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# Diet of snapper (*Chrysophrys auratus*) in green-lipped mussel farms and adjacent soft-sediment habitats

**Lucy H. Underwood**[1,2] | **Aimee van der Reis**[1] | **Andrew G. Jeffs**[1,2]

[1]School of Biological Sciences, University of Auckland, Auckland, New Zealand

[2]Institute of Marine Science, University of Auckland, Auckland, New Zealand

**Correspondence**
Lucy H. Underwood, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand.
Email: lund481@aucklanduni.ac.nz

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## Abstract

Wild fish utilise aquaculture habitats for shelter and/or food resources. It is often assumed that fish respond to feed input, the abundance of the farmed species or the associated assemblage of biofouling which naturally colonises the structural habitats. However, few studies have directly analysed the composition of the diet of fish within aquaculture habitats, and of these most have focused on fed finfish aquaculture. Snapper are commonly present as adults within coastal mussel farms and tend to become a resident species of these farms. Therefore, they are a suitable case study species for exploring differences in diet between natural and aquaculture habitats. This study investigated the gut contents of snapper in soft-sediment habitats within and outside of New Zealand green-lipped mussel farms. Visual gut analysis and DNA metabarcoding methods were used to provide complementary analyses on the composition of gut contents between the mussel farm and natural (i.e., control) sites. Snapper within mussel farms were consistently found to have consumed different prey groups compared to the control snapper. Prey groups identified from mussel farm snapper gut contents could be directly linked to species commonly present in the farms, that is cultured green-lipped mussels, blue mussels and barnacle biofouling. There was good alignment between the visual gut and genetic analyses for the key species identified. Overall, the results show that the highly abundant prey groups consumed by snapper in mussel farm habitats are likely to be beneficial to the snapper population, reducing foraging effort and potentially supplying more nutritious prey. These findings provide evidence towards the supporting services of mussel farm habitats through the provision of food resources.

KEYWORDS
aquaculture, fish nutrition, habitat

## 1 | INTRODUCTION

Coastal aquaculture structures operate as artificial habitats for fish through the provision of shelter and food resources (Alleway et al., 2019; Callier et al., 2018; Theuerkauf et al., 2022). Aquaculture habitats are known to attract and aggregate some fish species (e.g. Siganidae species in seaweed farms [Hehre & Meeuwig, 2016] and Sparidae species in mussel farms [Šegvić-Bubić et al., 2011]), enabling them to reach greater abundance within farms compared to sites without aquaculture structures (Barrett et al., 2022). Some fish species are also known to settle and recruit directly into non-fed aquaculture structures, utilising the structure as a nursery habitat (Underwood & Jeffs, 2023). It is often assumed that the aggregation of fish at aquaculture structures is a response to the input of feed, the abundance of the farmed species or the associated assemblage of potential prey items due to the natural colonisation of aquaculture structures (e.g. biofouling) (Barrett et al., 2022; Callier et al., 2018; Sanchez-Jerez et al., 2011; Theuerkauf et al., 2022). Research in this area has largely focused on differences in the abundance of the functional groups of fish living within or in surrounding aquaculture habitats, to infer responses to the food resources which may be available (Clynick et al., 2008; Theuerkauf et al., 2022). However, few studies have directly analysed the composition of the diet of fish within aquaculture habitats, and of these most have focused on fed finfish aquaculture (Anyango et al., 2017; Fernandez-Jover & Sanchez-Jerez, 2015; Fernandez-Jover et al., 2009; Hayden, 1995; Hehre & Meeuwig, 2016; Šegvić-Bubić et al., 2011; Skog et al., 2003).

In non-fed aquaculture practices (e.g. shellfish), studies have focused on the predatory pressures that wild fish can have during the early stages of production, particularly predation of mussel spat (Peteiro et al., 2010; Šegvić-Bubić et al., 2011; Stenton-Dozey & Broekhuizen, 2019). For example, members of the Sparidae family (seabreams and snapper) are commonly sighted in large schools stripping recently seeded mussel lines (Gerlotto et al., 2001; Peteiro et al., 2010; Stenton-Dozey & Broekhuizen, 2019). These fish are generalist predators and tend to feed based on prey availability rather than selectively (Šegvić-Bubić et al., 2011; Usmar, 2012). The gut contents of seabream sampled from mussel (*Mytilus galloprovincialis*) farms identified mussels as the dominant prey (69.7% biomass), although a diverse range of other prey items (e.g. gastropods, fishes and macroalgae) were also found (Šegvić-Bubić et al., 2011). This provides evidence of what a member of the Sparidae family may feed on within mussel farms, and highlights the concurrent and/or selective epibiota consumption.

Methods to analyse fish diet have traditionally relied on gut dissections and morphology as a precise method to determine the possible food items that are sourced from local habitats, and to compare the differences among habitats (Amundsen & Sánchez-Hernández, 2019; Baker et al., 2014; Braga et al., 2012). However, several other methods exist including visual observations, stable isotope analysis, fatty acid analysis and DNA metabarcoding (Braga et al., 2012; Castro et al., 2008; Udy et al., 2019). Visual observations of the feeding behaviour of fish usually provide high-level information on patterns of feeding but little detail on the relative components of their diet (Amundsen & Sánchez-Hernández, 2019; Braga et al., 2012; Cole, 2010; Morton et al., 2008). Non-lethal methods are less common and have only been trialled on few fish species (Barnett et al., 2010; Castro et al., 2008; Kamler & Pope, 2001; Rennó Braga et al., 2017; Trkov & Lipej, 2019). Non-lethal methods are species specific, are invasive and require technical extraction experience. However, they can still result in mortality and generally are not suitable for most studies of fish diet. Stable isotope and fatty acid analyses are better suited to inferring broad-scale trophic linkages and changes to diet over time rather than identifying specific dietary components (Boecklen et al., 2011; Budge et al., 2006; Udy et al., 2019). DNA metabarcoding is suited to the latter and can identify digested gut content items previously 'unidentifiable' due to the lack of morphological structure (Berry et al., 2015).

A range of methods can be used to sort and quantify gut contents, including numerical frequency of prey items, gravimetric methods and volumetric composition (Amundsen & Sánchez-Hernández, 2019; Baker et al., 2014; Mahesh et al., 2018). Although there have been attempts to standardise the quantification metrics among visual gut content investigations, suitable metrics vary based on the objectives of the study, and each quantification metric has biases making some more suitable metrics for certain species than others. For example, numerical frequency methods are most suitable for fish that consume similar sized prey items to reduce bias towards smaller numerically dominant species, which may have limited volumetric importance (Amundsen & Sánchez-Hernández, 2019). The relative gut fullness method is a modified volumetric method that allows for each prey grouping to be proportionately allocated, while also standardising the entire contents by the fullness of the gut. Therefore, it is an effective approach when samples have varied gut fullness, and for accommodating a range of prey types. DNA-based methods (e.g. DNA metabarcoding) can increase taxonomic accuracy by allowing for the identification of the presence of prey items when their remains are visually unidentifiable in gut contents (O'Rorke et al., 2012; van der Reis et al., 2020). However, the results are semi-quantitative (Deagle et al., 2019), and species-level identification is reliant on the availability of an adequate taxonomic DNA reference library (Amundsen & Sánchez-Hernández, 2019; van der Reis & Lavery, 2020). Therefore, the combination of visual gut analysis and DNA-based methods is considered an effective approach for analysing gut contents.

The overall aim of this research was to compare the diet of snapper (*Chrysophrys auratus*), a common coastal demersal fish species, within and outside established green-lipped mussel longline farms (*Perna canaliculus*) in the Coromandel Harbour, Firth of Thames, New Zealand. Snapper are commonly present as adults and tend to become resident within coastal mussel aquaculture sites in New Zealand (Gibbs, 2004; Stenton-Dozey & Broekhuizen, 2019). These fish are generalist predators that have marked ontogenetic shifts in their diets that are regulated by age and the habitat within which they reside (Usmar, 2012). Therefore, it could be expected that their diet would reflect their use of the surrounding habitat. This research aimed to test whether the diet of snapper collected from mussel farms would differ from those collected in adjacent areas, with the differences likely

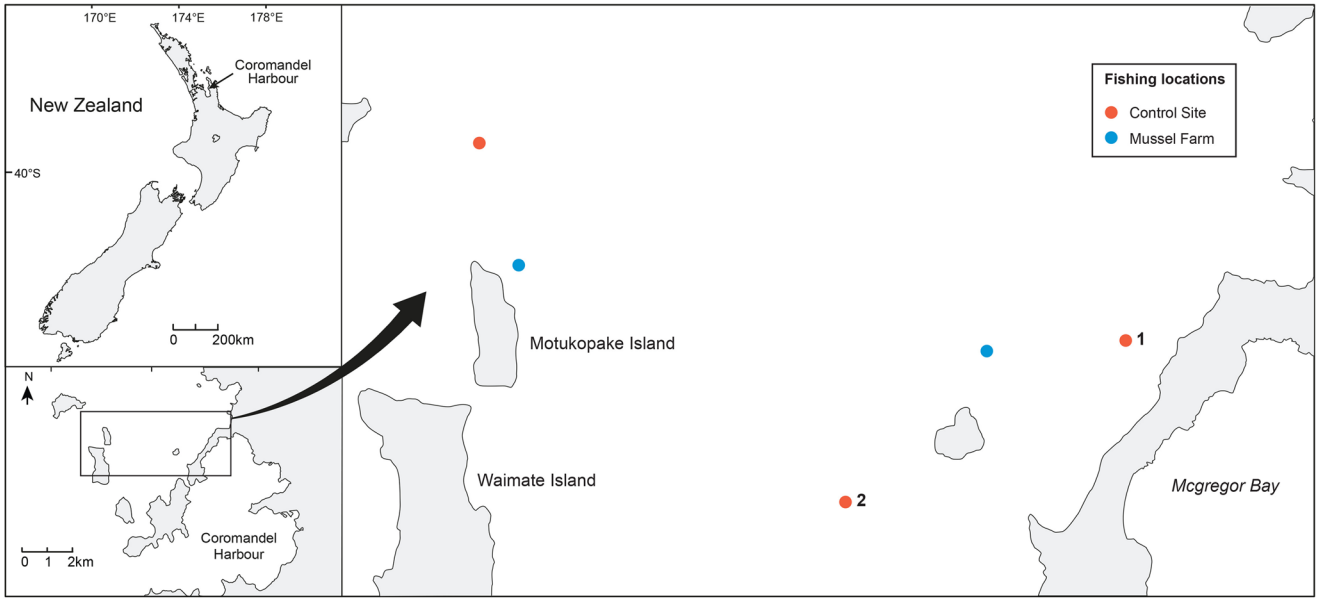


**FIGURE 1** The sampling sites within the Coromandel Harbour, Firth of Thames where snapper were captured using hook and line fishing methods from the seafloor within mussel farms and from nearby control sites at both Motukopake Island and Rat Island.

relating to prey items sourced directly or indirectly from the mussel aquaculture infrastructure.

