Core Intestinal Microbiome Richness of Coral Reef Damselfishes (Actinopterygii: Pomacentridae) Reflects Trophic Guild

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Research Article

Keywords: Damselfish, microbiome, gastrointestinal, coral reef, bacteria, Pomacentridae

Posted Date: December 15th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-126554/v1

Abstract

Background: Fish harbour diverse microbiomes within their gastro-intestinal system that affect the host's digestion, nutrition and immunity and facilitate resource partitioning in coral reef ecosystems. Despite the great taxonomic diversity of fish, little is understood about fish microbiome diversity and the factors that determine its structure and composition. Damselfish are important coral reef fish species that play a strong role in determining algae and coral structure of reefs. Broadly, damselfish belong to either of two trophic guilds based on whether they are planktivorous or algae-farming. In this study, we use 16s rRNA sequencing to interrogate the intestinal microbiome of 10 damselfish species (Pomacentridae) from the Great Barrier Reef to compare the composition of their intestinal bacterial assemblages across the planktivorous and algae-farming trophic guilds.

Results: We identify core intestinal bacterial taxa for each host fish species. Gammaproteobacteria, belonging to the genus *Actinobacillus,* were detected in 80 % of sampled individuals and suggests a possible core member of pomacentrid microbiomes. Core microbiomes of algae-farming species were more diverse than planktivorous species with farming species sharing 35 ± 22 ASVs and planktivorous sharing 7 ± 3 ASVs. We also provide evidence for significant shifts in bacterial community composition along the intestines. We show that Bacteroidia, Clostridia and Mollicutes bacteria are more abundant in the anterior intestinal regions while Gammaproteobacteria are generally highest in the stomach. Finally, we highlight differences in microbiomes associated with both trophic guilds. Algae-farming and planktivorous damselfish host species significantly differed in their composition of bacteria belonging to Vibrionaceae, Lachnospiraceae and Pasteurellaceae.

Conclusions: Our results demonstrate that the richness of the core intestinal bacterial communities of damselfish reflects host species diet and trophic guild, whereby algae-farming hosts have larger and more diverse core microbiomes than planktivorous hosts. We suggest that algae-farming damselfish within the same species share bacterial taxa that reflect their specialised diets.

Background

Fishes represent the greatest taxonomic diversity of vertebrates, and despite our understanding of the importance of intestinal microbiota of terrestrial vertebrates, we still lack an understanding of fish microbiome diversity and functioning [3]. Largely, fish microbiome studies have centred around species with commercial value, including trout, salmon and carp [4]. For example, gastrointestinal fish microbiomes are known to be important in intestinal cell proliferation [5, 6], nutrition [3, 7] and immunity [8–10]. These studies show that the intestines of fishes harbour a large abundance and diversity of bacteria [11] and regulation of this diversity is important in the maintenance of host health through a complex set of microbe - microbe interactions and microbe - host interactions [1, 2].

There are many factors that affect the structure of fish gastrointestinal microbiomes [3, 4]. These include host-related factors such as genetic attributes, size, age, sex [12–14], host phylogeny [15–

17],environmental factors (such as water quality) [18–20] and host diet [15, 17, 18]. Studies that investigate intestinal microbiome changes have mostly been concentrated on the impact of fish foods on species of aquaculture importance [21, 22], although a few studies have investigated wild fish populations [15, 23]. Bacterial symbiont diversification in wild herbivorous surgeonfish intestines is thought to be an important driver of host niche-partitioning [24, 25], suggesting that intestinal microbiomes can influence the trophic ecology of coral reefs and facilitate resource partitioning in these hyper-diverse ecosystems.

There is increasing evidence that herbivorous fishes have distinct microbiomes as compared to omnivorous and carnivorous fishes [26]. Herbivorous and carnivorous fish diets are known to cause shifts in intestinal microbiomes; fishes with plant-based diets have intestinal microbiomes dominated by Firmicutes, such as *Clostridium*, while fishes with fat-based diets have microbiomes dominated by protease producing Proteobacteria [27–30]. In addition, the diversity of herbivorous fish intestinal microbiomes is higher than omnivorous and carnivorous host species under similar environmental conditions [31], suggesting that host feeding behaviour has a significant effect on fish intestinal microbiomes.

Damselfishes (Pomacentridae) are a diverse and abundant group of coral reef fishes [32, 33], and they are among the most widely studied family of reef fishes [34, 35]. Broadly, damselfishes are grouped into either planktivorous or herbivorous trophic guilds, although some herbivorous species may also feed on zooplankton [36]. Many herbivorous damselfishes that inhabit reef crest environments are territorial, and they cultivate palatable algae within their territories, which they aggressively defend from other species. Territorial species can be differentiated based on the algal composition within their territories, and they are divided into several behavioural guilds, including indeterminate grazers, extensive grazers, and intensive grazers [37–40]. Indeterminate and extensive grazers have territories where the benthic algal community is not distinct from the surrounding turf, while intensive grazers maintain distinct areas of turf algae through selective grazing and weeding of unpalatable algae [37, 38]. Intensive grazing damselfish are also referred to as algae-farmers. Research on territorial grazers has focused on competition [41], patterns of co-existence [36, 42, 43], behavioural interactions [44, 45], and their role in structuring algae and coral communities [40, 46–51].

In this study, we investigate the intestinal microbial diversity of ten species of planktivorous and algae-farming damselfishes, two guilds of damselfishes that significantly impact coral reef trophic dynamics. Planktivorous damselfishes play a key role transferring energy from the plankton to higher tiers of food chain, while algae-farming damselfishes influence sediment and algae dynamics on coral reefs and may increase the presence of coral disease associated pathogens within their territories [35, 40, 49, 52–54]. The aim of this study is to describe the taxonomic composition of planktivorous and algae-farming damselfish intestinal microbiomes. Thus, we hypothesise that differences in intestinal microbial communities will reflect the differences between these two feeding guilds. Specifically, across the different host species and feeding guilds, we examined (1) the phylogenetic differences in microbial

communities, (2) the core microbial members, and (3) the changes in microbial community structure along the length of the intestinal tract.

Methods

Species collections and dissections

Fishes were collected from the Heron Island lagoon in the southern Great Barrier Reef, Australia (23°26’53”S, 151°56’52”E) in January and February of 2015. Collections occurred at a depth of 1–8 m adjacent to the Heron Island Research Station. Three individuals of ten damselfish species of similar lengths were randomly collected across the two trophic guilds(Table 1). Each trophic guild was represented by five species and 15 individuals. Collections were conducted on SCUBA, and the planktivorous species were collected using a barrier net, while the algae-farming species were collected by speargun. Following collections, the fishes were immediately placed on ice and transported to Heron Island Research Station. In the laboratory under sterile conditions, fishes were weighed, measured and photographed, then the gastrointestinal tract was removed, and the gut length was recorded and photographed. The entire gut was fixed in 4% DNA/RNA free paraformaldehyde and sterile phosphate-buffered saline for 12 hours, then it was stored in DNA/RNA free water.

Table 1
List of planktivorous and algae-farming damselfish collected in this study with morphological characteristics. Three individuals from each species were collected. Inferred trophic level data was obtained from www.fishbase.us.

Species	Tail length (cm; ± sd)	Inferred trophic level (± se)
Planktivorous		
Abudefduf sexfasciatus	8.8 ± 1.7	2.7 ± 0.3
Abudefduf whitleyi	16.4 ± 1.4	2.6 ± 0.3
Acanthochromis polyacanthus	8.4 ± 0.8	2.7 ± 0.3
Chromis atripectoralis	9.5 ± 0.6	3.1 ± 0.1
Pomacentrus moluccensis	6.5 ± 1.5	2.4 ± 0.1
Algae-farming		
Dischistodus perspicillatus	18.2 ± 2.5	2.0 ± 0.0
Dischistodus pseudochrysopoecilus	14.6 ± 0.6	2.0 ± 0.0
Pomacentrus wardi	7.5 ± 1.0	2.0 ± 0.0
Stegastes apicalis	12.7 ± 1.6	-
Stegastes nigricans	13.1 ± 1.0	2.2 ± 0.0

DNA extraction and 16S rRNA MiSeq Illumina Sequencing

Samples were transported to James Cook University for subsampling along each intestinal tract and DNA extraction. Under sterile conditions, standardized biopsy cores were taken and from four locations along the intestinal tract: the stomach, the anterior intestine, the mid-intestine, and the posterior intestine. DNA was extracted from tissue biopsies using a QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) following the manufactures guidelines. A nanodrop was used to record the quality (260/280 ratio) and quantity (ng/μL) of DNA from each extraction.

