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Seasonal habitat drives intestinal microbiome composition in anadromous Arctic char (Salvelinus alpinus)

Geraint Element, Katja Engel, Josh D. Neufeld, John M. Casselman, Peter van Coeverden de Groot, Charles W. Greer and Virginia K. Walker
1Department of Biology, Queen's University, Kingston, ON, K7L 3N6, Canada.
2Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1, Canada.
3National Research Council Canada, Energy, Mining and Environment Research Centre, Montreal, QC, H4P 2R2, Canada.
4School of Environmental Studies, Queen's University, Kingston, ON, K7L 3N6, Canada.

Summary
Intestinal microbial communities from 362 anadromous Arctic char (Salvelinus alpinus) from the high Arctic Kitikmeot region, Nunavut, Canada, were characterized using high-throughput 16S rRNA gene sequencing. The resulting bacterial communities were compared across four seasonal habitats that correspond to different stages of annual migration. Arctic char intestinal communities differed by sampling site, salinity and stages of freshwater residence. Although microbiota from fish sampled in brackish water were broadly consistent with taxa seen in other anadromous salmonids, they were enriched with putative psychrophiles, including the nonluminous gut symbiont Photobacterium iloliscaricum that was detected in >90% of intestinal samples from these waters. Microbiota from freshwater-associated fish were less consistent with results reported for other salmonids, and highly variable, possibly reflecting winter fasting behaviour of these char. We identified microbiota links to age for those fish sampled during the annual upriver migration, but little impact of the intestinal content and water microbiota on the intestinal community. The strongest driver of intestinal community composition was seasonal habitat, and this finding combined with identification of psychrophiles suggested that water temperature and migratory behaviour are key to understanding the relationship between Arctic char and their symbionts.

Introduction
Although Arctic char (Salvelinus alpinus) is a valued subsistence fish for the traditional peoples of the Arctic (Stewart, 2005), its popularity elsewhere has prompted efforts to make the species more commercially profitable by improving aquaculture performance (Nilsson et al., 2010). However, despite these efforts, Arctic char are among the most extreme and perplexing of all salmonids (Klemetsen et al., 2003), with a northern range exceeding that of any other freshwater-dwelling fish and with a phenotypic plasticity that may be unparalleled among vertebrates, resulting in surprising distributions and unpredictable adaptability (Duston et al., 2007; Klemetsen, 2010).

Typically residing in oligotrophic lakes, northern populations display anadromous behaviour, taking advantage of nutrient-rich marine waters during ice-free weeks in summer and returning to freshwater habitats in autumn (Berg and Berg, 1993). Like all sea-going salmonids, Arctic char must cope with enormous environmental changes associated with traversing a salinity gradient. Unlike most other salmonids, anadromous char in the extreme north must escape the annual super-cooling of winter Arctic seas. This necessitates physiological, behavioural, dietary and immune adaptations to varying salinity. For example, osmoregulatory capacity increases in early spring, prior to seaward migration, and decreases again in the weeks following the autumn return to freshwater (Nilssen et al., 1997; Aas-Hansen et al., 2005). The primary stimulus for these osmoregulatory changes appears to be associated with varying photoperiod, but other variables, including temperature and genetics, may also influence the seasonal ability of char to tolerate higher salinities (Finstad et al., 1989; Delabbio et al., 1990; Amesen et al., 1992). Although the mechanisms for physiological changes...
balance of solutes and water are well established, a complete model of the biological and environmental factors that contribute to fitness in saltwater, compared with freshwater, is lacking (Arnesen et al., 1993; Duston et al., 2007).

Along with salinity, anadromous fish are exposed to distinct aquatic microbial communities that inhabit marine and freshwater environments (Lozupone and Knight, 2007). Similar to other vertebrates, teleost fish form complex relationships with their gut microbiota, with implications for development, metabolism, the innate immune response and behaviour (Rawls et al., 2004; Bates et al., 2006; Balcízar et al., 2007; Borrelli et al., 2016), but unlike better studied animals little is known of the function of individual taxa within wild fish intestinal communities. Wang et al. (2018) linked dysbiosis in the intestinal community of Atlantic salmon (Salmo salar) to the likelihood of host disease, with diseased fish harbouring greater proportions of Vibrio and Aliivibrio, genera that include notable fish pathogens. For anadromous fish, a turnover of gut microbiota occurs with the transition between distinct salinity environments (Ringo and Strom, 1994; Wong and Rawls, 2012; Schmidt et al., 2015; Dehler et al., 2017). In wild Atlantic salmon, the gut microbiota of marine adults is characterized by a low bacterial diversity dominated by members of Mycoplasma, Photobacterium and Aliivibrio, in contrast to the more diverse microbial communities associated with the intestines of young freshwater phases, including Proteobacteria, Tenericutes, Bacteroidetes, Firmicutes, Actinobacteria and Verrucomicrobia (Llewellyn et al., 2015). Despite this turnover during development in different habitats, Atlantic salmon may harbour a small number of "core" taxa from the Tenericutes and Firmicutes that persist between environments and thus could represent important functional members of the gut community (Rudi et al., 2018).

