Molluscan Research

ISSN: 1323-5818 (Print) 1448-6067 (Online) Journal homepage: www.tandfonline.com/journals/tmos20

Gut microbiome of New Zealand abalone (*Haliotis iris*): a Chatham Islands case study

Jinchen Guo, Leonie Venter, Shaneel S. Sharma, Stephen D. J. Archer, Donnabella C. Lacap-Bugler & Andrea C. Alfaro

To cite this article: Jinchen Guo, Leonie Venter, Shaneel S. Sharma, Stephen D. J. Archer, Donnabella C. Lacap-Bugler & Andrea C. Alfaro (09 Jun 2026): Gut microbiome of New Zealand abalone (*Haliotis iris*): a Chatham Islands case study, Molluscan Research, DOI: 10.1080/13235818.2026.2680807

To link to this article: https://doi.org/10.1080/13235818.2026.2680807

MOLLUSCAN RESEARCH
https://doi.org/10.1080/13235818.2026.2680807

Gut microbiome of New Zealand abalone (*Haliotis iris*): a Chatham Islands case study

Jinchen Guo [a], Leonie Venter [b], Shaneel S. Sharma [a], Stephen D. J. Archer [c], Donnabella C. Lacap-Bugler [a] and Andrea C. Alfaro [d]

[a]School of Science, Faculty of Health and Environmental Sciences, Auckland University of Technology, Auckland, New Zealand; [b]Biomedical and Molecular Metabolism Research, Faculty of Natural and Agricultural Science, North-West University, Potchefstroom, South Africa; [c]AgResearch Ltd, Grasslands Research Centre, Palmerston North, New Zealand; [d]Department of Animal Science and Coastal Marine Sciences Institute, University of California Davis, Davis, CA, USA

ABSTRACT

The New Zealand black-footed abalone, *Haliotis iris*, holds significant ecological, economic, and cultural value. Abalone from the Chatham Islands fisheries contribute substantially to the national catch, yet populations show marked variability in growth rates. To investigate whether this variability relates to the gut microbiome, sub-adult and adult abalone from four sites were assessed using morphometrics and 16S rRNA gene Illumina *MiSeq* sequencing. Adult abalone collected from Ascots Beach and Wharekauri were heavier, longer and had larger tissue areas than those from Owenga Harbour and Point Durham, whereas sub-adults showed no size differences. Gut content analysis revealed that fast-growing populations consumed more red algae and less green algae, while brown algae dominated digestion across all sites. Although alpha-diversity did not differ significantly among sites or ages (except at Point Durham), microbial beta-diversity varied significantly by both factors. Core taxa included *Psychrilyobacter*, *Mycoplasma*, *Vibrio*, *Propionigenium*, unassigned Bacilli, and *Blastopirellula*. Site- and age-associated microbiome differences may reflect seaweed availability and nutritional quality, warranting further investigation through targeted feeding trials. This study provides a reference baseline for future gut microbiota research on wild *H. iris* and highlights how algal nutrients and gut-bacteria-mediated digestion may contribute to population-level growth patterns, supporting sustainable fishery management.

ARTICLE HISTORY
Received 2 February 2026
Final version received 25 May 2026

KEYWORDS
Abalone gut microbiota; seaweed; 16S rRNA; Illumina sequencing; Chatham Islands; New Zealand gastropod fishery

Introduction

The commercial abalone fishery in New Zealand (NZ) is managed under the quota management system based on the 1996 Fisheries Act. The total allowable commercial catch is set within seven main regional quota management areas. The Chatham Islands pāua fishery (PAU4), based mainly on *Haliotis iris* and secondarily on *H. australis*, contributes a significant proportion (35.5%) of the national catch. Even though there is limited information on the status of PAU4, it is classified as a productive and abundant fishery (Fisheries`New`-Zealand 2021). Despite the $50 million (NZ dollars) revenue contributed by this economic sector annually (Stenton-Dozey *et al.* 2021), the growth and physiological performance of the wild abalone are not well documented. Wild *H. iris* grows asymptotically, with rapid growth in the early stages and slows down after maturation. There are at least two fundamental considerations when classifying the growth rates of wild *H. iris*. First, abalone growth patterns and rates need to be compared to the expected asymptotic growth trajectory of this species based on historic morphometric assessments. Second, comparing mean shell length and growth increments per year per age structure across different populations is also useful to define slow- and fast-growing populations (Ryder *et al.*, 2025).

Abalone populations in NZ exhibit high variability in growth rates, manifested in significant demographic variability across different spatial scales (McShane *et al.* 1994b; Naylor *et al.* 2006). Various factors, such as water temperature (Naylor *et al.* 2006), food availability, wave exposure and habitat structure (McShane *et al.* 1994b; McShane and Naylor 1995), are known environmental drivers influencing growth rates of abalone. Histopathological analyses of *H. iris* indicated that the differentiated growth rates could be associated with poor nutritional status among the local abalone populations (Copedo *et al.* 2024). In addition, metabolic pathway analysis showed that the fast-growing adult abalone utilised carbohydrates as their main energy source (Venter *et al.* 2022). These observations indicate

CONTACT Donnabella C. Lacap-Bugler donnabella.lacapbugler@aut.ac.nz; dlacapbu@aut.ac.nz School of Science, Faculty of Health and Environmental Sciences, Auckland University of Technology, Private Bag 92006, Auckland 1142, New Zealand

Supplemental data for this article can be accessed online at https://doi.org/10.1080/13235818.2026.2680807.

Published online 09 Jun 2026

that seaweed's dietary nutritional value, among all factors, is a main consideration for abalone growth and physiology. Not all seaweed species are equal in their nutritional value for abalone. In general, seaweed with low protein, high indigestible components or a single seaweed species is considered to have low nutritional value. Previous studies have demonstrated that seaweed species such as *Palmaria palmata* (Roussel *et al.*, 2019), *Undaria pinnatifida* (Troell *et al.*, 2006), *Gracilaria* spp. (Yusup *et al.*, 2020), and *Ulva* spp. (Naidoo *et al.*, 2006) often promote better abalone growth. Moreover, abalone fed with mixed seaweed diets of red and green algae or red and brown algae often showed better growth performance than single seaweed type diets (Naidoo *et al.*, 2006). Considering the influence of nutrition on growth differences and a complete digestive system in abalone, microorganisms (especially bacteria) in the gut of abalone (gut microbiota) could have a substantial influence on food digestion and may eventually contribute to abalone growth differences among different populations around the Chatham Islands.

The digestive-tract-associated microbiota contributes to nutrient digestion and absorption (Poore 1972) and the development of mucosa (Villasante *et al.* 2020). Moreover, the microbiota of abalone's digestive system contains abundant and diverse microorganisms, primarily bacteria, essential to the nutrient digestion of the hosts (Erasmus *et al.* 1997). Since most of the biochemical digestion occurs in the gastrointestinal region (gut) of the host's digestive system, it is useful to focus on the gut bacterial communities for compositional profiling and prediction of potential driving factors that may influence the bacterial communities. Unlike in oysters where parental influences have been observed in the gut microbiota of offspring (Schei *et al.* 2017; Unzueta-Martínez *et al.* 2022), wild abalone gut bacterial profiles are mostly prone to changes due to environmental parameters, such as seawater temperature (Wang *et al.* 2020), heavy metal concentrations (Yao *et al.* 2024), and diet (Bullon *et al.* 2025). Living in coastal intertidal and subtidal regions with fluctuating ambient oceanographic and habitat conditions makes wild abalone more susceptible to anthropogenic disturbances. In particular, the seaweed type consumed by abalone contributes to the shaping of the gut bacterial composition and diversity (Hur *et al.* 2023). For example, the Pacific abalone (*H. discus hannai*) in Japan and South Korea fed with brown macroalgae were observed to have abundant bacterial strains in the genera *Bifidobacterium*, *Lactobacillus*, *Clostridium*, *Psychrilobacter*, *Vibrio*, and *Mycoplasma* in their intestines (Sawabe *et al.* 1995; An *et al.* 2013; Hur *et al.* 2023). Abalone that grazed on red macroalgae tend to have significantly high abundance of *Bacillus*, *Faecalibacterium*, *Escherichia*, and *Shigella* in their digestive tract (Hehemann *et al.* 2012; Gerasimidis *et al.* 2020; Corino *et al.* 2021; De Vos *et al.* 2022). Since abalone gut bacteria are often specialised in digesting seaweed, evaluating the gut bacterial composition and diversity can provide insight into the types of algae processed, the adequacy of nutrient-digesting bacteria for seaweed metabolism, and whether the host experienced fasting or malnutrition, which can affect growth (Guo *et al.* 2026a).

The aim of this study was to characterise the microbiome of sub-adult and adult New Zealand black-footed abalone (*Haliotis iris*) from slow-growing ('stunted' or partially fished) and fast-growing (intensively fished) collection sites around the Chatham Islands and to quantitatively evaluate the algal gut composition. Characterising the gut microbial community of the wild abalone populations on the Chatham Islands can provide valuable information on the health and nutritional status of abalone and support management efforts towards the local fishery.

