

# FORAGING ECOLOGY OF RED-CAPPED PLOVERS IN THE COORONG, SOUTH AUSTRALIA



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## **Declaration**

I certify that the work in this thesis contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge, contains no material previously published or written by another person, except where due reference has been made.

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November 2021

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## Abstract

Globally shorebird species are declining due to the extensive degradation and loss of wetland habitat. In South Australia, the Coorong, a wetland of international importance, composed of two lagoons (south lagoon and north lagoon) has experienced major habitat degradation from reduced flows and drought; particularly in the south lagoon. This habitat degradation is currently affecting many species, due to reduced prey availability. Management in the Coorong is needed to improve habitat quality and prey availability for shorebirds, including a common resident species, the Red-capped Plover (*Charadrius ruficapillus*). This requires an understanding of the foraging ecology and diet of the Red-capped Plover, which I studied this to help inform future management.

My study analysed the foraging behaviour of Red-capped Plovers at three sites in the Coorong, two in the south lagoon and one in the north lagoon, and determined their autumn diet at these three sites, using DNA metabarcoding on scat samples. I found that foraging behaviour was influenced by large scale effects, such as site and season and small scale effects such as location on beach. The two more northern sites provided better foraging habitat, indicated by the higher numbers of Red-capped Plovers, higher peck rates (pecks/s) and increased proportion of foraging birds. This indicates that these two sites have higher prey abundances, which coincides with past benthic invertebrate studies. Less favourable foraging conditions were present in Winter, due to decreased foraging habitat from high water levels. The Red-capped Plover diet largely consisted of Chironomidae (non-biting midges) (78% of the sequences) and no significant differences were found with the abundance of Chironomidae between the three sites. Other prey taxa identified were from the families Muscidae (House fly), Platycephalidae (Anchovy), Engraulidae (Flat head) and Scombridae (Mackerel, Tuna and Bonito). Red-necked Stint DNA (Scolopacidae) was also present in the results, which is likely from incorrect scat samples.

Future research and management needs to determine the importance of Chironomidae to Red-capped Plover diet throughout the year and location within the Coorong. Specifically, management needs to conserve habitat quality for foraging shorebirds, which involves ensuring water levels are not too high for sustained periods, as this reduces available foraging area, as well as controlling occurrence of algae blooms, which can also reduce foraging area and suppress Chironomid emergence.

# 1. Introduction

## 1.1 Global wetland decline

Wetlands are one of the most ecologically productive ecosystems on Earth, yet they are also one of the most threatened. Globally they have experienced as much as a 50% loss in area over the last century (Bildstein *et al.* 1991; Dudgeon *et al.* 2006; Davidson 2014; Leadley *et al.* 2014). The main impacts threatening wetlands are: (i) climate change; (ii) habitat loss and degradation from development and land use change; (iii) pollution; (iv) overharvesting of species; (v) water resource development and overextraction; and (vi) invasive species (Davidson 2014; Kingsford *et al.* 2016). Climate change is predicted to have a huge impact on wetland ecosystems through increased temperatures, changes in precipitation, and rise in sea levels (Finlayson *et al.* 2013; Junk *et al.* 2013). By 2100 it is estimated that there will be a 50cm increase in sea level, resulting in a loss of 46-59% of coastal wetlands across the globe (Spencer *et al.* 2016). Additionally, the increasing human population is very likely to have an impact on these wetland ecosystems through the increase in infrastructure development leading to the further loss of wetland habitat (Davidson 2014). In Australia one of the major drivers of wetland degradation is the alteration of flow regimes due to upstream water extraction, largely for agriculture (Finlayson *et al.* 2013; Brandis *et al.* 2018). Water extraction reduces wetland inundation, which can have many negative effects on the wetland community and can result in permanent wetland loss (Nielsen *et al.* 2013).

Wetland degradation and loss of habitat has major effects on shorebirds; a diverse group of birds that generally feed on the mudflats of intertidal habitats and inland wetlands (Sorensen *et al.* 2020). Shorebirds rely on wetland habitats for breeding and foraging and are fast becoming one of the most threatened bird groups globally (Clemens *et al.* 2016). In Australia the degradation and loss of wetland habitat has major effects on both the resident shorebirds and the migratory species who reside in Australia during their non-breeding months. Long term declines have been recorded in both of these groups, where between the 1980s and 2006 there has been a decline of 81% in resident Australian shorebirds (Nebel *et al.* 2008). This decline in resident shorebird populations emphasises the current extent of wetland degradation that is occurring in Australia (Nebel *et al.* 2008; Clemens *et al.* 2016).

Many-small and large-scale conservation treaties, conventions, and agreements incorporate aspirations to improve the status of wetlands. For example, The Ramsar Convention on Wetlands was established in 1971 and currently has 2,413 listed sites, which are helping to protect wetlands of international importance. However, fewer than half of these declared Ramsar wetlands have developed and

implemented management plans (Ramsar 2018). Coupled with the current state of shorebird decline, this suggests that more conservation and management of wetlands is needed. Understanding shorebird ecology, particularly how they utilise their habitat, can play an important role in this progress.

### *1.2 Decline in the ecological condition of the Coorong*

The Coorong, Lower Lakes and Murray Mouth (CLLMM), is listed as a wetland of international importance under the Ramsar Convention for several different criteria, including its importance to waterbird populations (Colloff *et al.* 2015). The Coorong is a saline lagoon that is located at the mouth of the Murray-Darling Basin, Australia. It is highly dependent on adequate and appropriately timed flows of freshwater from the Basin (Paton *et al.* 2009). Without these adequate inflows the habitat quality of the Coorong is affected through the loss or alteration of ecological processes and species interactions that the water flow promotes (Kingsford 2000). The Coorong is composed of two lagoons, the north and south lagoon, which are ecologically distinct and have minimal exchange of water (Dittmann *et al.* 2015a). The salinity of the Coorong increases with distance from the Murray mouth, resulting in higher salinity levels in the south lagoon (Paton *et al.* 2009). Between the two lagoons there is a change in waterbird community structure and also prey availability (Paton *et al.* 2009).

Water extraction has significantly affected the ecological character of the Coorong, through the construction of weirs and dams, and diversion of water upstream for agriculture and human consumption (Paton *et al.* 2009; Kingsford *et al.* 2016). This water extraction has resulted in habitat degradation through the loss of inundation and reduced flooding (Kingsford 2000; Finlayson *et al.* 2013). Specifically, it has had major effects on the South Lagoon through increased salinities and reduced flows. The Millennium Drought (1999 to 2010) has also been a major contributor to this habitat degradation (Paton *et al.* 2009). Furthermore, water extraction significantly exacerbates the effect of droughts, which are increasing due to climate change.

Since the 1980s, the Coorong has experienced declines of >50% in populations of multiple waterbird species (Paton *et al.* 2009). This habitat degradation caused by both water extraction and drought has been the main contributor to these declines. Together, they have altered the food availability and habitat quality that the waterbirds rely on (Paton *et al.* 2009). Specifically, the drought caused declines in abundance and biomass of macroinvertebrates, due to the hypersaline conditions and lowered water levels (Dittmann *et al.* 2015a; Paton *et al.* 2019). This food availability is perceived to be the predominant driver of reduced waterbird numbers (Paton *et al.* 2009).

In recent years filamentous green algae blooms have significantly affected the Coorong, and currently are a major threat facing the southern Lagoon (Paton *et al.* 2020; Brookes *et al.* 2021). The algae can form large mats on the shoreline, which interferes with the foraging behaviour of shorebirds (Brookes *et al.* 2021). Additionally, it affects one of the key food resources of shorebirds through suppressing the emergence of chironomids (Brookes *et al.* 2018; Paton *et al.* 2018)

Australia's obligations under the Ramsar agreement state that the ecological character of the site must be maintained. However, given the reduced inflows this goal to maintain the ecological character of the Coorong is becoming difficult to achieve (Brookes *et al.* 2021). Management is needed in the Coorong to protect and conserve these habitats that waterbirds depend on. Particularly it is needed to ensure that organisms, including Red-capped Plovers (*Charadrius ruficapillus*) persist into to the future, specifically given the future climate conditions that involve increased sea level rise and increased temperatures (Paton *et al.* 2019; Brookes *et al.* 2021).

### *1.3 Shorebird foraging ecology*

Food availability, specifically the prey type, density, size and accessibility, is a driving factor in habitat selection for shorebird foraging (Yates *et al.* 1993). One of the primary factors influencing shorebird habitat selection is the location of sites where they can easily meet their energetic and nutritional requirements (Van Gils *et al.* 2003). Other factors that also influence their foraging habitat selection include predation risk, the intensity of competition and proximity to roost site (Finn *et al.* 2007; Castillo-Guerrero *et al.* 2009). The foraging ecology of shorebirds, specifically the amount of time allocated to foraging, can indicate habitat quality (van der Kolk *et al.* 2019). The time a bird spends foraging reflects the ability of the individual bird to harvest its food requirements in a specific habitat (Van Gils *et al.* 2003; Van gils *et al.* 2005). However, it is not always straightforward to interpret foraging behaviour; shorebirds that spend more time foraging may be experiencing poorer food resources and therefore have to feed longer to meet their energetic and nutritional requirements (Paton *et al.* 2019). Nonetheless, shorebirds in particular can spend most of their day foraging, and some decreased foraging time has been related to lower habitat quality (Bellio and Kingsford 2013).



#### 1.4 Red-capped Plovers

Red-capped Plovers are a non-migratory bird that are widespread across Australia. They inhabit a range of littoral, estuarine and terrestrial wetlands, including the arid regions of Australia (Marchant and Higgins 1993). They have a foraging technique that is unique to the Charadriidae family, which differs to most other shorebirds (Marchant and Higgins 1993), this is the ‘run-stop-peck’ foraging technique (Thomas *et al.* 2006). This technique involves the bird visually searching for their prey and once detected they run to the prey item and rapidly peck at it (Pienkowski 1983; Turpie and Hockey 2008). Their short-billed morphology indicates that they would primarily take surface-living or shallow-dwelling invertebrates (Barbosa and Moreno 1999; Martin and Piersma 2009). The specific foraging technique and bill morphology of Red-capped Plovers means they utilise their foraging habitat slightly different to other shorebird species. Most shorebirds forage in shallow water (a few centimetres deep) or at the waterline, whereas Red-capped Plovers are known to primarily forage on exposed mudflats, particularly damp mudflats (Paton *et al.* 2019).



Figure 1 – Female Red-capped Plover on left and male on right (image source: Stephen Garth).

The diet of Red-capped Plovers has not been studied intensively. A study conducted by Abensperg-Traun & Dickman (1989) analysed the gut composition of four male Red-capped Plovers in Western Australia. They found that it contained dytiscidae beetles, ants and polychaete worms, indicating that Red-capped Plovers are primarily insectivorous birds (Abensperg-Traun and Dickman 1989). This limited information on the diet of Red-capped Plovers represents a key knowledge gap. In addition,

Red-capped Plovers can be aged and sexed based on plumage characteristics. This creates a novel opportunity to investigate sex-specific foraging patterns, which is not possible for most other shorebird species that are sexually monomorphic.