## 2 | METHODS

### 2.1 | Site locations

Snapper were sampled from four sites in the Firth of Thames. Two were long established longline green-lipped mussel farms and two were sites without mussel farms (i.e. control sites), all located over similar unstructured soft sediment habitat of similar depth (8–13 m) (Figure 1). The farm sites were Motukopake Island (36° 45′ 2.87″ S, 175° 25′ 22.8″ E), and Rat Island (36° 45′ 25.9194″ S, 175° 26′ 59.99″ E). The control sites were at least 500 m away from the farms and were Motukopake Island control site (36° 57′ 32.03″ S, 175° 28′ 51.59″ E) and Rat Island control site (36° 45′ 23.03″ S, 175° 27′ 39.6″ E; 36° 45′ 46.07″ S, 175° 26′ 49.2″ E). Two locations, with the same habitat features, were collectively used for the Rat Island control site due to the low numbers of fish caught at the first control site. The mussel aquaculture operations comprised a series of paired parallel backbone lines held near the surface by large plastic floats that support suspended loops of dropper ropes covered with attached mussels. The dropper ropes extended 6–8 m below the surface floats and were held several metres above the soft-sediment seafloor.

### 2.2 | Sample collection and processing

Adult snapper only within a size range of 26–42 cm fork length were sampled in May and June 2022. Hook and line fishing methods were used, with plastic soft-bait and lures utilised as much as possible to avoid possible contamination of gut contents by bait. Where natural bait was used, a minimal amount of bait was deployed of a readily recognisable species that were not common in the area (i.e. pilchard, squid or mullet). Crepuscular periods of snapper feeding activity were primarily targeted for the sampling to increase the probability of capturing snapper with gut contents. Sixteen fish were obtained from each of three sampling sites (Motukopake Island mussel farm, Motukopake Island control site and Rat Island mussel farm), while 13 snapper were sampled from Rat Island control site. Immediately upon capture, all fish were humanely euthanised according to animal ethics approval (NZ Animal Welfare Act 1999, UoA-AEC Approval # 21619), labelled and put into salted ice slurry. Each snapper was measured (fork length) and weighed back on land (2–6 h after capture) before being frozen for subsequent gut analyses. Previous studies have indicated that freezing snapper gut contents was an appropriate method of preservation that facilitates both visual, molecular genetic and biochemical analysis of gut contents to be undertaken (Drummond, 2020; Supono et al., 2021; Third, 2022).

### 2.3 | Snapper gut dissection

Frozen snapper samples were thawed at room temperature and then dissected. The alimentary tract was removed via incisions at the oesophageal opening and at the anus. The foregut and hindgut were separated and each opened. A gut fullness score was estimated for both the foregut and the hindgut, with 0 being completely empty and 10 being completely full (Third, 2022). Subsequently, the gut fullness score was averaged between the hindgut and the foregut, so that there was only one score per snapper gut. Similarly, a digestion score was

calculated to provide an estimate of the extent of digestion for the prey items for the hindgut and the foregut individually, with a digestion score of 0 being no digestion and 5 being fully digested (Third, 2022). Subsequently, the digestion score was averaged between the hindgut and the foregut, so that there was only one score per snapper gut. Any bait identified in the foregut was removed, and not considered further in this study other than for exclusion from any DNA signal detected. The remaining material in the foregut was weighed, followed by the foregut lining after the gut contents had been emptied. The process was repeated for the hindgut.

## 2.4 | Visual analysis of snapper gut contents

Gut contents for the foregut and hindgut were combined and then spread onto a sterile tissue culture dish (2 × 2 cm grid) and sorted into groups of similar prey items, for each sample. Distilled water was used to separate and clean contents. Each prey group was classified to the lowest practical taxonomic level. An adapted version of the relative-fullness method was used to quantify the proportions of each prey grouping in each individual snapper gut (Amundsen & Sánchez-Hernández, 2019; Baker et al., 2014; Binning & Chapman, 2010). The relative-fullness method then uses proportions of each prey group and standardises by the fullness of the gut to calculate 'points' for each individual prey group within the snapper gut contents. This was undertaken by firstly estimating the two-dimensional (2D) coverage of each prey group in the culture dish (e.g. Bivalvia covering two squares). All prey groups were then summed together to estimate the total coverage within each snapper gut, including the unidentifiable digested material which had its own category. This estimate was then used to calculate the proportion of each prey group within the gut of the sampled snapper. Proportions were multiplied by the average fullness estimate (foregut and hindgut calculated independently but averaged to produce one fullness score) to calculate the relative proportion of each prey group within each individual snapper, thereby standardising the relative proportion of each prey group to facilitate comparisons among samples. For example, if the proportion of the gut content of a snapper was 0.8 (i.e. 80% coverage of the gut contents in the culture dish) for green-lipped mussel (*P. canaliculus*) and the average fullness estimate was 3, this would be calculated as 0.8 × 3 which equals a relative proportion of 2.4 points.

## 2.5 | Genetic analysis of snapper gut content

### 2.5.1 | Gut content removal and DNA extraction

Once gut contents for individual fish had been extracted and analysed for visual gut analysis, the gut contents were mixed and a 2-mL subsample was preserved in 90% ethanol (2 mL cryovial) and stored at −20°C. All dissection tools were sterilised between handling individual snapper.

DNA was extracted from the subsample using the Nucleospin Tissue DNA extraction kit (Macherey-Nagel) following the manufacturer's instructions. Controls were included in the extractions, that is DNA extraction blank where no content was added. The DNA quality and quantity were viewed on 0.8% agarose, visualised with gel red (Biotium) using a Gel Doc XR+ (Bio-Rad).

### 2.5.2 | DNA amplification

Species identified microscopically from the gut contents were compared to sequences available on GenBank. The results indicated that cytochrome oxidase one (COI) would have the best coverage, and thus COI was targeted for amplification during the polymerase chain reactions (PCRs). The universal primer pair selected was M1COIintF (forward; Leray et al., 2013) and jgHCO2198 (reverse; modified replacing 'I' with 'N'; Geller et al., 2013). Illumina Nextra adapters were added to the primer sequences. The PCRs were done in triplicate using 6.25 μL of MyTaq Red Mix (Bioline; Meridian Bioscience), 0.25 μL of each primer (10 μM), 1 μL of bovine serum albumin (1%) and 4.75 Ultra-Pure DNase/RNase free water (Invitrogen; Thermo Fisher Scientific). Each PCR cycle included the DNA blanks and a PCR blanks (no DNA added) to check for possible contamination. The PCR protocol was a modified version of Lobo et al. (2013): 94°C for 60 s, 35 × [94°C for 30 s; 54°C for 90 s; 72°C for 60 s] and a final extension at 72°C for 5 min (van der Reis et al., 2023). Amplification was checked by running the PCR products on a 1.6% agarose, and visualised as outlined above.

### 2.5.3 | Sequencing and bioinformatics

PCR triplicates were pooled per sample and then cleaned using AMPure XP (Beckman Coulter), following the Illumina 16S protocol (Illumina, 2013). The concentration of the cleaned PCR products was determined using Qubit dsDNA HS Assay Kit (Invitrogen) following the manufacturer's instructions and subsequently diluted to 2 ng/μL before sequencing. Sequencing was done by Auckland Genomics and undertaken on an Illumina MiSeq (2 × 250 paired-end nano run; single lane used).

Cutadapt v3.5 (Martin, 2011) was used for primer removal, retaining only those sequences with exact primer matches anchored at the beginning of the sequence. Qiime2 v2022.2 (Bolyen et al., 2019) was used to assess the sequence quality prior to truncation in DADA2 (Callahan et al., 2016; utilised within Qiime2). High-quality amplicon sequence variants (ASVs; Callahan et al., 2017) were produced by filtering for sequence quality, retaining only sequences that merged and that were identified non-chimeric.

ASVs were run through GenBank's BLAST database v2022-07 using the MegaBLAST function within the BLASTn suite (Benson et al., 2013; minimum *E*-value threshold of 0.001 and a minimum percentage identity of ≥70%). Resulting taxonomic assignments were then run through the World Register of Marine Species (WoRMS) database (Horton

et al., 2022) to confirm taxonomy and filter to retain only marine species (i.e. remove any spurious terrestrial sequences). R v4.0.4 (R Core Team, 2021) was used for final sequence filtering and quality assurance of the taxonomic assignments. Potential contamination was accounted for by a proportional subtraction of the controls (DNA and PCR blanks were sequenced). Sequences for snapper (*C. auratus*) (host species) were removed. Only sequences >280 base pairs that had ≥85% identity match to the taxonomic sequence were used. Sequences were also filtered to retain only those with ≥90% query coverage.

## 2.6 | Statistical analyses

All statistical analyses and plots were produced using R (R Core Team, 2021). All analyses used a significance of $\alpha = 0.05$. All means are presented as mean ± standard error. Plots were prepared with ggplot (package version 4.0.4, ggplot2 function).

### 2.6.1 | Snapper metrics

To compare differences in snapper length, a two-way analysis of variance (package version 4.0.4, aov function) was used with the main factors of Location (Motukopake Island or Rat Island) and Treatment (mussel farm or control). Data were plotted and visually assessed to confirm parametric assumptions were met. Tukey's honest significant difference (package version 4.0.4, TukeyHSD function) post hoc tests were conducted if an overall significant difference was identified. This approach was repeated to compare the differences in the wet weight (g) for each of foregut and hindgut contents separately. Only foregut wet weight data needed to be transformed prior to analyses by using a log($x$ + 1) transformation. A generalised-linear model (package version 4.0.4, glm function) was used to compare the differences in the combined average digestion score for the hindgut and the foregut, with a quasi-Poisson distribution and the main factors of Location and Treatment. If an overall significant difference was identified, 'emmeans' (package version 1.5.5-1, emmeans function) post hoc analyses were used.

### 2.6.2 | Visual gut analysis

To compare the amount of prey groups at a broad taxonomic level in snapper gut contents among samples, non-metric multidimensional scaling (NMDS) was conducted with a Bray–Curtis distance and a permutation value of 999, using points data derived from the relative-fullness method. An ordination plot was produced for a 2D representation of the ordination, separated by Treatment. A permutational multivariate analysis of variance (PERMANOVA) was undertaken using points data of prey groups at the broad taxonomic level, with the Bray–Curtis distance and the main factors of Location and Treatment. Where the overall model results were significant, a Wilcoxon test was used to determine differences in the amount (quantified as 'points' from the relative-fullness method) of prey groups at the broad taxonomic level consumed by snapper among sites. A Bonferroni adjustment was used for *p*-values to account for potential error inflation from multiple comparisons.