Anadromous Arctic char in Nunavut typically make their first migration 3–6 years after hatching and commonly spend 4–8 weeks of the summer in the sea (Gilbert et al., 2016, and citations therein). Individuals from high Arctic populations can live up to three decades, and thus they may endure repeated seasonal microbial recolonization of their intestines. Changes to Arctic temperatures could affect the frequency and duration of sea residency in anadromous Arctic char and have a detrimental impact on fitness (Gjedrem and Gunnes, 1978; Berg and Berg, 1989; Delabbio et al., 1990; Jonsson and Jonsson, 2009; Finstad and Hein, 2012; IPCC, 2014). However, an increase in mean temperature could mean a longer sea-fishing season due to an extended ice-off period. The latter consideration prompted an interest in an exploratory commercial fishery for the region, with the Hunters and Trappers Association of the community of Gjoa Haven in the Kitikmeot region of Nunavut, Canada, advising on the fishing sites. Previously, we reported that intestinal microbial communities of anadromous Arctic char in this region differ between freshwater and saline environments (Hamilton et al., 2019). Here we expanded our research using nearly twice as many samples, and with a thorough habitat coverage to further elucidate the effects of seasonal habitat and other physiological and environmental factors. We know of no other investigations that explore the effects of seasonal migrations on the microbiota of anadromous Arctic char. The results of this study will be important for furthering the understanding of the dynamic nature of anadromous salmonid gut microbiomes and could also provide insight into the roles that psychrophilic microorganisms play within Arctic salmonid gut communities.

Results

Samples and microbial community composition

After controls for contamination and community reproducibility were implemented, a total of 362 intestinal samples yielded useable 16S rRNA sequence communities, 309 of which were considered statistically comparable (Data S3, S4). Samples came from nine distinct fishing sites and could be assigned to one of four types of seasonal habitat, based on the site geography, salinity and time of year (Table 1).

The first phase of the autumn upriver migration where Arctic char were caught near the sea shore before entering river systems was designated as the autumn brackish run (ABR), with those sampled further up river defined as the autumn freshwater run (AFR). Collections made from a frozen lake (site 1) represented winter freshwater overwintering (WFO) habitat, and those from this lake as well as others under thick ice were designated as spring freshwater overwintering (SFO) habitat. Of the autumn samples, 13 were caught from a site that was not analyzed for water conductivity and thus could not be confidently assigned, but were nonetheless retained for analysis. As expected, autumn fish showed more evidence of feeding and parasitism in their intestines than overwintering fish (Data S3). Less than 8% of the OTUs from any fishing site were shared between the water and intestinal sample communities (Fig. S1).

Taxonomic assessment of averaged intestinal communities revealed clear differences between seasonal habitats (Fig. 1). Mycoplasma and other OTUs associated with the family Mycoplasmataceae made up 18%–25% of the average microbial community in ABR sites, 45%–76% in the AFR sites (excluding Site 20 where n = 1) and had variable proportions <21% in fish caught from overwintering sites (Fig. 1). Taxa affiliated with Photobacterium dominated the ABR site samples, representing 34% and 21% of the average community in samples from...
SIMPER analysis was used to identify bacteria that defined each seasonal habitat, and the results largely supported the taxonomic assessment in Fig. 1, with most of the similarity between ABR, AFR and overwintering samples contributed by Vibrionaceae (predominantly Photobacterium), Mycoplasmataceae (predominantly Mycoplasma) and Pseudomonas respectively (Data S5).

The highly correlated factors of sample site, salinity, season and seasonal habitat, all explained some dissimilarity between intestinal communities (Fig. 2). In general, most variation (14%) was explained by fishing site; however, overall differences between sites were small (pseudo-$F = 5.5$) compared with seasonal habitat, which provided a better factor to define communities, explaining nearly as much variation as sample site (12%) but with much more separation between factor groups (pseudo-$F = 12.9$). Clustering of samples from Site 3 with other autumn (AFR) samples suggested that all 13 were derived from freshwater-caught fish. In total, ABR, AFR, WFO and SFO communities contained 1037, 1103, 887 and 1458 unique OTUs, respectively, out of 4,485 OTUs. Of these, only 4% (176 OTUs) were shared between all habitats (Fig. S2). PERMDISP testing showed that groups based on seasonal habitat had significantly different dispersions ($F = 4.7, p = 0.003$). Since PERMANOVA testing may be vulnerable to the effects of heterogeneous dispersion of factor groups when the experimental design is unbalanced (Anderson and Walsh, 2013), random subsampling to achieve sample size parity (61 samples per habitat) was performed with the WFO group (only 44 samples) excluded.

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**Table 1.** Fishing sites sampled between 2016 and 2017, their geographic location, water conductance, sampling time and the number of Arctic char intestinal communities obtained.