Although Red-capped Plovers are not listed as a threatened species, they are at risk of population declines and local extinctions (Lamanna 2019). The continuing degradation of Australian wetlands and coastal habitat may see major population declines of this species. The Coorong has traditionally supported at least 1% of the global population of Red-capped Plovers, yet they have experienced continuing declines in their abundance across the Coorong, particularly in the southern lagoon (Brookes et al. 2018; Hunt et al. 2019). This emphasises the need to study the diet and foraging ecology of Red-capped Plovers in the Coorong. It will help to inform how ongoing changes in the habitat quality of the Coorong may affect them, helping to assist with future management of the Coorong and how it can be optimised to benefit Red-capped Plovers and other shorebird species.

### *1.5 Research Aims*

My research looked at the foraging ecology of Red-capped Plovers in the Coorong, South Australia, during two seasons (autumn and winter). Additionally, I determined their autumn diet using DNA metabarcoding at three sites in the Coorong.

The aims of my study were to:

1. Understand how foraging behaviour (time spent foraging) and foraging activity (pecking rate) changes with distance to the waterline and variable local conditions (e.g., season, time of day, location, wind speed, temperature); and,
2. Quantify changes in foraging behaviour/activity and dietary composition between sites, including sites in different regions of the Coorong (i.e., between the north and south lagoons).
3. Characterise the diet of the Red-capped Plover at different sites along the Coorong, using non-invasive DNA metabarcoding of scat samples.

## 2. Materials and Methods

### 2.1 Study area

My study was conducted in the Coorong National Park, located in South Australia. Three study sites were chosen based on the known occurrence of Red-capped Plovers from previous surveys (Paton *et al.* 2020). Additionally, the sites were chosen based on their location in the Coorong, specifically whether they were located in the south or north Lagoon. The two lagoons are separated by Parnka point (Figure 2). Two of my sites were located in the south Lagoon (Salt Creek and Villa del Yumpa) and one site was located in the north Lagoon (Parnka North) (Figure 2). Salt Creek is a small beach approximately 250m long, has a regulated freshwater creek and has rocky areas that are suitable for roosting by shorebirds. Villa del Yumpa and Parnka North are both beaches that are approximately 2km long and have suitable rocky roosting habitat. I completed sampling in autumn (16th - 24th March) and winter (6th – 15th June).

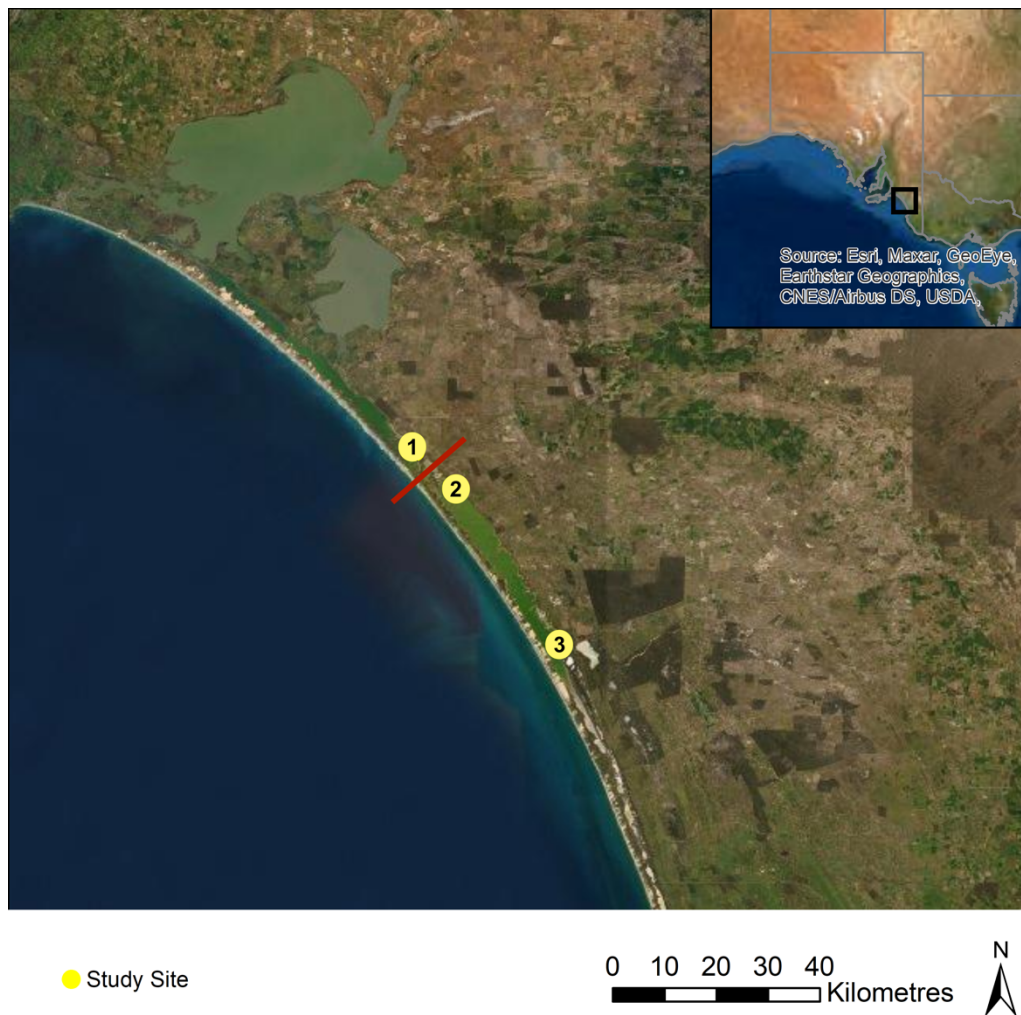


Figure 2 – Study site location in the Coorong, South Australia: (1) Parnka North, (2) Villa del Yumpa and (3) Salt Creek. The boundary between the north Lagoon and south Lagoon (which is at Parnka

*Point*) is depicted by the red line. Inset shows the location of the study region (black rectangle) in relation to South Australia.

## 2.2 Red-capped Plover foraging behaviour

I analysed the foraging behaviour of Red-capped Plovers from the time they spent foraging and where they foraged in relation to the waterline. To assess where the birds spent most of their time foraging, each site was stratified into three sections: (i) at the waterline or within 1m from the waterline; (ii) on the beach 1-6 metres from the waterline; and (iii) on the beach >6 metres from the waterline. This stratification was chosen based on the different types of prey that the Red-capped Plovers may be consuming (e.g., close to waterline mainly being benthic invertebrates and past 6 meters being terrestrial invertebrates). These sections were not always easy to identify as some waterlines were highly heterogenous and on occasions some areas in the 1-6m and >6m from waterline were still wet due to high winds. In these cases, it was identified as 'waterline' when the birds were foraging in puddles and >6 meters when otherwise. To quantify allocation of foraging effort to these strata, the total number of Red-capped Plovers within each distance band was observed and the number of birds that were foraging and resting was recorded. This was completed hourly throughout the seven-hour field days. Additional information was also recorded for each count, including weather variables (temperature (°C), wind speed (km/h) and wind direction) and if other bird species were present.

## 2.3 Red-capped Plover foraging rate

Foraging rate was recorded as the pecking rates of foraging Red-capped Plovers. I chose birds haphazardly and viewed them with a spotting scope (20 - 60x magnification) for 1-minute (timed using a stopwatch). For each bird, the sex (male/female), age (adult/immature), foraging location (waterline, 1-6m and <6m) and number of observed pecks were recorded. Male Red-capped Plovers were identified by red colouring on their head and immature birds were identified by their paler and more scaly colouring. This was completed for every individual Red-capped Plover at the study site and was conducted within each of the hour observations. On occasions, multiple peck rate records were completed for the same individual bird. In these cases, an average was calculated for each individual bird. If a Red-capped Plover flew off before the 1-minute observation was completed, the number of completed pecks was still recorded, along with the number of seconds that the bird was observed for. From these observations I calculated the peck rate (number of pecks/second) of Red-capped Plovers.

## 2.4 Diet analysis

### 2.4.1 Scat collection

Scats were collected from 10-15 Red-capped Plovers at each study site, during the two sampling periods (autumn and winter). Collection was achieved by one of two methods: (1) visually watching a plover through a spotting scope until they were seen defecating and/or (2) from roost sites where Red-capped Plovers were seen roosting previously with no other bird species. Scats were collected using a spatula and fine forceps and were stored in *RNAlater* in 2.5 ml microcentrifuge tubes at  $-20^{\circ}\text{C}$  until analysis. To the best of my ability, scats were collected from multiple Red-capped Plovers and not from the same individual bird to maximise the sample representation of the population diet of Red-capped Plovers.

#### 2.4.2 DNA extraction and amplification

DNA extraction was completed on each individual scat sample and next-generation sequencing library preparation, using Illumina chemistry, was performed by the Molecular Sciences Laboratory at SARDI Aquatic Sciences. A Red-capped Plover blocking primer was designed, optimised and added to the Polymerase Chain Reaction (PCR) mastermix to reduce amplification of predator DNA while allowing for amplification of prey DNA. The pooled library was then sent to Australian Genome Research Facility (AGRF) for sequencing on the Illumina MiSeq using a 250bp paired-end read system.

#### 2.4.3 DNA sequence processing and taxonomic assignment

The forward and reverse sequences for every sample were initially paired and primers were removed by AGRF. I then completed all other bioinformatic steps using the software MOTHUR (Schloss *et al.* 2009). Sequences with ambiguous base calls and low-quality reads were removed. Identical sequences (PCR duplicates) were merged to a single consensus sequence for identification, but sequence counts were retained for relative abundance analysis. Prior to classification, sequences where the number of base differences  $<2$  were clustered to collapse possible PCR errors and chimeras in the dataset were removed.

To facilitate taxonomic classification of each read, a reference library was used that contained all available Australian Cytochrome c oxidase subunit I (COI) sequences from the Barcode of Life Data System (Ratnasingham and Hebert 2007). After trimming the database, sequences were aligned against the high-quality MIDORI COI sequence database to retain the COI barcode only (Leray *et al.* 2018). BOLD sequences with an alignment length  $<240\text{bp}$  were removed, along with any sequences with ambiguous base calls. This left 104,217 BOLD sequences in the reference database.

This reference library was also augmented with additional sequences of invertebrate samples from the Coorong, including species of Diptera (Chironomidae, Dolichopodidae, Stratiomyidae), Crustaceans (Amphipoda, Ostracoda) and Annelids (Capitellidae, Serpulidae, Phyllodocida). The extraction and sequencing of these reference library samples was performed by the Molecular Sciences Laboratory at SARDI Aquatic Sciences. Extraction was completed with a Qiagen DNeasy Blood and Tissue Kit following manufacturer's directions and PCR was completed with universal COI primers (mICOIntF/jgHC02198 – see Leray *et al.* 2013). Gel electrophoresis was used to visualise PCR amplification before positive samples were sent to AGRF for PCR clean-up, Sanger sequencing in F & R direction using the Leray primers (Leray *et al.* 2013), and sequencing clean-up.