Materials and methods

Site descriptions and sample collections

Wild abalone were collected in March 2020 by commercial divers from four sites around the Chatham Islands (Figure 1): Ascots Beach (44°00′59.0″S 176°23′11.7″W; site 1), Wharekauri (43°42′18.9″S 176°35′04.7″W; site 2), Owenga Harbour (44°01′28″S 176°21′56″W; site 3), and Point Durham (44°00′24.8″S 176°40′54.2″W; site 4). Based on historic catch data, sites 1 and 2 (Ascots Beach and Wharekauri) are classified as high-fished areas (fast-growing), while sites 3 and 4 (Owenga Harbour and Point Durham) are classified as low recovery areas that are not fished often (slow-growing) (Naylor and Fu, 2016). At each collection site, 10 adults and 10 sub-adults (sexually mature with a shell length below 100 millimetres [mm]) *Haliotis iris* individuals were randomly collected by the diver and placed in a net bag. The animals were transported to a nearby holding tank facility in a bucket of fresh seawater and released into a flow-through system for interim housing while sampling was completed. Animals were collected under special permit (720, client number 9791209) issued by Fisheries New Zealand.

Abalone processing

Animals were processed within 15 min after they were collected, targeting one life stage collected from one collection site at a time. Abalone was removed from the tanks using a chipping technique, where a plastic blade was slid beneath the animal to prevent clamping. Abalone was weighed to the nearest 0.01 gram (g), and the shell lengths, widths and heights were measured to the nearest 0.10 mm using callipers.

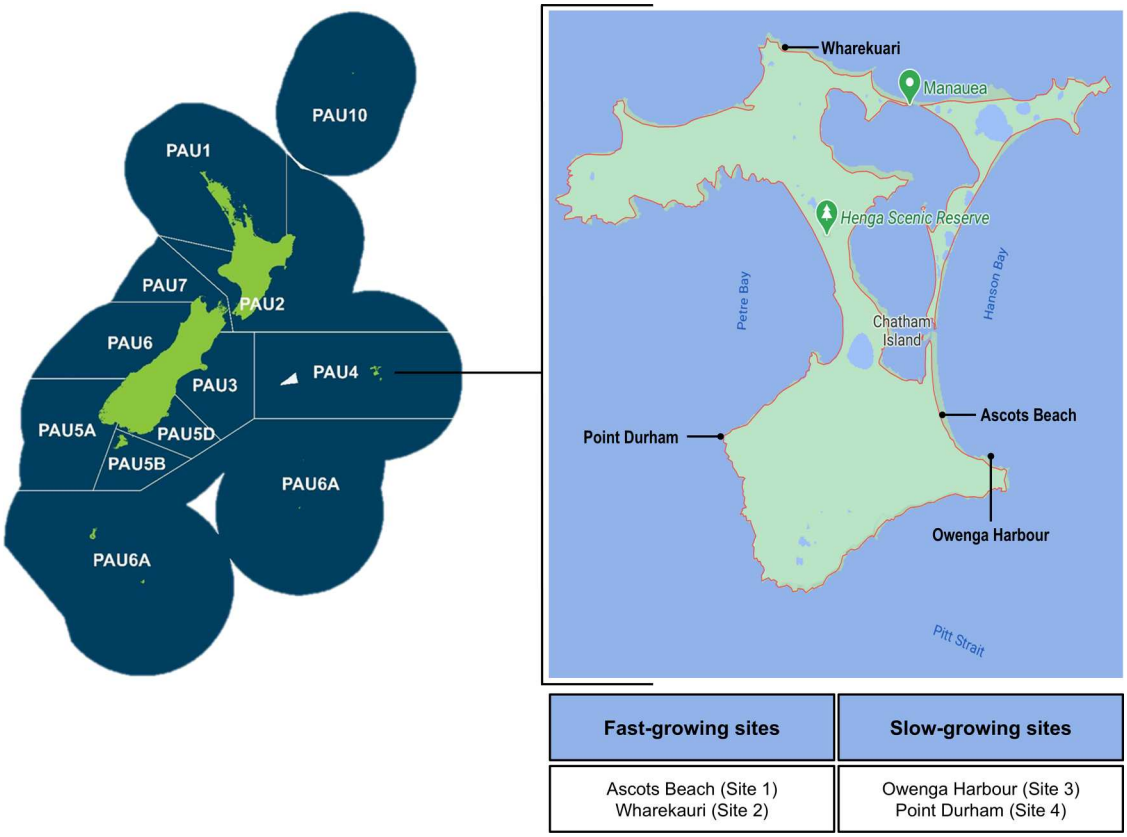

Figure 1. Abalone quota management areas around New Zealand with the PAU4 illustrating the Chatham Islands where abalone were collected from site 1 – Ascots Beach, site 2 – Wharekauri, (fast-growing abalone sites), site 3 – Owenga Harbour and site 4 – Point Durham (slow-growing abalone sites).

Gender annotations were made after shucking based on gonad colour (which is green in females and white/cream in males). Additionally, images were collected for soft tissue measurements. Before gut content with the lining tissue was retrieved from the digestive tract, the area was rinsed three times with 70% ethanol and phosphate-buffered saline. Up to 5 g of gut material was obtained by carefully extruding the posterior intestine material from the anus, using sterile forceps, into individual sterile 2 mL cryo-vials with 200 µL of RNAprotect tissue reagent, followed by snap-freezing in liquid nitrogen and temporary storage at −80°C until DNA extraction at Auckland University of Technology.

Soft tissue imaging

Soft tissue measurements of abalone gonad, adductor muscle and foot muscle were made from photographs collected during the sampling procedure. These images were used to determine tissue area using ImageJ® (version 1.53t24), a Java-based image processing program.

Gut content composition

Four grams of the gut content were collected from the posterior intestine of each of the adult abalone ($n = 10$ per site) and fixed in formalin for algal scoring assessments. Due to the nature of feeding by the abalone and algal deterioration within the gut, it was impossible to determine each specific species of seaweed. However, based on visual and morphological characteristics unique to each of the classes of seaweed, the algal content within the gut was divided into the three main classes of seaweed (green, red, and brown). Green seaweed was characterised by thin blades and had a green hue, red seaweed was characterised by thin blades and a reddish hue, and brown seaweed was characterised by thick blades and brown hue. The gut content was emptied into individual petri dishes and was gently agitated to ensure that the gut content lay in one layer flat on the petri dish. The dishes were then photographed, and scores were obtained by visually observing the proportion of organic fragments and sand occupying the petri dish. Values per seaweed colour were represented as follows: 0: 0% cover; 1: 1–20% cover; 2: 21–40% cover; 3: 41–60% cover; 4: 61–80% cover; 5: 81–100% cover.

16S Ribosomal RNA (rRNA) gene sequencing

Genomic deoxyribonucleic acid (gDNA) of the animal gut (10 adults and 10 sub-adults per site) samples (used for the gut content compositional analysis)

were extracted using the DNeasy PowerSoil Pro Kit (Catalogue No. 47014, Qiagen, Germany) following the manufacturer's protocol, quantified using the Qubit™ dsDNA HS Assay Kit (Catalogue No. Q32854, Thermo Fisher Scientific, USA), and normalised to 3 nanograms/microlitre (ng/μL) for two-step polymerase chain reaction (PCR) assays. The entire genetic amplicon library preparation and Illumina sequencing laboratory workflow followed the protocol written by (Guo *et al.* 2026b). Briefly, the first-round PCR amplification was conducted in triplicates with a pair of customised 16S rRNA primers (Life Technologies Japan Ltd, Japan; forward: 5′-CCTACGGGNGGCWGCAGG-3′; reverse: 5′-GACTACHVGGGTATCTAATCC-3′), and the triplicated amplicon product was pooled by sample, purified with an optimised SpeedBead carboxylate purifying reagent (Catalogue No. 651521050511502910485 5, Cytiva, USA) previously tested by Li *et al.* (2024) and Guo *et al.* (2026b), quantified with the Qubit assays, and normalised to 3 ng/μL for the second-step PCR amplification. The second-step PCR amplification utilised Illumina indexed primers, or 'barcodes', to distinguish the 16S rRNA genetic product produced in the first-round PCR amplification. The barcoded PCR amplicon was purified with the same customised purification reagent used in the post-PCR cleaning after the first-round PCR. The DNA concentration of the final indexed PCR product was quantified and normalised to 10 nanomolars (nM). Lastly, 5 μL of each sample was pooled, purified and quantified using the Bioanalyzer High Sensitivity DNA Kit (Agilent, USA) for quality control. Finally, the quantified libraries were sequenced on an Illumina *MiSeq* platform using a v3 600-cycle sequencing kit (Illumina, USA) following the manufacturer's protocol at the University of Auckland (Auckland, New Zealand).