Amplification of the 16S V1-V3 rRNA gene region was done using the primers 27F (5'-AGRGTTTGATCMTGGCTCAG-3') [55] and 519R (5'-GTNTTACNGCGGCKGCTG-3') [56] with barcodes on the forward primer. These genes were amplified in a 30 cycle PCR (5 cycle used on PCR products) using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94 °C for 3 minutes, followed by 28 cycles of 94 °C for 30 seconds, 53 °C for 40 seconds and 72 °C for 1 minute, after which a final elongation step at 72 °C for 5 minutes was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together (e.g., 100 samples) in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Then the pooled and purified PCR products were used to prepare a DNA library by following Illumina TruSeq DNA library preparation protocol. Sequencing was performed at the Molecular Research LP (MR DNA; Texas, USA) on a MiSeq™ System following the manufacturer's guidelines.

Amplicon sequence data were sorted by the sample and demultiplexed using *demux* for QIIME 2 (version 2018.11; Bolyen *et al.* 2018). Sequences were screened for quality, trimmed at 450 bp after removal of primer sequences and assigned as amplicon sequence variants (ASVs) [58] using DADA2 [59]. Taxonomy of the ASVs was determined using a pre-trained, naïve Bayes classifier [60] and the q2-feature-classifier plugin [61]. The classifier was trained on the target 480 bp region of sequences in the Greengenes 13_8 99% database. ASV clusters were arranged in a phylogenetic tree using FastTree [62, 63] and visualised using Interactive Tree of Life [64]. The feature table, metadata and taxonomic classifications were exported from QIIME 2 in .biom format [65], and the rooted phylogenetic tree was exported in .nwk format. The closest known sequences and the origin of selected ASVs were identified through a BLASTN-based search against the GenBank nr/nt database.

Statistical analysis

The exported feature table and phylogenetic tree were imported into R version 3.5.2 (R Core Team 2019) and stored as a *phyloseq* object [66] for downstream analyses. All ASVs not assigned to phylum were filtered from the data, and those designated as chloroplasts or cyanobacteria were removed and stored as a separate object for further analysis. Samples were rarefied to minimum sampling depth for diversity analyses; however, non-rarefied data were used for multivariate modelling [67, 68]. Multivariate generalised linear models were used to test for significant differences in bacterial communities among host fish species, trophic guild and location along intestines using *mvabund* in R [69, 70]. Bacterial taxa

were grouped by class when examining microbiome changes along the length of the intestinal tract. Bacterial community data were fitted to negative binomial distributions and tested using log-likelihood ratios (LRT) via 999 simulations using Monte Carlo resampling. A nested analysis of variance (ANOVA) used to test the role of gut location when accounting for species variation. Traditional distance-based ordination methods to visualise variation across communities, such as non-metric multidimensional scaling (NMDS) and principal coordinate analysis (PCoA) may confound trends [71]. To avoid these issues, the R package *boral* [72] was utilised to explain the bacterial community composition of each sample through a set of latent variables. Bacterial community data were fitted to a negative binomial distribution, and the model was run with two latent variables to account for residual variation for each of the major phyla detected in the samples. Venn diagrams were produced using the *VennDiagram* package [73].

Results

A total of 1,254,909 sequences were detected in 119 samples after denoising and trimming of all chloroplast, mitochondria sequences and host DNA. Among these sequences, 3,776 ASVs were detected; 39.4% of which belonged to the Phyla Proteobacteria, 26.2% to Bacteroidetes, 13.4% to Firmicutes and 12.6% to Planctomycetes. The 20 most abundant ASVs accounted for 41% of the total number of detected sequences. The most common ASV belonged to the genus *Actinobacillus* and accounted for 9.9% of the total detected sequences (Table 2). A further two unknown members of Mollicutes and Pasteurellacea accounted for 9.9 and 3.8% of sequences, respectively.

Table 2
Sequence abundance and taxonomy for each ASVs representing more than 1% of total sequences. Accession numbers for closest GenBank sequences (similarity given in brackets) are supplied.

ASV	Phylum	Lowest taxonomic division	Number of Sequences	Proportion of total (%)	GenBank Accession Number
b727	Proteobacteria	*Actinobacillus* sp.	124,499	9.9	KT952745 (97.5%)
5647	Tenericutes	Mollicutes	87,057	6.9	HG971018 (96.3%)
94ba	Proteobacteria	Pasteurellacea	47,527	3.8	KT952745 (93.5%)
3023	Firmicutes	Ruminococcaceae	26,355	2.1	MG488771 (98.8%)
6350	Tenericutes	Mycoplasmataceae	24,219	1.9	LN612674 (91.5%)
9b2f	Proteobacteria	Pasteurellacea	24,219	1.9	KT952745 (91.9%)
d532	Proteobacteria	Alteromonadales	23,877	1.9	KT952746 (100.0%)
5a8a	Proteobacteria	*Vibrio ponticus*	22,112	1.8	MG524941 (100%)
7936	Proteobacteria	Alteromonadales	15,147	1.2	KT952746 (99.8%)
596f	Proteobacteria	Gammaproteobacteria	14,436	1.2	LC121875 (88.4%)
73d1	Proteobacteria	*Vibrio* sp.	13,977	1.1	KT952854 (98.7%)
6013	Proteobacteria	Pasteurellacea	13,435	1.1	KT952745 (92.3%)
af86	Firmicutes	*Clostridium colinum*	13,177	1.1	KC993540 (94.2%)

Different levels of ASV richness were detected for each host fish species. *Dischistodus perspicillatus* has the greatest mean richness of ASVs, with a total of 322 ± 17 ASVs per individual. The species with the lowest ASV richness are *C. atripectoralis* and *A. sexfasciatus* with 47 ± 21 and 30 ± 8 ASVs per individual, respectively (Fig. 1). Shannon diversity is greatest for three algae-farming species *D. perspicillatus, Stegastes nigricans* and *S. apicalis* and lowest for the planktivorous species *C. atripectoralis, A. sexfasciatus* and *Pomacentrus moluccensis.*

An ordination analysis revealed that most host fish species have distinct Proteobacteria, Bacteroidetes and Firmicutes communities (Fig. 2). *Abudefduf sexfasciatus* and *Abudefduf whitleyi* displayed high

variation in Proteobacteria communities while the two trophic guilds have similar community composition. Bacteroidetes are distinct for *A. sexfasciatus* and *Stegastes apicalis*, with no discernible patterns between the two trophic guilds. Communities of Firmicutes are the most distinct between host species, although some host species, such as *S. apicalis*, *Chromis atripectoralis* and *A. whitleyi*, are variable in composition. However, there is reasonable separation of the two trophic guilds in terms of Firmicutes community composition .

Core Microbiomes

Most ASVs occur in less than 30% of sampled individuals across all host species (Fig. 3a). 13 bacterial ASVs are found in more than 30% of sampled individuals; therefore, they may represent core members of pomacentrid microbiomes (Table 3). The most common ASV in this study belongs to the genus *Actinobacillus*, which occurs in more than 80% of sampled individuals, albeit at a low abundance in many individuals, with the highest abundances in the planktivorous damselfishes *Acanthochromis polyacanthus* and *P. moluccensis*.

Table 3
Taxonomic composition of core ASVs occurring in more than 80% of sampled individuals. Accession numbers for closest GenBank sequences (similarity given in brackets) are supplied. Occurrence and relative abundances were generated from rarefied data.