Sequences were aligned to the reference taxonomy under the Wang method, which looks at the query sequence kmer to kmer. The Cut-off value was set to 80%, which retains taxonomy of a sequences that has a bootstrap value greater than 80%. This method classified some of the sequences to species level, but other sequences to only higher order taxonomic levels.

## 2.5 Statistical analysis

### 2.5.1 Foraging analysis

The number of foraging Red-capped Plovers observed for each site, season, temperature and time of day was analysed by fitting a generalised linear mixed model (GLMM) with a negative binomial distribution using Template Model Builder (glmmTMB) in the R software environment for statistical and graphical computing (Team 2013) version 3.6.3. Observation period was treated as a random effect to reduce overdispersion in the model. Predicted values from the model were generated using the emmeans package to produce a figure displaying the results (Lenth *et al.* 2021).

The proportion of Red-capped Plovers foraging was calculated from observations where Red-capped Plovers were present. I then fitted a GLMM with a binomial distribution and observation as random effect to analyse how the proportion of birds foraging changed between site, season, time and temperature.

The proportion of foraging Red-capped Plovers within the three distance bands was analysed against season and site. A multinomial model using mclogit function was fitted, accounting for random variation in response across observation periods (Martin 2021). Predicted values were then produced from the model to create a figure displaying the results.

I additionally analysed the peck rate (pecks/s) of foraging Red-capped Plovers against site, season and distance band by fitting a Generalised Linear Model (GLM) with a gaussian link function. Peck rate was square root transformed in the model and transformed back when producing predicted values for interpretation. For this analysis the Red-capped Plover age and sex observations were grouped together due to their uniform peck rates (see appendix 1).

### 2.5.2 Diet analysis

Relative read abundance (the proportion of the total number of sequences accounted for by a given prey taxon) was calculated for each scat collected. The relative abundance of key dietary taxa was compared between sites and periods using beta regression models. Differences between sites was visualised with a Non-metric Multi-dimensional Scaling (NMDS) plot with the Vegan package (Oksanen *et al.* 2007). An analysis of distance (ANODIS) was used to assess whether statistically significant differences in dietary composition existed between the three sites (Skalski *et al.* 2018). ANODIS is a multivariate analysis that considers members of the diet ‘community’ simultaneously whereas the beta regression model only investigates single prey taxa at a time.

### 3. Results

#### 3.1 Foraging behaviour

The number of Red-capped Plovers changed between both site and season (Figure 3). There was large variability in the number of birds per observation. Although some observations had >30 birds, the majority of the observations had low numbers, including observations with no birds.

Autumn had significantly higher numbers of Red-capped Plovers compared to winter (GLMM; estimate = -1.77, Std error = 0.59, p-value = 0.002) (Figure 3). Salt Creek had significantly lower numbers of Red-capped Plovers on average in both seasons, in comparison to Parnka North (GLMM; estimate = -1.17, Std error = 0.40, p-value = 0.003) (Figure 3). No significant difference was found between the abundance of birds at Villa del Yumpa compared to Parnka North (GLMM; estimate = -0.30, Std error = 0.40, p-value = 0.66) and between the abundance of birds Salt Creek compared to Villa de Yumpa (GLMM; estimate = -0.87, Std error = 0.44, p-value = 0.12) (Figure 3). No relationship was detected between the abundance of Red-capped Plovers and time of day ( $t = 1.08$ ,  $df = 2$ , p-value = 0.58) or temperature ( $t = 0.63$ ,  $df=1$ , p-value = 0.43).

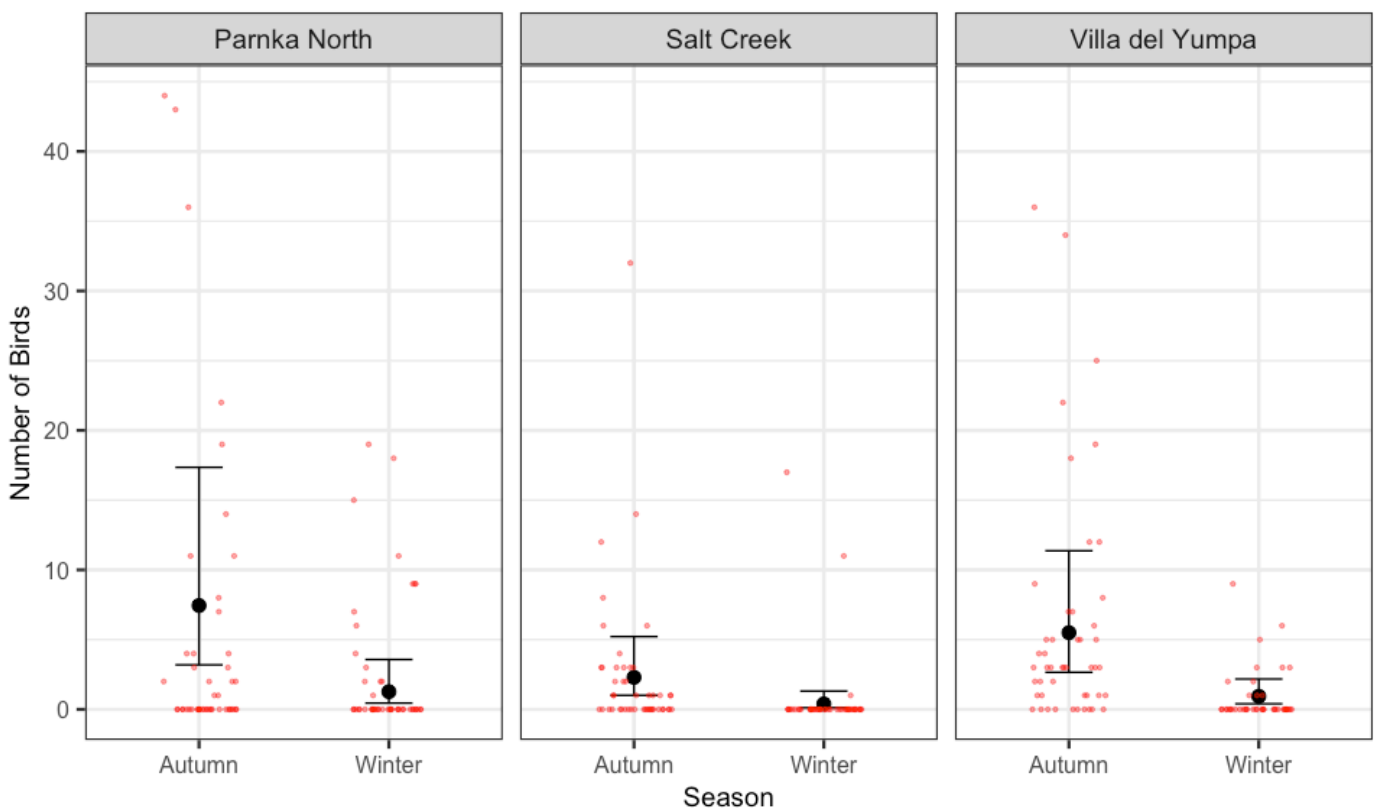


Figure 3 – Total number of Red-capped Plovers at the three study sites (Parnka North, Salt Creek, Villa del Yumpa), across the two seasons (autumn and winter). Mean and standard error predicted values from generalised linear mixed effect model. Plotted points are raw data of abundance of Red-capped Plovers at three sites, across the two seasons.



When Red-capped Plovers were present at a site, the proportion of birds foraging at the site was highly variable between foraging observations (Figure 4). No seasonal effect was found for the proportion of foraging Red-capped Plovers, although there was a site effect. The proportion of foraging Red-capped Plovers was significantly lower at Salt creek compared to Parnka North (GLMM; estimate = -3.943, Std error = 1.659, p-value = 0.0175) and similarly lower at Villa del Yumpa (GLMM; estimate = -3.656, Std error = 1.535, p-value = 0.0171) (Figure 4). No relationship was found between the proportion of foraging birds with time of day ( $t = 2.30$ ,  $df = 2$ ,  $p\text{-value} = 0.316$ ) and temperature ( $t = 3.79$ ,  $df = 1$ ,  $p\text{-value} = 0.0515$ ).

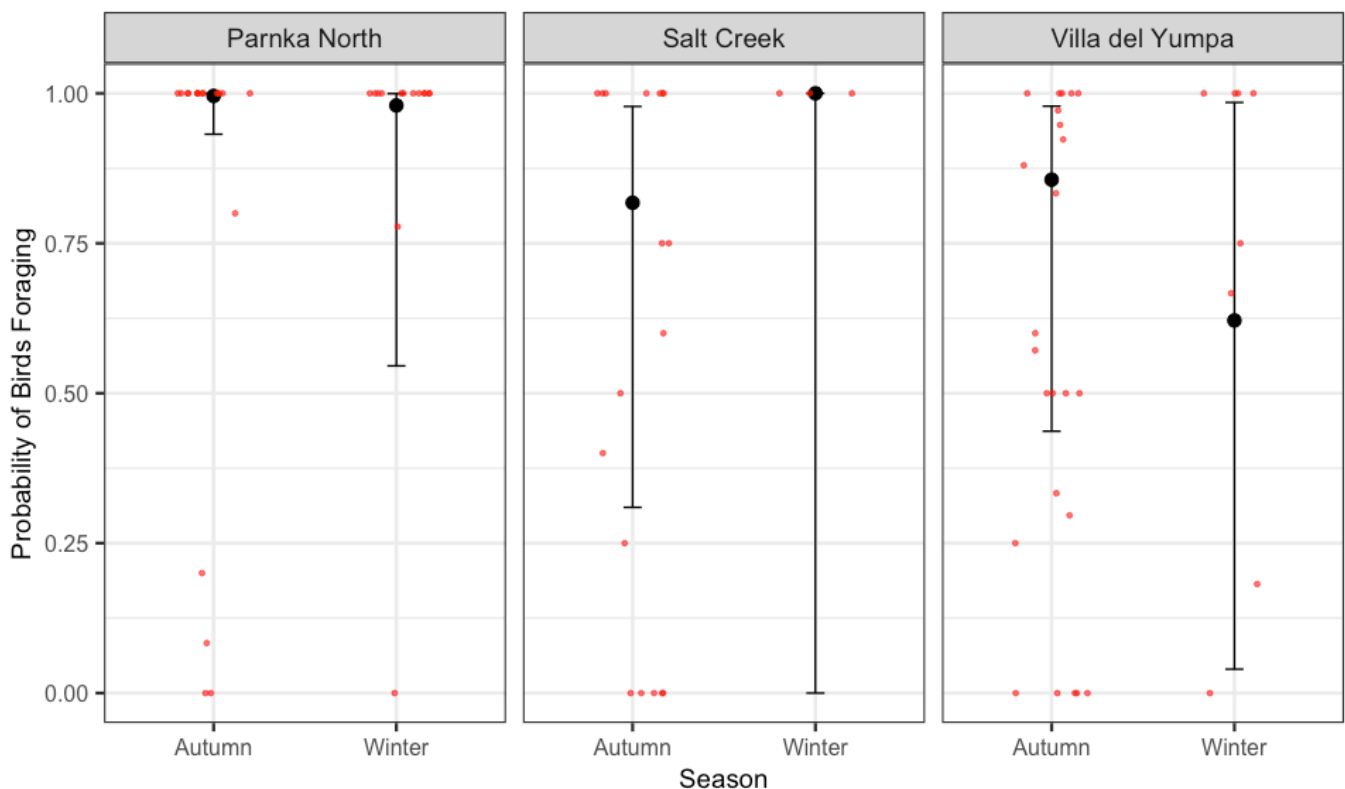


Figure 4 – Probability of Red-capped Plovers foraging at the three study sites (Parnka North, Salt Creek, Villa del Yumpa), across the two seasons (autumn and winter). Mean and standard error predicted values from generalised linear mixed effect model. Plotted points are proportion of Red-capped Plovers foraging per observation at the three sites and across the two seasons.