Data processing and statistical analyses

Morphometrics: Statistical analysis of animal weight, shell length, shell height, and shell width measurements were carried out in R Studio (version 1.4.1103). All data were checked for normality and heterogeneity. Two-way analysis of variance (ANOVA) was used to analyse morphometric data, with weight, shell length, shell width, and shell height as the response variables, and life stage (two levels: adult and sub-adult) and growth type (two levels: fast- and slow-growing) as factors. Weight, shell length, shell width, and shell height data were square-root-transformed when necessary to meet assumptions of normality and homogeneity of variances. Data were also partitioned into four 'growth type' categories (adult fast-growing, adult slow-growing, sub-adult fast-growing, sub-adult slow-growing) and one-way ANOVA with site as a factor was used to examine statistical differences in weight, shell length, shell width, and shell height in animals from different sites. Soft tissue measurements of the adductor muscle, foot muscle, and gonad were analysed using two-way ANOVA, with collection site (four levels: Ascots Beach, Wharekauri, Owenga Harbour and Point Durham) and life stage (two levels: adult and sub-adult) as factors. A principal component analysis (PCA) was conducted to illustrate variation in all morphometric measurements. For all analyses, Tukey pairwise comparisons were used to examine differences between factor levels, and α was set at 0.05.

Algal composition in the gut: Abalone gut content ranking scores were analysed using a two-way ANOVA, with study site (four levels: Ascots Beach, Owenga Harbour, Point Durham, and Wharekauri) and growth type (slow-growing and fast-growing) as factors. For all analyses, Tukey pairwise comparisons were used to examine differences between factor levels, and α was set at 0.05.

16S rRNA gene sequencing data: Illumina sequences were processed through a modified DADA2 data processing pipeline (Archer *et al.* 2020) in R (version 4.2.2) to generate amplicon sequence variants (ASVs), construct an ASV abundance table, and assign taxonomic information to the representative ASVs. Briefly, the 16S rRNA primers were removed from the forward and reverse pair-ended sequences using the 'Cutadapt' command (Martin 2011), followed by filtering out erroneous sequences and removing chimeras. Contaminant sequences in the DNA extraction and PCR negative control samples were removed using the 'decontam' R package (Davis *et al.*, 2018). The pair-ended, quality-filtered sequences were merged to form representative ASVs after being dereplicated. Microbial taxonomic information was assigned to the ASVs using the SILVA high-quality ribosomal RNA database (Version 138) (Quast *et al.* 2012). Chloroplast ASVs were removed to reduce the molecular interference from the macroalgal cells.

To reduce reference database limitations, enhance ecological interpretability, and improve comparability with previous studies, statistical analysis focused on microbiota comparisons at the prokaryotic genus level between the adult and sub-adult gut samples at all sampling sites. Briefly, the microbial genus abundance data were normalised through the total sum scaling method to account for the slightly different sequencing depths across all groups. Alpha-diversity estimators (Chao1 richness and Shannon's diversity index) were calculated and plotted using the 'vegan' package (Oksanen *et al.* 2013) in R (version 4.2.2) and the MicrobiomeAnalyst portal (Lu *et al.*, 2023). Relative abundance bar charts of the most abundant microbial genera were presented to illustrate the gut microbial compositions of the abalone gut samples at each site. Furthermore, the microbial data were transformed

into a Bray–Curtis dissimilarity matrix to compare beta-diversity patterns. A principal coordinate analysis (PCoA) plot was generated using the 'vegan' R package to illustrate microbial compositional similarities between the age groups at each study site. Permutational Multivariate Analysis of Variance (PERMANOVA) and dispersion tests were conducted to detect statistical significance of microbial diversity. Lastly, the core microbiota of all samples was presented in a heatmap with a detection threshold of 10% and a sample prevalence of 20%.

Results

Morphometrics

The collective morphometric data are presented as principal component analysis (PCA) scores in Figure 2. Herein, the animal weight, shell length, shell width, shell height, adductor muscle area, foot muscle area, and gonad area (as determined from imaging) from adult (42.5% were females and 57.5% were males) and sub-adult (22.5% females, 25.0% males, and 52.5% undetermined) abalone collected across the four collection sites are plotted. A clear separation between adult and sub-adult abalone is seen when considering PC1 (99.63%), which is mainly attributed to the animals' weight, shell length, shell height, shell width, foot area, and adductor muscle area measurements of abalone. Adult abalone collected from the fast-growing sites (Ascots Beach and Wharekauri) showed the largest variation among the measured morphometrics attributed to individual differences. Conversely, adult abalone from the slow-growing sites (Owenga Harbour and Point Durham) showed closer clustering of individuals, suggesting similarity within the population.

While the sub-adult abalone showed insignificant differences in all measured morphometric parameters across all study sites, the adult abalone showed a greater variation among the collection sites (Table S1). In general, adult abalone collected from the fast-growing sites (Wharekauri and Ascots Beach) were significantly heavier (ANOVA, Growth Type, $p < 0.001$), longer (ANOVA, Growth Type, $p < 0.001$), wider (ANOVA, Growth Type, $p < 0.001$) and higher (ANOVA, Growth Type, $p < 0.001$) shells than those from the slow-growing sites (Owenga Harbour and Point Durham). Within the adult abalone, pairwise comparisons between collection sites showed that Wharekauri had the largest and heaviest individuals, while Owenga Harbour had the smallest and lightest adult population. There were no significant differences amongst the weight (Tukey, $p = 0.92$), shell length (Tukey, $p = 0.85$) or shell height (Tukey, $p = 0.95$) of adult abalone collected from Ascots Beach and Point Durham.

Soft tissue imaging (Figure 2) showed the significant differences between adductor muscles, foot muscles and gonad tissue areas between adult and

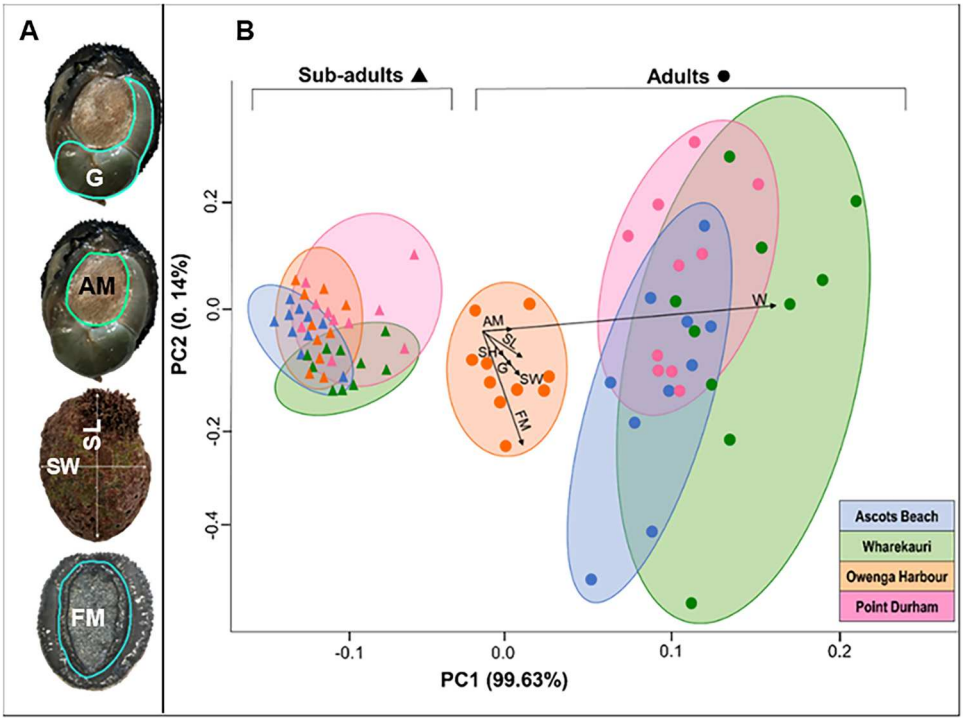

Figure 2. Dorsal-ventral view of the external anatomy (A) and PCA plot (B) of the sampled adult (•) and sub-adult (▲) *Haliotis iris* collected from four sites (Ascots Beach, Wharekauri, Owenga Harbour and Point Durham) based on measured morphological features [shell length (SL), shell height (SH), shell width (SW), weight (W), adductor muscle (AM) area, gonad area (G), and foot muscle area (FM)]. Groupings based on collection sites and life stage of the abalone. Ellipses represent 95% confidence intervals around group centroids.

sub-adult abalone (ANOVA, Growth Type, $p < 0.001$). As expected, adult populations from all collection sites had larger adductor muscle, foot muscle, and gonad tissue area compared to sub-adults from all sites. Amongst the sub-adult abalone, there were no significant differences in all the measured parameters (ANOVA, Collection Site, $p = 0.95$, $p = 0.89$, and $p = 0.90$ for adductor muscle, foot muscle, gonad area, respectively). Within the adult samples, the adductor muscle, foot muscle and gonad tissue areas were significantly different (ANOVA, Growth Type, $p < 0.001$, $p < 0.001$, and $p < 0.001$ for adductor muscle, foot muscle and gonad area, respectively) between the fast-growing and slow-growing sites. Pairwise comparisons between collection sites showed adult individuals collected at Wharekauri (fast-growing site) had the largest adductor muscle, foot muscle and gonads, whereas adult individuals collected at Owenga Harbour (slow-growing site) had the smallest tissue areas.