<table>
<thead>
<tr>
<th>Site ID</th>
<th>Latitude, Longitude</th>
<th>Time of year fished</th>
<th>Surface specific conductance (μS/cm)</th>
<th>PSU estimated from conductivity*</th>
<th>Seasonal habitat</th>
<th>Intestinal sequence communities obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N69°33′28.764&quot;, W97°26′13.884&quot;</td>
<td>May 2016, 2017</td>
<td>286</td>
<td>&lt;1</td>
<td>SFO</td>
<td>101</td>
</tr>
<tr>
<td>2</td>
<td>N69°33′28.764&quot;, W97°26′13.884&quot;</td>
<td>May 2016</td>
<td>880</td>
<td>&lt;1</td>
<td>SFO</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>N68°32′5.1&quot;, W96°12′45.899&quot;</td>
<td>May 2016</td>
<td>671</td>
<td>&lt;1</td>
<td>SFO</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>N68°28′51.24&quot;, W96°17′37.32&quot;</td>
<td>Aug 2017</td>
<td>240</td>
<td>4.6</td>
<td>ABR</td>
<td>48</td>
</tr>
<tr>
<td>5</td>
<td>N67°27′22.2&quot;, W95°21′38.6&quot;</td>
<td>Sep 2016</td>
<td>8240</td>
<td>&lt;2</td>
<td>AFR</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>N67°31′17.8&quot;, W96°26′21.8&quot;</td>
<td>Sep 2016</td>
<td>3450</td>
<td>&lt;2</td>
<td>AFR</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>N68°34′1.2&quot;, W93°22′37.452&quot;</td>
<td>Sep 2017</td>
<td>225</td>
<td>&lt;1</td>
<td>AFR</td>
<td>37</td>
</tr>
<tr>
<td>8</td>
<td>N66°57′30.7&quot;, W95°18′5.20&quot;</td>
<td>May 2017</td>
<td>19</td>
<td>&lt;1</td>
<td>SFO</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>N68°55′58.08&quot;, W96°13′10.2&quot;</td>
<td>Aug 2017</td>
<td>214</td>
<td>&lt;1</td>
<td>AFR</td>
<td>27</td>
</tr>
<tr>
<td>10</td>
<td>N67°9′17.35&quot;, W95°21′21.41&quot;</td>
<td>Sep 2017</td>
<td>662</td>
<td>&lt;1</td>
<td>AFR</td>
<td>16</td>
</tr>
<tr>
<td>11</td>
<td>N67°6′38.65&quot;, W95°18′29.63&quot;</td>
<td>Sep 2017</td>
<td>NA</td>
<td>NA</td>
<td>AFR</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>N67°8′18.6612&quot;, W95°17′47.0436&quot;</td>
<td>Sep 2017</td>
<td>NA</td>
<td>NA</td>
<td>AFR</td>
<td>1</td>
</tr>
</tbody>
</table>

*Salinity in practical salinity units (PSU) was estimated directly from specific conductance (Fofonoff and Millard Jr, 1983).

*In some cases, water-specific conductance could not be measured, such as for Site 3, as this area was fished in August by a local fisherman, with coordinates indicating that fish were in a river flowing into the main lake that was sampled in May, but close enough to the sea that these samples could not be confidently assigned to fresh or brackish water. Sites 19 and 20 do not have conductivity measurements, but these sites were both adjacent to, and up river of Site 18, and so it was reasonable to assume that they were similarly freshwater sites.

*Site 17 conductance was not measured in the same year that samples were collected, but later in August 2018.
PERMANOVA testing confirmed that the other three groups were represented by distinct centroids ($R^2 = 0.12$, pseudo-$F = 12.7$, $p < 0.001$).

The hypothesis that seasonal habitat is a driver of community composition was also tested using all samples, including those that were removed from the data set as contaminants (Data S4), and with an unfiltered OTU table. The OTU table was rarefied to the lowest number of reads in a sample (6,098), then PERMANOVA was performed. The results, showing dissimilarity between seasonal habitats, were highly similar to those derived from the filtered table ($R^2 = 0.12$, pseudo-$F = 14.9$, $p < 0.001$), indicating that removal of contaminant OTUs did not impact the overall conclusions.

**Investigation of a core microbiome within seasonal habitats**

Although a core microbiome has been described for Atlantic salmon across different stages of migration (Rudi et al., 2018), this may be less true for Arctic char; only two OTUs were detected in 50% of samples, corresponding to an unclassified bacterium and *Photobacterium* (Table 2). Within seasonal habitats, the identification of shared taxa was more feasible. For example, within ABR fish, one OTU associated with *Photobacterium* was present in 90% of these samples (Table 2), it seemed likely that a single species was represented. Cross-referencing this OTU with the EzBioCloud database (Yoon et al., 2017) revealed 100% identity with the species *Photobacterium iliopiscarium*; no other OTU from Table 2 could be resolved to a lower taxonomy.

