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

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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 iliopiscarium* 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 autumn 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; Arnesen *et al*., 1992). Although the mechanisms for physiological

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; Balcázar *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 (Ringø and Strøm, 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

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.

Site ID	Latitude, Longitude	Time of year fished	Surface specific conductance (μS/cm)	PSU estimated from conductivity[a]	Seasonal habitat	Intestinal sequence communities obtained
1	N69°33'28.764", W97°26'13.884"	May 2016,2017	286	<1	SFO	101
1	N69°33'28.764", W97°26'13.884"	Dec 2016	NA[b]	NA	WFO	54
2	N68°40'13.62, W95°56'57.408"	May 2016	880	<1	SFO	11
3	N68°32'5.1", W96°12'45.899"	May 2016	671	<1	SFO	5
3	N68°28'51.24", W96°17'37.32"	Aug 2017	NA[b]	NA	Autumn run, salinity undeterminable	13
6	N67°27'27.2", W95°21'38.6"	Sep 2016	8240	4.6	ABR	48
7	N67°31'17.8", W96°26'21.8"	Sep 2016	3450	<2	ABR	17
8	N68°34'1.2", W93°22'37.452"	Sep 2017	225	<1	AFR	50
13	N66°57'30.70", W95°18'5.20"	May 2017	19	<1	SFO	37
17	N68°55'58.08", W96°13'10.2"	Aug 2017	214[c]	<1	AFR	27
18	N67°9'17.35", W95°21'21.41"	Sep 2017	662	<1	AFR	16
19	N67°6'38.65", W95°18'29.63"	Sep 2017	NA[b]	NA	AFR	14
20	N67°8'18.6612", W95°17'47.0436"	Sep 2017	NA[b]	NA	AFR	1

[a]Salinity in practical salinity units (PSU) was estimated directly from specific conductance (Fofonoff and Millard Jr, 1983).
[b]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.
[c]Site 17 conductance was not measured in the same year that samples were collected, but later in August 2018.

Site 6 and 7, respectively, compared with <1%–6% from all other sites. Although *Brevinema* was present in all autumn collection sites (4%–19%), these bacteria were present at lower levels in overwintering char (<1%–2%). Generally, *Pseudomonas* OTUs were more abundant in overwintering fish (1%–18%) than in autumn samples (<1%–2%). Except for the WFO site, and the five SFO samples from Site 3, all averaged site communities contained various levels (2%–24%) of an unclassified taxon in the order *Fusobacteriales* (Family Silva nomenclature: *Hados*.*Sed*.*Eubac*.).

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.
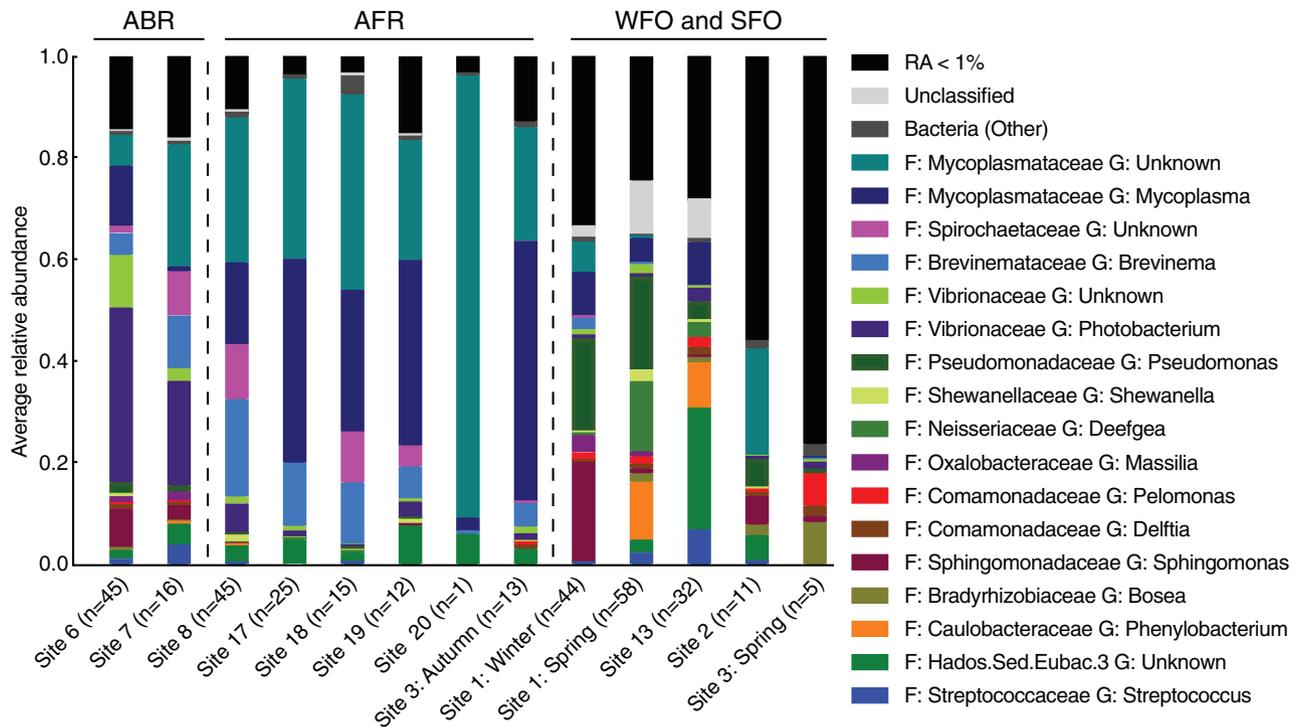
Fig 1. Stacked bar graph showing relative abundance of reads observed for different genera associated with fish caught at various sites including those representing the ABR, the AFR, the WFO and the SFO habitat.
Each bar represents average of proportions from all Arctic char intestinal communities obtained from that site with "*n*" referring to the number of samples pooled. Note that Site 20 and Site 3 (spring) have sample sizes <10. Samples from Sites 2 and 3 (spring) are included for interest but were not used for subsequent statistical analyses as the sequencing plate could not be assessed for PCR bias (see Experimental Procedures). RA < 1% (black) includes all genera that made up less than 1% of the total data set.