Gut content composition

Assessment of the preserved gut content (consisting largely of algal debris and sand) from adult abalone was conducted at each study site. In general, the composition of red algae in abalone from fast-growing sites (Ascots Beach and Wharekauri) was between 1.27 and 3.39 units higher than at the slow-growing sites (Owenga Harbour and Point Durham; two-way ANOVA, Growth Type, $p = 0.005$); the composition of green algae at fast-growing sites was between 0.16 and 2.62 units lower than at slow-growing sites (two-way ANOVA, Growth Type, $p = 0.03$); there were no significant differences observed for brown algae (two-way ANOVA, Growth Type, $p = 0.7$) and composition of sand (two-way ANOVA, Growth Type, $p = 0.9$). In contrast, the gut content compositions were significantly different at individual sites. Specifically, the abundance of sand was significantly lower than the three algal types at Wharekauri (two-way ANOVA, Study Site, $p = 0.001$); the abundance of red algae was significantly lower at Owenga Harbour (two-way ANOVA, Study Site, $p = 0.008$); the abundance of both red algae and sand was significantly lower at Point Durham (Figure 3).

Gut bacterial microbiota

More than 11 million 16S rRNA gene sequence reads were generated from the 40 adult (~5.6 million reads) and 40 sub-adult (~5.5 million reads) abalone gut samples across the four sampling sites (10 individuals per site). 5.4 million reads were merged and passed the quality filtering steps (Table S2). There were 823 amplicon sequence variants (ASVs) identified from all gut samples. The observed 16S rRNA ASVs were taxonomically assigned to 28 phyla, 45 classes, 102 orders, 129 families, and 200 genera. The core microbiome (detection threshold of 10% and sample prevalence of 20%) in the samples consisted of *Psychrilyobacter*, *Mycoplasma*, *Vibrio*, *Propionigenium*, Bacilli,

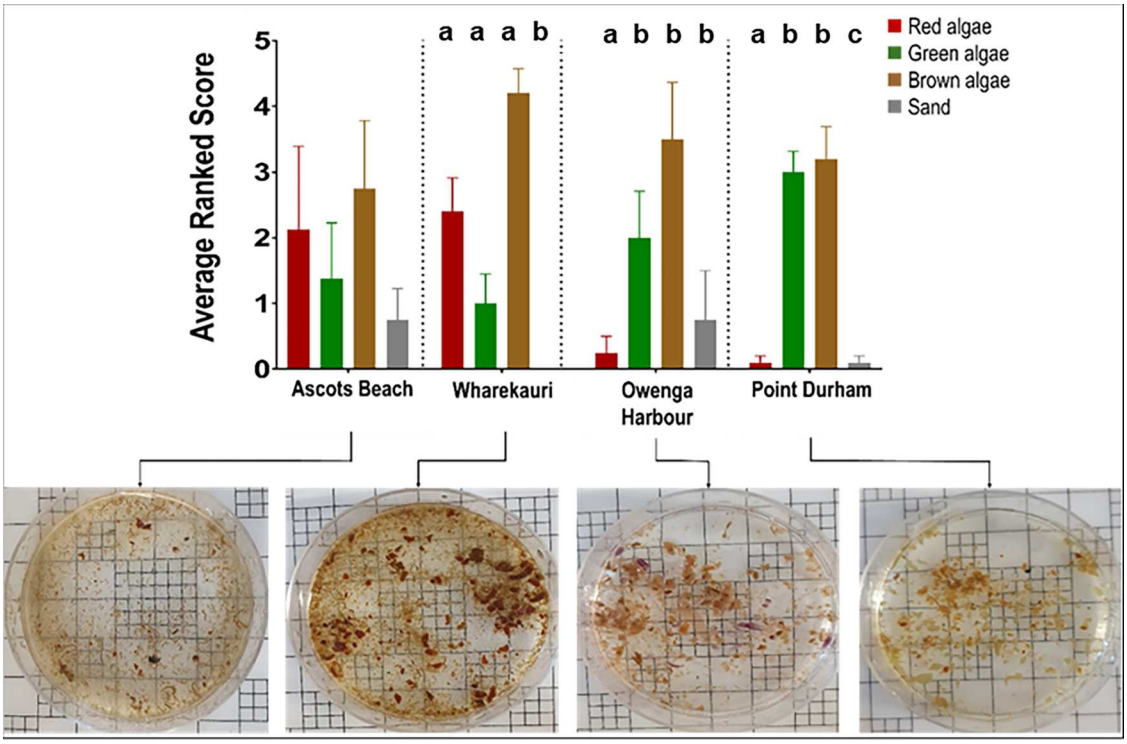

Figure 3. Gut content scoring (average ± SE) by algae type (red, green, and brown algae) and sand in adult abalone collected from Ascots Beach, Wharekauri, Owenga Harbour and Point Durham. Letters above the bars indicate statistical significance.

SAR324 clade (Marine group B), and *Blastopirellula* (Figure S1).

Gut microbial alpha-diversity estimators were compared at the microbial genus level between the adult and sub-adult gut samples collected across the four sites. There was no significant difference in the Chao1 estimator across the sampling sites as well as between the adults and sub-adults (Figure S2; Table 1). Shannon's diversity index calculated at the microbial genus level also showed no significant differences across the four sites and between the age groups except at Point Durham where Shannon's diversity indices were significantly different between the adults and sub-adults (Figure S2; Tables 1 and S3).

Abalone gut bacterial composition and beta-diversity were evaluated at the genus level and showed study site differences. While *Psychrilyobacter*, *Mycoplasma*, and *Vibrio* were the most abundant bacterial genera observed in adults and sub-adults at all study sites, less abundant bacterial genera were different across the sites (Figure S3). For example, *Propionigenium* was seen to be more abundant at Wharekauri and Owenga Harbour in both adult and sub-adult abalone, while the unassigned Bacilli were absent at Owenga Harbour, yet found at all the other sites, at both life stages. Furthermore, the unassigned SAR324 clade (Marine group B) and *Blastopirellua* were more evident in adult abalone, and the sub-adults showed higher levels of *Vibrio*. Interestingly, *Psychromonas* were absent in the adult and sub-adult abalone collected at Ascots Beach and in the adults from Point Durham.

Based on the Similarity Percentage (SIMPER) pairwise analysis, the beta-diversity at the bacterial genus level showed 84% and 83% similarities between the fast-growing sites (Ascots Beach and Wharekauri) and the slow-growing sites (Owenga Harbour and Point Durham), respectively (Table S4). The observed beta-diversity was noticeably different between the adult and sub-adult abalone at each site (Figure 4). Moreover, the differentiated microbial compositions by site and age group were statistically significant (Table 2) but not due to between-group variations (Table S5). The gut microbial compositional differentiation across the study sites and between the age groups at individual sites was also indicated by clustering heatmaps (Figure S1).

Table 1. Two-way ANOVA test results in main factors of site and sample type on Chao1 richness and Shannon's diversity estimators of the gut samples collected from the four study sites (Ascots Beach, Wharekauri, Owenga Harbour, and Point Durham). The non-significant interaction term was removed. The alpha-diversity estimators were calculated at the microbial genus level.

	Chao1 Richness					Shannon's Diversity				
	df	Sum of Square	Mean of Square	*F*-statistic	*p*-value	df	Sum of Square	Mean of Square	*F*-statistic	*p*-value
Site	3	271	90.5	0.28	0.840	3	0.718	0.239	2.572	0.06
Age	1	265	265.0	0.82	0.368	1	0.844	0.844	9.069	0.03
Residuals	75	24,243	323.2			75	6.977	0.093		
Total	79	49,834				79	8.667			

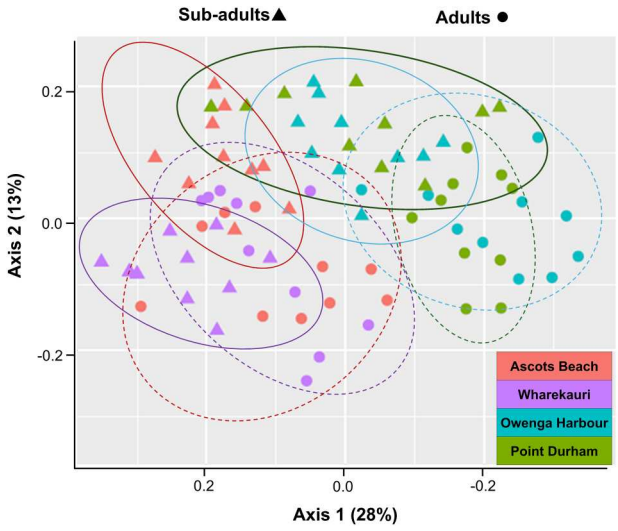

Figure 4. Principal coordinate analysis (PCoA) plots showing the microbial compositions of the adult and sub-adult abalone gut samples collected from the four study sites. Solid and dashed eclipses, representing 95% confidence intervals around group centroids, are for the sub-adults and adults, respectively. Read abundance data at the microbial genus level were normalised and converted to a Bray-Curtis dissimilarity matrix.