**Diversity and growth differences by habitat and age**

Using all available data, stepwise model selection revealed that minimum acceptable models required only seasonal habitat as a factor to explain variation in both Chao1 and Shannon index measurements of bacterial community diversity (Data S6). Here, Chao1 diversity was generally greater in ABR fish compared with other habitats, and
Shannon index tended to be the lowest in AFR fish while the other two habitats were more similar (Fig. S3). To both test for the presence of resident populations of Arctic char and explore the effects of variable fish growth on gut community diversity, a standard growth at age curve was constructed which identified any individual growth deviation (Fig. S4), and these were significantly greater for AFR compared with WFO fish ($p = 0.023$), but not when compared with AFR ($p = 0.059$) (Fig. S5A). However, both ABR sites were located on the mainland where fish showed a 12% greater positive deviation from the growth standard than for King William Island fish ($p < 0.001$). In contrast, there were no significant growth deviations between habitats within the Back River migration route on the mainland (Fig. S5B). Perhaps unsurprisingly, young fish tended to have greater positive and negative deviations from the growth standard than older individuals (Fig. S4B). When the age data were trimmed to remove younger ages according to Nordeng (1983) who defined ages 1–8 years as young char (after which all eco-types, resident or anadromous, should be expected to have achieved reproductive maturity) and also trimmed to remove very old fish (>21 years) due to few samples, deviations from the growth standard stabilized (Fig. S4B) and no relationship was found between intestinal microbial community diversity and deviation from the growth standard. Using the age trimmed data, a positive relationship was found between age and Chao1 diversity for AFR fish only ($R^2 = 0.07$, $p = 0.018$), although not significant for Shannon index diversity ($p = 0.064$). Furthermore, when only actively feeding fish were considered (judged by the presence of faeces = 1–3), the strength of this relationship was increased (Chao1 $R^2 = 0.31$, $p = 0.015$; Shannon $R^2 = 0.16$, $p = 0.046$; Fig. 3). Considering that feeding appeared to impact Chao1 diversity, functional interrogation of the taxa using FAPROTAX was undertaken, but this resulted in many unassigned OTUs (Fig. S6).

### Discussion

Anadromous Arctic char must cross salinity barriers more times in their lives than other fish populations that live in less demanding ecosystems. We hypothesized that these changing seasonal habitats would have an impact on their gut microbiota. In turn, specific microorganisms can affect fish host fitness (Wang et al., 2018), further urging an understanding of the response of microbiota to host migration. Here, we assumed that the sampled Arctic char were anadromous because all fishing sites were within sea-migration routes, and this assumption was supported by Inuit traditional knowledge in addition to the absence of major growth differences between brackish and freshwater fish, as would be expected if there was a mixture of resident and anadromous Arctic char eco-types (Fig. S5B; Rikardsen et al., 2000). Although King William Island fish populations appear to be slower growing than mainland fish, this could be due to the reproductive isolation of these two populations (Li et al., submitted).

### Changing community composition

Intestinal community composition changed as Arctic char transitioned between different stages of their annual migration (Figs. 1 and 2). Although this turnover might be attributed to colonization by microbiota characteristic of marine or freshwater environments, this hypothesis is overly simplistic because no more than 8% of the OTUs at any site were shared between the water and intestinal communities, and at most fishing sites this was even less (Fig. S1). Thisconcurs with a previous report showing the independence of the teleost microbiome from the water microbial community (Schmidt et al., 2015). Additionally, community differences were found between the stages of the Arctic char’s freshwater residence, and at least one marine bacterium, *Photobacterium*, was observed in the intestines of migrating freshwater fish,

### Table 2. Taxonomies corresponding to OTUs found in varying percentages of Arctic char intestinal communities obtained from different seasonal habitats (abbreviated here as indicated in Table 1), with some taxa shown with a multiplier where more than one unique OTU was observed for that taxon.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Present in 50% of samples</th>
<th>Present in 60% of samples</th>
<th>Present in 70% of samples</th>
<th>Present in 80% of samples</th>
<th>Present in 90% of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>[Unclassified][2], <em>Photobacterium</em></td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>AFR</td>
<td>[Unclassified], <em>Photobacterium</em> X2, <em>Sphingomonas</em>, <em>Photobacterium</em> X4, <em>leigoni subsp. mandapamensis</em></td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>WFO</td>
<td>[Unclassified], <em>Pseudomonas</em></td>
<td>[Unclassified]</td>
<td>[Unclassified]</td>
<td>[Unclassified]</td>
<td>[Unclassified]</td>
</tr>
<tr>
<td>SFO</td>
<td>[Unclassified], <em>Phenylobacterium</em> X4</td>
<td>[Unclassified]</td>
<td>[Unclassified]</td>
<td>[Unclassified]</td>
<td>None</td>
</tr>
</tbody>
</table>

*Habitat abbreviations are as in Table 1

*In all cases, [Unclassified] is a specific OTU sequence that could not be assigned a taxonomy by the SILVA database.

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which is also consistent with previous studies of migrating Yukon River salmon (Budsberg et al., 2003), and suggesting that there may be a gradual transition of the microbiome during habitat change. Overall, it is clear that any environmental changes that influence the frequency or duration of sea-residency in these Arctic char would also be expected to affect their gut microbiome composition as a consequence. Gut community changeover likely...

Fig 2. Non-metric multidimensional scaling graphs showing Bray–Curtis dissimilarity of Arctic char intestinal communities, with different colours representing different factors.

A. Sample site explains the most variation between samples ($R^2 = 0.14$, pseudo-$F = 5.5$, $p < 0.001$).
B. The brackish water sites appear distinct from other sites ($R^2 = 0.04$, pseudo-$F = 11.2$, $p < 0.001$).
C. The season in which samples were caught explains some variation ($R^2 = 0.08$, pseudo-$F = 13.6$, $p < 0.001$).
D. Seasonal habitat explains nearly as much variation as sample site but with more separation between factor groups ($R^2 = 0.12$, pseudo-$F = 12.9$, $p < 0.001$).

