Interspecific comparisons of trophic morphology for sympatric bull trout (solid circles and solid line; N = 49) and Dolly Varden (open circles and broken line; N = 37) in terms of (a) mouth width (slopes: F = 4.8, P = 0.03) and (b) gill-raker spacing (slopes: F = 7.14, P = 0.009). See Appendix 1 for relationship equations.



We found a linear and significant negative relationship between the densities of the two species ($t_{0.05(1),6}$ = 2.79, P < 0.025, r^2 = 0.61, Fig. 8). Further, we found no significant difference between the observed slope (β = –0.94) and that predicted by complete density compensation (Fig. 8, β_o = –1.0, $t_{0.05(1),6}$ = 0.19, P > 0.50). The average total density of the four sympatric reaches (11.43 fish 100·m^{-2}) was not significantly different ($t_{0.05(2),6}$ = 0.32, P > 0.50) from that of the three allopatric reaches (12.99 fish·100 m^{-2}).

Discussion

Adaptation to different resource environments

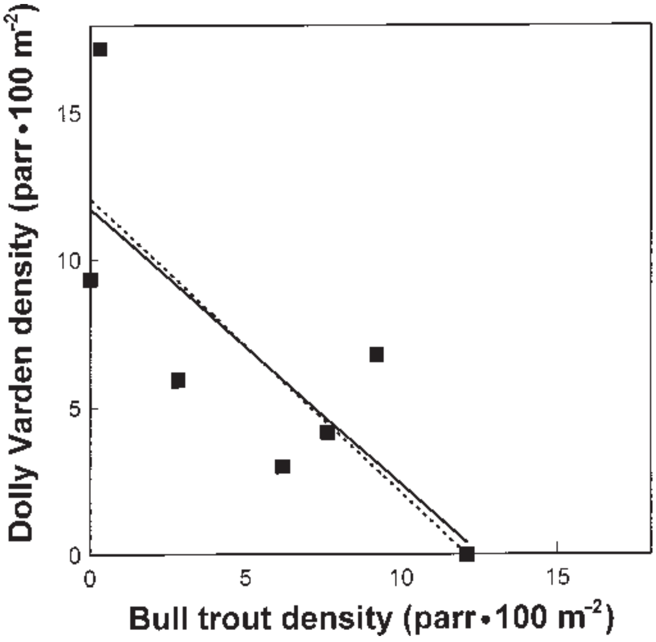
In Thutade Lake's tributary streams, the resource environments of Dolly Varden and juvenile bull trout in strict sympatry could be distinguished statistically, but the differences were subtle. Because the distributions of all the resource-use variables showed extensive interspecific overlap with no evidence of discrete niches, there is little indication that Dolly Varden and juvenile bull trout are adapted to divergent resource environments within the tributary streams. Consequently, our data suggest no obvious basis for natural selection against intermediate, hybrid phenotypes during the stream-resident phase of their life history.

Table 2. Thutade Lake tributary reaches used in analysis of density compensation of one species for missing individuals of the other species (by ordinary least squares regression).

			Average reach densities 1995–1998		
Reach	Category	No. of sites in reach	Bull trout (BT) ($100\ m^{-2}$)	Dolly Varden (DV) ($100\ m^{-2}$)	Total ($100\ m^{-2}$)
North Kemess Creek	Sympatry	4	9.22	6.78	16.00
Upper North Kemess Creek	Allopatry DV	2	0.31	17.20	17.51
Kemess Creek	Sympatry	7	7.64	4.16	11.80
Upper Attichika River	Allopatry BT	1	12.12	0.00	12.12
Tributary 4	Sympatry	3	6.18	2.98	9.16
South Pass Creek	Sympatry	2	2.83	5.93	8.76
Attycelley Creek	Allopatry DV	2	0.00	9.35	9.35
Attichika River (outlier)	Sympatry	1	2.98*	1.95*	2.46*
Niven River (outlier)	Sympatry	5	0.70*	1.13*	0.91*

*Reaches of larger main stems, considered as outliers (see text for explanation).

Fig. 8. Density compensation within independent stream reaches of the Thutade Lake watershed, British Columbia. The slope of the regression of observed Dolly Varden densities on bull trout densities (solid line; $N = 7$, $r^2 = 0.61$, $\beta = -0.94$) was significantly different from the null hypothesis slope of zero ($t = 2.79$, $P < 0.025$) but was not different statistically from the null hypothesis slope of –1 (complete density compensation, broken line; $t = 0.19$, $P > 0.50$).