Table 2. Permutational Multivariate Analysis of Variance (PERMANOVA) test results of the main factors of site and age group on microbial composition of the gut samples collected from the four study sites (Ascots Beach, Wharekauri, Owenga Harbour, and Point Durham). The non-significant interaction term was removed. Read abundance data at the bacterial genus level were normalised and converted to a Bray-Curtis dissimilarity matrix, and the PERMANOVA test was conducted using 999 permutations.

	df	Sum of Square	*F* Statistics	*p*-value
Site	3	2.25	7.70	0.005
Age Group	1	0.80	8.24	0.009
Residual	75	7.02		
Total	79	10.73		

Core gut microbiota

Bacteria in genera *Psychrilyobacter*, *Mycoplasma*, *Vibrio*, *Propionigenium*, unassigned Bacilli, unassigned SAR324_clade, and *Blastopirellula* formed the core gut microbiota of all abalone gut samples collected from the four study sites (Figure 5) based on the set detection threshold (10%) and sequencing read prevalence (20%) to account for microbial variability of wild *H. iris* populations, capture biologically meaningful and recurrent microbial members, ensure consistent microbial taxa identification across groups, and minimise the inclusion of rare or transient taxa.

Discussion

Molluscan gut microbiota performs important functions (e.g., assisting host food digestion) and can be influenced by various genetic and environmental factors such as diet type (Kang *et al.*, 2022). While abalone gut microbiome research is expanding, knowledge pertaining to the gut microbial composition of *Haliotis iris* with different growth populations and dietary backgrounds is limited, creating a gap within population management strategies. This study provides the first exploratory analysis into the gut microbiome of adult and sub-adult abalone collected from four Chatham Islands sites with varying growth and dietary seaweed backgrounds. We characterised the gut microbial composition and diversity of local wild abalone populations and examined their relationship to algal gut content. The findings suggest that seaweed type and nutritional profile may significantly influence abalone gut microbiota and ultimately contribute to differences in growth and morphometric traits.

The differentiated morphometric measurements (i.e., sizes of adductor, foot muscles, and gonad tissues) among the adult abalone collected from the fast-growing and slow-growing sites in the present study were previously observed among wild *H. iris* populations in New Zealand (McShane *et al.* 1994a; Naylor *et al.* 2006). Abalone in New Zealand are known to have varying growth rates, and these differences can be mostly associated with food availability and the nutrients (ultimately energy) absorbed from abalone's diets (Nguyen *et al.* 2023). The microbial community dwelling in the gut of abalone is an important component in efficiently assisting with food digestion (Erasmus *et al.* 1997). Therefore, understanding the changes in abalone's gut microbial composition and diversity can provide indications on how the microbial function of food digestion could be influenced.

Differentiated abalone gut microbiota and seaweed type

Adult and sub-adult abalone from the slow- and fast-growing sites in this study showed differentiated gut microbial compositions despite having similar alpha-diversity estimators across the sites. Evidence in other abalone species also suggested differentiated gut microbial richness and diversity in different age groups. For instance, adult *H. diversicolor* (Huang *et al.* 2010) and *H. midae* (Nel *et al.* 2018) exhibited

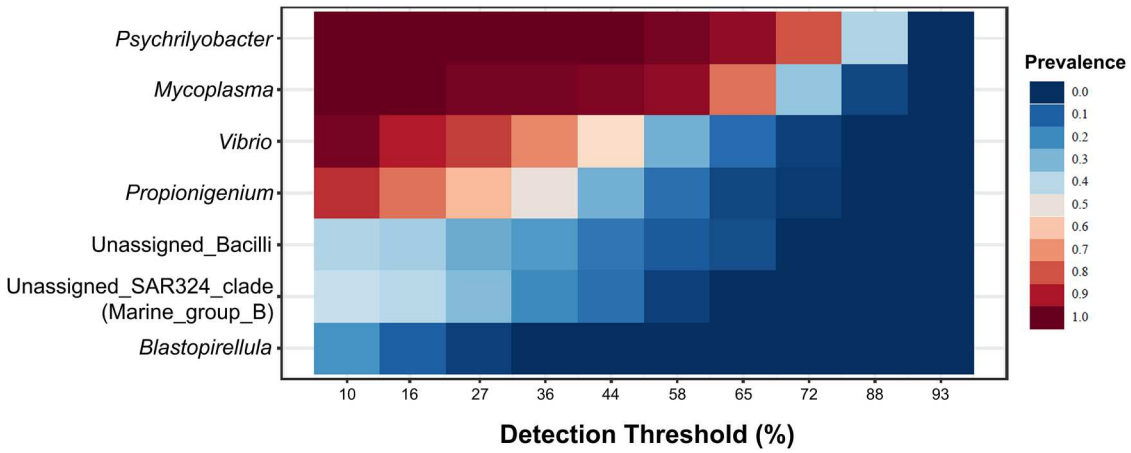

Figure 5. Core microbiota of all gut samples collected from the four study sites (Ascots Beach, Wharekauri, Owenga Harbour, and Point Durham). The detection threshold was set to 10% and the sequencing read prevalence was set to 20%.

higher intestinal microbial diversity than the juvenile samples. The differentiated abalone gut microbiota could be related to the type of seaweed (i.e., brown, red, and green seaweed) that is consumed by wild abalone species like *H. discus hannai* (Tanaka *et al.* 2003), *H. midae* (Nel *et al.* 2018), and *H. tuberculata* (Gobet *et al.* 2018). For example, microorganisms in the gut of abalone fed on brown seaweed such as kelp (*Ecklonia maxima* and *Sargassum horneri*) are often dominated by *Mycoplasma*, fermentative *Clostridia* bacteria, *Psychrilobacter*, and *Vibrio* (Nel *et al.* 2017). Moreover, the proliferation of beneficial bacterial strains like *Mycoplasma* sp. and *Vibrio halioticoli* was found to be associated with the addition of red seaweed into abalone diets (Iehata *et al.* 2014); and the inclusion of *Ulva* spp. in abalone diets has been linked to an increase of beneficial *Vibrio* and *Mycoplasma* strains (Guo 2017). The gut algal content analysis in the present study showed that wild *H. iris* consumed different proportions of mixed seaweed types at all study sites, which could explain why some of the core gut bacterial genera, such as *Psychrilobacter*, *Mycoplasma*, and *Vibrio*, had different relative abundance across the study sites. In fact, researchers have previously documented that *H. iris* prefers to eat drifting brown algae over red and green algae, but they ultimately consume different groups of algae depending on their availability and accessibility (Cornwall *et al.* 2009). Comparatively, on a short-term basis (24 h), *H. diversicolor* were reported to consume more leathery brown algae than red and green, while on a long-term basis (20 days), the consumption of more green algae was documented (Alcantara and Noro 2005). Literature suggests that abalone digests preferred algal species within 24 h, while less digestible algal species remain identifiable in abalone gut for more than 48 h (Britz *et al.* 1996). As such, abalone digestibility might be related to the nutritional characteristics (e.g., carbohydrates) of each seaweed type, which could influence the abalone's gut microbial composition and diversity.

Effects of seaweed carbohydrates on abalone gut microbiota

Each of the three types of seaweed (i.e., brown, red, and green) has specific carbohydrate (mostly polysaccharides) profiles that often require specific bacterial taxa to break down. As a result, abalone gut microbiota could be shaped by seaweed polysaccharides. Brown seaweed contains a high amount of alginate, laminarin, and fucoidan. In Pacific abalone (*H. discus hannai*) fed on *Ecklonia maxima* and *Sargassum horneri,* gut bacteria such as *Mycoplasma*, fermentative *Clostridia*, *Psychrilobacter*, and *Vibrio* have been biochemically shown to hydrolyse these polysaccharides (Hur *et al.* 2023). Similarly, bacterial strains capable of digesting fucoidan-rich seaweed (e.g., *Ecklonia maxima*) were notably abundant in the gut of the South African abalone *H. midae* (Barkai and Griffiths 1986). In addition to the most abundant bacterial strains, some minor bacterial genera, such as the unassigned marine group B SAR324 clade, also play an important role in seaweed polysaccharide digestion and were previously seen to be dominant in the intestines of *Haliotis diversicolor* and *H. tuberculata* (Gobet *et al.* 2018; Huang *et al.* 2021). Genome-centric metagenomics suggested that some SAR324 bacteria strains possess adenosine triphosphate binding cassette transporters for carbohydrate metabolism (Huang *et al.* 2021). Such metabolomic characteristics are in line with previous findings where certain bacteria, including the SAR324 group, have been described as key in the degradation of complex polysaccharides such as alginate, which is a major component of brown algae (Hyun *et al.* 2013; Tanaka *et al.* 2016). Since the gut content composition analysis showed that brown seaweed was the most abundant seaweed type digested by the adult abalone in this study, it is reasonable to observe alginate-digesting bacteria, including the SAR324 strains, in the gut of the local wild abalone.