For prey groups at the lowest taxonomic level, only presence/absence data were used for each prey group identified within each individual snapper. NMDS was conducted with a Bray–Curtis distance and a permutation value of 999, using presence/absence data of the different prey groups at the lowest taxonomic level. An ordination plot was produced for a 2D representation of the ordination, separated by Treatment. A PERMANOVA was undertaken for prey groups at the lowest taxonomic level using presence/absence data, with the Bray–Curtis distance and the main factors of Location and Treatment. Where the overall model results were significant, a Fisher's exact test was used to determine whether the frequency of each prey group at the lowest taxonomic level found within snapper gut contents was significantly different between Treatment and Location. A Bonferroni adjustment was used for *p*-values to account for potential error inflation from multiple comparisons.

### 2.6.3 | DNA metabarcoding analysis

The presence/absence data of prey groups identified from DNA metabarcoding at the family level and class level for gut content of all individual snapper were analysed separately. A PERMANOVA was undertaken on the presence/absence data for detected prey separately for the family level and at the class level with the Bray–Curtis distance using the main factors of Location and Treatment. If significance was detected, a Fisher's exact test was undertaken using presence/absence data of the prey groups. Family-level comparisons among the samples of gut content excluded 13 snapper samples where no families were identified (Motukopake Island control site = 2, Motukopake Island mussel farm = 3, Rat Island control site = 3 and Rat Island mussel farm = 5). Class-level comparisons excluded nine snapper samples where zero taxonomic classes of prey were identified (Motukopake Island control site = 3, Motukopake Island mussel farm = 1, Rat Island control site = 2 and Rat Island mussel farm = 3).

# 3 | RESULTS

## 3.1 | Snapper metrics

Snapper length ranged from 27.4 to 41.2 cm at Rat Island mussel farm, 27.0 to 41.6 cm at Motukopake Island mussel farm, 25.8 to 30.2 cm at Rat Island control site and 26.8 to 37.4 cm at Motukopake Island control site (Figure 2). There were differences in mean snapper length for the sampled snapper for Treatment, that is mussel farm versus control site ($F_{(1, 57)} = 5.41$, $p = 0.024$) and the interaction Treatment × Location ($F_{(1, 57)} = 9.51$, $p = 0.0032$), but not for Location, that is Motukopake Island versus Rat Island ($F_{(1, 57)} = 1.04$, $p = 0.31$) (Figure 2). Post hoc analyses identified that snapper sampled at the Motukopake Island

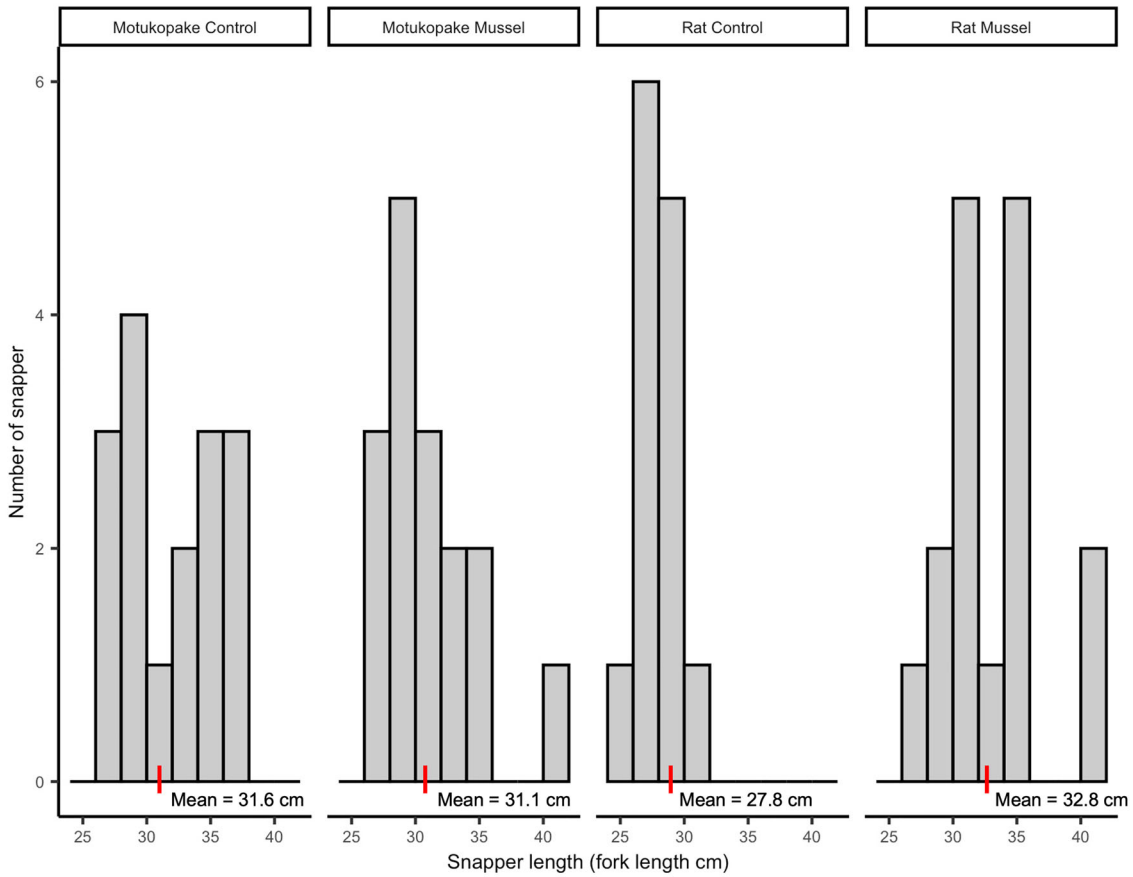


**FIGURE 2** Size frequency of snapper (*Chrysophrys auratus*) length (as fork length [cm]) (bin width = 2 cm) sampled at four sites in the Firth of Thames (Motukopake Island control site, $n = 16$; Motukopake Island mussel farm, $n = 16$; Rat Island control site, $n = 13$; Rat Island mussel farm, $n = 16$). Red lines indicate mean snapper length for each site.

control site were larger than at the Rat Island control site ($p = 0.024$; 31.6 ± 1.0 and 27.8 ± 0.3 cm, respectively), while snapper from Rat Island mussel farm were larger than those sampled from the Rat Island control site ($p = 0.002$; 32.8 ± 1.0 and 27.8 ± 0.3 cm, respectively).

The gut contents of snapper sampled at mussel farm sites had a range of 1–5 in digestion scores, and control sites had a range of 2–5 in digestion scores (Figure 3). There were overall significant differences in mean digestion scores between the two Locations and Treatments ($F_{(57,60)} = 2.79, p = 0.04$). The main factor effects of Location and Location × Treatment were significant ($p = 0.04$ and $p = 0.01$, respectively), but not the main factor Treatment ($p = 0.42$). Post hoc analyses identified that Rat Island control site had a significantly higher digestion score compared to Motukopake Island control site ($p = 0.04$; 0.9 ± 0.24 and 3.3 ± 0.16) and Rat Island mussel farm ($p = 0.005$; 3.9 ± 0.24 and 2.9 ± 0.29).

Total wet weight of snapper gut contents ranged from 1.26 to 30.08 g at Rat Island mussel farm, 1.29 to 17.99 g at Motukopake Island mussel farm, 3.26 to 15.33 g at Rat Island control site and 2.17 to 4.50 g at the Motukopake Island control site. Every snapper had some gut contents present; however, the majority of gut contents were in the hindgut. There were no differences in the mean wet weight of the foregut contents between Treatments ($F_{(1,57)} = 0.16$, $p = 0.69$), Location ($F_{(1,57)} = 0.75$, $p = 0.39$), or for the interacting effect of Treatment × Location ($F_{(1,57)} = 2.56$, $p = 0.12$) (Figure 3). Similarly, there were no differences in mean wet weight of the hindgut contents between Treatments ($F_{(1,57)} = 1.36, p = 0.25$), Location ($F_{(1,57)} = 0.004$, $p = 0.95$), or for the interacting effect of Treatment × Location ($F_{(1,57)} = 0.89, p = 0.35$) (Figure 3).

## 3.2 | Gut content composition in visual analysis

The gut contents of snapper were moderately to highly digested for all four sites (Figure 3), which made the identification of prey groups difficult, and required piecing together parts of the organism to identify what taxa it was. Most of the material came from the hindgut, with 25% of snapper in mussel farm sites and 17% of snapper in control sites with no material in the foregut. The Rat Island control site also had a higher total digestion score (average of hindgut and foregut) and a higher proportion of digested material compared to other sites, which is likely due to time since prey consumption rather than types of prey consumed. Overall, the digestion of the gut contents meant that the taxonomic level to which prey groups could be identified was most often limited. For example, the remains of Decapoda within the gut contents

