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SHORT COMMUNICATION

The biogeography of the atlantic salmon (*Salmo salar*) gut microbiome

Martin S Llewellyn[1,2,3], Philip McGinnity[4], Melanie Dionne[5], Justine Letourneau[2], Florian Thonier[2], Gary R Carvalho[1], Simon Creer[1] and Nicolas Derome[2]

[1]*Molecular Ecology and Fisheries Genetics Laboratory, Bangor University, Environment Centre Wales, Bangor, UK;* [2]*Institut de Biologie Intégrative et des Systèmes, Université Laval, Quebec City, Quebec, Canada;* [3]*Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, UK;* [4]*School of Biological, Earth and Environmental Sciences, University College Cork, Cork, Ireland and* [5]*Ministère des Ressources Naturelles et de la Faune, Quebec City, Quebec, Canada*

Although understood in many vertebrate systems, the natural diversity of host-associated microbiota has been little studied in teleosts. For migratory fishes, successful exploitation of multiple habitats may affect and be affected by the composition of the intestinal microbiome. We collected 96 *Salmo salar* from across the Atlantic encompassing both freshwater and marine phases. Dramatic differences between environmental and gut bacterial communities were observed. Furthermore, community composition was not significantly impacted by geography. Instead life-cycle stage strongly defined both the diversity and identity of microbial assemblages in the gut, with evidence for community destabilisation in migratory phases. *Mycoplasmataceae* phylotypes were abundantly recovered in all life-cycle stages. Patterns of *Mycoplasmataceae* phylotype recruitment to the intestinal microbial community among sites and life-cycle stages support a dual role for deterministic and stochastic processes in defining the composition of the *S. salar* gut microbiome.
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Introduction

Atlantic Salmon (*Salmo salar*) are non-obligate anadramous salmonids of significant commercial, cultural and recreational importance. Growth, development and migration in anadromous *S. salar* involves a radical shift across an ecological and trophic spectrum (Jacobsen and Hansen, 1999; Orlov *et al*., 2006). Accompanying the physiological, behavioural and dietary adaptations necessary to cope with transition between freshwater and marine environments (McCormick *et al*., 2013), significant and potentially adaptive shifts in host-associated microbiota might be expected.

The advent of culture-free microbial metasequencing techniques means that bacterial communities can be profiled in unprecedented detail. As such, teleost-associated intestinal microbiota are increasingly subject to scrutiny (Llewellyn *et al*., 2014). Salmonids have received particular attention in view of their importance in aquaculture (for example, Zarkasi *et al*., 2014) and the urgent need for innovation in feed sources (Green *et al*., 2013). Attempts to establish the natural identity and stability of commercially exploited teleost intestinal microbiomes have been limited to focal studies from single aquaculture facilities (for example, Zarkasi *et al*., 2014) and single sites in the wild (Star *et al*., 2013). The ecological succession of gut bacterial phylotypes during wild teleost development and migration is an excellent system in which to explore the relative contribution of host and environmental factors to shaping microbiome recruitment, especially in euryhaline species (Schmidt *et al*., 2015). Furthermore, exploration of the biogeography of microbiome composition among species with wide geographic distributions is required to form a sound baseline for experimental study. In this study, we set out to explore microbiome ontogeny and biogrography in wild *S. salar.*

Materials and methods

Bacterial 16S SSU rRNA (V4 region) diversity was profiled from the intestinal contents of 96 wild *S. salar* on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) and analysed using Mothur (Schloss *et al*., 2009) and QIIME (Caporaso *et al*., 2010) (full methods in Supplementary Data S1). Juveniles and returning adults were sampled in eastern Canada and western Ireland. Marine adults were sampled in cooperation with the West Greenland subsistence fishery (www.nasco.int) (Supplementary Table S1).