The proportion of Red-capped Plovers foraging varied between the three distance bands (Figure 5). At Parnka North the greatest proportion of foraging birds was found at the waterline for both seasons (Figure 5). At Salt Creek the proportion of foraging birds in both seasons was the greatest at the waterline and at 1-6m from waterline, with some birds foraging at >6 m in autumn (Figure 5). At Villa del Yumpa Red-capped Plovers were found predominantly foraging at >6 m from waterline in autumn and in winter it varied between distance bands, although the highest proportion was found at >6 m and secondly at the waterline (Figure 5). No statistically significant differences were found between

seasons, however some site differences were found. Villa del Yumpa had a greater proportion of birds foraging at >6m in comparison to birds foraging at Parnka North, where they predominantly foraged at the waterline (mblogit; estimate = 4.867, std error = 1.341, p value = 0.00029) (Figure 5). Similarly Salt Creek had a higher proportion of birds foraging at 1-6m compared to birds foraging at Parnka North (mblogit; estimate = 2.314, std error = 0.562, p value = 3.77e-05) (Figure 5).

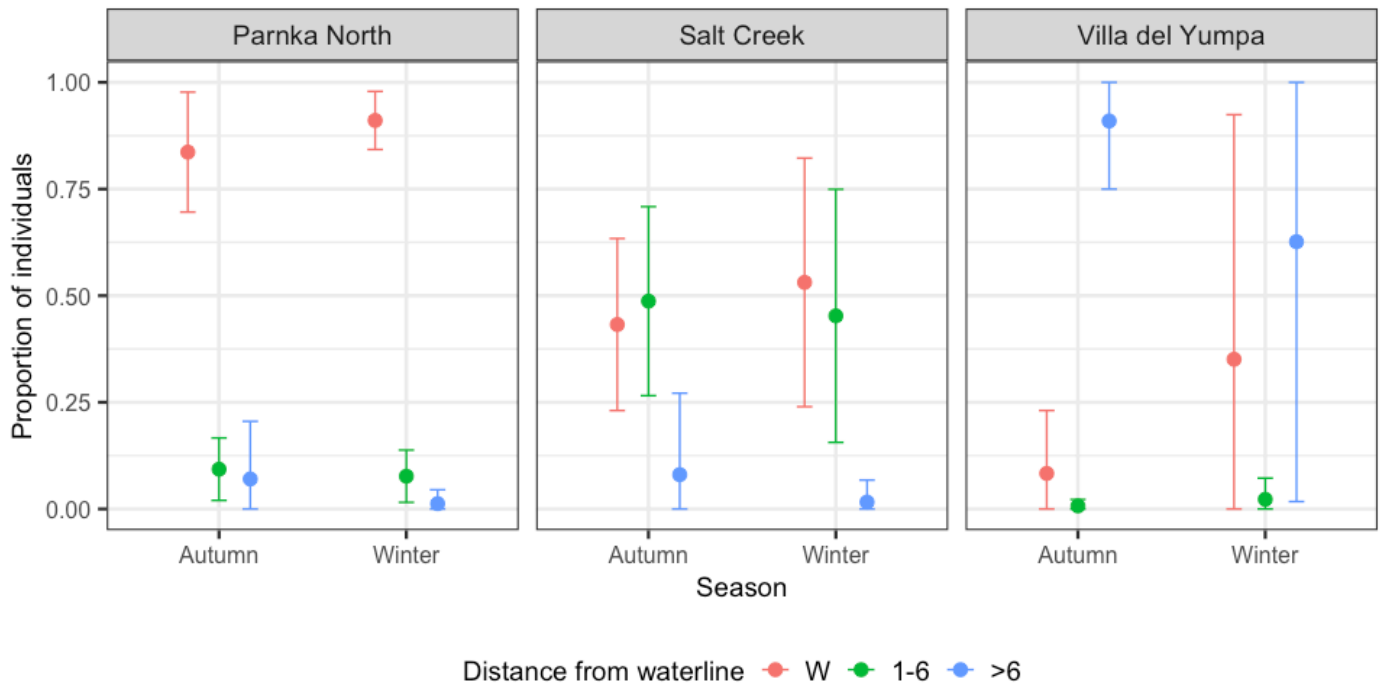


Figure 5 – Proportion of foraging Red-capped Plovers within the three distance bands (waterline, 1-6 meters from waterline and >6 meters from waterline) for all three study sites (Parnka North, Salt Creek, Villa del Yumpa), across the two seasons (autumn and winter). Mean and standard error predicted values from multinomial regression model.

### 3.2. Foraging Rate

The peck rate of foraging Red-capped Plovers varied between distance bands, site and season (Figure 6). Across all sites, winter had higher peck rates compared to autumn (GLM; estimate = 0.105, std error = 0.0388, p value = 0.00691) and higher peck rates were observed at >6m distance band compared to the waterline (GLM; estimate = 0.0911, std error = 0.0345, p value = 0.00868) (Figure 6). Villa del Yumpa had slightly higher peck rates over the three distance bands compared to Parnka North (GLM; estimate = 0.0814, std error = 0.0399, p value = 0.042), although no significant difference was found between Parnka North and Salt Creek (GLM; estimate = 0.08, Std error = 0.04, p-value = 0.057) (Figure 6). Salt Creek had lower peck rates at >6m compared to at the waterline (GLM; estimate = -0.16, std error = 0.07, p value = 0.034) and Villa del Yumpa had lower peck rates at 1-6m compared to at the waterline (GLM; estimate = -0.24, std error = 0.07, p value = 0.0005) (Figure 6). In autumn

Parnka North had higher peck rates at >6m compared to at the waterline (GLM; estimate = 0.09, Std error = 0.03, p-value = 0.016).

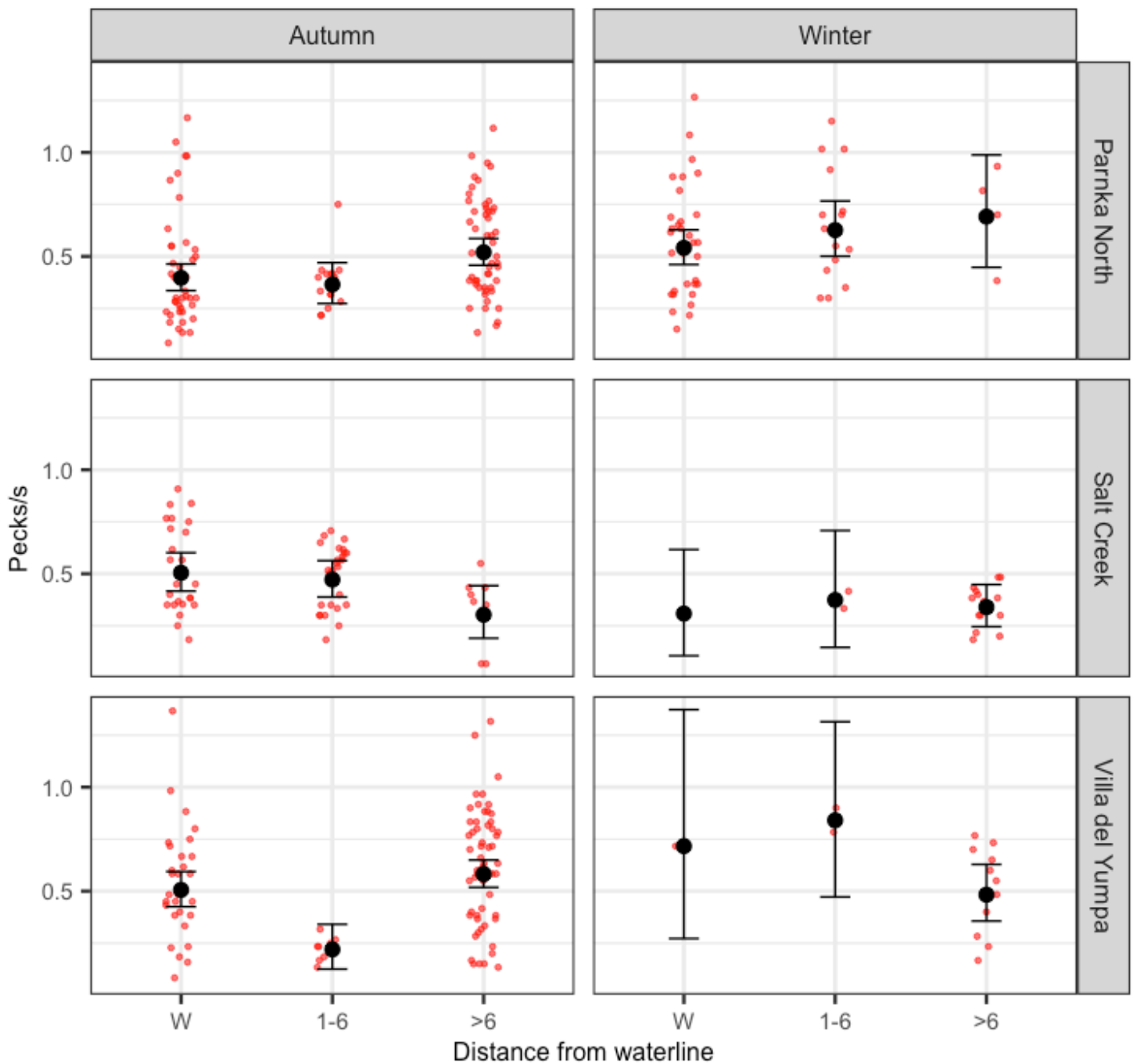


Figure 6 – Peck rate (pecks/s) of foraging Red-capped Plovers at the three study sites (Parnka North, Salt Creek, Villa del Yumpa), across the two seasons (autumn and winter). Mean and standard error predicted values from generalised linear model. Plotted points are raw data of Red-capped Plover peck rates at three sites, across the two seasons.