ASV	Phylum	Lowest taxonomic division	Occurrence (%)	Relative Abundance	GenBank Accession Number
b727	Proteobacteria	*Actinobacillus* sp.	83.3	0.083	KT952745 (97.5%)
94ba	Proteobacteria	*Actinobacillus* sp.	53.3	0.017	KT952745 (93.5%)
9bd9	Proteobacteria	*Photobacterium damselae*	43.3	0.013	CP035457 (100%)
5647	Tenericutes	Mollicutes	40.0	0.022	HG971018 (96.3%)
a832	Proteobacteria	*Photobacterium damselae*	40.0	0.008	CP018297 (100%)
73d1	Proteobacteria	*Vibrio* sp.	40.0	0.010	KT952854 (98.7%)
9b2f	Proteobacteria	*Actinobacillus porcinus*	40.0	0.018	KT952745 (91.9%)
6c33	Proteobacteria	Spirobacillales	37.7	0.002	KU578602 (100%)
dc1c	Proteobacteria	*Vibrio* sp.	37.7	0.004	CP033144 (100%)
5a8a	Proteobacteria	*Vibrio ponticus*	37.7	0.019	MG524941 (100%)
762a	Bacteroidetes	*Lutimonas* sp.	30.0	0.001	MG488523 (99.6%)
ca47	Proteobacteria	*Vibrio harveyi*	30.0	0.009	CP033144 (100%)
6013	Proteobacteria	Pasteurellaceae	30.0	0.007	KT952745 (92.3%)

Significant variation in the richness of core bacterial assemblages for each fish species (defined as ASVs that are shared between all sampled individuals for each species) were also detected (Fig. 3b). For example, there are 70 bacterial ASVs shared between the three sampled individuals of *D. perspicillatus* and only two ASVs shared between the three *A. sexfasciatus* individuals. Core microbiomes within host species are more rich in algae-farming species than planktivorous species, with algae-farming host species sharing 35 ± 22 ASVs and planktivorous species sharing only 7 ± 3 ASVs (Wilcox test W = 25, p = 0.008).

Core ASVs that occur in all three sampled individuals of a host fish species belong to the bacterial phyla Bacteroidetes, Firmicutes, Tenericutes, Spirochaetes, Planctomycetes, Proteobacteria and Verrucomicrobia. The core ASVs *Coraliomargarita* sp. and Verruco-5 (Verrucomicrobia), Pirellulaceae (Planctomycetes) and Desulfovibrionaceae (Deltaproteobacteria) occur in all three sampled *D. perspicillatus* individuals (Supplementary Fig. 1). There is high richness of core Pasteurellaceae and Vibrionales ASVs, with 10 and 21 core members, respectively. High diversity of an unknown clade of Gammproteobacteria is also reported for *P. moluccensis* and *Pomacentrus wardi* hosts .

There are 61 core ASVs belonging to the Bacteroidetes, 28 of which occur in *S. apicalis* and 38 in *P. perspicillatus* (Supplementary Fig. 2). An unknown clade of Flavobacteriales and a diverse consortium of Rikenellaceae are core members of *S. apicalis*, while *P. perpicillatus* has a diverse core assemblage of ASVs belonging to the family Flavobacteriaceae. One ASV belonging to Spirochaetes, *Brevinema andersonii*, is a core member of *S. nigricans* and *C. atripectoralis*, while a Tenericutes ASV belonging to Mollicutes is a core member of all host species except the planktivorous damselfishes *A. polyacanthus* and *A. sexfasciatus* (Supplementary Fig. 3). There is a rich consortium of core Firmicutes ASVs for *S. apicales* and *S. nigricans*, which includ members of the Erysipelotrichaceae, Ruminococcaceae and Lachnospiraceae families.

Bacterial shifts along the intestinal tract

Bacterial communities significantly shift in composition along the intestinal tract (LRT = 1263, P = 0.001; Supplementary Table 1). Nine classes of bacteria have significantly change in abundance across the different fish species and locations along the intestinal tract ($P < 0.05$; Fig. 4). Members of Gammaproteobacteria were especially common throughout the all the planktivorous host species' intestinal tracts, but also in high abundance along all locations of the *D. perspicillatus*, *D. pseudochrysopoecilus* and *P. wardi* intestines. Gammaproteobacteria are less common in the intestines of *S. apicalis*, making up only a small component of the posterior intestines in this algae-farming host. In intestinal locations where Gammaproteobacteria are uncommon, members of Bacteroidia and Clostridia are generally found at higher abundances – especially for algae-farming host species. Members of Mollicutes and Planctomycetia are more common throughout the intestinal tracts of algae-farming hosts than planktivorous hosts although their abundances are generally lowest within the stomach region. The stomach has 286 unique bacterial ASVs, the anterior intestine 753, while 1139 and 656 ASVs are only found in the mid and posterior intestines, respectively. Only 19 ASVs are common to the stomach and posterior intestine while 152 ASVs are found throughout the intestine (Fig. 5).

Effect of trophic guild on microbiomes

There is a significant difference between trophic guilds and microbiome composition (LRT= -0.021, P = 0.001). Most bacterial ASVs are unique to either of the trophic guilds of the host fish, with only 124 ASVs common to both guilds (Fig. 5). 78 bacterial ASVs, belonging to 20 families, are important drivers of this relationship. There are marked differences in abundances of ASVs belonging to Vibrionaceae, Lachnospiraceae and Pasteurellaceae. Two *Vibrio* sp. (Vibrionaceae) are more common in planktivorous host species, and five members of *Actinobacillus* are more abundant in algae-farming host species. None of the ASVs occurred exclusively in the planktivorous or algae-farming damselfishes, suggesting that this relationship is driven by host species rather than trophic guild.

Discussion

This study reveals that algae-farming damselfish species have taxonomically richer core microbiomes than planktivorous species. This result is likely attributable to the specialised feeding behaviour of these species where they largely consume a narrow range of turf algae species [37, 39, 49], unlike planktivorous species which are adapted to a more opportunistic feeding strategy. We also provide evidence that algae-farming damselfish tend to have more diverse intestinal microbiomes than planktivorous species. These results show that microbiome structure of host fish species that have specialised feeding behaviour have acquired specialised intestinal bacteria and further research is needed to investigate how microbiome specialisation effects host digestion and metabolism.

Like many other species of marine fish, the intestinal microbiomes, the damselfish microbiomes presented here were dominated by members of Proteobacteria, Bacteroidetes, Firmicutes and Planctomycetes. For example, surgeonfish, parrotfish and rabbitfish intestinal microbiomes from the Red Sea also consist of diverse assemblages of Firmicutes and Proteobacteria [15]. Another dominant ASV in the damselfish microbiome belonging to Mollicutes (Tenericutes) resembled bacteria detected in rabbitfish intestines [23]. The number of highly similar bacterial ASVs shared among pomacentrids, acanthurids and siganids may reflect the similar feeding behaviours of these coral reef fishes. For instance, algae-farming damselfishes may also ingest prey items other than algae, such as zooplankton [36] or other invertebrates [75]. The functional roles of these seemingly important microbial taxa warrant further attention in order to understand the potential consequences on host metabolism and health.

Damselfish microbiomes were largely dominated by Gammaproteobacteria of the Pasteurellaceae, with one ASV occurring in more than 80% of sampled fishes and representing almost 10% of total detected sequences. Although this ASV currently represents an unknown species of the *Actinobacillus* genus, a 98% similar sequence has been collected from the intestines of surgeonfishes in Saudi Arabia [25], suggesting these taxa are important components of reef fish microbiomes. Members of Pasteurellaceae have also been recorded in high abundances in adult damselfishes and cardinalfishes collected around Lizard Island, Australia [76], and they are deemed important components of tropical planktivorous fish gut microbiomes [77]. Gammaproteobacteria are also very abundant on the skin of many coral reef fishes

[78]. The prevalence of Pasteurellaceae amongst the damselfishes in this study, as well as other reef fishes, provides additional evidence that Pasteurellaceae are important members of coral reef associated fish microbiomes.

Algae-farming damselfishes had larger core microbiomes than the planktivorous damselfishes, and these core microbiomes were specific to each host species. For example, *P. wardi* and *P. moluccensis* had diverse, but different strains of Gammaproteobacteria, while *D. perspicillatus* and *S. apicalis* had large Bacteroidetes core communities but were dominated by Flavobacteriaceae and Rikenellaceae, respectively. Different species of territorial damselfishes farm and consume different species of algae [37, 49], and the large differences in their specialized microbiomes may reflect these narrow dietary preferences. Conversely, the small core microbiomes of the planktivorous damselfishes may reflect the high variation in consumed plankton of each species, suggesting these fishes have opportunistic feeding behaviours. These results, however, do not support the notion that fish with greater diet variability have more diverse microbiomes [26]. In fact, the damselfish with narrow, algae-farming feeding behaviours tended to have the greatest diversity of intestinal bacterial, suggesting that the host may select microbial populations that include specialised bacteria that enhance the digestion and absorption of nutrients from specific algal diets.