All abbreviations for habitat are as described in Table 1, and NAs refer to samples caught from Site 3 in autumn of 2017 where salinity could not be determined. A single outlier from Site 8 was removed to improve visualization.
influences Arctic char behaviour, and it is noteworthy that the transfer of farmed Arctic char to seawater results in a period of appetite reduction that is apparently unrelated to development of osmoregulatory capacity (Usher et al., 1991; Arnesen et al., 1993; Duston et al., 2007) and here transition of the gut microbiota may be implicated, since these are known to regulate appetite in teleosts (Borrelli et al., 2016; Falcinelli et al., 2016).

**Major genera in the char intestinal community**

Bacterial taxa affiliated with the Vibrionaceae family contributed more than 50% of the similarity between intestinal communities of Arctic char from the ABR habitat, and in this family, Photobacterium predominated (Fig. 1), contributing ~40% of similarity (Table S1), with P. iliopiscarium as the most frequently occurring species. This bacterium is a known psychrophilic intestinal resident of cold-water marine fish including herring, coal fish, cod and salmon (Onarheim et al., 1994; Urakawa et al., 1999). Indeed, P. iliopiscarium is thought to be a symbiotic or commensal similar to most other members of its phylogenetic clade (Urbanczyk et al., 2011). Other species in this clade produce bioluminescence in the light-emitting organs of fish, but P. iliopiscarium is non-luminous, due to ancestral loss of the lux genes (Asth and Dunlap, 2005). The relationship between P. iliopiscarium and Arctic char may be commensal, and possibly saprophytic, as the bacterium has also been isolated from spoiled cod (Asth and Dunlap, 2005; Flodgaard et al., 2005). Additionally, Photobacterium species produce a number of antimicrobials (Mansson et al., 2011) and thus P. iliopiscarium may benefit Arctic char primarily by occupying space on the intestinal epithelium and inhibiting the growth of opportunistic pathogens. Notably, some of these opportunists may be members of the same family, as other Vibrionaceae species were present (Fig. 1). Although no specific fish pathogens were detected, several OTUs could be classified no lower than Vibrionaceae, and thus these fish may harbour low levels of opportunistic pathogens. The presence of *P. iliopiscarium* in Arctic char is of possible concern to fisheries because these bacteria have the potential to cause histamine food poisoning in spoiled or even seemingly fresh fish, and histamine production increases as temperatures rise above 4°C (Torido et al., 2012; Takahashi et al., 2015). This may be of special concern in a warming Arctic where adequate refrigeration is often lacking.

*Mycoplasma* was found in intestinal communities in all seasonal habitats but was most prominent in the riverine fish during the autumn up-river migration (Fig. 1) where it contributed more than 30% of similarity between samples (Table S1). *Mycoplasma* are well adapted to parasitism owing to their small size and lack of a cell wall, allowing them to tightly adhere to host epithelial cells (Razin and Jacobs, 1992). This may explain their persistence in the different habitats, especially during the AFR phase when the Arctic char appears to have shed many of the cells belonging to other bacterial species (Fig. 1 and Fig. S3B). After *Mycoplasma*, *Brevinema* was the next greatest contributor to similarity in the AFR habitat (>10%) and
has previously been isolated from the lower intestines of rainbow trout (*Oncorhynchus mykiss*) (Ricaud *et al.*, 2018) as well as whitefishes from the Coregonoids (Belkova *et al.*, 2017). Thus, this taxon may be a common colonizer of the salmonid gut, and because *Brevinema* species have denitrifying capacity (Yang *et al.*, 2018), they could contribute to nitrogen metabolism in Arctic char.

Taxa affiliated with *Pseudomonas* dominated overwintering habitat intestinal communities overall (contributing 11%-21% of similarity; Table S1), whereas *Phenylobacterium* OTUs were most abundant in SFO sites. The *Pseudomonas* spp. are common, with at least 202 identified species, including pathogens to both plants and animals (Özen and Ussery, 2012), and with some species being psychrophilic (Reddy *et al.*, 2004; Sinha *et al.*, 2017). Bacteria affiliated with the genus *Sphingomonas* contributed to community similarity at the WFO site, and to a lesser extent at the ABR sites, making the habitat specificity of this genus unclear, but they are known to contribute to gut mucosa and modulate the immune response in mammals (Caballer and Pamer, 2015). *Deelfnea* representatives contributed some similarity (5%) in the SFO sites, and they are known psychrophilic chitinolytic bacteria and have been previously isolated from freshwater fish (Jung and Jung-Schroers, 2011; Bel’Kova *et al.*, 2015; Etyemez and Balcázar, 2015; Dai *et al.*, 2016). In this regard, the ability to metabolize chitin is consistent with the gut health of an omnivorous fish such as Arctic char.