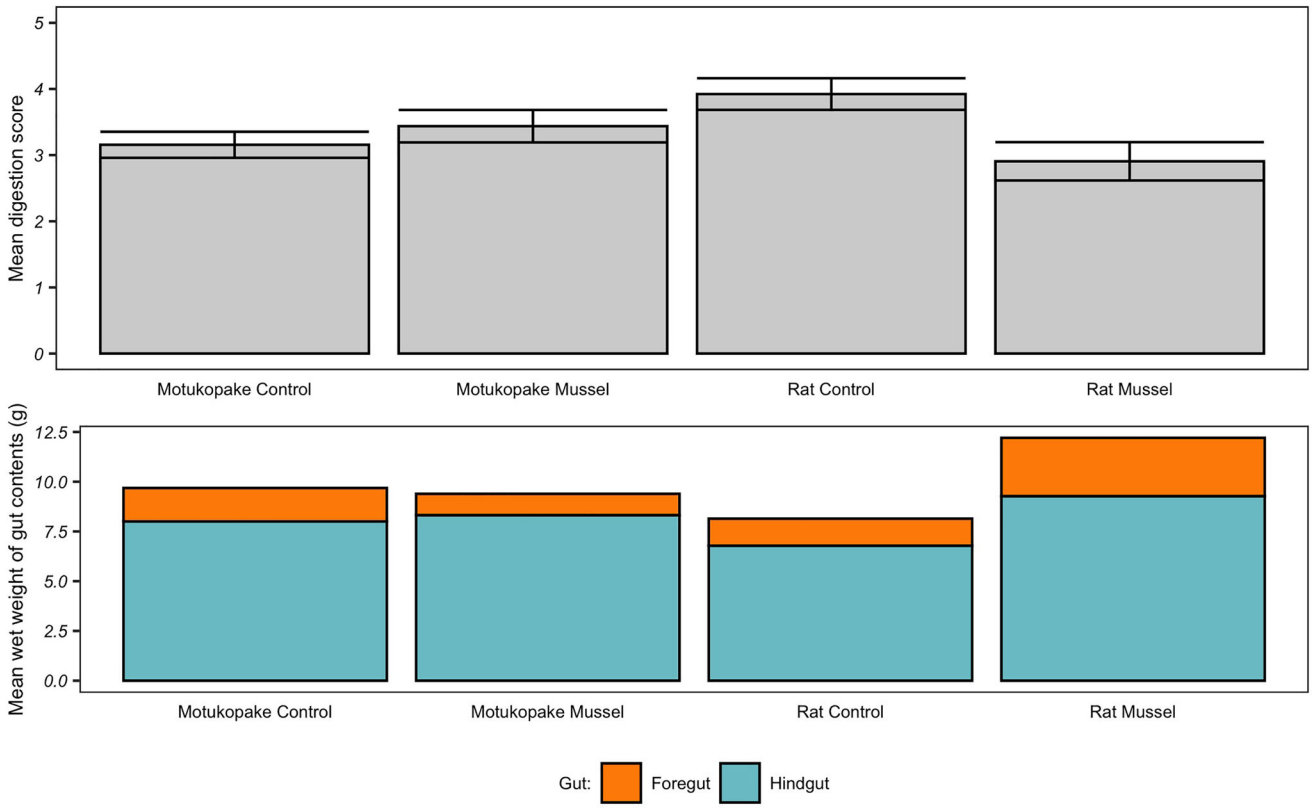


**FIGURE 3** Top figure mean digestion score of gut contents of snapper sampled from each of the four sites (Motukopake Island control site, Motukopake Island mussel farm, Rat Island control site and Rat Island mussel farm). A digestion score of 0 represents completely undigested material and a score of 5 represents fully digested material (error bars represent standard error). Bottom figure mean wet weight (g) of snapper foregut and hindgut contents for the four sites (Motukopake Island control site, $n = 16$; Motukopake Island mussel farm, $n = 16$; Rat control site, $n = 13$; Rat Island mussel farm, $n = 16$).

of snapper sampled at Rat Island control sites could not be identified to lower taxonomic levels, for example Caridea, Brachyura and Paguroidea.

### 3.2.1 | Gut content analysis for prey groups at broad taxonomic level

Bivalvia were the dominant prey group in snapper gut contents, especially for the two mussel farm sites (Figure 4; Table 1). This was due to the high proportion of mussels present in the gut contents (i.e. 29% in Rat Island mussel farm and 18% in Motukopake Island mussel farm vs. 0% for both the Motukopake Island and Rat Island control site), which are within the Bivalvia category at the broad taxonomic level (Figure 4). In contrast, Decapoda were a common component of the gut contents at one control site but not at either of the mussel farm sites (i.e. 22.5% in Rat Island control site vs. 0% in Rat Island mussel farm) (Figure 4). Other Crustacea (i.e. excluding those prey groups that were classified into different Crustacea taxa such as Caridea, Decapoda, Brachyura and Paguroidea) were higher in mussel farm sites (i.e. 14.6% in Rat Island mussel farm vs. 1.4% in Rat Island control site), and Paguroidea was higher in control sites (i.e. 9.5% in Motukopake Island control site vs. 0% in the Motukopake Island mussel farm) (Figure 4; Table 1).

The ordination plot of the amount of prey groups at the broad taxonomic level (derived from relative proportion data) in the snapper gut contents identified a partial separation between control and mussel farm sites; however, there is still considerable overlap between treatments (Figure 5). PERMANOVA identified significant differences in the composition of gut contents for Treatment (pseudo$F_{(1, 57)} = 6.39$, $p = 0.001$) and Treatment × Location (pseudo$F_{(1, 57)} = 3.12$, $p = 0.01$), but not for Location (pseudo$F_{(1, 57)} = 1.46$, $p = 0.18$). Post hoc analyses indicated that the prey groups at a broad taxonomic level driving the differences were the categories Bivalvia, other Crustacea, Decapoda and Paguroidea (Table 2). Specifically, snapper sampled at Rat Island control site had consumed fewer Bivalvia compared to snapper sampled from all other sites (Table 2). Additionally, snapper from Motukopake Island mussel farm consumed more other Crustacea than both control sites. Rat Island control site snapper consumed more Decapoda compared to both mussel farm sites. Motukopake Island control site snapper consumed more Paguroidea compared to Motukopake Island mussel farm (Table 2).

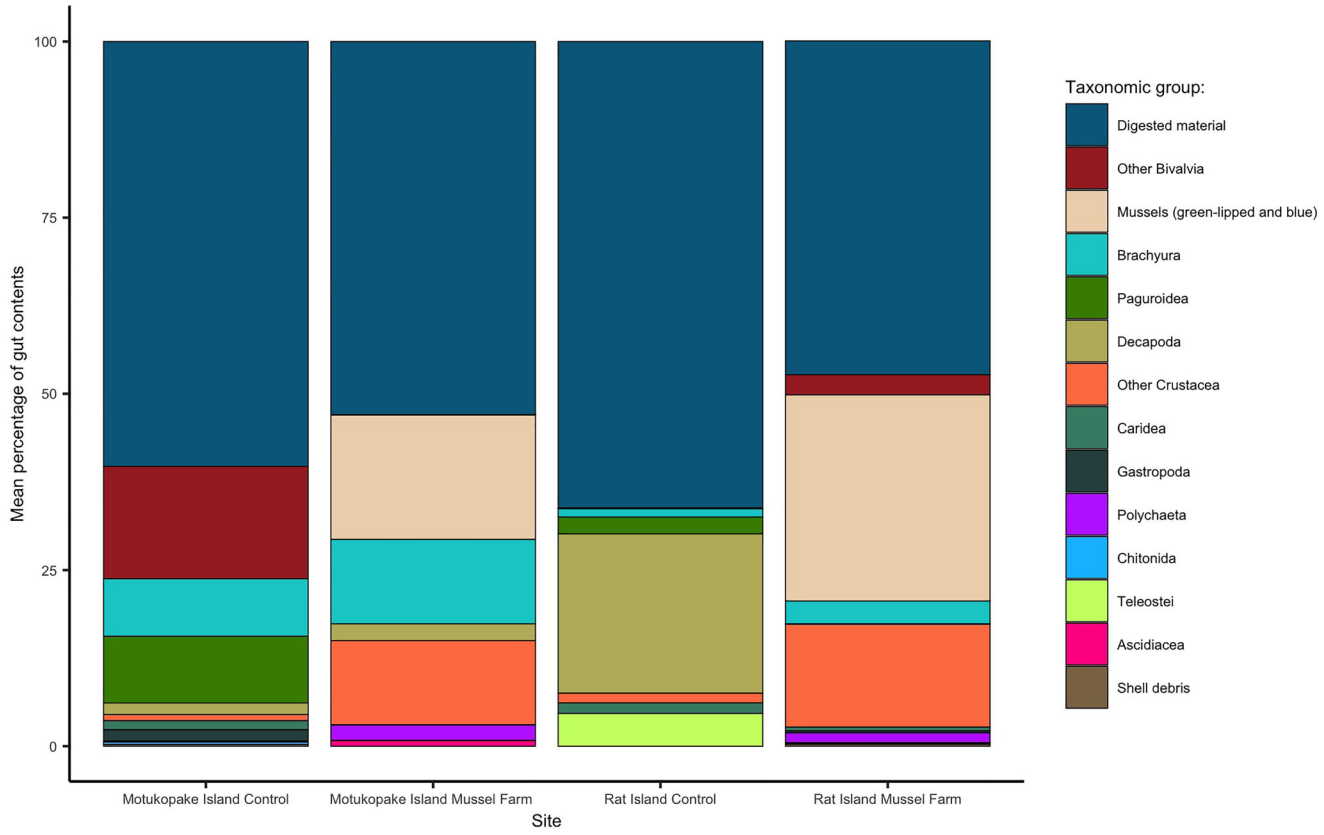


**FIGURE 4** Mean percentage of prey groups at the broad taxonomic level present in snapper gut contents (hindgut and foregut combined), as a relative proportion of each prey group based on the total gut contents within each individual snapper. The relative proportions of each broad taxonomic group in individual snapper were averaged for each of the four sampling sites (Motukopake Island control site, Motukopake Island mussel farm, Rat Island control site and Rat Island mussel farm). Mussels (green-lipped and blue) extracted from other Bivalvia for visual purposes only and not as part of the statistics at the broad taxonomic level.

**TABLE 1** Total amount of prey groups at the broad taxonomic level in gut contents of snapper sampled from each of four sites (Motukopake Island control site, $n = 16$; Motukopake Island mussel farm, $n = 16$; Rat Island control site, $n = 13$; Rat Island mussel farm, $n = 16$)

| Prey groups at the broad taxonomic level in snapper gut contents | | Motukopake Island control site | Motukopake Island mussel farm | Rat Island control site | Rat Island mussel farm |
|---|---|---|---|---|---|
| Digested material | | 18.94 | 15.34 | 14.48 | 13.25 |
| Mollusca | Bivalvia | **4.71** | **6.53** | 0.075 | **12.01** |
| | Gastropoda | 0.52 | 0.00 | 0.00 | 0.00 |
| | Chitonida | 0.11 | 0.00 | 0.00 | 0.20 |
| Arthropoda | Brachyura | 3.83 | 4.16 | 0.38 | 1.15 |
| | Paguroidea | 3.37 | 0.00 | 0.83 | 0.02 |
| | Decapoda | 0.53 | 0.94 | **5.99** | 0.00 |
| | Other Crustacea | 0.35 | 3.33 | 0.42 | 6.07 |
| | Caridea | 0.51 | 0.00 | 0.40 | 0.11 |
| Annelida | Polychaeta | 0.04 | 0.88 | 0.00 | 0.52 |
| Chordata | Teleostei | 0.00 | 0.00 | 2.42 | 0.00 |
| | Ascidiacea | 0.00 | 0.32 | 0.00 | 0.00 |
| Shell debris | | 0.1 | 0.00 | 0.00 | 0.16 |

*Note*: Prey were classified into a broad taxonomic level, and the relative proportion measure (points) identifies the contribution of each group standardised by the gut fullness score, as calculated by the modified relative-fullness method. Bolded values represent the highest prey group (excluding digested material) per site.