Recent evidence suggests a high degree of resource partitioning in fish communities which is a key mechanism that facilitates the high diversity of coral reefs [79, 80]. The largely distinct microbiomes of each host species presented in this study may reflect the high degree of resource partitioning found in coral reef communities, whereby different species of damselfish may be consuming different size classes of zooplankton [79], farm different algal species [37, 49] or occupy different trophic niches [80]. The similarity between closely related host species and microbiomes, such as *P. wardi* and *P. moluccensis*, also demonstrates that phylogeny may influence the intestinal microbiomes of damselfishes [15–17, 78].

Interestingly, *Photobacterium damselae, Vibrio harveyi, Vibrio ponticus* and other *Vibrio* sp. were prevalent amongst the damselfishes sampled in this study. These bacteria represent potential pathogenic members of Vibrionacaea and have been detected in many fishes of aquaculture importance, including *Chromis punctipinnis* [81], *Lutjanus argentimaculatus* [82], *Seriola dumerili* [83], *Scophthalmus maximus* [84, 85], *Sparus aurata* [86], *Solea quinqueradiata* [87] and *Solea senegalensis* [88]. Although identified as *Vibrio harveyi* in the GreenGenes database, GenBank revealed there was high similarity of these sequences to other members of the Harveyi clade, such as *Vibrio owensii* [83]. There are thought to be up to 11 species of *Vibrio* belonging to this clade [89], most of which are pathogens of fish, shrimp and coral [90–92]. Given the apparent healthy state of the sampled fishes and the high abundances of potentially pathogenic *Vibrionacaea* in the fish guts, we provide support to the idea that these organisms are natural components of healthy fish microbiomes and are opportunistic pathogens in fishes only under specific conditions [82, 93].

The facultative anaerobic bacterial classes Bacteroidia, Clostridia and Mollicutes were generally in higher abundance in the mid and posterior intestinal regions than the stomach. Differences in microbiomes

along the intestinal tract have been recorded in the rabbitfish *Siganus fuscescens* [94], with midgut communities more representative of the environmental sources and hindguts hosting a microbiome more specialised to anaerobic conditions and fermentation [95]. The increase in Bacteroidia, Clostridia and Mollicutes along the intestines may be due some members of the class being mutualistic components of the fish gastrointestinal flora. Some members of Bacteroidetes are known to breakdown polysaccharides and metabolise the derived sugars [96], while members of *Clostridium* are known to metabolise cellulose [30]. Our results confirm the increased prevalence of anaerobic bacteria in the hindgut of damselfishes, which probably consists of taxa responsible for the fermentation and metabolism of complex molecules before being absorbed by the host [3].

Conclusions

In this study, we demonstrate that damselfishes have diverse intestinal microbial communities whereby the core members of a species reflect diet and trophic guild. We show that algae-farming damselfishes have more rich core microbiomes, which may reflect the more specialised diets of these species. We also provide evidence that damselfish mid and posterior intestines have higher abundances of facultative anaerobic bacteria that are known to play important roles in fermentation and cellulose breakdown. These findings add to a growing body of literature that suggests that host fish feeding behaviour has a strong influence on the composition of intestinal microbiomes.

Declarations

Ethics approval and consent to participate

All work was authorized by James Cook University, permitting limited impact research under the university's research accreditation in the Great Barrier Reef Marine Park.

Consent for publication

Not applicable

Availability of data and material

The Illumina MiSeq datasets for each damselfish species are available at the Sequence Read Archive (NCBI) repository under BioProject accession number PRJNA638998, https://www.ncbi.nlm.nih.gov/sra. Data and R-scripts used in this study are available at https://github.com/ChrisKav/WildDamselfishMicrobiomes.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable

Authors' contributions

CRJK analysed and interpreted the amplicon sequence data and was the major contributor in writing the manuscript and preparing figures and tables. JMC undertook the fieldwork and collected all specimens, performed gut dissections, tissue biopsies and provided feedback on the manuscript. JHC was involved with the initial synthesis and design of this study and provided feedback on the manuscript. WL and TDA were involved with the initial synthesis and design of this study, provided the facilities to undertake laboratory work and provided feedback on the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank Philip Munday for his involvement with the synthesis and design of this study. We also thank Simon Brandl, Ben Chapman, Alejandra Hernández Agreda, César Herrera, and the staff at Heron Island Research Station for field and logistical support.

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Figures

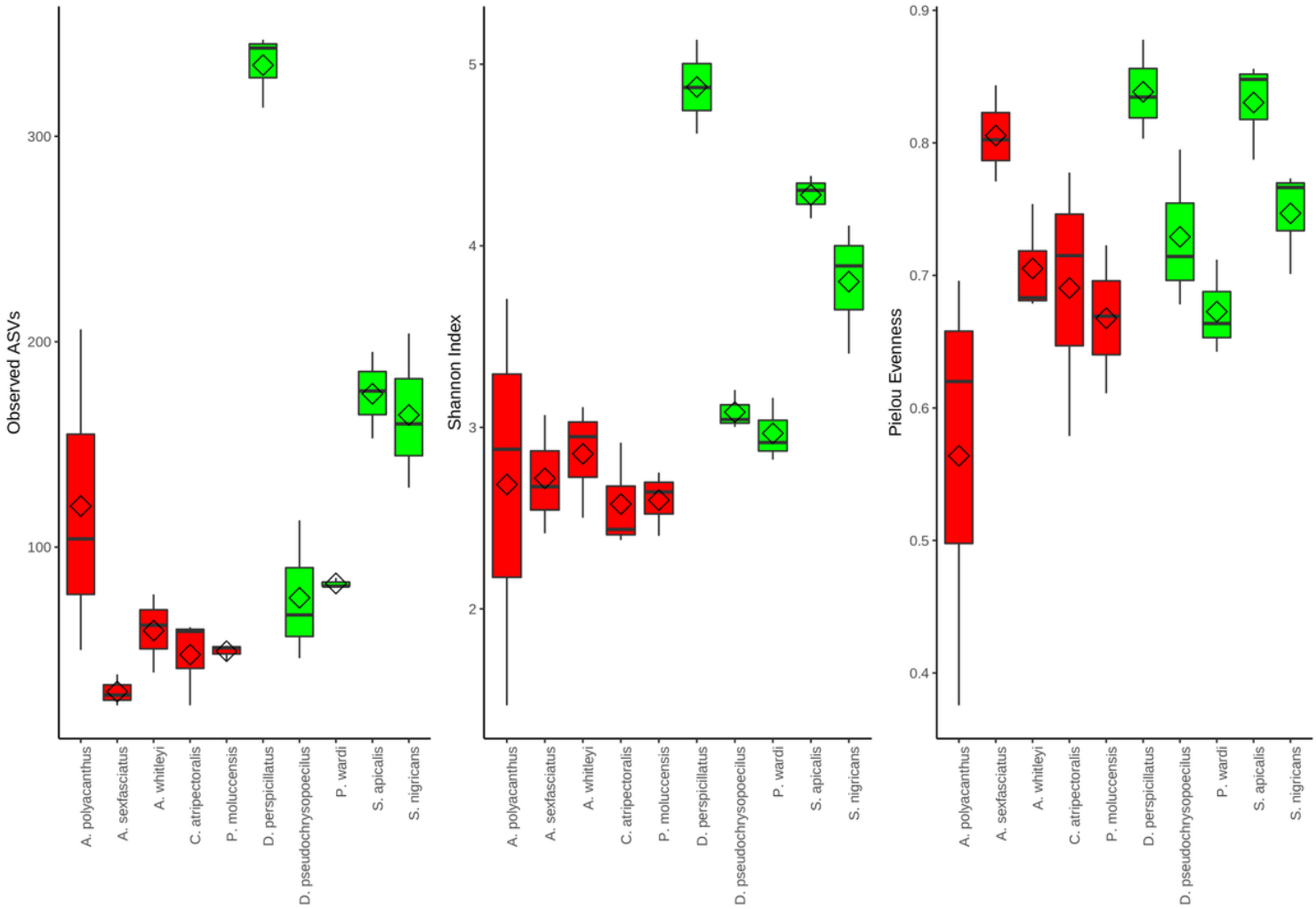
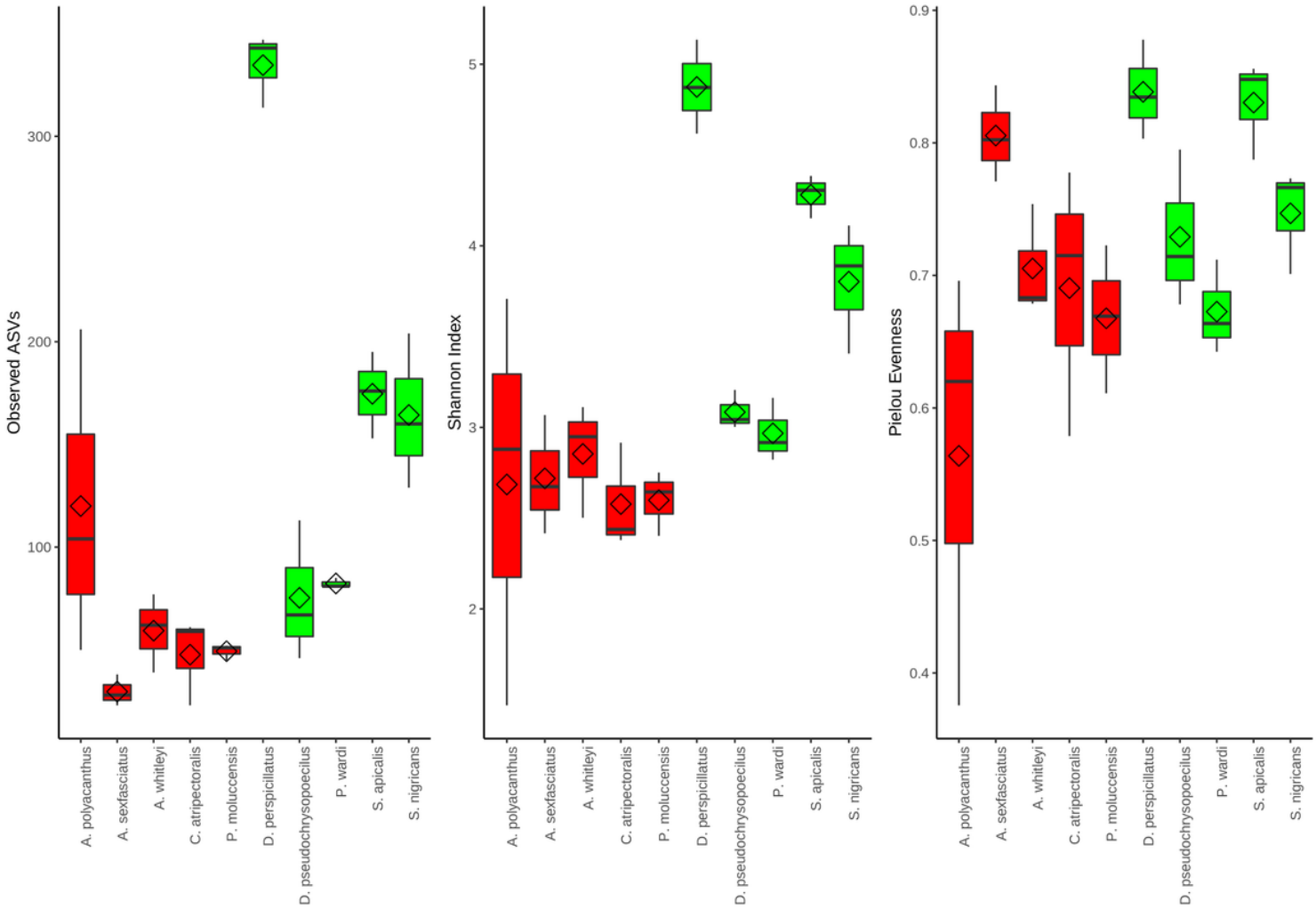