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 the samples, whereas an unclassified sequence was found in 80% of samples from both overwintering habitats, with another associated with *Mycoplasmataceae* found in 70% of AFR samples. Because *Photobacterium* appeared to be a key taxon in the chars' brackish phase, and a single OTU within this genus appeared 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

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.

Habitat[a]	Present in 50% of samples	Present in 60% of samples	Present in 70% of samples	Present in 80% of samples	Present in 90% of samples
All	[Unclassified][b], *Photobacterium*	None	None	None	None
ABR	Vibrionaceae X2, *Sphingomonas*, *Photobacterium* X4, *leiognathi* subsp. *mandapamensis*	Vibrionaceae, *Photobacterium* X2	*Photobacterium*	*Photobacterium*	*Photobacterium*
AFR	Mycoplasmataceae X2, *Mycoplasma*, *Photobacterium*, *Brevinema*	Mycoplasmataceae	Mycoplasmataceae	None	None
WFO	[Unclassified], *Pseudomonas*	[Unclassified]	[Unclassified]	[Unclassified]	None
SFO	[Unclassified], *Phenylobacterium*	[Unclassified]	[Unclassified]	[Unclassified]	None

[a]Habitat abbreviations are as in Table 1
[b]In all cases, [Unclassified] is a specific OTU sequence that could not be assigned a taxonomy by the SILVA database.