Correspondence: MS Llewellyn, Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow G12 8QQ, UK.
E-mail: martin.llewellyn@glasgow.ac.uk
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Results

Study site (or country of origin) had no discernible effect on microbiome identity among samples from freshwater life-cycle stages, including returning adults ($P=0.7264$, PERMANOVA UniFrac distances (PUd); Oksanen *et al.*, 2015—a full list of statistical tests applied and sample sizes are included in Supplementary Table S2). Nonetheless, study-site specific variation in intestinal operational taxonomic unit (OTU) richness (Chao1) was a consistent feature across juvenile life-cycle stages (Figure 1a, $P=0.003$, Kruskal–Wallis test (K-WT)). Diversity estimates (Shannon Index) corroborate this finding (Supplementary Figure S1, $P=0.009$).

In contrast to those between-study sites, clear differences were observed in the microbial community identity of the *S. salar* intestine between freshwater and marine life-cycle stages ($P<0.019$; PUd; Oksanen *et al.*, 2015; Figure 1b), in which returning adults retain a large proportion of their microbial assemblage from the marine environment. Microbiome identity within fresh and saltwater ecotopes was not impacted by life-cycle stage (smolt vs parr: $P=0.268$; marine vs returning adult $P=0.522$; PUd). Over study sites, inter-individual microbial composition was most stable among parr and marine life-cycle stages and least stable among smolts and returning adults (open and green bars, Figure 1c). OTU richness (Chao1) was dramatically affected by life-cycle stage across marine and freshwater environments ($P=0.0001$; K-WT) but not diversity (Shannon, $P=0.276$). By contrast, both OTU richness and diversity were purged during the transition from parr to smolt in freshwater (Chao1, $P<1.8\times10^{-5}$, Shannon, $P=0.0105$; K-WT; Figure 1a, Supplementary Figure S1).

Discussion

Phylum-level assignment of OTUs (Figure 2b) indicated the dominance of Proteobacteria among all samples. All life-cycle stages, especially marine adults, were enriched for Tenericutes (Genus *Mycoplasma* especially). In contrast, Firmicutes, Bacteriodetes and Actinobacteria—abundant in returning adults, smolt and parr—occur at negligible levels in marine adults. Both Tenericutes and Firmicutes OTUs occur in freshwater environmental samples but at minimal levels with respect to those recovered from fish. By contrast, Verrucomicrobia, common among freshwater samples, occurs at minimal levels in freshwater life-cycle stages. Although Verrucomicrobia is common in soil, freshwater and marine environmental samples (Freitas *et al.*, 2012), as well as a frequent member of mammalian gut flora (Zhang *et al.*, 2009), our study corroborates the low abundance of Verrucomicrobia found in fish found