### 3.3. Red-capped Plover Diet

From all Red-capped Plover scat samples collected in autumn, there were 1,768,208 sequences and 1336 unique taxonomies. From this database I subset out singletons (i.e. sequences present only once in a sample, which likely represent application or sequencing errors), which resulted in 1093 unique taxonomies. Finally, I removed sequences that were unclassified at order level, which left a total of 1635354 sequences and 936 unique taxonomies.

From this data I calculated the relative abundance (the proportion of the total number of sequence counts accounted for by a given family) of the top 20 most abundant families across the three sites (Figure7). The most abundant family was Chironomidae, with 78% of all sequences belonging to this family and this percentage of Chironomidae was similar between the three sites (Table 1). The second most abundant family was Scolopacidae, however this family is likely contamination from misidentified scats (Table 1). These scats were likely collected from Red-necked Stint, which were identified at species level in the results and were present in the Coorong during my sampling. Third most abundant family is Muridae, with only 1.10% of all sequences containing this family (Table 1) and the last 7 families are fish species that have low abundances (Table 1).

*Table 1 – Percent of sequences from the top 10 abundant families that were found in Red-capped Plover scats. Also, the percent of sequences of these most abundant families between the three study sites (Parnka North, Salt Creek and Villa del Yumpa).*

<b>Family</b>	<b>%</b>	<b>Parnka North</b>	<b>Salt Creek</b>	<b>Villa del Yumpa</b>
<i>Chironomidae</i>	77.71	77.15	86.07	67.73
<i>Scolopacidae</i>	7.78	0.13	4.50	17.46
<i>Muscidae</i>	1.10	2.04	0.09	1.68
<i>Engraulidae</i>	0.87	0.04	0.58	1.84
<i>Platycephalidae</i>	0.59	0.09	0.66	0.88
<i>Scombridae</i>	0.54	0.19	0.54	0.79
<i>Cepolidae</i>	0.47	0.61	0.40	0.46
<i>Berycidae</i>	0.40	0.36	0.36	0.48
<i>Carangidae</i>	0.39	0.18	0.27	0.69
<i>Sillaginidae</i>	0.35	0.54	0.14	0.47

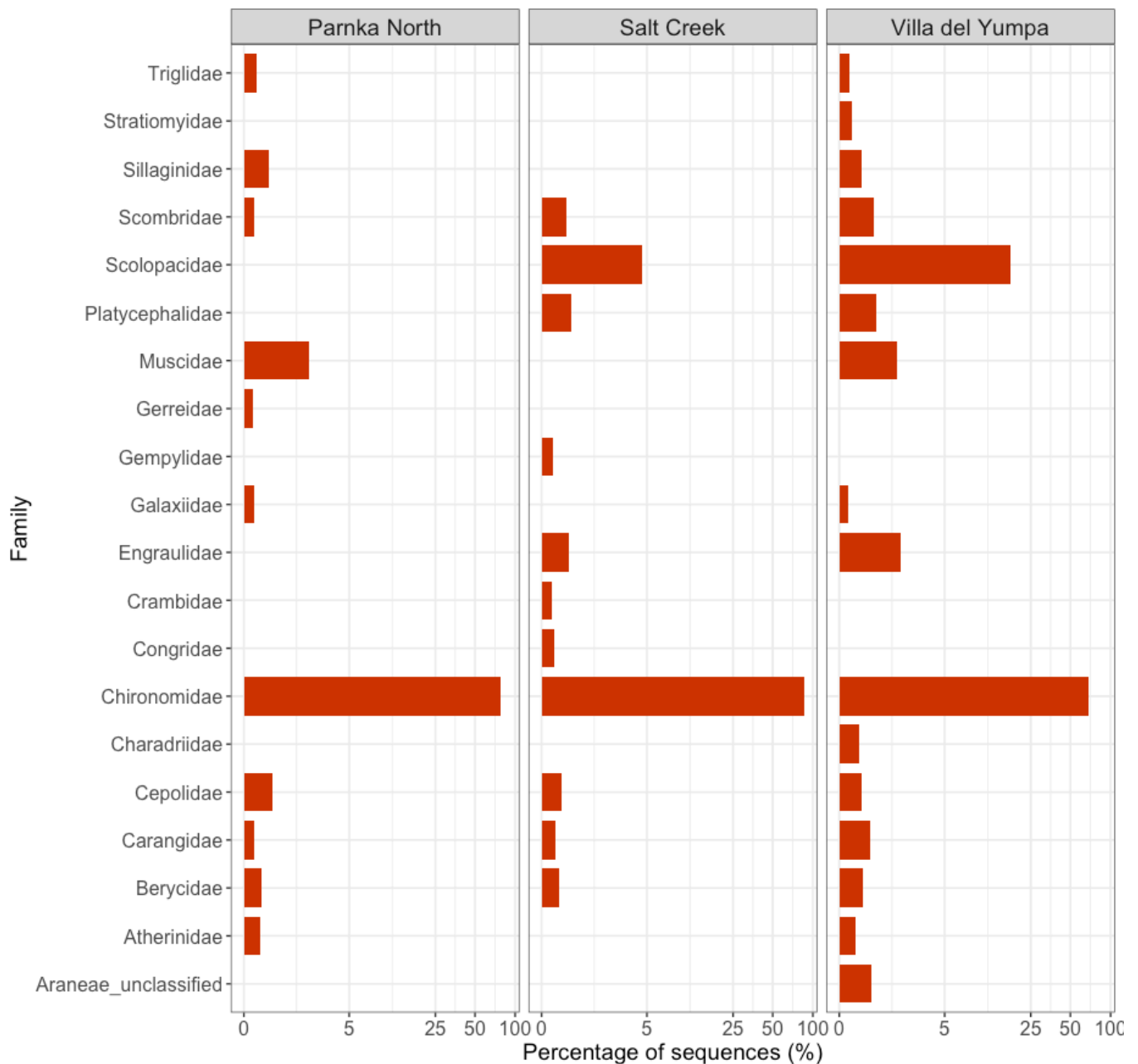


Figure 7 – Bar plot displaying the relative abundance of the 20 most abundant families within the Red-capped Plover scat samples. Split into the three site location samples (Parnka North, Salt Creek and Villa del Yumpa). Y-axis is on log scale to display the less abundant families.

Samples containing Scolopacidae were taken out to explore the effect they had on the prey composition. I recalculated the top 10 most abundant families using data from samples that contained no Scolopacidae sequences and these results are displayed in Table 2. Chironomidae (92% of all sequences) is still the most abundant family (Table 2). The remainder of the top 10 most abundant families did not change substantially, however an unclassified family of Araneae was present, though only at one site (Villa del Yumpa) (Table 2).

Table 2 – Percent of sequences from the top 10 abundant families that were found in Red-capped Plover scats, not including samples containing Scolopacidae. Also, the percent of sequences of these most abundant families between the three study sites (Parnka North, Salt Creek and Villa del Yumpa).

<b>Family</b>	<b>%</b>	<b>Parnka North</b>	<b>Salt Creek</b>	<b>Villa del Yumpa</b>
<i>Chironomidae</i>	91.27	93.21	95.27	66.96
<i>Muscidae</i>	1.39	2.55	0.04	3.97
<i>Engraulidae</i>	0.92	0.03	0.50	5.58
<i>Platycephalidae</i>	0.80	0.10	0.81	2.90
<i>Scombridae</i>	0.70	0.16	0.63	2.69
<i>Cepolidae</i>	0.58	0.72	0.26	1.64
<i>Sillaginidae</i>	0.53	0.64	0.18	1.78
<i>Berycidae</i>	0.49	0.28	0.41	1.52
<i>Araneae unclassified</i>	0.43	0.00	0.00	3.71
<i>Carangidae</i>	0.40	0.21	0.25	1.72

No significant difference was found between the relative abundance of Chironomidae and Site location ( $t = 1.86$ ,  $df = 2$ ,  $p$ -value = 0.39) and similarly between the scats of Red-capped Plover (RCP) and Red-necked Stint (RNS) ( $t = 0.15$ ,  $df = 1$ ,  $p$ -value = 0.69) (Figure 8). Between the next four most abundant families (*Muscidae*, *Platycephalidae*, *Engraulidae*, *Scombridae*), no significant difference was found between site location and type of scat (see appendix b).

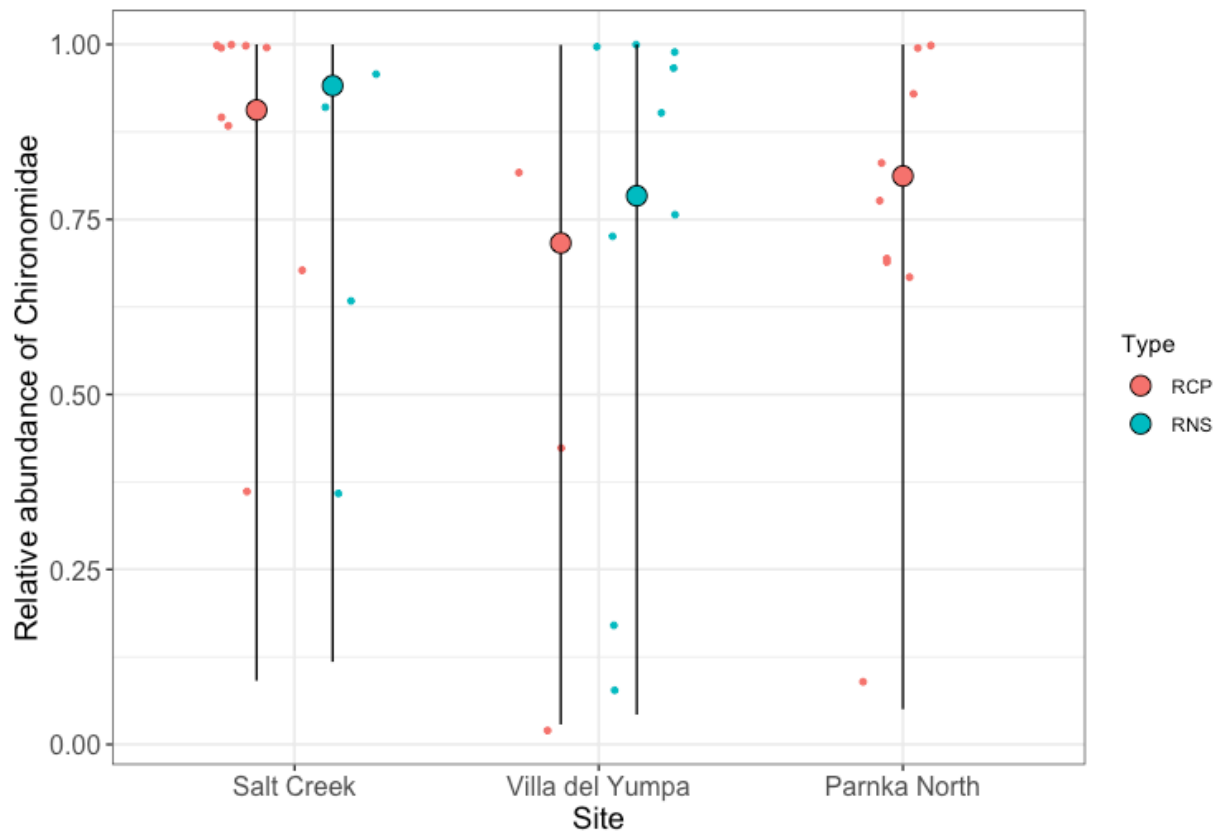


Figure 8 – Relative abundance of Chironomidae at the three study sites (Parnka North, Salt Creek, Villa del Yumpa) and between the two scat types (Red-capped Plover and Red-necked Stint). Mean and standard error predicted values from beta regression model. Plotted points are raw abundance of Chironomidae at the three study sites and between the two types.