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 deviations (Fig. S4), and these were significantly greater for ABR 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 ecotypes, 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 ecotypes (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). This concurs 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,
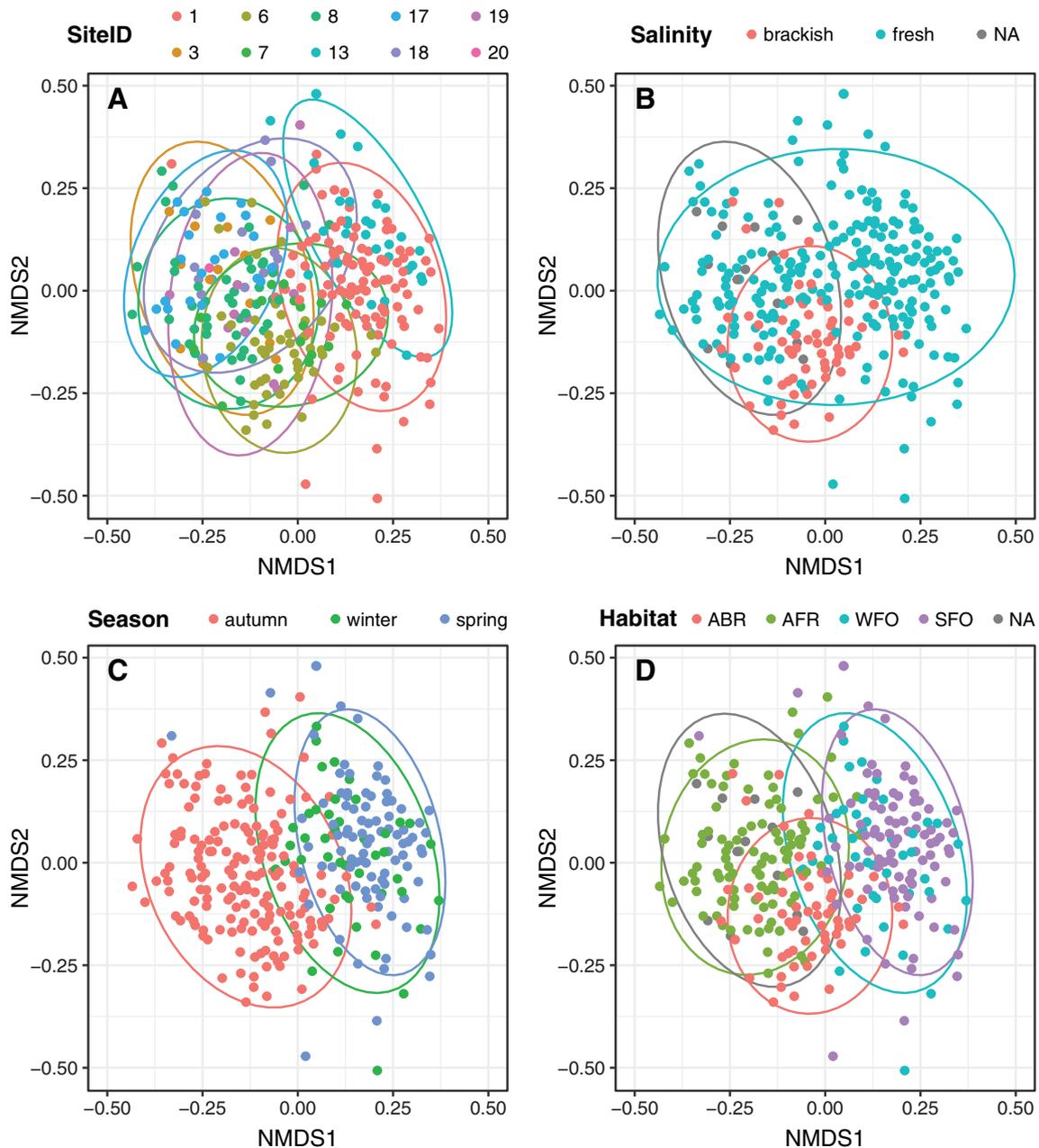
Fig 2. Non-metric multidimensional scaling graphs showing Bray–Curtis dissimilarity of Arctic char intestinal communities, with different colours representing different factors.
A. Sample site explains the most variation between samples ($R^2 = 0.14$, pseudo-$F = 5.5$, $p < 0.001$).
B. The brackish water 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.

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
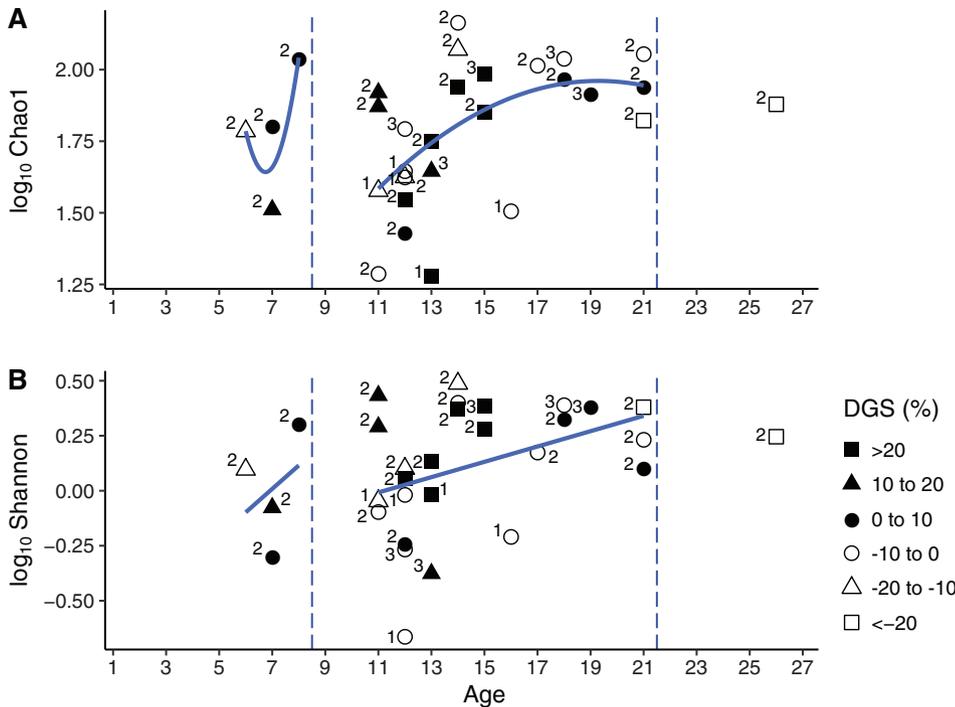
A

B

Fig 3. Scatter plots showing Chao1 and Shannon indices of Arctic char intestinal communities by age (years), for fish from the AFR habitat, with shape indicating deviation from the growth standard (DGS [%]).