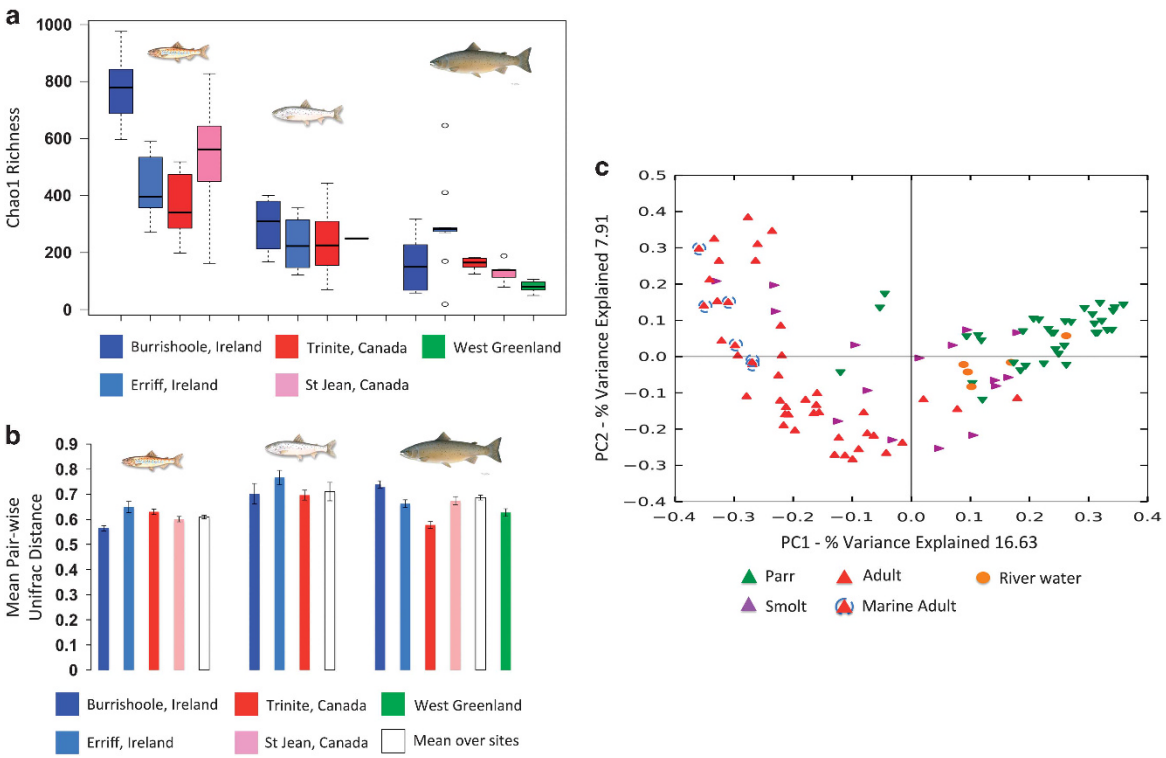

Figure 1 Alpha and beta diversity comparisons of Atlantic Salmon intestinal microbiota. (**a**) Box plot showing alpha diversity (Chao1 richness estimator) variation across sites and life-cycle stages: blue bars—Burrishoole, Ireland; pale blue bars—Erriff, Ireland; red bars—St Jean, Canada; pink bars—Trinite, Canada. Marine adults from West Greenland are represented in green. (**b**) Mean beta diversity distances (unweighted unifrac) among individuals grouped by site and life-cycle stage. Error bars represent s.e.m. (**c**) Principal coordinate analysis of pairwise unweighted unifrac distances between all salmon and environmental samples. Axes represent the two synthetic variables explaining the greatest proportion of variation in the data set.

previously (for example, Rawls *et al*., 2006). To buffer inter-sample variability among OTUs assigned to genus level, we first evaluated the diversity of core OTUs (defined here as those occurring in 85% of individuals) present among fish from each life-cycle stage (Figure 2a). OTUs assigned to genus *Yersinia* and other unclassified Enterobacteriacae dominate the core microbiota of freshwater parr. Some