Using a multivariate analysis, Red-capped Plover prey composition at family did not change significantly between site locations and type of scat sample (Figure 10) (Table 3). Similarly, no significant difference was found with prey composition between study site locations and scat type, at Order level (Figure 11) (Table 4). Although Villa de Yumpa appears to have a more diverse prey composition, whereas Parnka North and Salt Creek are more similar in their prey compositions (Figure 10 & 11). However, this could be influenced by the type of Scat sample.

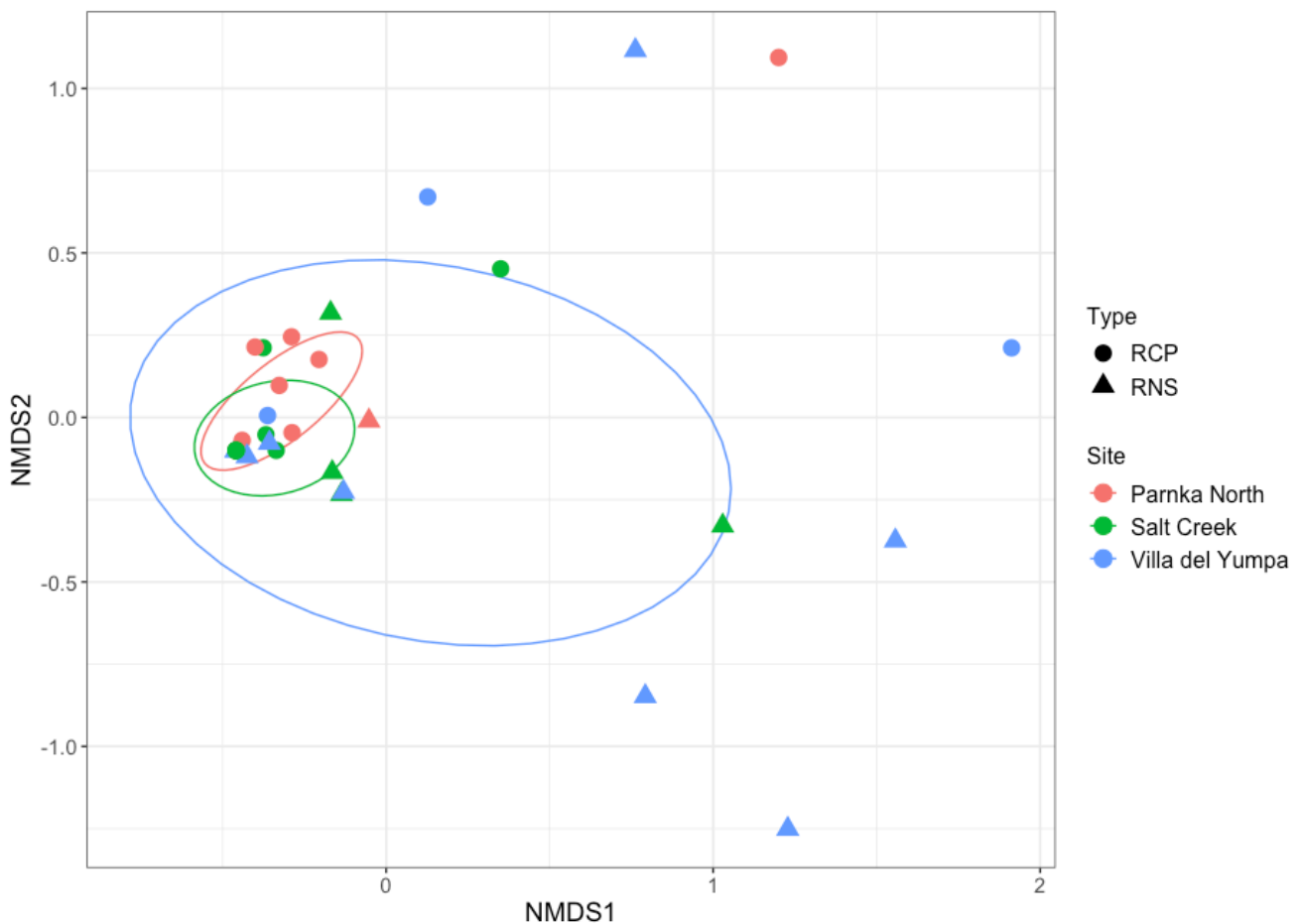


Figure 9 - Non-metric Multi-dimensional Scaling plot (NMDS) of Red-capped Plover prey composition, at Family level, between the three sites (Parnka North, Salt Creek and Villa de Yumpa). RCP is Red-capped Plover scat samples and RNS is Red-necked Stint scat samples.

Table 3 – Permutational Multivariate Analysis of Variance using Distance Matrices (ADONIS) results for Red-capped Plover prey composition at family level. Between the three sites (Parnka North, Salt Creek and Villa de Yumpa) and type of scat sample RCP is Red-capped Plover and RNS is Red-necked Stint.

Interaction	Df	SumOfSqs	MeanSqs	F. Model	R2	P-value
Site	2	0.57	0.28	2.0	0.11	0.06
Type	1	0.28	0.28	1.98	0.05	0.1
Site:Type	2	0.23	0.12	0.83	0.05	0.43

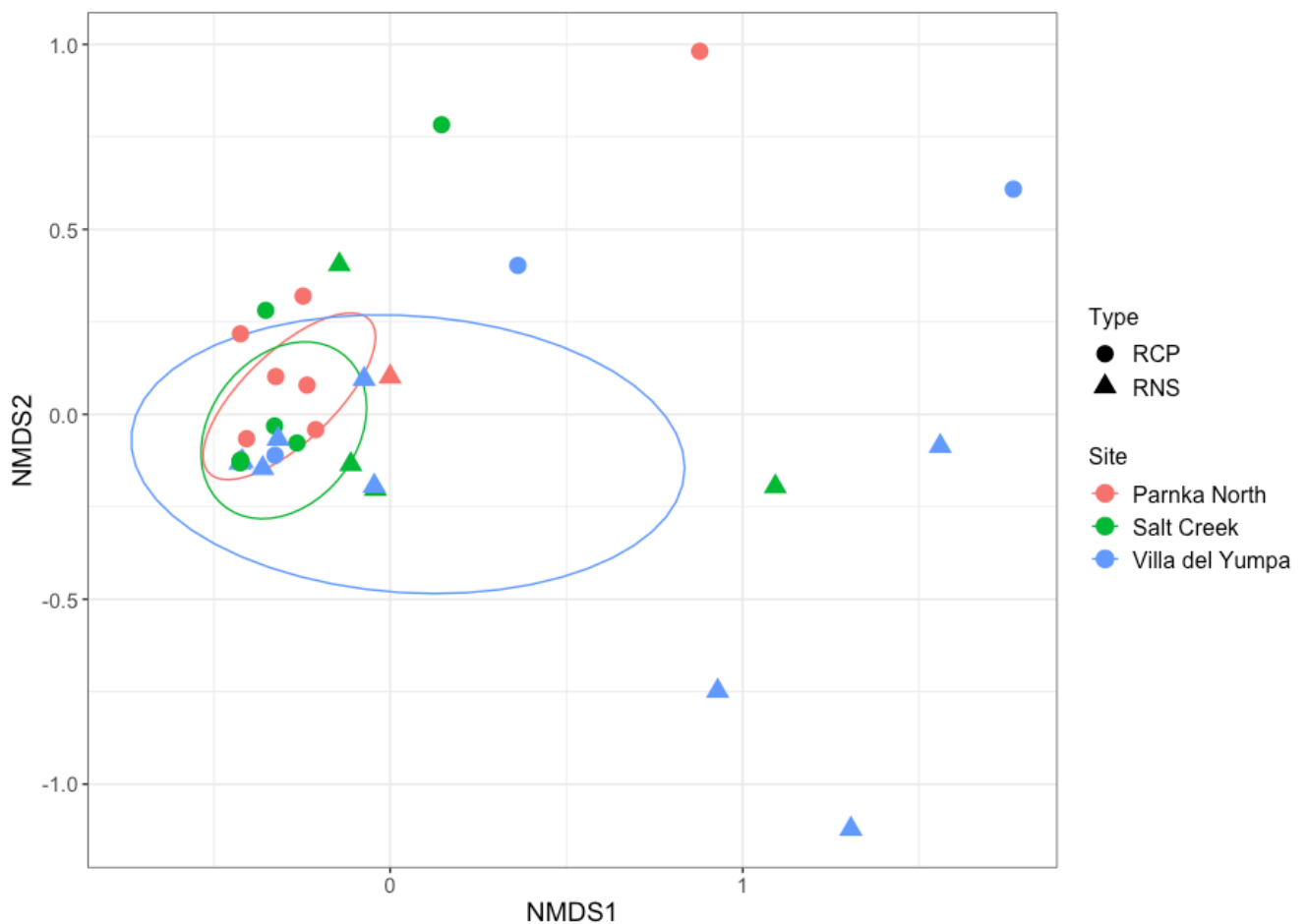


Figure 10 - Non-metric Multi-dimensional Scaling plot (NMDS) of Red-capped Plover prey composition, at Order level, between the three sites (Parnka North, Salt Creek and Villa de Yumpa).

Table 4 – Permutational Multivariate Analysis of Variance using Distance Matrices (ADONIS) results for Red-capped Plover prey composition at order level. Between the three sites (Parnka North, Salt Creek and Villa de Yumpa) and type of scat sample RCP is Red-capped Plover and RNS is Red-necked Stint.

Interaction	Df	SumOfSqs	MeanSqs	F. Model	R2	P-value
Site	2	0.45	0.22	1.81	0.1	0.1
Type	1	0.22	0.22	1.8	0.05	0.15
Site:Type	2	0.25	0.12	1	0.05	0.33



## 4. Discussion

I have provided the first detailed assessment of Red-capped Plover foraging behaviour and diet in the Coorong and found that aspects of the foraging ecology were influenced by large-scale effects, such as site and season, as well as small-scale effects, such as location on the beach.