Data are divided between the age ranges of 1–8, 9–21 and 22–28. Regression lines are second-order polynomials where these fit better than first order. Numbers next to shapes indicate the observed amount of faeces present in the intestine (on a scale of 0–3, with only actively feeding fish ≥1 considered). The regression in (A) is modelled by $y = 1.8 + 0.18x + 0.26x^2$, $n = 25$, $R^2 = 0.31$, $p = 0.015$. The regression in (B) is modelled by $y = -0.39 + 0.035x$, $n = 25$, $R^2 = 0.16$, $p = 0.046$.

DGS (%)

■ >20
▲ 10 to 20
● 0 to 10
○ -10 to 0
△ -20 to -10
□ <−20

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 symbiote 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 (Ast 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 (Ast 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 Coregonids (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 (Caballero and Pamer, 2015). *Deefgea* 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 reproliferate 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 ($\sim$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 ($\sim$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 Ultra-Clean 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
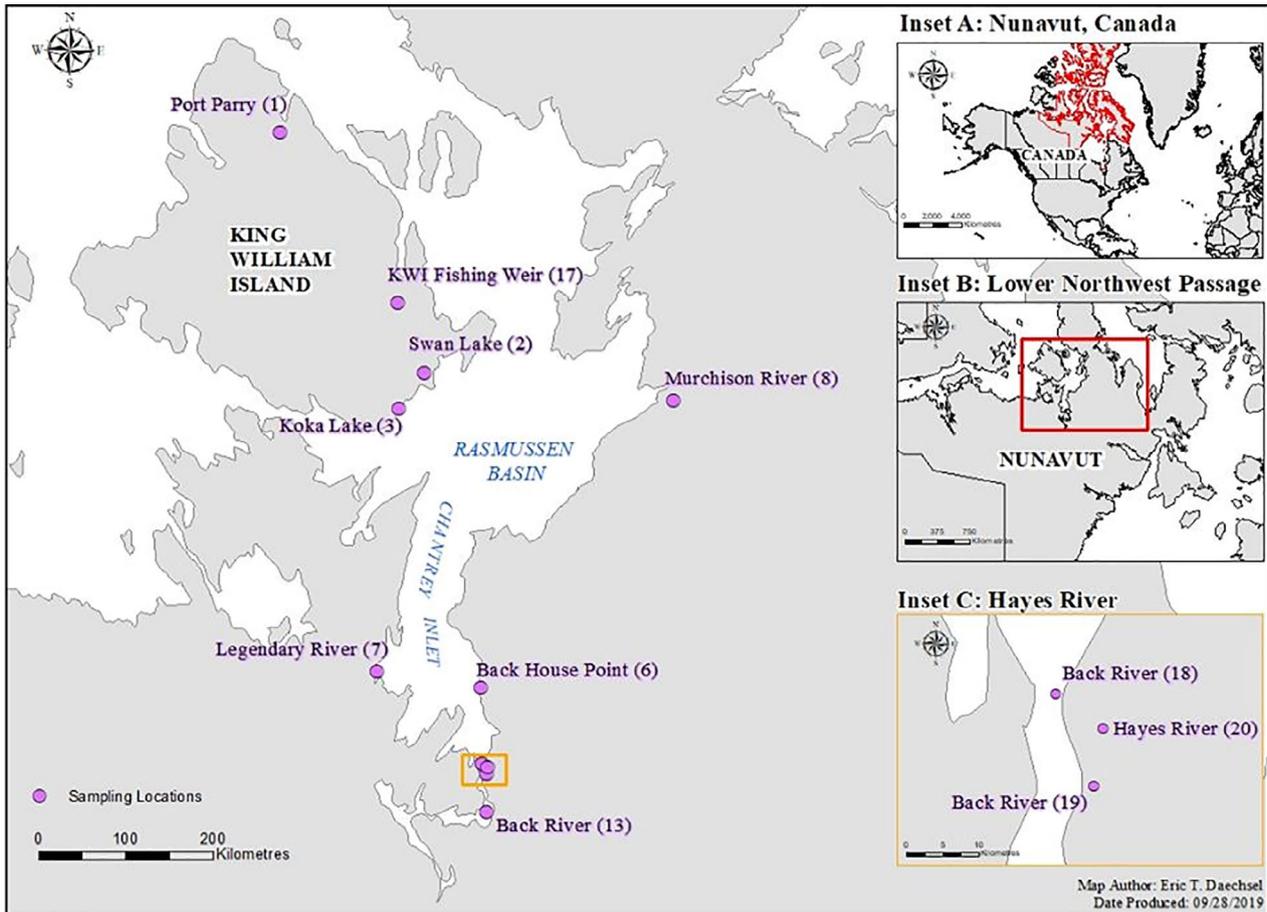
Fig 4. Map of Arctic char sampling sites showing additional sampling locations and codes.
Sites 18–20 were located close to each other with Site 20 situated in the mouth of a watershed (Hayes River) that empties into the Back River from the east, dividing Sites 18 and 19 into sites down-river and up-river of the mouth of Hayes respectively.
Source: Modified from Hamilton *et al*. (2019).