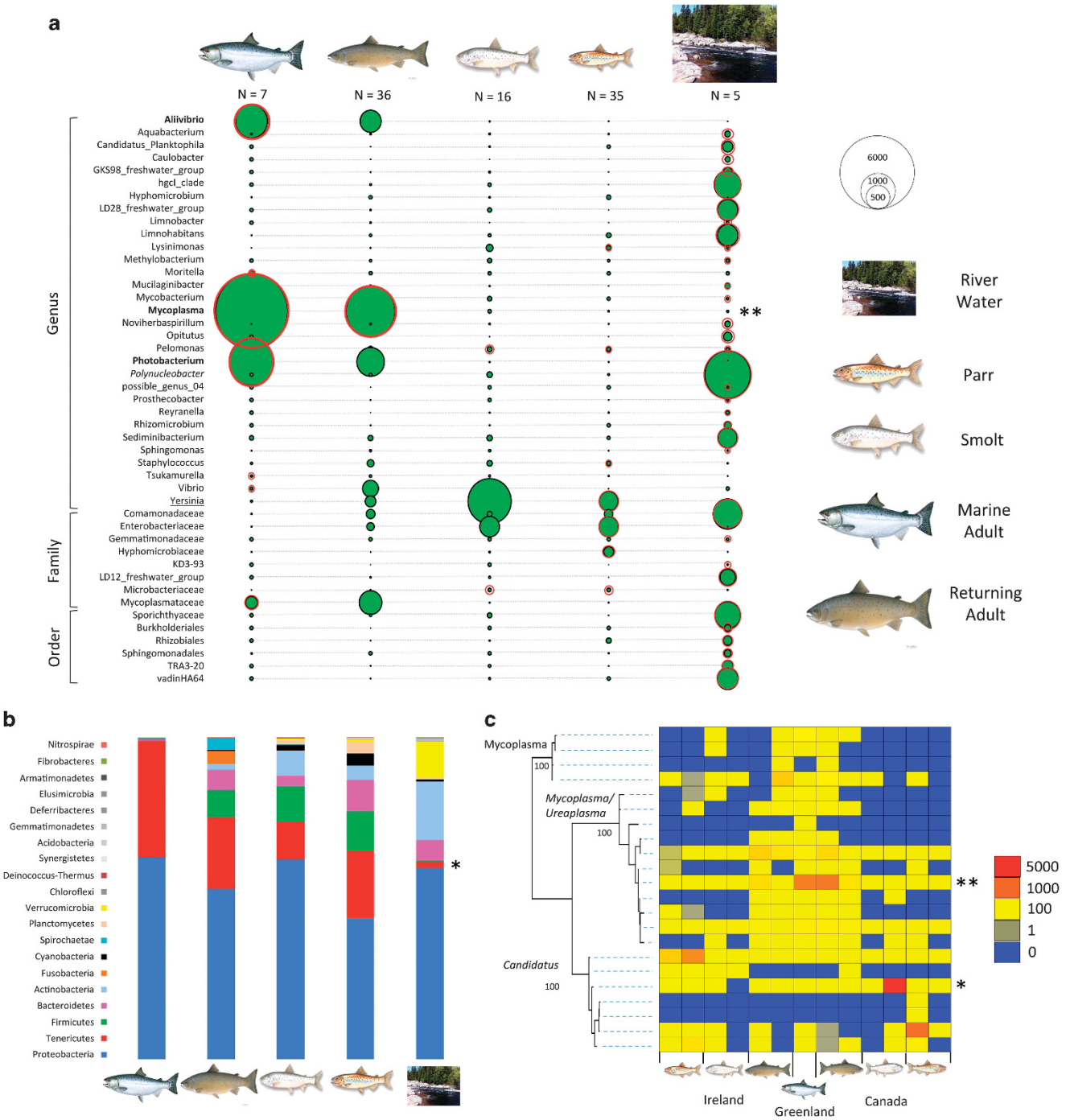

Figure 2 Taxonomic composition of the Atlantic Salmon intestinal microbiome. (**a**) Phlyum-level composition of total OTU abundances among distinct life-cycle stages and environmental samples. (**b**) Core (present in ⩾85% of individuals) 97% identity OTUs assigned to genus level from each life-cycle stage are represented by red-outlined ellipses. Black-outlined ellipses denote the presence of these 'core' OTUs among other life-cycle stages. Ellipse area is proportional to the mean abundance of OTUs assigned to each genus over all samples from each life-cycle stage. Core genera that occur at a mean frequency >1000 in each sample time are bold (adults), underlined (parr) or italicised (freshwater). (**c**) Heatmap displaying the frequency distribution of OTUs belonging to family Mycoplasmataceae across distinct life-cycle stages and countries of origin. Genera within the Mycoplasmataceae are indicated on the maximum likelihood phylogeny (left) on which values indicated the percentage of bootstrap support for the respective clades. Single asterisk indicates the *Candidatus* OTU also recovered from a sympatric environmental sample. Double asterisk indicates the most abundant core Mycoplasma OTU recovered from adult life-cycle stages.