Pacific basin salmon and trout (*Oncorhynchus* spp.) typically partition resources in streams with respect to microhabitat categories riffle versus pool (e.g., Taylor 1991), depth and velocity (e.g., Everest and Chapman 1972), diet and feeding behaviour (Johnson and Ringler 1980; Nakano and Kaeriyama 1995), and diel feeding periodicity (Johnson and Johnson 1981). Relative to *Oncorhynchus* spp., both Dolly Varden and bull trout are more closely associated with the stream bottom in terms of feeding behaviour, diet, and foraging microhabitat (e.g., Dolloff and Reeves 1990; Nakano et al. 1992). Similar microhabitat utilization occurs for Dolly Varden in the presence of a congener, the Asian white-spotted char (*Salvelinus leucomaenis*), in Japanese watersheds. The more aggressive *S. leucomaenis* feed almost exclusively from the drift, hold midwater focal points, and dominate Dolly Varden of the same size (Fausch et al. 1997). In sympatry with westslope cutthroat trout (*Oncorhynchus clarki lewisi*), bull trout are also strongly associated with a benthic foraging mode and habitat use (Nakano et al. 1992). In our investigation, then, it is perhaps not surprising that both Dolly Varden and juvenile bull trout in the tributary streams were nonaggressive and closely associated with the bottom—this is the feeding niche in which they have been observed during the above studies in allopatry. We did record higher drift foraging rates for both species than are known from these previous studies, but we suspect that the swift current of the steep mountain streams of the Thutade watershed may favour this more stationary foraging mode. The surprising limited resource partitioning by sympatric Dolly Varden and juvenile bull trout may be the result of any one of the following: historical factors either in terms of phylogenetic constraints owing to similar phenotypes or a lack of time since secondary contact for adaptation in sympatry to evolve; a lack of alternative ecological niches in the steep mountain streams of the Thutade Lake watershed; or natural selection for alternative niches that is weak during the juvenile stream-resident phase of char life history.

By contrast, when the entire life history of Thutade Lake watershed char is considered, it is clear that Dolly Varden and bull trout are exposed to very different resource environments for a portion of their lives—bull trout migrate from the tributary streams to Thutade Lake and become piscivorous, whereas Dolly Varden do not. In the lake, bull trout are exposed to a different predation regime, different food resources (primarily fishes compared with insects in the streams), and potential interspecific competition from rainbow trout, which were absent or rare in our tributary stream study sites. In fact, the major morphological characteristics used in species diagnoses of Dolly Varden and bull trout (bull trout have more branchiostegal rays, broader heads, and longer upper jaws) probably reflect adaptation to distinct "piscivore specialist" (bull trout) and "generalist" (Dolly Varden) feeding niches (Cavender 1978). These general ecomorphological and life-history differences have been observed in other areas of sympatry (McPhail and Taylor 1995; Bustard et al. 1999). Mouth size and gill-raker spacing set limits to the maximum and minimum size, respectively, of prey that salmonids can handle (Wankowski 1979). It appears, therefore, that the larger mouths and greater gill-raker spacing for bull trout recorded in this study are consistent with the idea that they are better adapted to exploit larger-bodied prey in lake or large river habitats and that Dolly Varden are better adapted to exploit the predominantly smaller-bodied prey in stream environments. Consequently, interspecific hybrids that are intermediate in morphology may be at a selective disadvantage when exploiting parental trophic niches (reviewed by Arnold and Hodges 1995).

Constraints to gene flow between species

The different resource environments that Dolly Varden and bull trout experience in the Thutade Lake watershed over their entire life cycle may contribute to constraining gene flow between the species in sympatry. The evolution of postmating isolation as a result of divergent natural selection has been demonstrated convincingly only in the laboratory (reviewed in Rice and Hostert (1993)). Results from field observations and experiments using other taxa, however, offer support for the hypothesis that the intermediate phenotypes of interspecific hybrids may be at a fitness disadvantage in parental niches (e.g., Arnold and Hodges 1995). The discontinuous nature of the Thutade watershed (lake and stream environments) may result in selection against hybrid genotypes and contribute to both pre- and post-mating isolation. First, the survival of hybrids in either lake or stream environments is unknown, but the smaller body size of the hybrids that had spent time in Thutade Lake observed in our study does suggest that hybrids, which are also intermediate in morphology (Haas and McPhail 1991), may be less proficient at exploiting the lake's larger-bodied prey fish resources.

Second, the adaptation of Dolly Varden and bull trout in the Thutade watershed to different life histories also provides a potential mechanism for strong premating isolation between the species. In salmonid taxa, individuals of both sexes typically select mates based on body size (e.g., Jonsson and Hindar 1982; Foote 1988). In the Thutade Lake watershed, then, the difference in body size alone between Dolly Varden and bull trout may promote assortative mating between species. The striking segregation of Dolly Varden and bull trout in terms of physical habitats for spawning is associated with large differences in body size at maturity and, therefore, provides an additional link between life-history differences and premating reproductive isolation. Spawning timing differences are likely important also. Potential causes of the timing differences include differing water-quality characteristics at suitable spawning sites and different egg sizes associated with female body sizes, although these have not been investigated directly. Premating isolation between Dolly Varden and bull trout is not completely effective, however, as evidenced by hybridization across a broad geographic range (Baxter et al. 1997; Taylor et al. 2001). The mechanism by which this gene flow occurs may be “sneak” spawning of the much smaller Dolly Varden males with bull trout pairs, a behaviour that has been documented for other salmonids (Maekawa and Onozato 1986; Wood and Foote 1996) and is consistent with genetic evidence (bull trout mitochondrial DNA introgression into Dolly Varden (Taylor et al. 2001)).