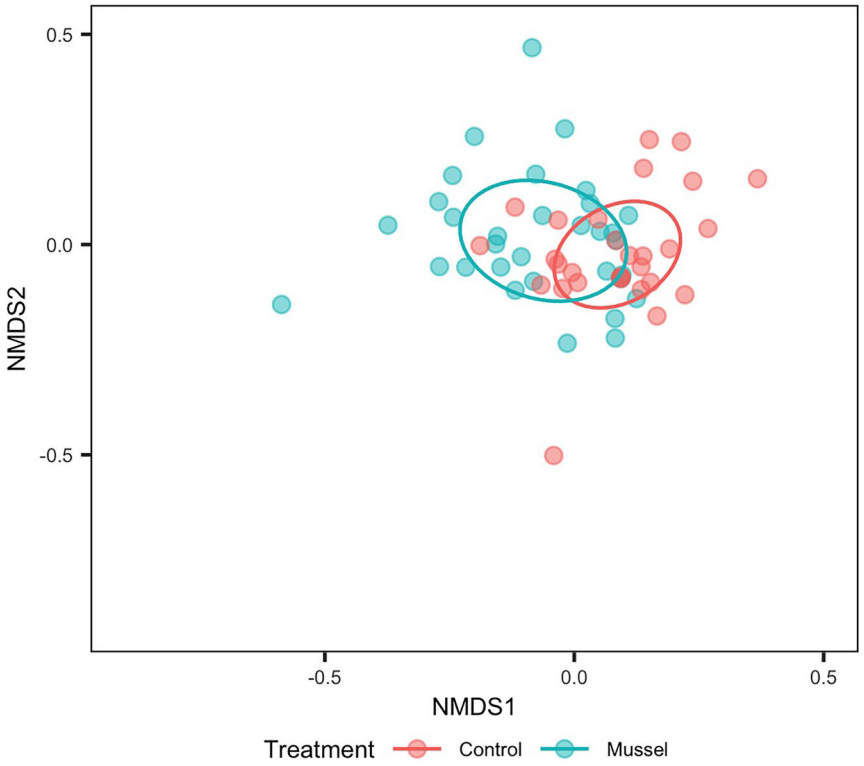


**FIGURE 5** Non-metric multidimensional scaling (NMDS) plot of the relative proportion measure data of each of 13 prey groups at a broad taxonomic level found in snapper gut contents at four sites and presented for the two control and two mussel farm sites (see Table 1). Confidence interval (50%) ellipses displayed over Treatments.

**TABLE 2** Wilcoxon test post hoc significant differences in the amount of prey groups at a broad taxonomic level consumed by individual snapper among the sites Motukopake Island mussel farm, Motukopake Island control site, Rat Island mussel farm and Rat Island control site

| Prey group at a broad taxonomic level | Comparison | Mean and standard error (points) | Adjusted *p*-value |
|---|---|---|---|
| Bivalvia | Rat Island control site < Motukopake Island control site | $0.006 \pm 0.006 < 0.29 \pm 0.07$ | 0.01 |
| | Rat Island control site < Rat Island mussel farm | $0.006 \pm 0.006 < 0.75 \pm 0.25$ | 0.03 |
| | Rat Island control site < Motukopake Island mussel farm | $0.006 \pm 0.006 < 0.41 \pm 0.13$ | 0.04 |
| Other Crustacea | Motukopake Island control site < Motukopake Island mussel farm | $0.02 \pm 0.02 < 0.21 \pm 0.06$ | 0.006 |
| | Rat Island control site < Motukopake Island mussel farm | $0.02 \pm 0.02 < 0.21 \pm 0.06$ | 0.04 |
| Decapoda | Motukopake Island mussel farm < Rat Island control site | $0.06 \pm 0.06 < 0.46 \pm 0.17$ | 0.02 |
| | Rat Island mussel farm < Rat Island control site | $0 \pm 0 < 0.46 \pm 0.17$ | 0.004 |
| Paguroidea | Motukopake Island mussel farm < Motukopake Island control site | $0 \pm 0 < 0.21 \pm 0.08$ | 0.04 |

*Note*: Prey were classified into a broad taxonomic level, and the relative proportion measure identifies the relative contribution of each prey group calculated from the modified relative-fullness method. Mean and standard error are from the raw data.

### 3.2.2 | Gut content analysis for prey groups classified to lowest taxonomic level

Prey identified to the lowest taxonomic level within snapper gut contents produced two sets of data. Firstly, the relative proportion measure derived from the proportions of prey items in gut content combined with the relative-fullness measure was used to identify the relative amount of each prey group consumed among snapper (Table 3). Secondly, presence/absence data were used to calculate the percentage of individual snapper within a site that had the prey group present (Table 3). The highest amount of prey consumed by Motukopake Island mussel farm snapper was green-lipped mussels (*P. canaliculus*); however, the most frequently present prey was the triangle barnacle (*Balanus trigonus*) (Table 3). At Rat Island mussel farm, the highest amount and most frequently present prey was green-lipped mussels (Table 3). The highest amount of prey consumed by Motukopake Island control site snapper was wrinkled swimming crab (*Liocarcinus corrugatus*) and the most frequently present prey was hermit crabs in the

**TABLE 3** Total amount (derived from relative proportion measure data) of prey groups classified to the lowest taxonomic level consumed by snapper, and total percentage of individual snapper with a prey group present (derived from presence/absence data), among the sites (Motukopake Island control site, $n = 16$; Motukopake Island mussel farm, $n = 16$; Rat Island control site, $n = 13$; Rat Island mussel farm, $n = 16$)

| Prey groups at the lowest taxonomic level in snapper gut contents | | | Motukopake Island control site | Motukopake Island mussel farm | Rat Island control site | Rat Island mussel farm |
|---|---|---|---|---|---|---|
| Digested material | | | 18.94 and 100% | 15.34 and 100% | 14.48 and 100% | 13.25 and 93% |
| Mollusca | Bivalvia | Unidentified Bivalvia | 0.48 and 6% | 0.00 | 0.00 | 0.04 and 6% |
| | | *Anomia trigonopsis* | 1.28 and 25% | 0.03 and 6% | 0.00 | |
| | | *Atrina zelandica* | 2.11 and 37% | 0.00 | 0.00 | 1.07 and 6% |
| | | *Austrovenus stutchburyi* | 0.34 and 6% | 0.00 | 0.00 | 0.00 |
| | | *Dosina* spp. | 0.59 and 19% | 0.00 | 0.00 | 0.00 |
| | | *Mytilus galloprovincialis planulatus* | 0.00 | 0.60 and 13% | 0.00 | 4.04 and 31% |
| | | Ostreidae spp. | 0.00 | 0.00 | 0.075 and 8% | 0.00 |
| | | *Paphies australis* | 0.08 and 6% | 0.00 | 0.00 | 0.00 |
| | | *Perna canaliculus* | 0.00 | **5.90** and 56% | 0.00 | **6.85 and 56%** |
| | Gastropoda | Trochidae spp. | 0.52 and 13% | 0.00 | 0.00 | 0.00 |
| | Chitonida | Unidentified chiton | 0.11 and 6% | 0.00 | 0.00 | 0.043 and 6% |
| | | *Cellana ornata* | 0.00 | 0.00 | 0.00 | 0.16 and 6% |
| Arthropoda | Brachyura | Unidentified Brachyura | 0.1 and 6% | 0.61 and 19% | 0.38 and 8% | 0.17 and 6% |
| | | *Halicarcinus innominatus* | 0.00 | 1.49 and 6% | 0.00 | 0.00 |
| | | *Halicarcinus* spp. | 0.00 | 0.18 and 6% | 0.00 | 0.00 |
| | | *Liocarcinus corrugatus* | **3.70** and 13% | 0.00 | 0.00 | 0.00 |
| | | *Nepinnotheres novaezelandiae* | 0.00 | 0.20 and 6% | 0.00 | 0.25 and 6% |
| | | *Notomithrax* spp. | 0.04 and 13% | 0.86 and 13% | 0.00 | 0.72 and 6% |
| | | *Pilumnus novaezelandiae* | 0.00 | 0.77 and 13% | 0.00 | 0.00 |
| | | Portunidae | 0.00 | 0.057 and 6% | 0.00 | 0.00 |
| | Paguroidea | Paguridae spp. | 2.86 and **38%** | 0.00 | 0.83 and 23% | 0.02 and 6% |
| | | *Lophopagurus* spp. | 0.50 and 19% | 0.00 | 0.00 | 0.00 |
| | Decapoda | Unidentified decapod | 0.53 and 13% | 0.94 and 6% | **5.99** and **62%** | – |
| | Crustacea | *Balanus trigonus* | 0.00 | 3.33 and **68%** | 0.42 and 15% | 5.76 and 44% |
| | | *Epopella plicata* | 0.00 | 0.00 | 0.00 | 0.32 and 6% |
| | | Meiura | 0.35 and 6% | 0.00 | 0.00 | 0.00 |
| | Caridea | Unidentified Caridea | 0.02 and 6% | 0.00 | 0.33 and 15% | 0.11 and 6% |
| | | *Alpheus richardsoni* | 0.00 | 0.00 | 0.07 and 8% | 0.00 |
| | *Biffarius filholi* | 0.48 and 6% | 0.00 | 0.00 | 0.00 | |
| Annelida | Polychaeta | Unidentified polychaete | 0.04 and 6% | 0.00 | 0.00 | 0.05 and 6% |
| | | *Eulalia microphylla* | 0.00 | 0.45 and 6% | 0.00 | 0.00 |
| | | Serpulidae spp. | 0.00 | 0.43 and 38% | 0.00 | 0.47 and 13% |
| Chordata | Teleostei | *Forsterygion* spp. | 0.00 | 0.00 | 2.42 and 8% | 0.00 |
| | Ascidiacea | *Styela clava* | 0.00 | 0.32 and 6% | 0.00 | 0.00 |
| Shell debris | | Misc. shell debris | 0.1 and 6% | 0.00 | 0.00 | 0.16 and 6% |

*Note*: The relative proportion measure identifies the relative contribution of each prey item calculated by the modified gut fullness method. The percentages represent the proportion of snapper samples that had the prey item present, out of the total number of individuals within each site. Bolded values represent the highest value (excluding digested material) per site.