### 4.1 Foraging habitat quality between sites

The value of a site for foraging Red-capped Plovers differed between my study locations, with the two more northern sites providing better foraging habitat conditions. Villa de Yumpa had significantly higher peck rates compared to Salt Creek, suggesting that Red-capped Plovers encountered and consumed more prey at this site. Red-capped Plovers are visual-foragers foraging birds meaning they can achieve capture success approaching 100% and they need a large number of small prey to maintain resting metabolic rates (Rose and Nol 2010). I assumed individual Red-capped Plovers in my study to have equally high capture success, meaning a higher peck rate is likely to indicate a high rate of prey encounter and accordingly greater prey biomass at a site. Surveys of benthic invertebrates in the Coorong have found that Chironomids, the main prey of the Red-capped Plovers analysed in my study, are more abundant in the central parts of the Coorong (close to Villa de Yumpa and Parnka North) in comparison to the extreme southern end of the Coorong (close to Salt Creek) (Dittmann *et al.* 2015b; Paton *et al.* 2016). In addition to the peck rate evidence, the abundance of birds at Parnka North and Villa de Yumpa were also significantly higher than the number of Red-capped Plovers at Salt Creek. The distribution of shorebirds is strongly influenced by the distribution and accessibility of prey (Yates *et al.* 1993; Ribeiro *et al.* 2004; Spruzen *et al.* 2008), so the higher numbers of Red-capped Plovers at these sites likely reflects relatively higher prey availability.

The proportion of foraging birds can also indicate the quality of a habitat for foraging and subsequently the abundance of prey. At Parnka North a high proportion of birds foraging at any given time (as opposed to roosting and other behaviours) was observed relative to the other two sites. This could be due to multiple factors: firstly, it may indicate that the abundance of prey at Parnka North is lower, meaning the Red-capped Plovers are allocating more time to foraging to sustain their food requirements (Van gils *et al.* 2005; van der Kolk *et al.* 2019); or secondly, it could be due to prey depletion from high abundances of foraging Red-capped Plovers (Fonseca Parra and Navedo 2020); or lastly, it could indicate that the abundance of prey at Parnka North is high and the Red-capped Plovers prioritise this site as a foraging site and go to other sites for roosting (Bellio and Kingsford 2013). Although some studies hypothesise that a high proportion of birds foraging indicates birds are

struggling to meet their resource requirements (van der Kolk *et al.* 2019), other studies indicate that birds use wetlands that provide high quality foraging resources for foraging and roost elsewhere (Bellio and Kingsford 2013). Together, my findings suggest that the Red-capped Plovers are using Parnka North primarily as a foraging site, and along with Villa de Yumpa these two sites provide better foraging conditions than Salt Creek for Red-capped Plovers.

#### *4.2 Foraging habitat quality between seasons*

The conditions of a season can provide more suitable foraging habitat for a bird. My research suggests autumn provides better conditions for foraging Red-capped Plovers, compared to winter, and this could largely be due to the water levels observed. The water level in winter was much higher than in autumn and this can have effects on Red-capped Plovers, through decreased foraging habitat. The higher abundances of Red-capped Plovers in autumn at all three sites would support this interpretation. Winter on average also had higher peck rates than autumn, which could relate to prey density, but could also indicate that during cold weather Red-capped Plovers need to consume more food to maintain their resting metabolic rates (Vézina *et al.* 2011). Additionally, the lower number of occurrences that were seen during winter caused more variability in the precision around the estimates of the number of birds foraging, which consequently cannot accurately provide a representation of the overall probability of Red-capped Plovers foraging and their peck rates. I also found that this increase in water levels observed affected where Red-capped Plovers chose to forage on the beach. It resulted in Red-capped Plovers choosing to forage more often at the waterline, possibly to reduce predation risk by being too close to vegetation back on the beach or it could just relate to their being higher abundance of prey at waterline. This indicates that water level can have a significant effect on the foraging behaviour of Red-capped Plover and could potentially be controlled to maximise foraging habitat.

#### *4.3 Foraging location on beach infers prey abundance*

Where Red-capped Plover chose to forage on the beach differed between the three sites and this could infer the abundance of benthic prey vs terrestrial prey. Red-capped Plovers were found to predominantly forage at the shoreline at two of the site locations (Parnka North and Salt Creek) and at the other site (Villa de Yumpa) they were found predominantly foraging on exposed mudflats/dry sand (>6m from the shoreline). This foraging area selection is likely influenced primarily by the abundance of prey at the waterline vs exposed mudflats/dry sand, with benthic invertebrates more abundant when Red-capped Plovers predominantly foraged at the waterline (i.e., at Parnka North and Salt creek), and either benthic invertebrates less abundant or terrestrial invertebrates more abundant when they foraged predominantly >6m from the waterline (i.e., at Villa del Yumpa). However, to accurately infer this,

research is needed to determine the benthic invertebrate abundance and diversity at the three sites during my sampling periods. Other factors could also influence this, including the presence of other shorebirds foraging on the beach which can cause heterospecific attraction. The range of a birds feeding habitat and food sources is dependent on their morphology (Baker 1979; Norazlimi and Ramli 2015). For shorebirds their leg length can influences what water depth they can forage in and their bill length/curvature effects what substrate they forage on and the prey they consume (Norazlimi and Ramli 2015). Specifically for Red-capped Plovers they consume surface dwelling invertebrates and tend to forage less exclusively at the shoreline, in comparison to most other shorebird species (Martin and Piersma 2009; Paton et al. 2019). Therefore, the presence of other shorebirds, that forage primarily at the waterline, could influence Red-capped Plovers to alter their foraging area selection. However more research would need to be completed to analyse this effect of other bird species on the foraging area selection of Red-capped Plovers.

Specifically for Salt Creek the area that Red-capped Plovers chose to forage, can infer the prey abundance at the site. At Salt Creek they had less preference of what distance band they foraged at, in comparison to the other two sites. This could indicate that there was not enough prey in just one section of the beach, which resulted in their varied foraging area selection. This statement coincides with the lower abundance of Chironomids that is found in this southern area of the Coorong compared the more central areas (Dittmann *et al.* 2015b). This means that the Red-capped Plovers may need to utilise most of the foraging area at Salt Creek to meet the feeding requirements, which could have implications for future habitat management, as completing habitat management in a particular section of the beach may affect them.

#### *4.4 Prey composition of Red-capped Plover diet between sites*

Chironomids were found to be the most dominant food source of Red-capped Plovers (78% of all sequences); however, it is not known whether they are consuming Chironomids by preference or just because they are dominant. In the Southern lagoon insect larvae makes up 50 -70% of all benthic invertebrates and specifically Chironomid larvae and pupae is one of the most abundant (Dittmann et al. 2019; Ye et al. 2020). To accurately infer the importance of Chironomids to Red-capped Plover diet, scat samples would need to be analysed from more northern sites, where the benthic invertebrates are more diverse and abundant.

The composition of Red-capped Plover prey can infer the habitat quality of a specific site. Although, no significant differences were found in my study between the composition of prey DNA in scats at

the three sites, Villa de Yumpa did appear to have a more diverse composition of prey DNA. Additionally, Villa de Yumpa scat samples did have slightly lower relative abundance of Chironomids, inferring that the Red-capped Plovers possibly chose to consume other food sources as well. This could also be due to Villa de Yumpa having lower abundance of chironomid larvae, however past invertebrates studies do not indicate this (Dittmann *et al.* 2015b). Nevertheless, Villa de Yumpa having a more diverse composition of prey DNA compared to the two sites, which were quite similar in the composition, could reflect the higher foraging habitat quality that was found at Villa de Yumpa. With significantly higher peck rates and greater numbers of Red-capped Plovers, it is possible that this species prefers the prey composition at this site. Additionally, the lower proportion of birds foraging might mean the prey available at this site is also more profitable, as a reduced foraging time can mean that the prey is not thinly spread or difficult to harvest (Paton *et al.* 2019).

#### *4.5 Importance of Chironomids in Red-capped Plover diet*

While my study cannot demonstrate that Red-capped Plovers specifically chose to prey on Chironomids, their importance to their diet during autumn in the Southern Coorong is clear. This means that future management needs to consider that a reduction in Chironomid abundance could have an impact on Red-capped Plover populations in this part of the Coorong. The DNA metabarcoding method cannot determine whether the Red-capped Plovers were consuming adult chironomids or their larvae. However, as I found that Red-capped Plovers forage nearly equally at the waterline (where larvae are generally found) and back on the beach, it is probable that they consume both life stages of the Chironomids. Chironomids in the past have been negatively impacted by decreased flows during the Millennial drought and more recently by filamentous algae blooms (Dittmann *et al.* 2015a; Paton *et al.* 2019). The emergence of algae suggests that the system is becoming eutrophic. This can be caused from reduced flooding, leading to increased accumulation of nutrients in the system, or increased important of external nutrients (Brookes *et al.* 2021). It is believed that in the Coorong increased flow of water from the Southeast and its associated nutrients may be causing these algae blooms (Brookes *et al.* 2021). These algae blooms not only reduce the foraging habitat of shorebirds but they also suppress the emergence of Chironomid larvae (Brookes *et al.* 2021). During my winter sampling, algae was present on some of the sites, most likely due to increased water levels that were present. The occurrence of algae blooms in the future has the potential to negatively affect the abundance of Chironomids and subsequently could affect the population of Red-capped Plovers in the Coorong.



*Figure 11 – Algae mats reducing the foraging area of Female Red-capped Plover in the Coorong, during winter sampling.*

#### *4.6 Presence of other DNA*

DNA from fish families were detected in the scats of Red-capped Plovers at the study sites. This could be contamination from the sediment or water where the scats were collected, however, fish could also be part of their diet. Past studies have identified juvenile fish and eggs as being part of other shorebird diets, along with detrital remains that wash up onto the mudflats (Gerwing *et al.* 2016). On one occasion during this study a Red-capped Plover was observed eating a small fish, which suggests that fish juveniles and eggs make up part of their diet. However, to accurately confirm this additional study would need to be completed on what DNA is present in the sediment and in the water at my study sites to determine if it is contamination or if Red-capped Plover do regularly consume fish species.

Some of the scat samples collected contained a high abundance of Red-necked Stint DNA. It is therefore possible that Red-necked Stint scats were collected instead of Red-capped Plovers. However, I believe this may also be due to some contamination, as Red-necked Stint were not always present during the sampling periods, and scats were specifically collected in a way to minimise the probability of mis-identification. Although there is always the possibility and future study should take this into consideration. It is not always possible to collect scats where Red-capped Plovers occur alone, especially during summer when the abundance and diversity of shorebirds in the Coorong is much higher. But collecting more scat samples from each site would help by producing larger sample sizes,

to account for scats being collected from non-target species. To accurately determine whether Red-necked Stint scats were collected; the sequences would need to be re-run without the Red-capped Plover blocker and the relative abundance of the Red-capped Plover sequence and Red-necked Stint sequence can be compared. In all true Red-capped Plover scats, the relative abundance of the Red-capped Plover sequence should be more abundant.