**Intestinal community diversity**

Assessments of intestinal bacteria diversity showed that, on average, Arctic char in freshwater appear to harbour slightly fewer intestinal bacterial species than those in brackish water (Fig. S3A). This is in contrast to a comparison of Atlantic salmon pre-smolt and marine adult life stages (Llewellyn *et al.*, 2015) as well as freshwater and saltwater acclimated pre-smolts (Dehler *et al.*, 2017). However, anadromous Arctic char are known to enter a state of voluntary fasting during the freshwater overwintering period (Jørgensen *et al.*, 2013), and this is supported by our assessments of intestinal faeces content (Data S3; Fig. S7). This and the fact that the fish must cease drinking water as they migrate up river could contribute to decreased microbiota diversity. When assessing microbiota using the Shannon index, which considers the evenness of species abundance, a more complex picture emerges, where evenness seems to decline as fewer OTUs become more representative of the community during the transition between brackish and freshwater habitats (represented here by the AFR habitat) and then returns to a similar diversity after a longer fish residence in freshwater (represented by the SFO habitat) (Fig. S3B). It should be noted that very little of the variation in either species richness or Shannon index were explained by seasonal habitat ($R^2 = 0.08$ and 0.06 respectively). However, at least for the Shannon index, the observation that the community becomes dominated by fewer taxa in the AFR habitat is strongly supported by the taxonomic analysis, where average AFR communities consist mostly of species from the family *Mycoplasmataceae* (Fig. 1). These results support a model in which Arctic char are recolonized by some bacterial taxa in accordance with their life history and are consistent with reports of gut-dwelling *Mycoplasmataceae* from all life stages of migrating Atlantic salmon (Llewellyn *et al.*, 2015), again suggesting that this family has a well-established relationship with salmonids compared with other members of the gut community.

Measurements of bacterial diversity increased with age in mature (9–21 years) and actively feeding (inferred from the faeces content) fish from the AFR habitat, and with most of the variation accounted for by fish at the younger ages, 10–13 years (Fig. 3). Nitrogen isotope analysis indicates that diet changes with char size (Hobson and Welch, 1995), and we found that deviations from the standard growth curve stabilized at around the age of 8–9 years (Fig. S4), which roughly coincided with the width of the annual increments in the otoliths (not shown). Thus, it is possible that anadromous Arctic char take advantage of alternative and increased food sources around this age, which may either facilitate the uptake of new bacteria or promote individual taxa for digestion. Alternatively, accumulation of new bacterial species in the gut may generally increase as a function of age, regardless of feeding behaviour, but decline in abundance during overwinter fasting, reducing them to levels that were not detectable. Such low-abundance cells could then repopulate upon resumption of feeding. Functional predictions of the intestinal taxa using FAPROTAX were generally not informative (Fig. S6) with a majority (63%) of the OTUs as unassigned, likely a result of a paucity of information about wild Arctic fish bacteria. It is also apparent that the identified intestinal bacteria are likely involved in biofilm formation and are aerobic or at least facultatively aerobic. Of the 37% of the OTUs that were assigned, samples from the majority of sites were classified as functioning in chemoheterotrophy, either aerobic or unspecified, as well as in fermentation. Little is known about digestion in these fish but in *O. mykiss* the rate of $O_2$ uptake in the gut, which only accounts for ~4% of the mass of the fish, represented more than 11% of the whole animal uptake, and can increase substantially following feeding and the transition to sea water (Brijs *et al.*, 2018 and references herein). Clearly, more needs to be learned about digestion in high Arctic char.

Overall, we have shown that the intestinal communities of anadromous Arctic char captured in the high Arctic...
reflect this dynamic environment and change when fish migrate between different seasonal habitats. The putative symbiote *P. iliopiscarium* was identified as an important member of the intestinal community while the Arctic char were in the marine environment. Additionally, the average diversity of bacteria found in the intestines of feeding Arctic char increases with maturity. Together, these findings highlight the need to further explore the specific symbiotic and parasitic interactions of Arctic char dwelling bacteria and their hosts. This has important implications for Arctic char fitness in different environments, especially with regard to feeding behaviour and the potential effects of changing water temperatures on psychrophilic members of the gut community. The implications of these for future expanded fisheries have also yet to be explored.

**Experimental procedures**

**Sample collection, DNA extraction and sequencing**

Traditionally important and distinct geographic fishing sites were identified by elders of Gjoa Haven (Fig. 4), thereby focusing sampling efforts on lakes, rivers and coastal regions located within, and proximal to, King William Island, Rasmussen Basin and Chantrey Inlet. Fish were netted and intestines removed aseptically and frozen at −20°C, as described, with all appropriate licenses (Data S1). Water samples (~1000 ml) were filtered in triplicate through 0.22 µm filters, which were then frozen at −20°C for shipping and −80°C for storage prior to analysis as previously described (Hamilton *et al*., 2019).

Dissected intestines were partially thawed to excise three slices within the distal large intestine (~2 cm from the vent), comprising a total of 5–100 mg of epithelial tissue, avoiding any faeces and connective tissue. The slices were pooled and DNA extracted using the UltraClean Tissue and Cells DNA Isolation Kit (Qiagen, Toronto, ON) following the manufacturer’s instructions, with the exception of changes to DNA elution steps as described (Data S1, S2). Genomic DNA extracts were assessed by agarose gel electrophoresis and quantified prior to preamplification using the variable V1–V9 region of the bacterial 16S ribosomal RNA (rRNA) gene, and subsequent nested polymerase chain reaction (PCR) was employed to amplify the V4–V5 region, with the products excised from agarose gel and purified before libraries were sequenced (MiSeq; Illumina, CA, USA) using a 2 × 250 cycle MiSeq Reagent Kit v2 (Illumina Canada) as detailed in supporting information (Data S1).