Figure 1

Mean observed richness, Shannon diversity and evenness for each fish species. Planktivorous host species are shaded red and algae-farming species shaded green. n=3.



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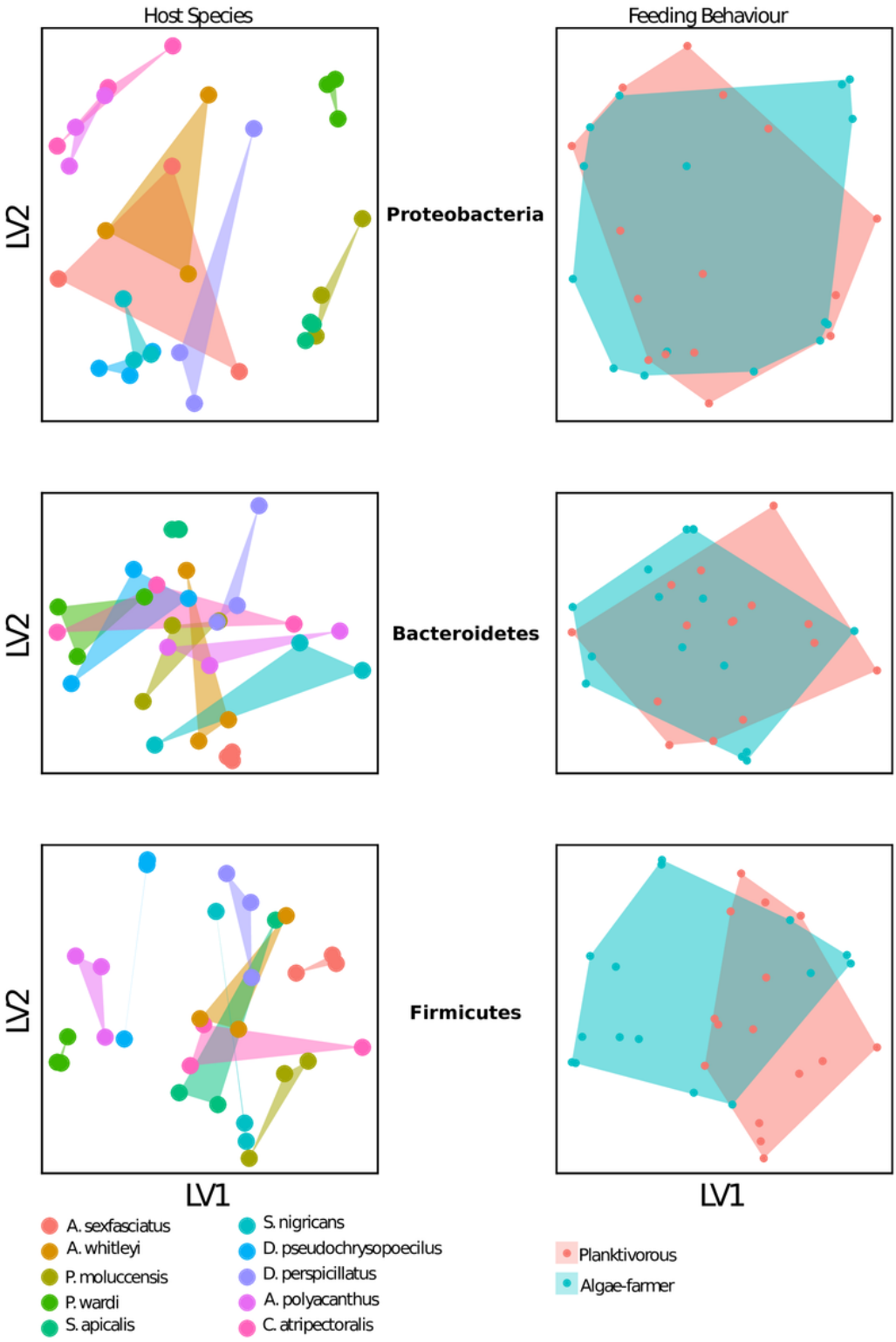
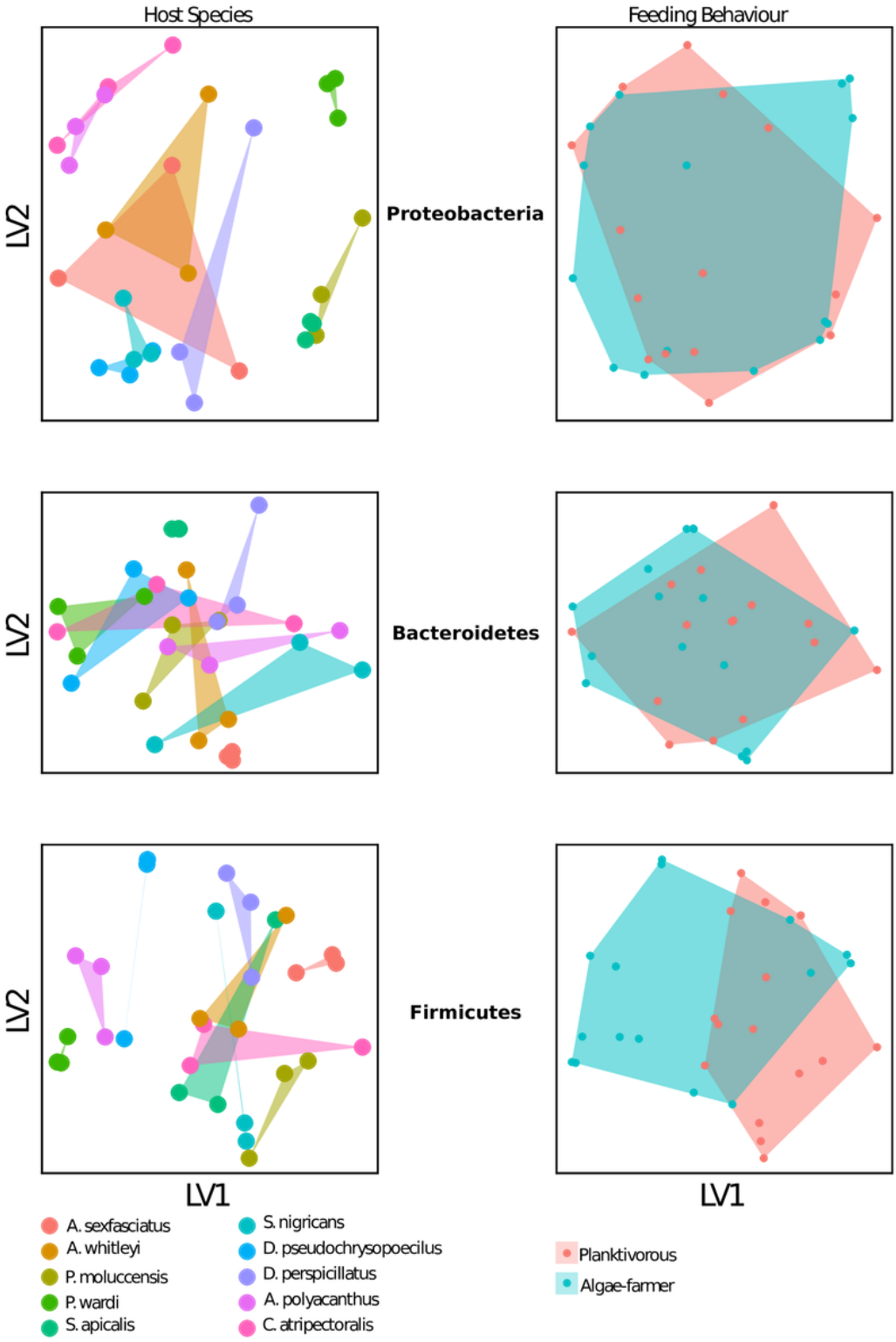