#### *4.7. Limitations*

There were a number of limitations in this study, which should be taken into consideration for future research. Firstly, my study site in the northern lagoon (Parnka North) cannot provide an overall representation of the northern lagoon, as it is located very close to Parnka Point (intersection between the two lagoons), and this can provide implications inferring the complete diet of Red-capped Plovers between the two lagoons. Benthic invertebrates, particularly polychaetes, are known to be more diverse and abundant in the northern end of the north lagoon and polychaetes have been listed in past studies as an important diet component of Red-capped Plovers (Abensperg-Traun and Dickman 1989). This suggests that the diet of Red-capped Plovers in the Coorong may be more variable than my study as indicated. Therefore, to accurately quantify the diet of Red-capped Plovers throughout the Coorong, scat samples would need to be analysed from additional northern sites. Similarly, my study was limited to sampling in autumn and winter, and I could only get DNA data from autumn due to constraints in the length of my project and this can provide implications regarding the change in diet of Red-capped Plovers throughout the year.

Furthermore, my study did not directly measure the invertebrate abundance at my study sites and therefore I can only speculate on the abundance and diversity of prey through my foraging observations. This means to accurately infer whether Red-capped Plovers are choosing a site based on benthic or terrestrial invertebrate abundance, research needs to measure the invertebrate abundance and diversity at the three sites during my sampling periods.

#### *4.8. Conclusion*

My study provides an insight into the foraging ecology of Red-capped Plovers over two seasons (autumn and winter) and documents their autumn diet in the southern Coorong. This information can not only be used to help inform future management in the Coorong, but can provide baseline information for future management in other areas of Australia that Red-capped Plovers inhabit. My research has shown that foraging behaviour and diet of Red-capped Plovers is strongly influenced by both site location, season and also by small scale effects, such as location on the beach. It is clear the

foraging conditions in the winter of 2021 were significantly worse compared to autumn and this was likely due to the water level. Therefore, where possible the amount of time that the Coorong experiences excessively high water levels could be limited to prevent severe restriction of foraging habitat for Red-capped Plovers and other shorebirds. Relating to the water level, algae blooms need to be controlled due to negative effects they have on Red-capped Plovers through reducing foraging area and interfering with the emergence of Chironomids, which I found to be an important food source for Red-capped Plovers. To ensure Red-capped Plovers and other shorebird species persist in the Coorong, South easterly flows need be controlled to reduce these occurrences of algae blooms. Salt Creek was identified as the least profitable site for foraging Red-capped Plovers and thus may not be able to support sufficient numbers of Red-capped Plovers. Therefore, future management should be considered at this site to improve the prey abundance and foraging habitat for Red-capped Plovers. There is a significant need to maintain and improve the foraging habitat of the Coorong to support not only Red-capped Plovers, but for a range of waterbirds. This requires sufficient management, to improve habitat quality and uphold the importance of this internationally significant wetland.

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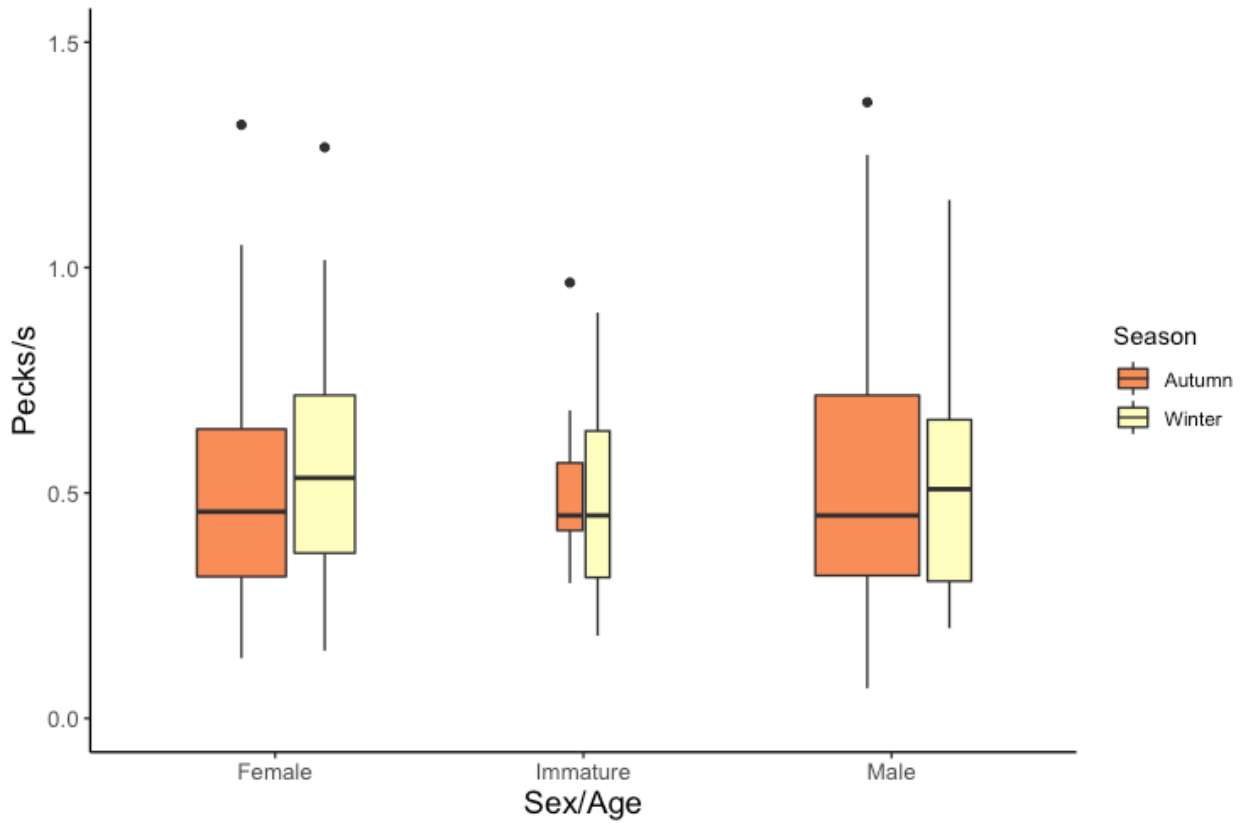
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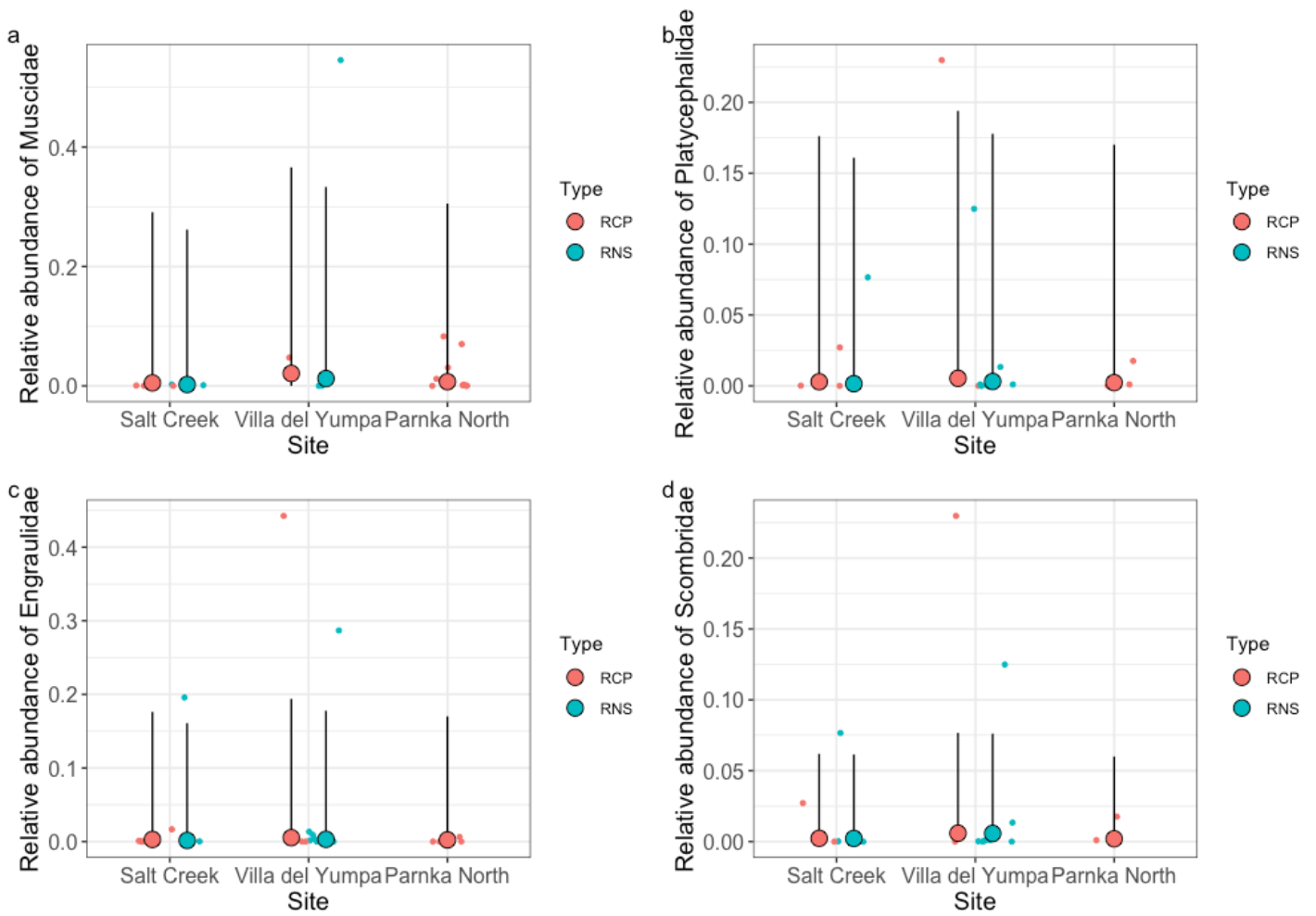
## Appendix

### Appendix A – Red-capped Plover foraging rate



*Appendix A - Boxplot comparing pecks/s between female, male and immature Red-capped Plovers for both autumn and winter. Mean peck rate does not differ between the groups, therefore they were grouped together for analysis.*

Appendix B – Red-capped Plover diet



Appendix B – Relative abundance of prey taxa families; a) Muscidae, b) Platycephalidae, c) Engraulidae and d) Scombridae, at the three study sites (Parnka North, Salt Creek, Villa del Yumpa) and between the two scat types (Red-capped Plover and Red-necked Stint). Mean and standard error predicted values from beta regression model. Plotted points are raw abundance of the families at the three study sites and between the two types.