Experimental trials had shown that this procedure was necessary to reduce amplification of the char mitochondrial DNA, and preliminary tests showed no bias compared with low cycle numbers. Sequence reads were demultiplexed using the MiSeq Reporter software, version 2.5.0.5, and assembled using the paired-end assembler for Illumina sequences (PANDAseq version 2.8; Masella *et al*., 2012) with a quality threshold of 0.9, an 8-nucleotide minimum overlap and 32-nucleotide minimum assembled read length. Once assembled, the reads were analyzed using Quantitative Insights Into Microbial Ecology (QiIME version 1.9.0; Caporaso *et al*., 2010). Sequences were clustered into operational taxonomic units (OTUs) using UPARSE algorithm USEARCH version 7.0.1090 (Edgar, 2013) at 97% identity and aligned with the Python Nearest Alignment Space Termination tool (PyNAST version 1.2.2; Caporaso *et al*., 2009). All representative sequences were classified using the Ribosomal Database Project (RDP version 2.2; Wang *et al*., 2007) with a stringent confidence threshold (0.8), and the SILVA database (release 128; Pruesse *et al*., 2007) was used to assign taxonomy. Chimeric sequences were filtered with UCHIME (Edgar *et al*., 2011). For functional analysis of the microbiome, OTU tables were also subjected to Functional Annotation of Prokaryotic Taxa (FAPROTAX; Louca *et al*., 2016).

**Qualitative assessment of intestinal samples and testing for ecotypes**

Feeding is known to affect bacterial proliferation in fish bowels (Montgomery and Pollak, 1988) and thus qualitative assessments of the amount of faeces present in samples used a devised scale (0–3), and evidence of piscivory was based on visual inspection for bones, scales and otoliths. Intestinal tracts of Arctic char host a variety of parasitic worms including roundworms, flatworms and thorny-headed worms (Hanek and Molnar, 1974; Mudry and McCart, 1976), and since it was unknown how these might affect the bacterial community, quantities were also estimated using a scale (0–3).

Since *S. alpinus* is known to have a plastic life-history (Klemetsen *et al*., 2003), it is possible that non-anadromous individuals could have been netted in some freshwater habitats. However, Arctic char from the Kitikmeot region with different life-histories are known to vary in growth, with resident (i.e., non-anadromous, despite having sea access) fish growing more slowly than anadromous individuals (Swanson *et al*., 2011). Testing for differences in incremental growth was accomplished by construction of a mean annual incremental growth curve (log-standardized fork length/age) for combined data and a comparison of deviations of individual values from this growth standard.

**Statistical analysis**

For multivariate statistical analysis, Arctic char intestinal communities from a rarefied OTU table were analyzed using a Bray–Curtis dissimilarity matrix (Bray and
Curtis, 1957) in the R package vegan. Non-metric multidimensional scaling plots were used to visually represent this dissimilarity in two-dimensional space. Significant differences between factor groups were determined by permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) using the vegan function adonis2 (McArdle and Anderson, 2001). Differences in dispersions between factor groups were tested using PERMDISP (Anderson, 2006) employed by the vegan function betadisper (Anderson et al., 2006). All PERMANOVA and PERMDISP tests used 999 permutations.

The univariate diversity metrics Chao1 and Shannon indices were used to assess OTU richness (Chao, 1984; Chao, 1987) and evenness (Shannon, 1948; Bik et al., 2006) respectively. All significant linear regression models were tested for normal distribution of residuals and homoscedasticity, using visual assessment of Q-Q and residuals versus fitted plots, and little to no autocorrelation of residuals using Durbin–Watson’s test. The leverage effects of outliers on models were also determined using Cook’s distance versus leverage plots. Stepwise model selection was done using the step function in R, which uses an analysis of variance (ANOVA) component to drop variables from a linear model provided that there are no significant differences in Akaike’s Information Criterion, in order to reveal the simplest acceptable regression model. ANOVA and Tukey’s honest significance difference (HSD) tests were performed to test for differences in means between factor groups using a 95% confidence level threshold for significance.

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References


Seasonal habitat drives Arctic char microbiome


Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Appendix S1: Supporting information

Table S1. Results of a similarity percentages (SIMPER) test of Arctic char intestinal communities from four seasonal habitats (abbreviated here as indicated in Table 1), where SIMPER attempts to identify the taxa (genus level) that contribute most to similarity within a group.

Fig. S1. Venn diagrams showing the number of unique and shared OTUs between water and intestinal samples for all fishing sites where water was sampled. For site 3 (autumn), site 13, and site 17, water samples were collected at
different times than fish, whereas for all other sites, water was filtered at the time of fishing.

**Fig. S2.** Venn diagram showing the numbers of unique intestinal community OTUs shared between Arctic char caught in different seasonal habitats. An OTU was present in a habitat if at least one Arctic char microbiome community from that habitat contained one or more sequence reads for the OTU. Diagram was produced using VENNY 2.0 (Oliveros, 2007).

All abbreviations for habitat are as described in Table 1.