Red seaweed, which contains a high amount of agar, carrageenan, and alginate, is said to provide a more diverse and stable gut microbial environment in abalone (Iehata *et al.* 2014; Gobet *et al.* 2018). A study by Kang and Kim (2015) showed that certain *Bacillus* strains could metabolise red seaweed agar and carrageenan to produce reduced sugars, which are critical to mollusks as an energy source (Kang and Kim 2015). In the present study, abalone from the fast-growing sites showed higher levels of red seaweed in the preserved gut content samples compared to their slow-growing counterparts. The presence of a relatively large proportion of the unassigned Bacilli in the gut of adult and sub-adult abalone at Ascots Beach and Wharekarui (fast-growing sites) could be associated with the consumption of red seaweed. In addition to Bacilli bacteria, *Psychromonas*, a genus of psychrophilic bacteria, was mostly found in the gut samples of abalone that were observed to consume a high volume of red seaweed in the present study. *Psychromonas* mainly contributes to the degradation of algal polysaccharides and has been associated with accelerated transportation of nutrients and the promotion of host digestion (Wu *et al.* 2023). The differentiated *Psychromonas* abundance observed across the study sites and between the age groups could be due to dietary changes from abalone's early life stages to juveniles and eventually adults, which can also lead to variations in the abundance and diversity of other bacterial species (Jiang *et al.* 2017; Hur *et al.* 2023).

Green seaweed, particularly from the genus *Ulva* spp., is another food source that can have a significant impact on the gut microbiota and microbial functionality in abalone and other mollusks (Bansemer *et al.* 2016). Many green seaweed species like *Ulva* spp. are rich in amino acids, fatty acids, polyphenols, and unique polysaccharides (Hagan and Fungwe 2023). These macronutrients can positively modulate the gut microbiota by promoting the growth of beneficial bacteria and enhancing short-chain fatty acid production (Shannon *et al.* 2021; Hagan and Fungwe 2023; Xu *et al.* 2023). Sub-adult abalone from all the collection sites in this study showed higher levels of *Vibrio* when compared to the adults. Several *Vibrio* strains are considered facultative anaerobes and can either be pathogens that trigger shellfish diseases or probiotics, as seen in the gastrointestinal tract of *H. midae* that support digestive tract functions and fermentation processes (Danckert *et al.* 2021). There could be two main reasons why the relative abundance of *Vibrio* bacteria could be differentiated in the life stages of abalone. First, different seaweed types can lead to variations in the availability of nutrients and substrates that support the growth of *Vibrio* spp., and this is mostly observed during dietary shifts between developmental stages (Cicala *et al.* 2018; Gobet *et al.* 2018). Second, the immune response of abalone at different developmental stages can also influence the abundance of *Vibrio* bacteria. For example, juvenile abalone have been observed to exhibit different immune responses compared to adults, which could affect their susceptibility to *Vibrio* infections and consequently alter the relative abundance of these bacteria in their gut (Huang *et al.* 2010; Wang *et al.* 2015).

Future research directions

The present study combined gut content microscopy and Illumina amplicon sequencing technologies to reveal the interplay between diet (seaweed) and wild abalone gut microbiota on the Chatham Islands. While our results highlighted the connections between dietary seaweed and the local abalone's gut microbial composition and diversity, it is also acknowledged that the environmental conditions (e.g., seawater temperature, salinity, dissolved oxygen levels, and pH) that were not available within this study due to the sampling site restrictions may also be influencing factors. Hence, it would be beneficial to incorporate environmental metadata into long-term gut microbiota investigations using the same methods at the same study sites for data validation. In addition, research activities in at least three other directions are needed to further test the hypothesis that seaweed type can significantly tailor the gut microbiota of wild *H. iris* to select microbial communities that specifically digest the seaweed nutrients. First, biological and ecological surveys on local seaweed composition, diversity, and morphology in abalone habitats around the Chatham Islands are recommended to understand seaweed availability and accessibility to the wild abalone populations. The health and diversity of seaweed communities are important for abalone growth and health. Studies have indicated that abalone populations recover when kelp restorations take place (Eger *et al.* 2022), with the opposite also being true where a decline in kelp forests triggers abalone mortality (Rogers-Bennett and Catton 2019). Second, targeted seaweed feeding experiments under controlled laboratory conditions are needed to specifically explore how abalone's gut microbial communities would respond to each type or species of seaweed. Lastly, field experimental translocations of wild abalone populations with different but known seaweed habitat characteristics could also be useful to investigate the dietary seaweed effects on wild *H. iris* that have the same genetic backgrounds. The growth differences in this study were largely documented in adult animals, with few differences seen between sub-adults from the same collection sites. Moreover, the gut microbial compositions of the sub-adult and adult abalone among the study sites were noticeably different. These findings indicate that *H. iris* could be housed at slow-growing sites until they reach the juvenile and sub-adult stages and then translocated to fast-growing sites to improve growth in adulthood. To some extent, the slow-growing sites could be considered spawning sites where the adults settled there will serve as breeding stock, while their offspring can be moved to other areas. It has been suggested that juveniles of around 30 mm are the best size to be released as part of stock enhancement programs, while enhancement by free-swimming larvae was classified as less successful (Cook 2023). The translocation survival of the Roe's abalone (*H. roei*) was much higher in adult than juvenile populations (Strain *et al.* 2019). Also, translocation of adult pink abalone (*H. corrugata*) resulted in successful dense aggregations of new populations (Taniguchi *et al.* 2013). Data collected from such translocation efforts will be constructive to determine if such initiatives will be valuable for the Chatham Islands fishery.

Abalone support valuable commercial, recreational and customary fisheries in New Zealand, and an understanding of the processes driving variation within different abalone fishing sites is crucial to ensure long-term conservation and responsible management of *H. iris*. While it has been noticed that anthropogenic, biological and climate-related stressors could disproportionately impact wild abalone habitats and abalone fishery stocks (Rogers-Bennett and Catton 2022), it is essential to continuously monitor the gut microbial

responses of this ecologically and economically valuable shellfish to seaweed and other environmental factors in New Zealand. A clear understanding of abalone gut microbial communities can be used to optimise feeding strategies, enhance growth and disease resistance to ultimately benefit abalone fishery management and support sustainable aquaculture operations.

Conclusions

This study provides novel insight into sub-adult and adult abalone morphometrics, gut microbiota, and digested seaweed proportional comparisons across four locations on the Chatham Islands. Abalone from fast-growing sites (Ascots Beach and Wharekauri) had more red algae and less green algae present in the gut compared to the slow-growing sites (Owenga Harbour and Point Durham). The corresponding microbes that digest these seaweeds may support early digestion, potentially enhancing feeding efficiency and growth over time. *Psychrilyobacter*, *Mycoplasma*, and *Vibrio* were the dominant bacterial genera forming the core microbiota across all samples. However, gut microbial composition varied between adult and sub-adult populations at all study sites, likely influenced by environmental factors, a pattern commonly observed in other abalone species. Differences in growth and gut microbiome may be linked to the types of seaweed digested by wild *H. iris*, alongside environmental factors. These findings suggest controlled feeding experiments using specific seaweed species and varying environmental conditions (e.g., temperature and sediment) are recommended to clarify how diet and environment influence gut microbiota and, ultimately, abalone growth.

Acknowledgements

Thank you to the Pāua Industry Council for logistical assistance and for supporting this research. Thank you to the divers and on-site sampling team. We are grateful to the Aquaculture Biotechnology Research Group and the technicians within the School of Science at Auckland University of Technology for intellectual and technical input.

Author contributions

JG, LV, SS and AA collected the abalone gut content and morphometric data; JG processed the gut microbiome samples, conducted the microbiome data analysis and wrote the manuscript; LV and AA designed the overall sampling scheme; LV analysed the morphometric data and wrote the manuscript; SS processed the gut algal content samples, analysed the gut algal content data and wrote the corresponding results; SA, DL and AA curated all data, reviewed the manuscript and provided feedback.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This project was co-funded by the Pāua Industry Council, New Zealand, the School of Science PhD funding and the Vice-Chancellor's Scholarship at Auckland University of Technology. This work was co-supported by Cawthron's Shellfish Aquaculture Platform (CAWX1801).

Data availability statement

All supplementary tables and figures are accessible online. The sequences and metadata presented in this study are available on the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/search) under Project Number PRJEB93823.

ORCID

Jinchen Guo http://orcid.org/0000-0001-5828-0859
Leonie Venter http://orcid.org/0000-0003-0019-3722
Shaneel S. Sharma http://orcid.org/0009-0007-7903-6127
Stephen D. J. Archer http://orcid.org/0000-0001-6054-2424
Donnabella C. Lacap-Bugler http://orcid.org/0000-0002-7481-6614
Andrea C. Alfaro http://orcid.org/0000-0003-0543-7212

References

Alcantara, L.B. & Noro, T. (2005) Effects of macroalgal type and water temperature on macroalgal consumption rates of the abalone *Haliotis diversicolor* Reeve. *Journal of Shellfish Research* 24, 1169–1177.