Figure 2

Biplots showing latent variable model (LVM) unconstrained ordinations of individual fish intestinal microbiomes for Proteobacteria, Bacteroidetes and Firmicutes. Ordinations are divided by host species (left) and trophic guild (right).



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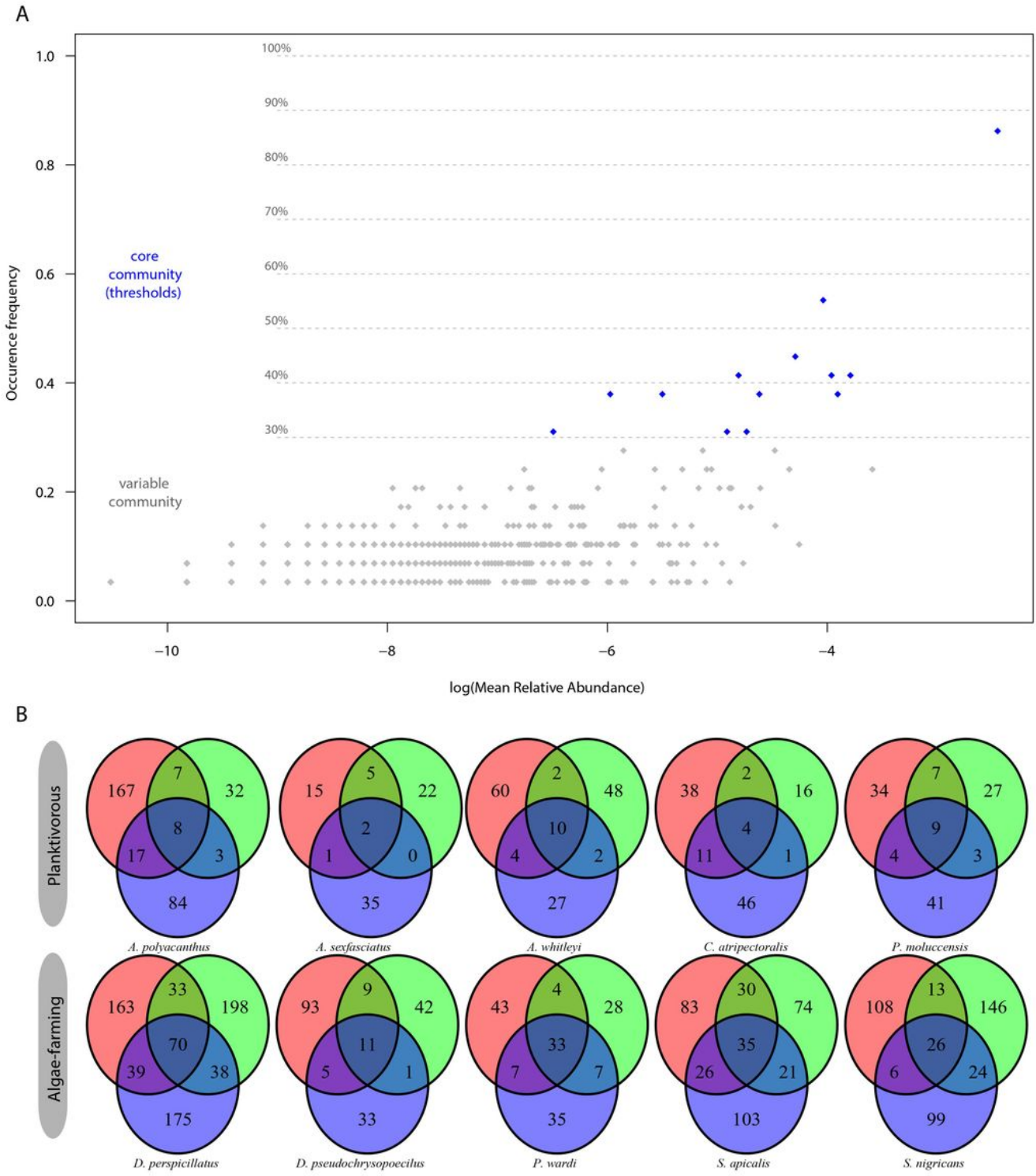
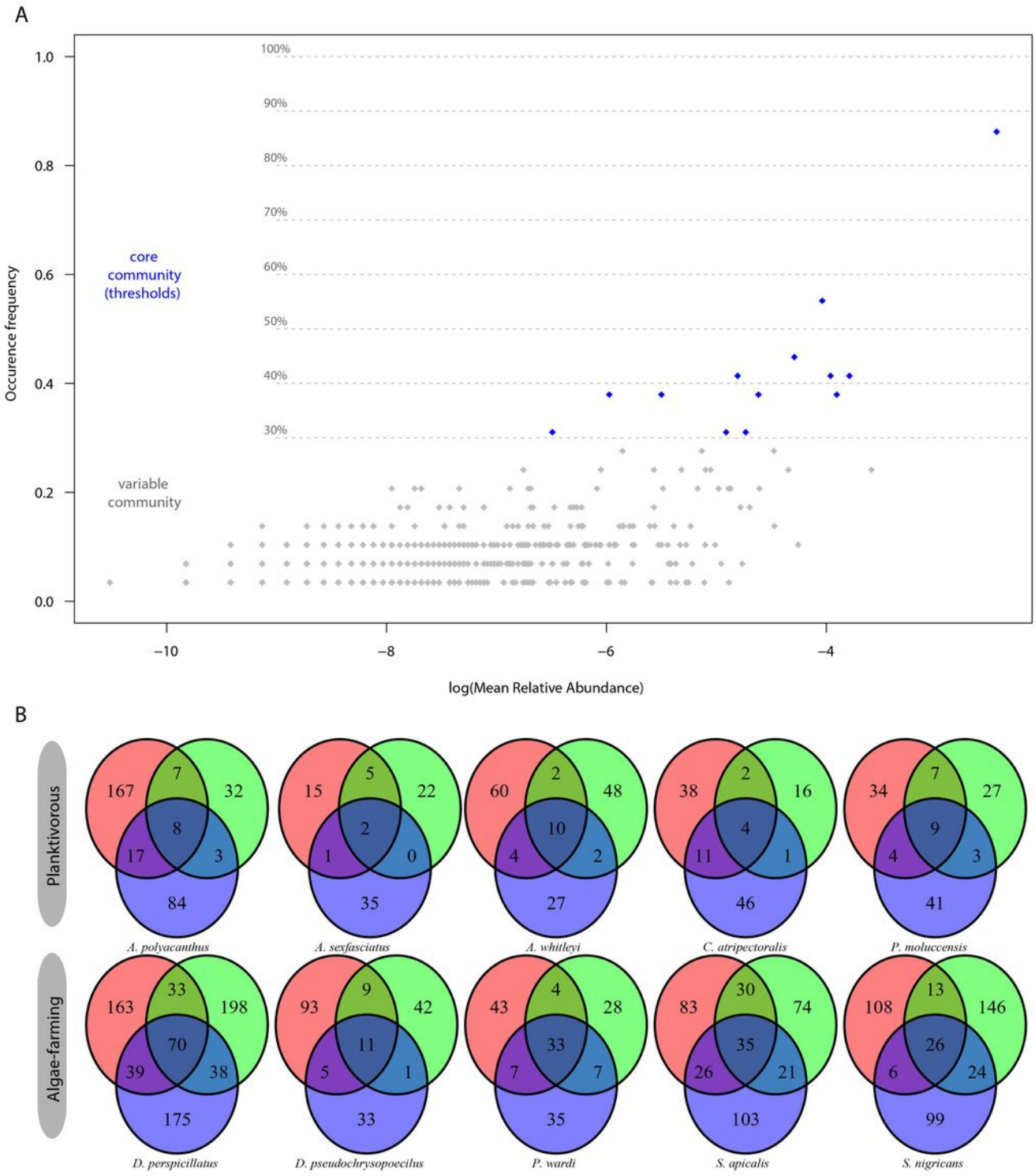