**Fig. S3.** Boxplots showing relationship between seasonal habitat and Arctic char intestinal community diversity, within the Back River migration system. The seasonal habitat is shown with “n” referring to the number of analyzed intestinal communities from individual Arctic char (represented by black dots). Graph A shows log10 transformed Chao1 diversity ($R^2 = 0.08$, $F = 4.3$, $p = 0.016$) where ABR and SFO have significantly different means from one another ($p = 0.014$). Graph B shows untransformed Shannon index ($R^2 = 0.06$, $F = 3.4$, $p = 0.037$) where ABR and AFR have significantly different means ($p = 0.030$). Habitat abbreviations are as described in Table 1.

**Fig. S4.** Incremental growth (fork length/age) of Arctic char of different ages and from different habitats (abbreviated as in Fig. 4). A shows growth (mm-year$^{-1}$) at each age. Black curve shows log standardized mean growth at age. For the log-log relationship (not shown), $R^2 = 0.88$, $p < 0.0001$. B shows deviation from the growth standard, divided into fast growing fish with positive deviation values (top line) and slow growing fish with negative deviation values (bottom line). Dashed lines indicated ages at which the data was later trimmed to reduce outlier effects. Habitat abbreviations are as described in Table 1.

**Fig. S5.** Boxplots showing deviation from the growth standard in the different seasonal habitats (abbreviations as indicated in Fig. 4 of Arctic char, as a percentage of the growth standard. A shows the amount that individuals deviate from the growth standard across all habitats and sites (positive values indicate that a fish shows greater growth than the standard at that individual’s age). Mean deviation of ABR samples is significantly greater than WFO ($p = 0.023$) and nearly significantly greater than AFR ($p = 0.059$). B shows the amount that individuals within the Back River system deviate from the growth standard. Here mean deviation is not significantly different between habitats. NAs refer to samples caught from site 3 in fall of 2017 where salinity was not determined. Habitat abbreviations are as described in Table 1.

**Fig. S6.** Diversity of sample sites based on 16S rRNA gene sequencing (top; at or above 4% abundance shown) and functional profiles based on FAPROTAX analysis (bottom; at or above 1% abundance shown). Sites refer to fishing sites as described in Table 1.

**Fig. S7.** Bar plots showing counts of Arctic char intestinal samples corresponding to graded qualitative states describing faeces and parasite content. For A, 0 = no faeces observed, 1 = trace amounts of faeces observed, 2 = intestine was moderately full of faeces, 3 = intestine was completely full of faeces. For B, 0 = no parasitic worms observed, 1 = 1–5 parasitic worms observed, 2 = >5 parasitic worms observed, 3 = intestine was completely full of parasitic worms. Number of samples that could not be confidently assessed due to small size are indicated with “NA”. Habitat abbreviations are as described in Table 1.

**Fig. S8.** Non-metric multidimensional scaling plot showing Bray-Curtis dissimilarity of fish intestinal communities and no-template-controls (NTCs). “Sample” refers to fish intestinal communities from a data set created over two years, containing Arctic char samples, as well as other salmonids (Coregonus spp. and Salvelinus namaycush). Inplate2017 refers to NTCs from three 2017 plates where controls were located in the first well of a 96 well PCR plate. OutplateEX2018 refers to NTCs from the four 2018 plates, where controls were run in the same PCR reaction as the 96 well plates, but in separate tubes. Unlike the 2017 controls, 2018 controls also used water that had been run through the entire extraction protocol to account for kit contamination.

**Fig. S9.** Bar plot showing the number of OTU reads for different genera observed in no-template control (NTC) samples. 2017 controls were in-plate (PCR reactions performed in the same 96 well plate as intestinal community samples) whereas 2018 controls were out-plate extraction controls (run through entire DNA extraction protocol and PCR amplification performed at the same time as intestinal community samples, but in separate PCR tubes). NTCs 11 and 16 are ddH$_2$O and MilliQ water samples respectively, from the same PCR plate. Only taxa making up >1% of the data set are shown.

**Fig. S10.** Rarefaction plot showing Chao1 diversity associated with increasing rarefaction depths of intestinal sample communities. Each colour corresponds to an individual intestinal community from the original multi-salmonid-species OTU table before sample filtering and final rarefaction. The table contained mostly S. alpinus samples, but also included Coregonus spp. and S. namaycush.

**Fig. S11.** Non-metric multidimensional scaling showing dissimilarity between A, PCR plates and B, replicate samples from different sequencing years. For graph A, plates Four2017 and Five2017 contained an even mixture of samples from different sample sites from that year, whereas plates One2018 and Two2018 contained an even mixture of samples from different sample sites from that year, and plate Three2018 contained a mixture of samples from 2017 and 2018. Plates Two2017 and Four2018 contained a small number of samples from their respective years. Plate Three2017 contained samples from sites 1, 2, and 3 collected during a separate sampling season from all other samples. For graph B, sample refers to Arctic char intestinal communities, whereas norm2017 and norm2018 refer to replicate Arctic char intestinal communities that were amplified and sequenced independently in both years.

**Fig. S12.** Bar plot showing abundances of different genera in replicate samples (from rarefied OTU table). Bar labels show the unique identifier number of an Arctic char intestinal sample, followed by the year in which 16S rRNA gene sequences were amplified and sequenced from that sample. Only taxa making up >1% of the data set are shown.