Curtis, 1957) in the R package vegan. Non-metric multi-dimensional 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.

References

Aas-Hansen, Ø., Vijayan, M.M., Johnsen, H.K., Cameron, C., and Jørgensen, E.H. (2005) Resmoltification in wild, anadromous Arctic char (*Salvelinus alpinus*): a survey of osmoregulatory, metabolic, and endocrine changes preceding annual seawater migration. *Can J Fish Aquat Sci* **62**: 195–204.

Anderson, M.J. (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecol* **26**: 32–46.

Anderson, M.J. (2006) Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* **62**: 245–253.

Anderson, M.J., and Walsh, D.C. (2013) PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? *Ecol Monogr* **83**: 557–574.

Anderson, M.J., Ellingsen, K.E., and McArdle, B.H. (2006) Multivariate dispersion as a measure of beta diversity. *Ecol Lett* **9**: 683–693.

Arnesen, A.M., Halvorsen, M., and Nilssen, K.J. (1992) Development of hypoosmoregulatory capacity in Arctic char (*Salvelinus alpinus*) reared under either continuous light or natural photoperiod. *Can J Fish Aquat Sci* **49**: 229–237.

Arnesen, A.M., Jørgensen, E.H., and Jobling, M. (1993) Feed intake, growth and osmoregulation in Arctic charr, *Salvelinus alpinus* (L.), transferred from freshwater to saltwater at 8°C during summer and winter. *Fish Physiol Biochem* **12**: 281–292.

Ast, J.C., and Dunlap, P.V. (2005) Phylogenetic resolution and habitat specificity of members of the *Photobacterium phosphoreum* species group. *Environ Microbiol* **7**: 1641–1654.

Balcázar, J.L., De Blas, I., Ruiz-Zarzuela, I., Vendrell, D., Gironés, O., and Muzquiz, J.L. (2007) Enhancement of the immune response and protection induced by probiotic lactic acid bacteria against furunculosis in rainbow trout (*Oncorhynchus mykiss*). *FEMS Immunol Med Microbiol* **51**: 185–193.

Bates, J.M., Mittge, E., Kuhlman, J., Baden, K.N., Cheesman, S.E., and Guillemin, K. (2006) Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. *Dev Biol* **297**: 374–386.

Bel'Kova, N.L., Denikina, N.N., and Dzyuba, E.V. (2015) Study of the microbiome of the intestine of the *Comephorus dybowski korotneff*, 1904. *Biol Bull* **42**: 463–469.

Belkova, N.L., Sidorova, T.V., Glyzina, O.Y., Yakchnenko, V. M., Sapozhnikova, Y.P., Bukin, Y.S., *et al.* (2017) Gut microbiome of juvenile coregonid fishes: comparison of sympatric species and their F1 hybrids. *Fund Appl Limnol* **189**: 279–290.

Berg, O.K., and Berg, M. (1989) The duration of sea and freshwater residence of the sea trout, *Salmo trutta*, from the Vardnes River in northern Norway. *Environ Biol Fishes* **24**: 23–32.

Berg, O.K., and Berg, M. (1993) Duration of sea and freshwater residence of Arctic char (*Salvelinus alpinus*), from the Vardnes River in northern Norway. *Aquaculture* **110**: 129–140.

Bik, E.M., Eckburg, P.B., Gill, S.R., Nelson, K.E., Purdom, E. A., Francois, F., *et al.* (2006) Molecular analysis of the bacterial microbiota in the human stomach. *Proc Natl Acad Sci U S A* **103**: 732–737.

Borrelli, L., Aceto, S., Agnisola, C., De Paolo, S., Dipineto, L., Stilling, R.M., *et al.* (2016) Probiotic modulation of the microbiota-gut-brain axis and behaviour in zebrafish. *Sci Rep* **6**: 30046.

Bray, J.R., and Curtis, J.T. (1957) An ordination of the upland forest communities of southern Wisconsin. *Ecol Monogr* **27**: 325–349.

Brijs, J., Gräns, A., Hjelmstedt, P., Sandblom, E., van Nuland, N., Berg, C., and Axelsson, M. (2018) In vivo aerobic metabolism of the rainbow trout gut and the effects of an acute temperature increase and stress event. *J Exp Biol* **221**: 180703. https://doi.org/10.1242/jeb.180703.