Figure 3

A) Core members of the microbiome (blue) at different threshold levels. The variable community represents ASVs occurring in less than 30% of sampled individuals. B) Venn diagrams depicting the number of ASVs shared between whole microbiomes of the three sampled individuals for each fish species. The top row represents planktivorous species and bottom row represent algae-farming species.



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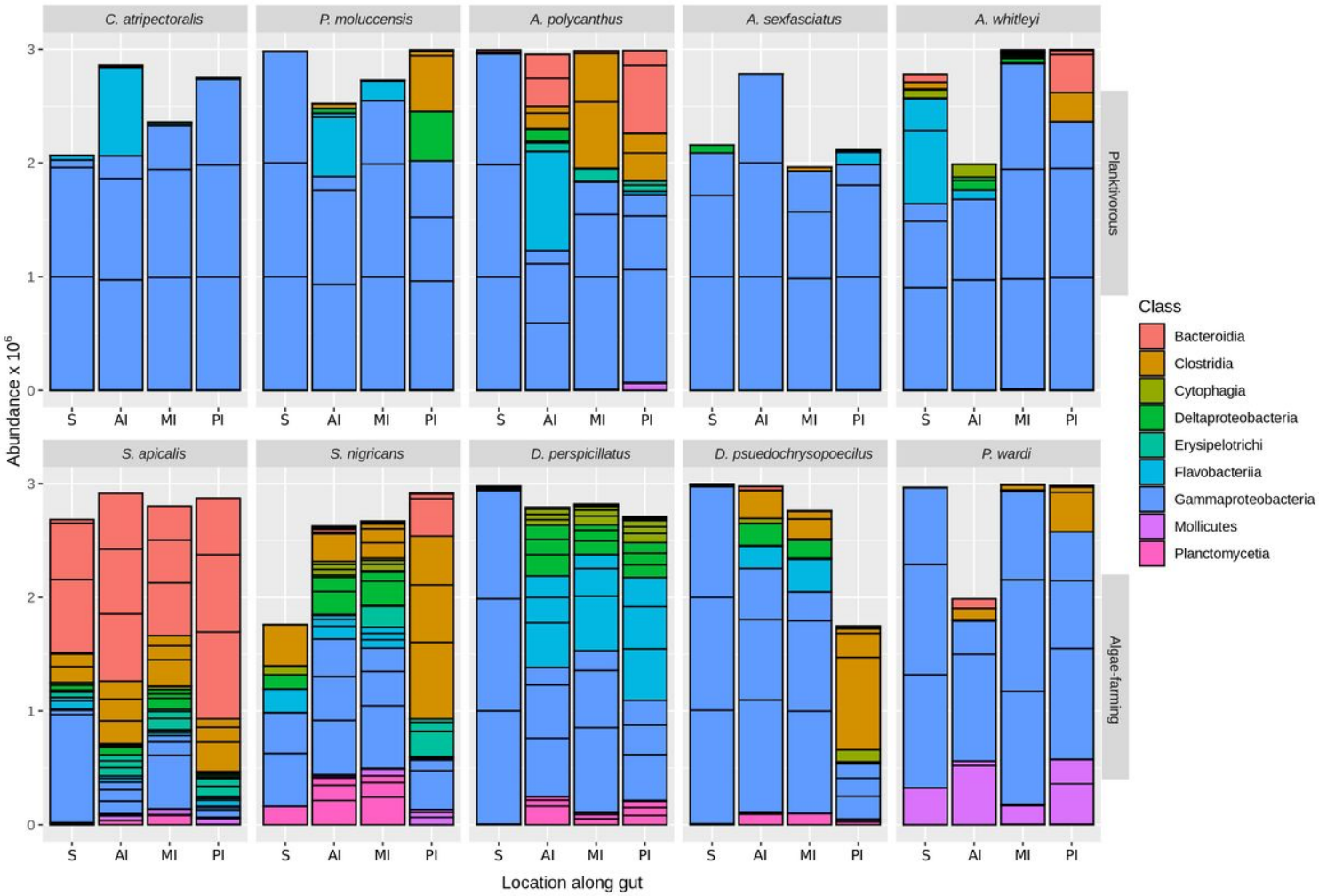
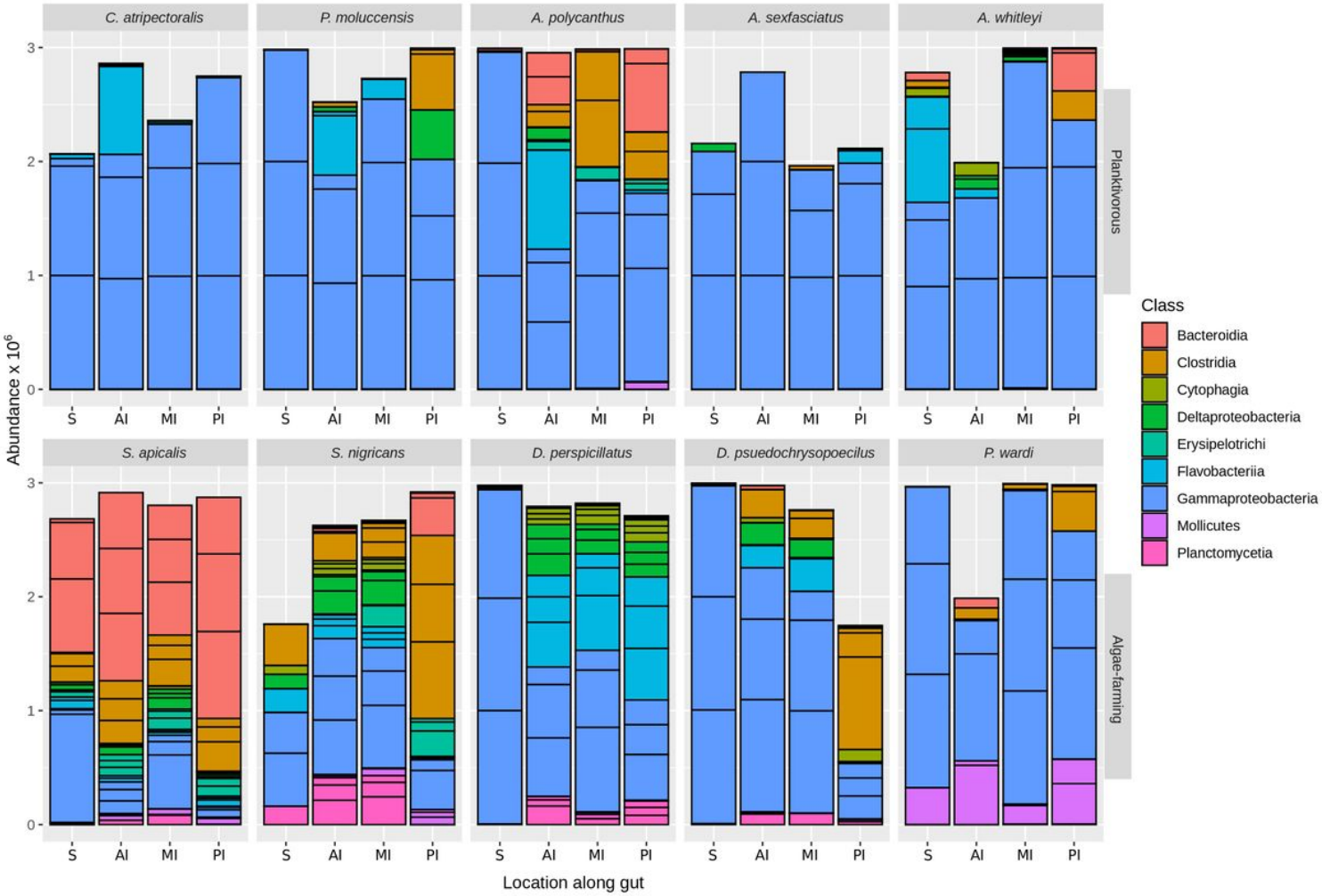