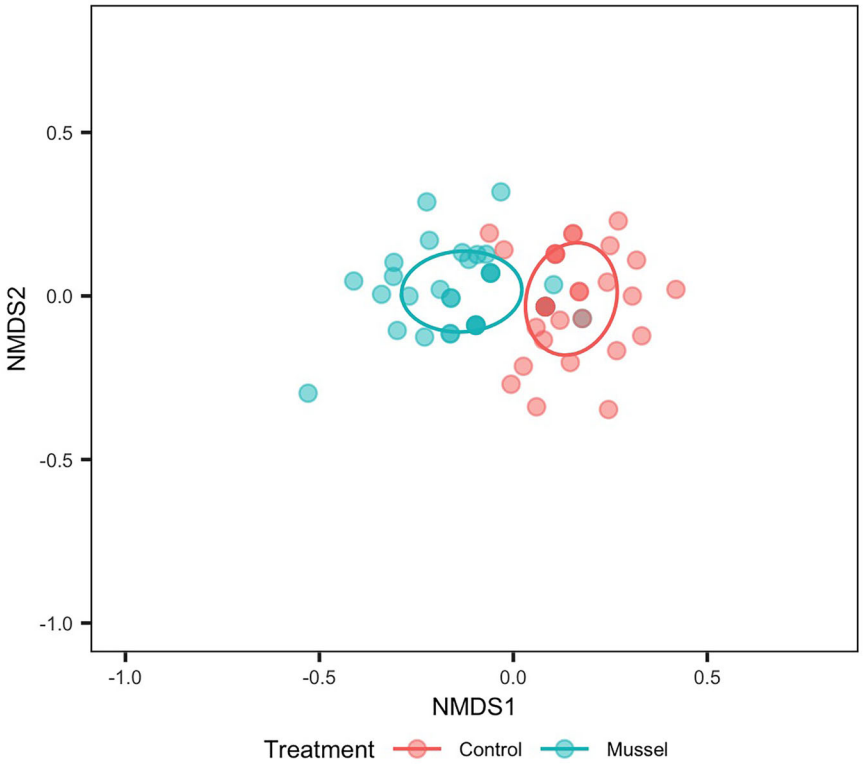


**FIGURE 6** Non-metric multidimensional scaling (NMDS) plot of presence/absence data for prey groups classified to the lowest taxonomic level within snapper gut contents at control and mussel farm sites (see Table 3). Confidence interval (50%) ellipses displayed over Treatments.

**TABLE 4** Fishers exact test post hoc significant differences in the presence/absence of prey groups classified to the lowest taxonomic level within snapper gut contents between the sites Motukopake Island mussel farm, Motukopake Island control site, Rat Island mussel farm and Rat Island control site

| Prey group at lowest taxonomic level | Comparison | Percent | Adjusted *p*-value |
|---|---|---|---|
| Triangle barnacle (*Balanus trigonus*) | Motukopake Island control site < Motukopake Island mussel farm | 0% vs. 68% | 0.002 |
| Green-lipped mussel (*Perna canaliculus*) | Motukopake Island control site < Motukopake Island mussel farm | 0% vs. 56% | 0.02 |
| | Rat Island control site < Rat Island mussel farm | 0% vs. 56% | 0.04 |
| | Motukopake Island control site < Rat Island mussel farm | 0% vs. 56% | 0.03 |
| | Rat Island control site < Motukopake Island mussel farm | 0% vs. 56% | 0.04 |
| Decapoda spp. | Rat Island mussel farm < Rat Island control site | 0% vs. 62% | 0.01 |

Paguridae family (Table 3). At the Rat Island control site, the highest amount and most frequently present prey in snapper gut contents was Decapoda (Table 3).

Statistical analysis for prey groups classified to the lowest taxonomic level was only completed on presence/absence data. The ordination plot for presence/absence data of prey groups at the lowest taxonomic level identified separation between control and mussel farm sites (Figure 6). PERMANOVA identified significant differences in the composition of snapper gut contents for Treatment (pseudo$F_{(1,57)}$ = 13.58, $p$ = 0.001) and Treatment × Location (pseudo$F_{(1,57)}$ = 3.46, $p$ = 0.001), but not for Location (pseudo$F_{(1,57)}$ = 2.11, $p$ = 0.05). Post hoc analyses indicated that the main prey groups at the lowest taxonomic level driving the differences were triangle barnacle, green-lipped mussel and other Decapoda (Table 4). Triangle barnacles and green-lipped mussels occurred more frequently in Motukopake Island mussel farm compared to the Motukopake Island control site (Table 4). Motukopake Island mussel farm also had a higher frequency of green-lipped mussels present in snapper gut contents compared to Rat Island control site. Similarly, the Rat Island mussel farm had a higher frequency of green-lipped mussels present in snapper gut contents compared to Rat Island control site and Motukopake Island control site. The Rat Island control site had a higher frequency of other Decapoda compared to the mussel farm site (Table 4).

## 3.3 | Gut content composition in genetic analysis

At Motukopake Island, there were 20 families identified at the four sites (Figure 7). The greatest proportion of species at both Treatments was within the Malacostraca (Crustacea) class (Figure 7). Key differences were the presence of a high proportion of Mytilidae (mussels)

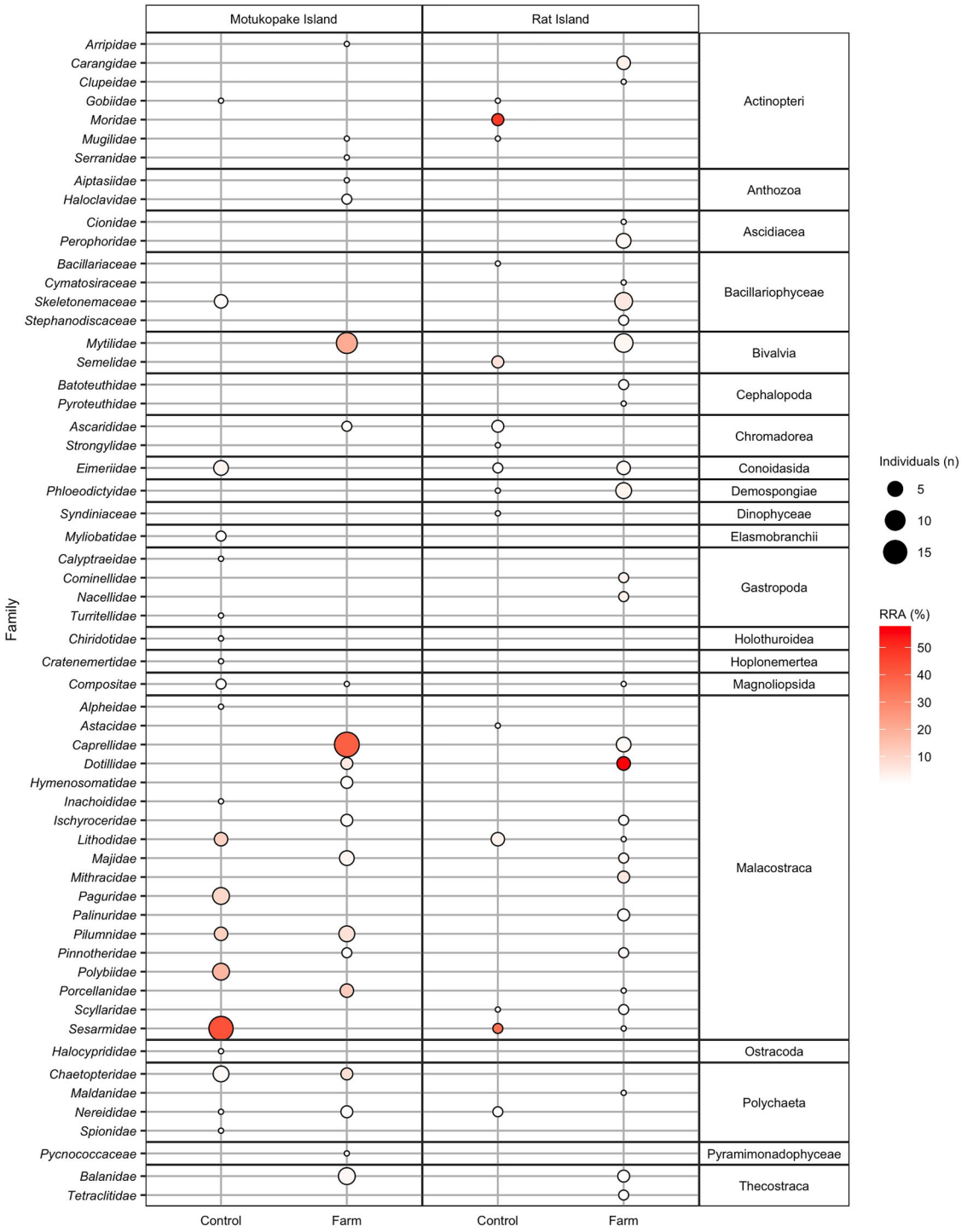


**FIGURE 7** Presence/absence plot for DNA sequences identified in snapper gut contents at Motukopake Island mussel farm ($n = 16$) and Motukopake Island control site ($n = 16$), and Rat Island mussel farm ($n = 16$) and Rat Island control site ($n = 13$) categorised by family level (left-hand side) and class level (right-hand side). The size of the circles indicates the number of individuals where the prey group was identified, and the colour is the relative read abundance (RRA) which is a semi-quantitative metric to understand dominance of the prey group within the snapper sample.

and Caprellidae (Amphipoda) in mussel farm sites and none in the control site (Figure 7). Sesarmidae (Brachyura) had the highest proportion in control sites with none present at mussel farm sites (Figure 7).

At Rat Island, there were 15 families identified at the control site and 29 families identified at the mussel farm (Figure 7). The greatest proportion of families at the control site was within the Actinopteri (ray-finned fish) and Malacostraca (Crustacea) class, and in the mussel farm it was Malacostraca (Figure 7). Mytilidae (mussels) and Skeletonemaceae (microalgae) were most present in the mussel farm, whereas Semilidae (clams) and Lithodidae (king crabs) were represented in greater number of samples in the Rat Island control site (Figure 7). The most dominant prey group (based on relative read abundance) in the mussel farm was the Dotillidae (crabs) and Moridae (cod fish) at the control site (Figure 7).