Budsberg, K.J., Wimpee, C.F., and Braddock, J.F. (2003) Isolation and identification of Photobacterium phosphoreum from an unexpected niche: migrating salmon. *Appl Environ Microbiol* **69**: 6938–6942.

Caballero, S., and Pamer, E.G. (2015) Microbiota-mediated inflammation and antimicrobial defense in the intestine. *Annu Rev Immunol* **33**: 227–256.

Caporaso, J.G., Bittinger, K., Bushman, F.D., DeSantis, T.Z., Andersen, G.L., and Knight, R. (2009) PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* **26**: 266–267.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., and Knight, R. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**: 335–336. https://doi.org/10.1038/nmeth.f.303.

Chao, A. (1984) Nonparametric estimation of the number of classes in a population. *Scand Stat Theory Appl* **11**: 265–270.

Chao, A. (1987) Estimating the population size for capture-recapture data with unequal catchability. *Biometrics* **43**: 783–791.

Dai, Y., Yan, Z., Jia, L., Zhang, S., Gao, L., Wei, X., *et al.* (2016) The composition, localization and function of low-temperature-adapted microbial communities involved in methanogenic degradations of cellulose and chitin from Qinghai–Tibetan Plateau wetland soils. *J Appl Microbiol* **121**: 163–176.

Dehler, C.E., Secombes, C.J., and Martin, S.A. (2017) Seawater transfer alters the intestinal microbiota profiles of Atlantic salmon (Salmo salar L.). *Sci Rep* **7**: 13877.

Delabbio, J.L., Glebe, B.D., and Sreedharan, A. (1990) Variation in growth and survival between two anadromous strains of Canadian Arctic charr (*Salvelinus alpinus*) during long-term saltwater rearing. *Aquaculture* **85**: 259–270.

Duston, J., Astatkie, T., and Murray, S.B. (2007) Effect of salinity at constant 10°C on grow-out of anadromous Arctic charr from Labrador. *Aquaculture* **273**: 679–686.

Edgar, R.C. (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* **10**: 996–998. https://doi.org/10.1038/nmeth.2604.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., and Knight, R. (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**: 2194–2200.

Etyemez, M., and Balcázar, J.L. (2015) Bacterial community structure in the intestinal ecosystem of rainbow trout (*Oncorhynchus mykiss*) as revealed by pyrosequencing-based analysis of 16S rRNA genes. *Res Vet Sci* **100**: 8–11.

Falcinelli, S., Rodiles, A., Unniappan, S., Picchietti, S., Gioacchini, G., Merrifield, D.L., and Carnevali, O. (2016) Probiotic treatment reduces appetite and glucose level in the zebrafish model. *Sci Rep* **6**: 18061.

Finstad, A.G., and Hein, C.L. (2012) Migrate or stay: terrestrial primary productivity and climate drive anadromy in Arctic char. *Glob Chang Biol* **18**: 2487–2497.

Finstad, B., Nilssen, K.J., and Arnesen, A.M. (1989) Seasonal changes in sea-water tolerance of Arctic charr (*Salvelinus alpinus*). *J Comp Physiol B* **159**: 371–378.

Flodgaard, L.R., Dalgaard, P., Andersen, J.B., Nielsen, K.F., Givskov, M., and Gram, L. (2005) Nonbioluminescent strains of *Photobacterium phosphoreum* produce the cell-to-cell communication signal N-(3-hydroxyoctanoyl) homoserine lactone. *Appl Environ Microbiol* **71**: 2113–2120.

Fofonoff, N. P., and Millard Jr, R. C. (1983). Algorithms for computation of fundamental properties of seawater. Endorsed by Unesco/SCOR/ICES/IAPSO Joint Panel on Oceanographic Tables and Standards and SCOR Working Group 51. Unesco Technical Papers in Marine Science, No. 44.

Gilbert, M.J., Donadt, C.R., Swanson, H.K., and Tierney, K. B. (2016) Low annual fidelity and early upstream migration of anadromous Arctic char in a variable environment. *Trans Am Fish Soc* **145**: 931–942.

Gjedrem, T., and Gunnes, K. (1978) Comparison of growth rate in Atlantic salmon, pink salmon, Arctic char, sea trout and rainbow trout under Norwegian farming conditions. *Aquaculture* **13**: 135–141.

Hamilton, E.F., Element, G., de Groot, P.V.C., Engel, K., Neufeld, J.D., Shah, V., and Walker, V.K. (2019) Anadromous Arctic char microbiomes: bioprospecting in the high Arctic. *Front Bioeng Biotechnol* **7**: 32 https://doi.org/10.3389/fbioe.2019.00032.

Hanek, G., and Molnar, K. (1974) Parasites of freshwater and anadromous fishes from Matamek River system, Quebec. *J Fish Res Board Can* **31**: 1135–1139.