An, C., Yazaki, T., Takahashi, H., Kuda, T. & Kimura, B. (2013) Diet-induced changes in alginate- and laminaran-fermenting bacterial levels in the caecal contents of rats. *Journal of Functional Foods* 5, 389–394.

Archer, S.D., Lee, K.C., Caruso, T., King-Miaow, K., Harvey, M., Huang, D., Wainwright, B.J. & Pointing, S.B. (2020) Air mass source determines airborne microbial diversity at the ocean–atmosphere interface of the Great Barrier Reef marine ecosystem. *The ISME Journal* 14, 871–876.

Bansemer, M.S., Qin, J.G., Harris, J.O., Howarth, G.S. & Stone, D.A. (2016) Nutritional requirements and use of macroalgae as ingredients in abalone feed. *Reviews in Aquaculture* 8, 121–135.

Barkai, R. & Griffiths, C. (1986) Diet of the South African abalone *Haliotis midae*. *South African Journal of Marine Science* 4, 37–44.

Britz, P., Hecht, T. & Knauer, J. (1996) Gastric evacuation time and digestive enzyme activity in abalone *Haliotis midae* fed a formulated diet. *South African Journal of Marine Science* 17, 297–303.

Bullon, N., Alfaro, A.C., Guo, J., Copedo, J., Nguyen, T.V. & Seyfoddin, A. (2025) Expanding the menu for New Zealand farmed abalone: dietary inclusion of insect meal and grape marc (effects on gastrointestinal microbiome, digestive morphology, and muscle metabolome). *New Zealand Journal of Marine and Freshwater Research* 59(1), 31–60. https://doi.org/10.1080/00288330.2024.2311376.

Cicala, F., Cisterna-Céliz, J.A., Moore, J.D. & Rocha-Olivares, A. (2018) Structure, dynamics and predicted functional role of the gut microbiota of the blue (*Haliotis fulgens*) and yellow (*H. corrugata*) abalone from Baja California Sur, Mexico. *PeerJ* 6, e5830.

Cook, P.A. (2023) Abalone reseeding and enhancement. In: P. A. Cook & S. E. Shumway (Eds.), *Developments in Aquaculture and Fisheries Science*. Vol. 42. pp. 331–346. Elsevier. https://doi.org/10.1016/B978-0-12-821537-0.00015-6.

Copedo, J.S., Webb, S.C., Ragg, N.L., Venter, L. & Alfaro, A.C. (2024) Histopathological investigation of four populations of abalone (*Haliotis iris*) exhibiting divergent growth performance. *Journal of Invertebrate Pathology* 202, 108042.

Corino, C., Di Giancamillo, A., Modina, S.C. & Rossi, R. (2021) Prebiotic effects of seaweed polysaccharides in pigs. *Animals* 11, 1573.

Cornwall, C.E., Phillips, N.E. & McNaught, D.C. (2009) Feeding preferences of the abalone *Haliotis iris* in relation to macroalgal species, attachment, accessibility and water movement. *Journal of Shellfish Research* 28, 589–597.

Danckert, N.P., Wilson, N., Phan-Thien, K.-Y. & Stone, D.A. (2021) The intestinal microbiome of Australian abalone, *Haliotis laevigata* and *Haliotis laevigata× Haliotis rubra*, over a 1-year period in aquaculture. *Aquaculture* 534, 736245.

Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018) Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome* 6, 226. https://doi.org/10.1186/s40168-018-0605-2.

De Vos, W.M., Tilg, H., Van Hul, M. & Cani, P.D. (2022) Gut microbiome and health: mechanistic insights. *Gut* 71, 1020–1032.

Eger, A.M., Marzinelli, E.M., Christie, H., Fagerli, C.W., Fujita, D., Gonzalez, A.P., Hong, S.W., Kim, J.H., Lee, L.C. & McHugh, T.A. (2022) Global kelp forest restoration: past lessons, present status, and future directions. *Biological Reviews* 97, 1449–1475.

Erasmus, J.H., Cook, P.A. & Coyne, V.E. (1997) The role of bacteria in the digestion of seaweed by the abalone *Haliotis midae*. *Aquaculture* 155, 377–386.

Fisheries`New`Zealand (2021) Paua South-East (Chatham Rise) (PAU4) [Online]. *Ministry of Primary Industries*. https://fs.fish.govt.nz/Page.aspx?pk=8&stock=PAU4&ey=2022.

Gerasimidis, K., Bryden, K., Chen, X., Papachristou, E., Verney, A., Roig, M., Hansen, R., Nichols, B., Papadopoulou, R. & Parrett, A. (2020) The impact of food additives, artificial sweeteners and domestic hygiene products on the human gut microbiome and its fibre fermentation capacity. *European Journal of Nutrition* 59, 3213–3230.

Gobet, A., Mest, L., Perennou, M., Dittami, S.M., Caralp, C., Coulombet, C., Huchette, S., Roussel, S., Michel, G. & Leblanc, C. (2018) Seasonal and algal diet-driven patterns of the digestive microbiota of the European abalone *Haliotis tuberculata*, a generalist marine herbivore. *Microbiome* 6, 60.

Guo, J. (2017) *Metabarcoding analyses of gut microbiome compositions in red abalone (Haliotis rufescens, Swainson, 1822) fed different macroalgal diets*, Thesis, California State University, Monterey Bay.

Guo, J., Venter, L., Lacap-Bugler, D.C. & Alfaro, A.C. (2026a) Abalone gut microbiome regulators: a review synthesizing gut physiology, microorganisms, diet, and the environment. *Aquaculture, Fish and Fisheries*, Submitted.

Guo, J., Alfaro, A. C., Venter, L., & Lacap-Bugler, D. C. (2026b) Bacterial microbiome composition and functional potentials across digestive regions of wild New Zealand abalone (Haliotis iris). *New Zealand Journal of Marine and Freshwater Research* 60(1), e70028.

Hagan, M. & Fungwe, T. (2023) Determining the effect of seaweed intake on the microbiota: a systematic review. *Functional Food Science-Online ISSN: 2767*-3146 3, 79–92.

Hehemann, J.-H., Kelly, A.G., Pudlo, N.A., Martens, E.C. & Boraston, A.B. (2012) Bacteria of the human gut microbiome catabolize red seaweed glycans with carbohydrate-active enzyme updates from extrinsic microbes. *Proceedings of the National Academy of Sciences* 109, 19786–19791.

Huang, Z., Petersen, J.M., You, W. & Shao, Z. (2021) A novel SAR324 bacterium associated with abalone, *Haliotis diversicolor*. *Aquaculture Research* 52, 1945–1953.

Huang, Z.B., Guo, F., Zhao, J., Li, W.D. & Ke, C.H. (2010) Molecular analysis of the intestinal bacterial flora in cage-cultured adult small abalone, *Haliotis diversicolor*. *Aquaculture Research* 41, e760–e769.

Hur, S.-W., Cadangin, J., Lee, S., Lee, J.-H., Park, S.-J., Jang, W.-J. & Choi, Y.-H. (2023) Dietary replacement of *Undaria pinnatifida* by *Sargassum horneri* in feed formulation for abalone *Haliotis discus hannai*: effect on growth, gut microbiota, and taste sensory profile. *Frontiers in Marine Science* 10, 1053240.

Hyun, D., Shin, N.-R., Kim, M.-S., Kim, P.S., Kim, J.Y., Whon, T.W. & Bae, J.-W. (2013) *Pseudoruegeria haliotis sp.* nov., isolated from the gut of the abalone *Haliotis discus hannai*. *International Journal of Systematic and Evolutionary Microbiology* 63, 4626–4632.

Iehata, S., Nakano, M., Tanaka, R. & Maeda, H. (2014) Modulation of gut microbiota associated with abalone *Haliotis gigantea* by dietary administration of host-derived *Pediococcus sp*. Ab1. *Fisheries Science* 80, 323–331.

Jiang, J.-Z., Zhao, W., Liu, G.-F. & Wang, J.-Y. (2017) Relationships between and formation dynamics of the microbiota of consumers, producers, and the environment in an abalone aquatic system. *PLoS One* 12, e0182590.

Kang, S. & Kim, J.K. (2015) Reuse of red seaweed waste by a novel bacterium, *Bacillus sp.* SYR4 isolated from a sandbar. *World Journal of Microbiology and Biotechnology* 31, 209–217.

Kang, W., Kim, P. S., Tak, E. J., Sung, H., Shin, N. R., & Hyun, D. W (2022) Host phylogeny, habitat, and diet are main drivers of the cephalopod and mollusk gut microbiome. *Animal Microbiome* 4. https://doi.org/10.1186/s42523-022-00184-x.

Li, S., Young, T., Archer, S., Lee, K., & Alfaro, A. C. (2024) Gut microbiome resilience of green-lipped mussels, Perna canaliculus, to starvation. *International Microbiology* 27(2), 571–580.