Yersinia species (*Y. ruckeri*, *Y. intermedia*) are important pathogens of salmon (Bruno *et al.*, 2013). However, the healthy state of the parr we sampled, as well as several single-nucleotide polymorphisms between the principal *Yersinia* OTU in our data set and the 16S V4 region of both *Y. ruckeri,* and *Y. intermedia* (data not shown), suggest that the *Yersinia* we sampled were likely to be commensals. Enterobacteriacae were also abundant among smolt, however, not among the core at 85%—unsurprising given raised beta diversity within this group (Figure 1b). Genus *Mycoplasma* phylotypes were the most abundant and consistently recovered phylotypes from adult salmon. More typical members of marine teloest intestinal microflora—Genera *Allivibrio*
and *Photobacterium* (for example, Sullam *et al.*, 2012)—were also well represented. OTUs attributable to family Mycoplasmataceae were also recovered in large numbers from other freshwater life-cycle stages. Clear differences in the frequency distribution of Mycoplasmataceae OTUs exist between adult and juvenile salmon (Figure 2c). Biogeographic differences in Mycoplasmataceae OTU distributions between Canada and Ireland were apparent in juveniles but not in returning adults (Figure 2c). With the exception of a single Mycoplasma OTU isolated from the Trinite river, Mycoplasmataceae OTUs abundant in parr and smolt were absent or rare ($<5/11\,000$) in local freshwater samples.

Both bacterial OTU richness and community stability declined over life-cycle stage in the intestine of *S. salar*, in stark contrast to mammals where community diversity increases after weaning and stabilises in early-to-late childhood (Yatsunenko *et al.*, 2012). Returning adult salmon were characterised by low richness, highly variable microbial assemblages in comparison to parr. Although poor in absolute numbers of OTUs (that is, richness), diversity estimates from returning adults were not significantly different from juveniles, suggesting a fairly even frequency distribution of those OTUs present. Dietary complexity in juvenile salmonids could explain rich associated microbial assemblages (Orlov *et al.*, 2006). Meanwhile physiological disturbances and fasting in migratory phases (smolt and returning adults) could underlie reduced community stability with respect to corresponding non-migratory phases (that is, parr and marine adults, Figure 1b). In particular, drinking rates increase during smoltification, as well as overall intestinal fluid re-absorption rates, perhaps affecting microbiome equilibrium (Stefansson *et al.*, 2008). Additionally, documented variation in the response of different intestine regions (midgut vs hindgut) during smoltification could impact associated microbiota accordingly, an interesting future avenue for investigation (Stefansson *et al.*, 2008). The microbial community of feeding marine adults was less rich and diverse than that of freshwater juveniles; perhaps attributable to the dominance of Mycoplasmataceae phylotypes among adult intestinal microbiota. Mycoplasmataceae, especially genera *Candidatus* and *Mycoplasma*, frequently colonise vertebrate and invertebrate mucosae, both as pathogens and commensals (Frey and Herrmann, 2002; Holben *et al.*, 2002; Nechitaylo *et al.*, 2009). Indeed, *Mycoplasma* have been isolated from *S. salar* in the past (Holben *et al.*, 2002; Zarkasi *et al.*, 2014). The abundance of Mycoplasmataceae (and individual OTUs, Figure 2c) among sites suggests an association with the salmon gut niche robust to developmental change and could point to some more complex level of interdependence with the host. In a recent laboratory study of microbiome ontology in euryhaline fishes, Schmidt *et al.* (2015) suggest a dominant role for deterministic forces (for example, niche appropriation) over neutral ones (for example, colonisation) (Schmidt *et al.*, 2015). Mycoplasmataceae abundance and diversity in this study suggest a dual role in the wild: geography and environment influence colonisation source (and thus a proportion of the microbiome variation at the genus level); however, the intra-host niche likely determines the abundance of Mycoplasmataceae in general across *S. salar*. More widely, a combination of deterministic host effects and stochastic environmental factors underpin diversity in the *S. salar* microbiome whereby the microbiota of freshwater juvenile and returning adults, while sharing many OTUs with local environmental samples, show radically different patterns of abundance and enrichment. Broad-scale shifts in the composition of key components of *S. salar* gut microbiomes pose fundamental questions in relation to functional significance of qualitative change. Such inferences demand an experimental approach to assess empirically the impact of microbiome diversity on fish health and survival in distinct environments, especially in the context of aquaculture.

Conflict of Interest

The authors declare no conflict of interest.

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