PERMANOVA identified significant differences in the presence/absence of prey at the family level in the gut contents of snapper for Treatment (pseudo$F_{(1, 44)}$ = 5.66, $p$ = 0.001), Location (pseudo$F_{(1, 44)}$ = 2.07, $p$ = 0.007) and Treatment × Location (pseudo$F_{(1, 44)}$ = 2.12, $p$ = 0.009). However, $p$-adjusted values in post hoc analyses could not detect any significant differences among the four sites. Non-adjusted $p$-values showed some differences in families between Treatments. For example, the higher presence of Mytilidae in snapper gut contents sampled from Motukopake Island mussel farm (54%) and Rat Island mussel farms (55%) was significantly greater than the control sites (0%) ($p$ = 0.002 and $p$ = 0.01, respectively). Sesarmidae had a higher presence in snapper gut contents from Motukopake Island control site (50%) compared to Motukopake Island mussel farm and Rat Island mussel farm (0%) ($p$ = 0.006 and $p$ = 0.04 respectively). Motukopake Island mussel farm had a higher presence of Balanidae (barnacles) (46%) compared to the Motukopake Island control site and Rat Island control site (0%) ($p$ = 0.006 and $p$ = 0.02, respectively). Also, Caprellidae (Amphipoda) were present in snapper gut contents sampled from Motukopake Island mussel farm (38%) but absent at the Motukopake Island and Rat Island control sites (0%) ($p$ = 0.02 and $p$ = 0.04, respectively).

PERMANOVA identified significant differences in the presence/absence of prey items at the class level in the gut contents for Treatment (pseudo$F_{(1, 48)}$ = 3.74, $p$ = 0.007) and Treatment × Location (pseudo$F_{(1, 48)}$ = 2.48, $p$ = 0.04), but not Location (pseudo$F_{(1, 48)}$ = 1.13, $p$ = 0.34). However, $p$-adjusted values in post hoc analyses could not detect any significant differences among sites. Non-adjusted $p$-values showed some differences in the presence/absence of the various classes of prey items in the gut contents between Treatment and Location. For example, snapper gut contents sampled from Motukopake Island mussel farm and Rat Island mussel farm had a greater presence of Bivalvia (including mussels) (47% and 46%) compared to Motukopake Island control site (0%) ($p$ = 0.007 and $p$ = 0.01, respectively). Motukopake Island mussel farm also had a greater presence of Thecostraca (e.g. barnacles) compared to both control sites (40% vs. 0%, $p$ = 0.02). Snapper gut contents from the Motukopake Island control site had a greater presence of Conoidasida (parasitic alveolates) (35%) compared to Motukopake Island mussel farm (0%) ($p$ = 0.03). Rat Island control site had a greater presence of Chromadorea (round worms) (36%) compared to Rat Island mussel farm (0%) and Motukopake Island control site (0%) ($p$ = 0.03).

## 4 | DISCUSSION

This study compared the difference in snapper gut contents between mussel farms and control sites to see whether prey groups identified in mussel farm snapper were sourced directly or indirectly from the mussel farm infrastructure. The results indicate that the snapper sampled from mussel farm habitat are consuming a distinctly different diet compared to those sampled from control sites. The differences in diet are due to the snapper utilising prey species made available through the presence of the mussel farm habitat, such as harvested and common biofouling species within the farm habitat, which are not available in nearby unstructured soft-sediment habitats (i.e. the control sites).

### 4.1 | Differences in snapper gut contents

#### 4.1.1 | Mussel farm versus control site snapper

The key differences in the gut contents of snapper between mussel farm and control sites were the significant contribution of mussels (green-lipped and blue) and barnacles in mussel farms and not in control sites. Genetic analyses also confirmed that there were differences in the presence of caprellid amphipods which were present in mussel farms but not control sites, but these soft bodied Amphipoda were not picked up in visual analyses. There were some differences with Brachyura families between mussel farm and control sites, such as for Sesarmidae which were more commonly present in snapper gut contents at both control sites. The crab family Sesarmidae is not a common Brachyura family in New Zealand and therefore likely represents a distinct Brachyura species not yet available in the DNA reference databases (Wilkens & Ahyong, 2015). The other key difference in control sites compared to mussel farms was the presence of Paguridae (hermit crabs). Paguridae are abundant across a range of soft-sediment and rocky reef locations, and have shown an association with mussel farm habitats overseas and in soft-sediment mussel reefs in New Zealand (D'Amours et al., 2008; McLeod et al., 2014; Sean et al., 2022). Therefore, it is unclear why the abundance of Paguridae was higher in control sites and not mussel farms. It is possible that the abundance of shells which Paguridae rely on for host shells was more abundant in the control site, as Paguridae prefer host shells from Gastropoda species rather than Bivalvia, and Bivalvia shells (from mussels) are most abundant on the seafloor underneath mussel farms (McLaughlin & Thiel, 2015; Wilding & Nickell, 2013).

The snapper consumption of/predation on cultured shellfish species (i.e. farmed green-lipped mussels) is consistent with the general global analyses of the diet of other farm-dwelling Sparidae species (Gerlotto et al., 2001; Hayden, 1995; Peteiro et al., 2010; Šegvić-Bubić et al., 2011; Tsuyuki & Umino, 2017). However, most of these studies focused on the spat stage rather than mature mussels on a grow-out cycle (Ger-

lotto et al., 2001; Hayden, 1995; Peteiro et al., 2010; Stenton-Dozey & Broekhuizen, 2019; Tsuyuki & Umino, 2017). Sparid species are known to be significant predators of farmed shellfish species, especially mussels. For example, the diet of gilthead sea bream (*Sparus aurata*) sampled from a Croatian mussel farm consisted of 70% cultured mussels, which was considerably higher than for the snapper sampled in the mussel farm sites in the current study, with up to 19% consumption of cultured green-lipped mussels. Snapper in the current study were also captured on the seafloor and so are likely consuming mussels which have fallen off the dropper lines as well as those present on the dropper lines. Additionally, the Croatian study showed no consumption of Crustacea by gilthead sea bream in mussel farms, which is surprising given that Crustacea are a dominant food resource for Sparidae and are commonly associated with mussel farms (Callier et al., 2018; McKindsey et al., 2011; Šegvić-Bubić et al., 2011; Usmar, 2012; Woods et al., 2012). Nonetheless, gilthead sea bream did consume Teleostei and more Gastropoda compared to the current study, suggesting that the site-specific differences in biofouling or the differences in diet between the two Sparidae species have impacted their respective diet compositions (Šegvić-Bubić et al., 2011).

Snapper in mussel farms also consumed blue mussels (up to 10.1%) and barnacles (up to 14%), which are key nuisance biofouling species in New Zealand green-lipped mussel farms (Forrest & Atalah, 2017; Woods et al., 2012; Zazzaro et al., 2018). Since 2009, there has been a dramatic increase in the amount of fouling of the triangle barnacle (*B. trigonus*) across mussel farms in the Hauraki Gulf (Zazzaro et al., 2018). Globally, it is estimated that aquaculture operations spend 5%–10% of total profits managing biofouling, and so if snapper are selectively predating on these nuisance fouling organisms, this could be of benefit to mussel farm operations (Zazzaro et al., 2018). Consequently, there is a potential trade-off between snapper consuming harvested green-lipped mussels, but also having some benefit from reducing nuisance biofouling within the farm. Given that the overall consumption of harvested mussels by snapper was a lot lower than previously documented for another sparid species (19% vs. 70%), the predation may not be significant enough to impact mussel production at the intermediate to final grow out stages.

Caprellids found in mussel farm snapper gut contents were likely present within the mussels on dropper lines as these are common biofouling species that associate with biogenic structure, for example mussels, hydroids and tunicates (Lim & Harley, 2018; South et al., 2019; Woods et al., 2012, 2014). Caprellids are particularly abundant on dropper lines during seed out of juvenile mussels (South et al., 2019). The presence of caprellids, mussels and barnacles within mussel farm snapper gut contents suggests that snapper are feeding on the dropper lines as well as the benthos, as these species are much more likely to be present on the structural substrate of the dropper lines (South et al., 2019; Woods et al., 2012; Zazzaro et al., 2018). Research that investigated the abundance and diversity of snapper within Coromandel mussel farms showed that snapper were observed at the surface 34% of the time and at the benthos 66% of the time, based on 89.6 h of footage (Underwood, 2023). These observations show that although snapper were present on the benthos for the majority of the time, they still visited the dropper lines presumably for feeding opportunities, as also confirmed by the composition of prey items in snapper gut contents.

There were other key indicator species that were present in mussel farm snapper but not control sites which confirmed that mussel farm snapper are consuming biofouling within the farm environment. These included *Notomithrax* and *Halicarcinus* Brachyura species which are both common inhabitants of mussel dropper lines (Woods et al., 2012). The pea crab (*Nepinnotheres novaezelandiae*) was also present in mussel farm snapper which is typically a parasitic crab only within blue and green-lipped mussels (Trottier & Jeffs, 2015a, 2015b; Trottier et al., 2012; Wilkens & Ahyong, 2015). Additionally, some Bivalvia which were present in control site snapper and not mussel farms, such as *Dosina* spp., cockle (*Austrovenus stutchburyi*) and pipi (*Paphies australis*), are more commonly found within soft, muddy and fine sand benthic habitats with no direct association with mussel farm benthic habitat where organic loading from the shellfish farming is typically higher (Powell, 1979; Wilding & Nickell, 2013).

### 4.1.2 | Comparison to broader Hauraki Gulf snapper population

Previous analyses on snapper gut contents within the Hauraki Gulf have covered broader habitats and snapper size ranges which has resulted in a wide range of species and taxonomic groups identified in gut contents (Colman, 1972; Drummond, 2020; Godfriaux, 1970; Russell, 1983; Third, 2022; Usmar, 2012). Snapper have been observed to consume nearly 100 different species across a range of soft-sediment sampling stations in the Hauraki Gulf, which is much higher than the 34 species identified in the visual gut analysis in the current study (Colman, 1972; Godfriaux, 1970). For example, higher proportions of Polychaeta (5%–10% vs. 0%–2%), Teleostei (5%–10% vs. 0%–4%) and Gastropoda (5%–15% vs. 0%–1.6%) have been previously identified in the gut contents of snapper living in natural habitats in the Hauraki Gulf (Colman, 1972; Drummond, 2020; Godfriaux, 1970; Third, 2022). Additionally, Echinodermata and Porifera were present in broader diet studies, with Echinodermata making up a large proportion of gut contents (9.6%–28.3%) (Colman, 1972; Godfriaux, 1970; Russell, 1983). These comparisons have confirmed the plasticity of snapper diet even within different habitats found within one marine region.