Lu, Y., Zhou, G., Ewald, J., Pang, Z., Shiri, T., & Xia, J. (2023) MicrobiomeAnalyst 2.0: Comprehensive statistical, functional and integrative analysis of microbiome data. *Nucleic Acids Research* 51(W1), W310–W318.

Martin, M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 17, 10–12.

McShane, P.E., Gorfine, H.K. & Knuckey, I. (1994a) Factors influencing food selection in the abalone *Haliotis rubra* (Mollusca: Gastropoda). *Journal of Experimental Marine Biology and Ecology* 176, 27–37.

McShane, P.E., Mercer, S.F. & Naylor, J.R. (1994b) Spatial variation and commercial fishing of New Zealand abalone (*Haliotis iris* and *H. australis*). *New Zealand Journal of Marine and Freshwater Research* 28, 345–355.

McShane, P.E. & Naylor, J.R. (1995) Depth can affect post-settlement survival of *Haliotis iris* (Mollusca: Gastropoda). *Journal of Experimental Marine Biology and Ecology* 187, 1–12.

Naidoo, K., Maneveldt, G., Ruck, K., & Bolton, J. (2006) A comparison of various seaweed-based diets and formulated feed on growth rate of abalone in a land-based aquaculture system. *Journal of Applied Phycology* 18(3-5).

Naylor, J., Andrew, N. & Kim, S. (2006) Demographic variation in the New Zealand abalone *Haliotis iris*. *Marine and Freshwater Research* 57, 215–224.

Naylor, R., & Fu, D. (2016) Estimating growth in pāua. *New Zealand Fisheries Assessment Report* 14, 76. https://www.mpi.govt.nz/dmsdocument/11815/direct/

Nel, A., Jones, C.L., Britz, P.J. & Landzela, S. (2018) The effect of juvenile abalone *Haliotis midae* (Linnaeus, 1758) weaning diet on gut-bacterial formation. *Journal of Shellfish Research* 37, 191–197.

Nel, A., Pletschke, B., Jones, C., Kemp, J., Robinson, G. & Britz, P. (2017) Effects of kelp *Ecklonia maxima* inclusion in formulated feed on the growth, feed utilisation and gut microbiota of South African abalone *Haliotis midae*. *African Journal of Marine Science* 39, 183–192.

Nguyen, v.T., Alfaro, A.C., Venter, L., Ericson, J.A., Ragg, N.L., McCowan, T. & Mundy, C. (2023) Metabolomics approach reveals size-specific variations of blackfoot abalone (*Haliotis iris*) in Chatham Islands, New Zealand. *Fisheries Research* 262, 106645.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R., Simpson, G.L., Solymos, P., Stevens, M.H.H. & Wagner, H. (2013) Package 'vegan'. *Community ecology package, version* 2, 1-295.

Poore, G.C. (1972) Ecology of New Zealand abalones, *Haliotis* species (Mollusca: Gastropoda). 1) Feeding. *New Zealand Journal of Marine and Freshwater Research* 6, 534–559.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. & Glöckner, F.O. (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41, D590–D596.

Rogers-Bennett, L. & Catton, C.A. (2019) Marine heat wave and multiple stressors tip bull kelp forest to sea urchin barrens. *Scientific Reports* 9, 15050.

Rogers-Bennett, L. & Catton, C.A. (2022) Cascading impacts of a climate-driven ecosystem transition intensifies population vulnerabilities and fishery collapse. *Frontiers in Climate* 4, 908708.

Roussel, S., Caralp, C., Leblanc, C., Le Grand, F., Stiger-Pouvreau, V., Coulombet, C., Le Goïc, N. & Huchette, S. (2019) Impact of nine macroalgal diets on growth and initial reproductive investment in juvenile abalone Haliotis tuberculata. *Aquaculture* 513, 734385. https://doi.org/10.1016/j.aquaculture.2019.734385

Ryder, F. J., Sainsbury, K. J., Hepburn, C. D., Pritchard, D. W., & Gnanalingam, G. (2025) Re-assessment of a blackfoot abalone (Haliotis iris) population in Peraki Bay, New Zealand, after 45 years. *New Zealand Journal of Marine and Freshwater Research* 59, 164–182.

Sawabe, T., Oda, Y., Shiomi, Y. & Ezura, Y. (1995) Alginate degradation by bacteria isolated from the gut of sea urchins and abalones. *Microbial Ecology* 30, 193–202.

Schei, K., Avershina, E., Øien, T., Rudi, K., Follestad, T., Salamati, S. & Ødegård, R.A. (2017) Early gut mycobiota and mother-offspring transfer. *Microbiome* 5, 1–12.

Shannon, E., Conlon, M. & Hayes, M. (2021) Seaweed components as potential modulators of the gut microbiota. *Marine Drugs* 19, 358.

Stenton-Dozey, J.M., Heath, P., Ren, J.S. & Zamora, L.N. (2021) New Zealand aquaculture industry: research, opportunities and constraints for integrative multitrophic farming. *New Zealand Journal of Marine and Freshwater Research* 55, 265–285.

Strain, L.W., Brown, J. & Hart, A.M. (2019) Recovering a collapsed abalone stock through translocation. Australian Seafood CRC Project No. 2011/762. *Department of Primary Industries and Regional Development, Western Australia*. 93pp. Fisheries Research Report No. 292.

Tanaka, R., Shibata, T., Miyake, H., Mori, T., Tamaru, Y., Ueda, M. & Bossier, P. (2016) Temporal fluctuation in the abundance of alginate-degrading bacteria in the gut of abalone *Haliotis gigantea* over 1 year. *Aquaculture Research* 47, 2899–2908.

Tanaka, R., Sugimura, I., Sawabe, T., Yoshimizu, M. & Ezura, Y. (2003) Gut microflora of abalone *Haliotis discus hannai* in culture changes coincident with a change in diet. *Fisheries Science* 69, 951–958.

Taniguchi, I.K., Stein, D., Lampson, K. & Rogers-Bennett, L. (2013) Testing translocation as a recovery tool for Pink (*Haliotis corrugata*) and Green (*Haliotis fulgens*) Abalone in Southern California. *Journal of Shellfish Research* 32, 209–216.

Troell, M., Robertson-Andersson, D., Anderson, R. J., Bolton, J. J., Maneveldt, G., Halling, C. & Probyn, T. (2006) Abalone farming in South Africa: An overview with perspectives on kelp resources, abalone feed, potential for on-farm seaweed production and socio-economic importance. *Aquaculture* 257(1-4), 266–281. https://doi.org/10.1016/j.aquaculture.2006.03.044

Unzueta-Martínez, A., Scanes, E., Parker, L.M., Ross, P.M., O'Connor, W. & Bowen, J.L. (2022) Microbiomes of the Sydney rock oyster are acquired through both vertical and horizontal transmission. *Animal Microbiome* 4, 32.

Venter, L., Alfaro, A.C., Van Nguyen, T. & Lindeque, J.Z. (2022) Metabolite profiling of abalone (*Haliotis iris*) energy metabolism: a Chatham Islands case study. *Metabolomics* 18, 52.

Villasante, A., Catalán, N., Rojas, R., Lohrmann, K.B. & Romero, J. (2020) Microbiota of the digestive gland of red abalone (*Haliotis rufescens*) is affected by withering syndrome. *Microorganisms* 8, 1411.

Wang, G., Zhang, Z., Lin, S., Zhang, L., Wang, B., Wang, S. & Wang, Y. (2015) A homologue of dermatopontin from *Haliotis diversicolor* and its response to pathogenic infection. *Aquaculture Research* 46, 1537–1549.

Wang, X., Tang, B., Luo, X., Ke, C., Huang, M., You, W. & Wang, Y. (2020) Effects of temperature, diet and genotype-induced variations on the gut microbiota of abalone. *Aquaculture* 524, 735269.

Wu, Z., Yu, X., Fu, Y., Guo, J., Pan, M., Guo, Y., Liu, J., Mai, K. & Zhang, W. (2023) Impacts of replacing dietary fish meal with poultry by-product meal on growth, digestive enzymes and gut microbiota, biomarkers of metabolic and immune response, and resistance to *Vibrio* challenge in abalone (*Haliotis discus hannai*). *Aquaculture* 576, 739871.

Xu, J., Liao, W., Liu, Y., Guo, Y., Jiang, S. & Zhao, C. (2023) An overview on the nutritional and bioactive components of green seaweeds. *Food Production, Processing and Nutrition* 5, 18.

Yao, T., Ye, L., Wang, S., Lu, J., Li, H. & Yu, G. (2024) Effects of cadmium exposure on gut microbiota and antibiotic resistance genes in *Haliotis diversicolor* abalone. *Chemosphere* 352, 141507.

Yusup, D. S., Mahardika, I. G., Suarna, I. W., & Giri, I. N. A. (2020) Feeding preference and growth response of early adult abalone, Haliotis squamata, on some macroalgae. *Biodiversitas* 21(9), 4369–4375. https://doi.org/10.13057/biodiv/d210945