Hobson, K.A., and Welch, H.E. (1995) Cannibalism and trophic structure in a high Arctic lake: insights from stable-isotope analysis. *Can J Fish Aquat Sci* **52**: 1195–1201.

IPCC (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, p. 151.

Jonsson, B., and Jonsson, N. (2009) A review of the likely effects of climate change on anadromous Atlantic salmon *Salmo salar* and brown trout *Salmo trutta*, with particular reference to water temperature and flow. *J Fish Biol* **75**: 2381–2447.

Jørgensen, E.H., Martinsen, M., Strøm, V., Hansen, K.E.R., Ravuri, C.S., Gong, N., and Jobling, M. (2013) Long-term fasting in the anadromous Arctic charr is associated with downregulation of metabolic enzyme activity and upregulation of leptin A1 and SOCS expression in the liver. *J Exp Biol* **216**: 3222–3230.

Jung, A., and Jung-Schroers, V. (2011) Detection of *Deefgea chitinilytica* in freshwater ornamental fish. *Lett Appl Microbiol* **52**: 497–500.

Klemetsen, A. (2010) The charr problem revisited: exceptional phenotypic plasticity promotes ecological speciation in postglacial lakes. *Freshw Rev* **3**: 49–75.

Klemetsen, A., Amundsen, P.A., Dempson, J.B., Jonsson, B., Jonsson, N., O'connell, M.F., and Mortensen, E. (2003) Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. *Ecol Freshw Fish* **12**: 1–59.

Llewellyn, M.S., McGinnity, P., Dionne, M., Letourneau, J., Thonier, F., Carvalho, G.R., *et al*. (2015) The biogeography of the atlantic salmon (*Salmo salar*) gut microbiome. *ISME J* **10**: 1280.

Louca, S., Parfrey, L.W., and Doebeli, M. (2016) Decoupling function and taxonomy in the global ocean microbiome. *Science* **353**: 1272–1277.

Lozupone, C.A., and Knight, R. (2007) Global patterns in bacterial diversity. *Proc Natl Acad Sci U S A* **104**: 11436–11440.

Mansson, M., Gram, L., and Larsen, T.O. (2011) Production of bioactive secondary metabolites by marine Vibrionaceae. *Mar Drugs* **9**: 1440–1468.

Masella, A.P., Bartram, A.K., Truszkowski, J.M., Brown, D.G., and Neufeld, J.D. (2012) PANDAseq: paired-end assembler for Illumina sequences. *BMC Bioinformatics* **13**: 31. https://doi.org/10.1186/1471-2105-13-31.

McArdle, B.H., and Anderson, M.J. (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* **82**: 290–297.

Montgomery, W.L., and Pollak, P.E. (1988) *Epulopiscium fishelsoni* NG, N. Sp., a protist of uncertain taxonomic affinities from the gut of an herbivorous reef fish 1. *J Protozool* **35**: 565–569.

Mudry, D.R., and McCart, P.J. (1976) Metazoan parasites of Arctic char (*Salvelinus alpinus*) from the north slope of Canada and Alaska. *J Fish Res Board Can* **33**: 271–275.

Nilssen, K.J., Gulseth, O.A., Iversen, M., and Kjol, R. (1997) Summer osmoregulatory capacity of the world's northernmost living salmonid. *Am J Physiol Regul Integr Comp Physiol* **272**: R743–R749.

Nilsson, J., Brännäs, E., and Eriksson, L.O. (2010) The Swedish Arctic charr breeding programme. *Hydrobiologia* **650**: 275–282.

Nordeng, H. (1983) Solution to the" char problem" based on Arctic char (*Salvelinus alpinus*) in Norway. *Can J Fish Aquat Sci* **40**: 1372–1387.

Oliveros, J.C. (2007–2015) Venny. An interactive tool for comparing lists with Venn's diagrams. http://bioinfogp.cnb.csic.es/tools/venny/index.html

Onarheim, A.M., Wiik, R., Burghardt, J., and Stackebrandt, E. (1994) Characterization and identification of two *Vibrio* species indigenous to the intestine of fish in cold sea water; description of *Vibrio iliopiscarius* sp. nov. *Syst Appl Microbiol* **17**: 370–379.

Özen, A.I., and Ussery, D.W. (2012) Defining the *Pseudomonas* genus: where do we draw the line with *Azotobacter*? *Microb Ecol* **63**: 239–248.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., *et al*. (2007) SILVA: A comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* **35**: 7188–7196.

Rawls, J.F., Samuel, B.S., and Gordon, J.I. (2004) Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proc Natl Acad Sci U S A* **101**: 4596–4601.

Razin, S., and Jacobs, E. (1992) Mycoplasma adhesion. *J Gen Microbiol* **138**: 407–422.