Overall, there is general consensus that mostly free-living Crustacea are the dominant prey group targeted by snapper, with all previous diet studies recording this taxa as the dominant component (Colman, 1972; Drummond, 2020; Godfriaux, 1970; Russell, 1983; Third, 2022; Usmar, 2012). This is generally consistent with the current study; however, the dominance of Bivalvia within mussel farm snapper gut contents exceeds the proportion of Crustacea at the Rat Island mussel farm (32% Bivalvia vs. 18.4% Crustacea), and is high but does not exceed the proportion of Crustacea within Motukopake Island mussel farm (18% Bivalvia and 26% Crustacea). Both control sites had a dominance of Crustacea—29% at Rat Island control site and 21.4% at Motukopake Island control site. Of the Crustacea, Brachyura was found to be the

dominant component in four previous studies of snapper diet in New Zealand (Colman, 1972; Godfriaux, 1970; Russell, 1983; Usmar, 2012); however, Paguroidea and Caridea were more dominant in two studies that focused on a smaller size range of adult snapper of 25–55 cm (Drummond, 2020; Third, 2022). Snapper typically start consuming Brachyura from 10 cm (fork length) and their diet becomes more varied from 25 cm to include Bivalvia, Paguroidea, Polychaeta, Gastropoda and Teleostei (Usmar, 2012). This is mostly due to the jaw crushing strength of the snapper which develops with age/increase in size, allowing snapper to successfully consume more hard-shelled organisms (Usmar, 2012). In the current study (which also used a refined size class of 26–42 cm), the dominant Crustacea component within mussel farm snapper were barnacles, mostly triangle barnacle (*B. trigonus*). This is an important finding as no other previous snapper study within the Hauraki Gulf has identified barnacles as a diet component (Colman, 1972; Drummond, 2020; Godfriaux, 1970; Third, 2022). For control sites, Paguroidea were dominant in Motukopake Island, aligning with the recent soft-sediment snapper studies (Drummond, 2020; Third, 2022). Decapoda spp. were dominant in Rat Island which could be from any of the Brachyura, Caridea and Paguridae families.

Of the Brachyura identified by our genetic and visual gut analysis methods, only four species had been previously recorded. The dwarf swimming crab (*Liocarcinus corrugatus*), identified in control sites, was present in two previous snapper gut studies and dominant in one (Drummond, 2020; Third, 2022). Other Brachyura found in mussel farm snapper were from the Majidae (decorator crab) family, including *Notomithrax* species which were previously found in most Hauraki Gulf snapper gut studies (Colman, 1972; Drummond, 2020; Godfriaux, 1970; Third, 2022). Similarly, *Halicarcinus* spp. were present in mussel farm snapper and are commonly consumed by snapper sampled from natural habitats in the Hauraki Gulf (Colman, 1972; Drummond, 2020; Godfriaux, 1970; Third, 2022; Usmar, 2012). Even though these crab species are present in natural soft-sediment habitats, there is likely to be an increased abundance of them within the mussel farm habitat (Wilkens & Ahyong, 2015; Woods et al., 2012).

The overall proportion of Bivalvia within the gut contents of mussel farm snapper largely exceeded the proportions in previous studies, for example 32%–18% in the current study versus 8%–13% (Godfriaux, 1970; Third, 2022). The Bivalvia species previously identified in the gut contents of snapper include *Dosina* spp., *Anomia* spp., *A. stutchburyi*, *Atrina zelandica*, *Tawera spissa*, *Soletellina nitida*, *Purpurocardia purpurata*, *Neilo australis*, *Gari stangeri* and *Linucula hartvigiana* (Colman, 1972; Drummond, 2020; Godfriaux, 1970; Third, 2022). These Bivalvia species previously observed in the gut contents of snapper sampled from natural habitats have four species in common with the control sites over soft-sediment natural habitat in this study, and only two in mussel farm sites. Interestingly, Motukopake Island control site snapper also had a high proportion of Bivalvia (excluding mussels) in gut contents (16%) exceeding previous studies (Godfriaux, 1970; Third, 2022). This is likely due to the availability of Bivalvia in the benthic habitat at this specific site, as snapper sampled from Rat Island only included 0.15% Bivalvia in their gut contents. Green-lipped mussels have been rarely reported in the gut contents of snapper sampled from natural habitats (Third, 2022; Usmar, 2012), but snapper are known to predate on them when they are available (Alder et al., 2021, 2022a, 2022b). Blue mussels have never been recorded in previous snapper diets in New Zealand (Colman, 1972; Drummond, 2020; Godfriaux, 1970; Russell, 1983; Third, 2022; Usmar, 2012). However, the detection of the consumption of mussels relies on the presence of this species in sufficient quantities within the habitat.

## 4.2 | Comparison of visual and genetic gut analysis results

Overall, there was good alignment between the visual and genetic gut analyses for the key species, such as mussels and barnacles. Additionally, many of the Brachyura families identified by the molecular genetic analysis precisely matched species of Brachyura identified by the visual gut analysis, such as Majidae (*Notomithrax* spp.), Hymenosomatidae (*Halicarcinus* spp.), Pilumnidae (e.g. *Pilumnus novaezelandiae*) and Pinnotheridae (*Nepinnotheres* spp.). The genetic analysis was also able to identify a broader range of taxa, including soft body and small organisms such as Ascidiacea (tunicates), Holothuroidea (sea cucumbers), Polychaeta and Hoplonemertea (worms) and amphipods (small crustaceans). Identifying these types of soft-bodied organisms to an informative level is often not possible using visual gut analysis due to their rapid digestion once consumed, masking their morphological structure needed for identification (O'Rorke et al., 2012; van der Reis et al., 2020). However, there were insufficient numbers of individual snapper for which DNA was detected from these prey groups to result in a significant difference between snapper from control versus mussel farm sites. Although amplification was achieved for the majority of samples, the DNA extracted from the gut contents was degraded (visible when running a 0.8% agarose DNA gel), likely hindering better amplification, and may be a limitation to identifying the full scope of taxa within the gut content in this study (O'Rorke et al., 2012; van der Reis et al., 2020). Although appropriate protocols were followed, the logistic constraints of field sampling and multiple sampling of gut contents (i.e. visual gut analysis and sampling for biochemical analysis) meant that gut contents could not be immediately preserved with chemicals (i.e. ethanol) upon capture of the snapper which would have been optimum for molecular genetic analyses (van der Reis & Lavery, 2020).

Taxonomic assignment of DNA sequences is reliant on a match that reaches the required minimum accuracy threshold that is typically established on the basis of the availability of sequences in the reference databases for the suspected prey types (van der Reis & Lavery, 2020). In New Zealand, there is a lack of references for Crustacea species in the DNA databases (particularly noticeable for crabs) (van der Reis et al., 2020). Therefore, a BLAST 85% identity match was chosen to be able to assign closest matches for these species (van der Reis et al., 2020). The lack of New Zealand Crustacea families sequenced has resulted in some crab misidentifications (five Malacostraca families) and thus few common coastal species were identified (Forest & McLay, 2001; Wilkens & Ahyong, 2015). Regardless, distinct

differences were found for crab families identified in one treatment and not the other.

### 4.3 | Implications for Hauraki gulf snapper population

Overall, the results show that snapper within mussel farms are consuming a unique diet, with prey groups linked to the mussel farm habitat, either underneath mussel farms and/or on dropper line habitats. In particular, the key prey groups which snapper were consuming in mussel farm habitats (mussels and barnacles) are highly abundant within mussel farms (Forrest & Atalah, 2017; Skelton et al., 2022; Woods et al., 2012; Zazzaro et al., 2018). Firstly, the green-lipped mussel industry produces approximately 1.78 billion adult mussels per year, with 30% of this production within the Coromandel region, which sums to approximately 530 million adults per year (Skelton et al., 2022). Secondly, blue mussels and barnacles are key nuisance biofouling species in Coromandel mussel farms as these can foul surfaces at high densities across mussel dropper line habitats (Forrest & Atalah, 2017; Woods et al., 2012; Zazzaro et al., 2018). Abundant prey groups can especially be targeted by snapper because of their omnivorous and highly diverse diets (Colman, 1972; Drummond, 2020; Godfriaux, 1970; Russell, 1983; Third, 2022; Usmar, 2012). Snapper have been observed at much higher densities within mussel farms compared to adjacent control sites (i.e. soft-sediment without mussel farms), with snapper up to five times more abundant underneath mussel farms (Underwood, 2023). Therefore, the higher abundances of snapper within mussel farms are likely to be partially due to the availability of prey given the direct link to prey species present in mussel farm habitats. A stable prey supply is important as it reduces the energy partitioned for foraging, so that there is more energy available for other biological processes such as growth and reproduction (Wootton, 2012). Furthermore, the prey groups targeted in mussel farms are energy rich, with high lipid content present in mussels and barnacles (Barclay et al., 2006; Barnes & Achituv, 1976). Consequently, snapper are likely receiving a nutritional benefit from feeding within mussel farm habitats. The highly abundant mussel farm habitats within the Coromandel have the potential to support the Hauraki Gulf snapper population through the supply of food resources. This is a direct outcome that can be used towards restorative aquaculture frameworks, to utilise farm habitats in a way that provides net positive ecological outcomes for coastal environments (Alleway et al., 2019; Froehlich et al., 2017; Gentry et al., 2020; Theuerkauf et al., 2019).

## 5 | CONCLUSIONS

The results from this study show that snapper living within mussel farms in the Hauraki Gulf are consuming a distinctly different diet to snapper in adjacent natural habitats without a mussel farm. The diet that snapper have consumed within mussel farms can be directly linked to the cultured species (i.e. green-lipped mussels) and the biofouling (i.e. blue mussels and barnacles). There was good alignment between the visual gut and genetic analyses for the key species identified. The DNA degradation within individual snapper samples has likely impacted the lack of additional differences identified. Overall, the results show that the highly abundant prey groups consumed by snapper in mussel farm habitats are likely to be beneficial to the snapper population, reducing foraging effort and potentially supplying more nutritious prey. These findings provide evidence towards the supporting services of mussel farm habitats through the provision of food resources. This evidence can support the restorative aquaculture objectives for the region, which aims to provide net positive ecological outcomes for wild fish populations.

### AUTHOR CONTRIBUTIONS

**Lucy H. Underwood**: Conceptualization; formal analysis; investigation; methodology; writing—original draft. **Aimee van der Reis**: Conceptualization; methodology; writing—review and editing. **Andrew G. Jeffs**: Funding acquisition; project administration; supervision; writing—review and editing.

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### ETHICS STATEMENT

This research was conducted in accordance with animal ethics approval under New Zealand's Animal Welfare Act 1999 (UoA-AEC Approval # 21619), which ensures the humane treatment of animals for research.

### CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

### DATA AVAILABILITY STATEMENT

The raw sequence data is available on NCBI's Sequence Read Archive (http://www.ncbi.nlm.nih.gov/bioproject/971684) with associated metadata. The underlying data for statistical analyses will be shared on reasonable request to the corresponding author.

### PEER REVIEW

The peer review history for this article is available at: https://publons.com/publon/10.1002/aff2.113

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