Figure 4

Changes in abundance of selected bacterial Classes along the four locations along the intestine for each species of damselfish as determined by nested multivariate generalised linear models. Intestinal locations include stomach (S), anterior intestine (AI), mid-intestine (MI) and the posterior intestine (PI).



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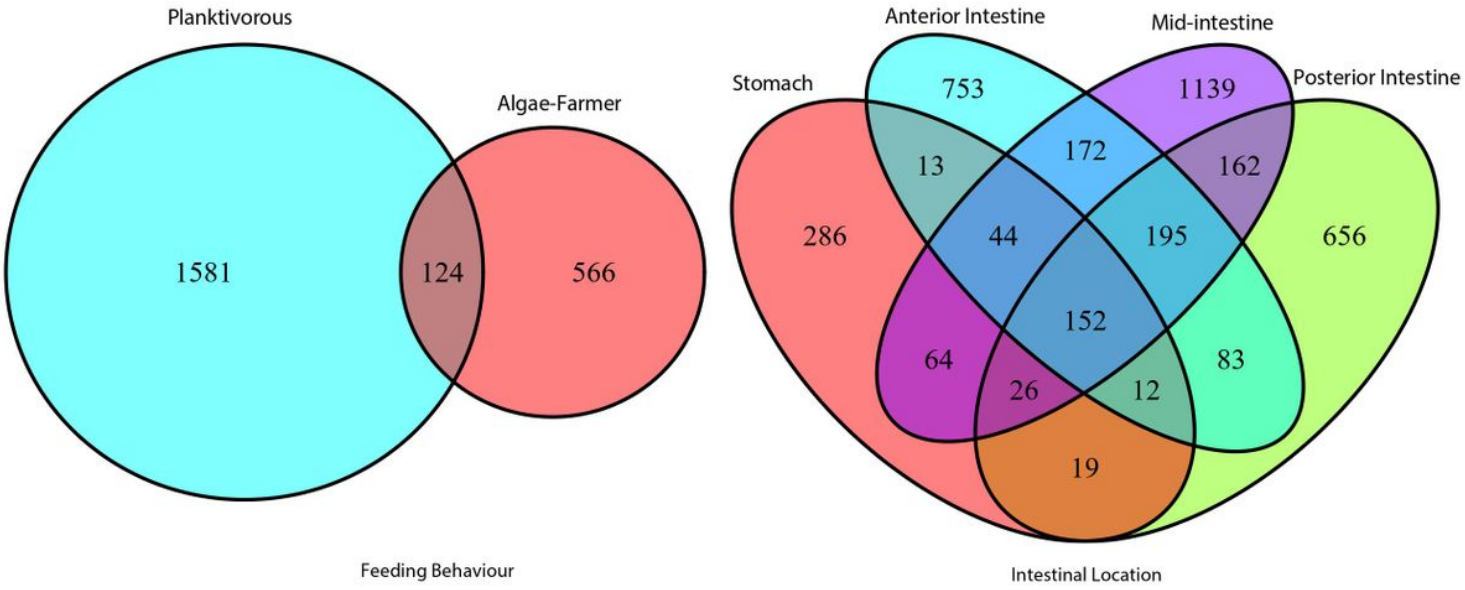
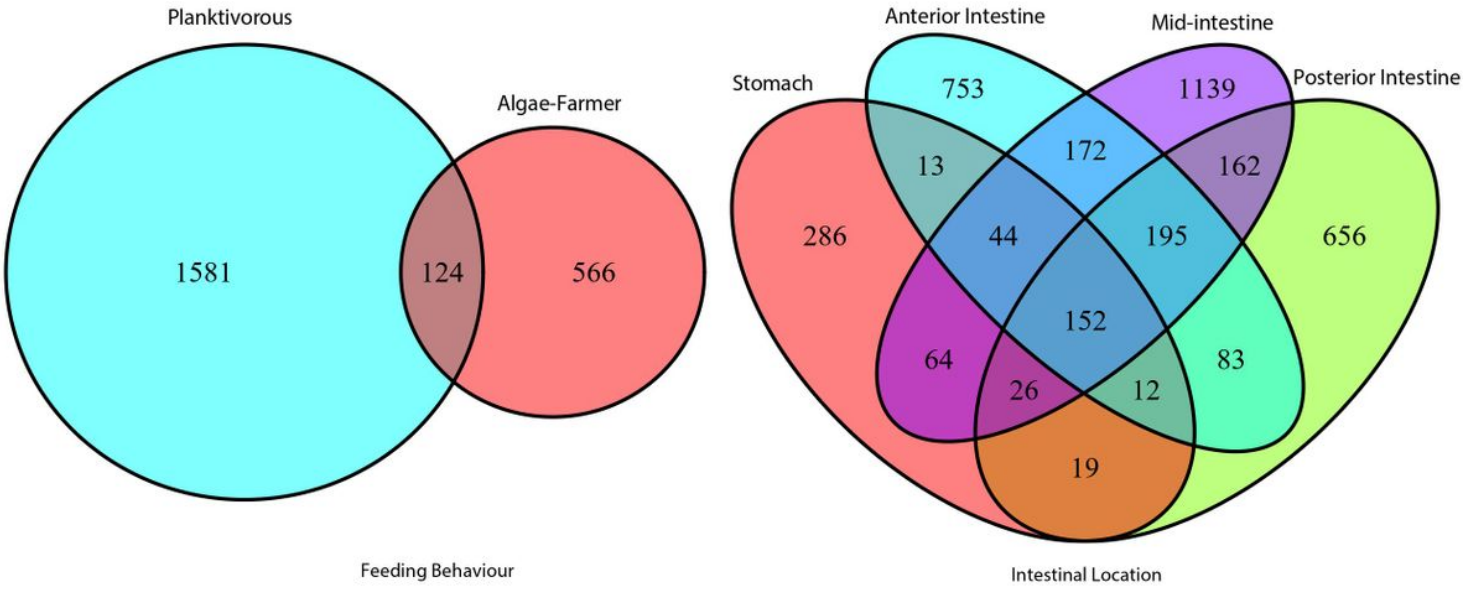


Figure 5

Venn diagrams depicting the number of shared ASVs for each trophic guild (left) and for each region of the intestine (right).



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Venn diagrams depicting the number of shared ASVs for each trophic guild (left) and for each region of the intestine (right).

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