Reddy, G.S., Matsumoto, G.I., Schumann, P., Stackebrandt, E., and Shivaji, S. (2004) Psychrophilic *pseudomonads* from Antarctica: *Pseudomonas antarctica* sp. nov., *Pseudomonas meridiana* sp. nov. and *Pseudomonas proteolytica* sp. nov. *Int J Syst Evol Microbiol* **54**: 713–719.

Ricaud, K., Rey, M., Plagnes-Juan, E., Larroquet, L., Even, M., Quillet, E., *et al*. (2018) Composition of intestinal microbiota in two lines of rainbow trout (*Oncorhynchus mykiss*) divergently selected for muscle fat content. *Open Microbiol J* **12**: 308.

Rikardsen, A.H., Amundsen, P.A., Bjørn, P.A., and Johansen, M. (2000) Comparison of growth, diet and food consumption of sea-run and lake-dwelling Arctic charr. *J Fish Biol* **57**: 1172–1188.

Ringø, E., and Strøm, E. (1994) Microflora of Arctic charr, *Salvelinus alpinus* (L.): gastrointestinal microflora of free-living fish and effect of diet and salinity on intestinal microflora. *Aquac Res* **25**: 623–629.

Rudi, K., Angell, I.L., Pope, P.B., Vik, J.O., Sandve, S.R., and Snipen, L.G. (2018) Stable core gut microbiota across the freshwater-to-saltwater transition for farmed Atlantic salmon. *Appl Environ Microbiol* **84**: e01974-17.

Schmidt, V.T., Smith, K.F., Melvin, D.W., and Amaral-Zettler, L.A. (2015) Community assembly of a euryhaline fish microbiome during salinity acclimation. *Mol Ecol* **24**: 2537–2550.

Shannon, C.E. (1948) A mathematical theory of communication. *Bell Syst Tech J* **27**: 379–423.

Sinha, R.K., Krishnan, K.P., Hatha, A.A.M., Rahiman, M., Thresyamma, D.D., and Kerkar, S. (2017) Diversity of retrievable heterotrophic bacteria in Kongsfjorden, an Arctic fjord. *Braz J Microbiol* **48**: 51–61.

Stewart, H. (2005) The fish tale that is never told: a reconsideration of the importance of fishing in Inuit societies. *Senri Ethnol Stud* **67**: 345–361.

Swanson, H., Gantner, N., Kidd, K.A., Muir, D.C.G., and Reist, J.D. (2011) Comparison of mercury concentrations in landlocked, resident, and sea-run fish (*Salvelinus spp.*) from Nunavut, Canada. *Environ Toxicol Chem* **30**: 1459–1467.

Takahashi, H., Ogai, M., Miya, S., Kuda, T., and Kimura, B. (2015) Effects of environmental factors on histamine production in the psychrophilic histamine-producing bacterium *Photobacterium iliopiscarium*. *Food Control* **52**: 39–42.

Torido, Y., Takahashi, H., Kuda, T., and Kimura, B. (2012) Analysis of the growth of histamine-producing bacteria and histamine accumulation in fish during storage at low temperatures. *Food Control* **26**: 174–177.

Urakawa, H., Kita-Tsukamoto, K., and Ohwada, K. (1999) Reassessment of the taxonomic position of *Vibrio iliopiscarius* (Onarheim *et al*. 1994) and proposal for *Photobacterium iliopiscarium* comb. nov. *Int J Syst Evol Microbiol* **49**: 257–260.

Urbanczyk, H., Ast, J.C., and Dunlap, P.V. (2011) Phylogeny, genomics, and symbiosis of *Photobacterium*. *FEMS Microbiol Rev* **35**: 324–342.

Usher, M.L., Talbot, C., and Eddy, F.B. (1991) Effects of transfer to seawater on growth and feeding in Atlantic salmon smolts (*Salmo salar* L.). *Aquaculture* **94**: 309–326.

Wang, Q., Garrity, G.M., Tiedje, J.M., and Cole, J.R. (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* **73**: 5261–5267.

Wang, C., Sun, G., Li, S., Li, X., and Liu, Y. (2018) Intestinal microbiota of healthy and unhealthy Atlantic salmon *Salmo salar* L. in a recirculating aquaculture system. *J Oceanol Limnol* **36**: 414–426.

Wong, S., and Rawls, J.F. (2012) Intestinal microbiota composition in fishes is influenced by host ecology and environment. *Mol Ecol* **21**: 3100–3102.

Yang, Z., Yang, L., Wei, C., Wu, W., Zhao, X., and Lu, T. (2018) Enhanced nitrogen removal using solid carbon source in constructed wetland with limited aeration. *Bioresour Technol* **248**: 98–103.

Yoon, S.H., Ha, S.M., Kwon, S., Lim, J., Kim, Y., Seo, H., and Chun, J. (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* **67**: 1613–1617.

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 *S. 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 (eluent 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.