Strong theoretical and experimental support exists for the idea that premating isolation can evolve or be maintained as a consequence of adaptation (e.g., Rice and Hostert 1993; Bush 1994), but specific conditions are required: divergent selection for alternative resource environments is strong relative to gene flow (in sympatry only) and the characters that are the focus of selection in alternative environments cause premating isolation themselves or else are strongly linked to other characters that do. One potential way in which resource exploitation may be linked to reproductive isolation is habitat-specific mating whereby mate choice takes place on or within a preferred habitat or host (Bush 1994). Assortative mating without habitat-specific mating can occur if the characters that reflect specializations to different resource environments also form the basis of premating isolation. For instance, in species pairs of threespine sticklebacks (*Gasterosteus aculeatus*) and in sockeye salmon (*Oncorhynchus nerka*) and kokanee, assortative mating is largely based on a considerable difference in adult body size between sympatric pairs. These are linked to the exploitation of zooplankton in open-water environments or macroinvertebrates in littoral areas by limnetic and benthic sticklebacks, respectively, and the anadromous (sockeye) and non-anadromous (kokanee) life histories (reviewed in Taylor 1999). These examples suggest that the link between body size and sexual selection may be important in promoting or maintaining reproductive isolation when ecological specialization leads to large body size differences. This linkage may, therefore, also contribute to reproductive isolation at the adult life-history stage in Dolly Varden and bull trout, which exhibit similar differences in size at maturity. Finally, although we observed few adult hybrids ($N = 3$), they were intermediate in body size to Dolly Varden and bull trout. Size-dependent spawning site selection in Dolly Varden and bull trout coupled with size-assortative mating behaviour in salmonids raises the hypothesis that natural and sexual selection, respectively, against intermediate-sized hybrid genotypes could also contribute to genetic distinction between sympatric char in spite of gene flow.

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Appendix 1. Regression equations for relationships between log-transformed resource-use variables and $\log_{10}$ body size and between measures of trophic morphology and body size. Interspecific comparisons of relationships were made via analysis of covariance (see Results). Where indicated, D represents daytime and N represents nighttime.

	Regression equations	
Dependent variable	Dolly Varden	Bull trout
Log depth at focal point (cm), D	$Y = 0.85X + 0.67$, $r^2 = 0.35$	$Y = 0.40X + 4.70$, $r^2 = 0.07$
Log depth at focal point (cm), N	$Y = 1.36X + 0.06$, $r^2 = 0.41$	$Y = 0.80X + 0.58$, $r^2 = 0.26$
Log velocity at focal point ($cm \cdot s^{-1}$), D	$Y = 1.63X + 0.01$, $r^2 = 0.26$	$Y = 0.24X + 8.05$, $r^2 = 0.02$
Log velocity at focal point ($cm \cdot s^{-1}$), N	$Y = 0.03X + 11.88$, $r^2 < 0.01$	$Y = -1.37X + 54.22$, $r^2 = 0.10$
Log drift foraging ($attempts \cdot 5\ min^{-1}$), D	$Y = -0.40X + 48.0$, $r^2 = 0.02$	$Y = 1.66X + 0.002$, $r^2 = 0.31$
Log drift foraging ($attempts \cdot 5\ min^{-1}$), N	$Y = 1.07X + 0.02$, $r^2 = 0.09$	$Y = 2.02X + 0.00032$, $r^2 = 0.31$
Log benthic foraging ($attempts \cdot 5\ min^{-1}$), D	$Y = 0.22X + 0.38$, $r^2 = 0.05$	$Y = -0.042X + 1.92$, $r^2 < 0.01$
Log benthic foraging ($attempts \cdot 5\ min^{-1}$), N	$Y = 0.16X + 0.83$, $r^2 < 0.01$	$Y = 1.83X + 0.001$, $r^2 = 0.18$
Log total foraging ($attempts \cdot 5\ min^{-1}$), D	$Y = -0.23X + 21.80$, $r^2 < 0.01$	$Y = 1.36X + 0.01$, $r^2 = 0.25$
Log total foraging ($attempts \cdot 5\ min^{-1}$), N	$Y = 1.14X + 0.014$, $r^2 = 0.08$	$Y = 0.28X + 0.002$, $r^2 = 0.26$
Log stomach content biomass (mg), D	$Y = 2.43X + 0.00$, $r^2 = 0.26$	$Y = 3.45X + 0.00$, $r^2 = 0.30$
Log stomach content biomass (mg), N	$Y = 2.17X + 0.0002$, $r^2 = 0.19$	$Y = 2.31X + 0.001$, $r^2 = 0.22$
Log mean prey item biomass (mg)	$Y = 0.18X + 0.72$, $r^2 = 0.02$	$Y = 0.81X + 0.05$, $r^2 = 0.18$
Mouth size (mm)	$Y = 0.065X + 0.58$, $r^2 = 0.81$	$Y = 0.077X + 0.42$, $r^2 = 0.96$
Gill-raker spacing (mm)	$Y = 0.0078X + 0.18$, $r^2 = 0.80$	$Y = 0.01X + 0.09$, $r^2 = 0.91$