The spatial dynamics of zooplankton communities in the Waikato River estuary, New Zealand

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Abstract

Estuaries are transitional areas between streams or rivers and the ocean. Estuaries are affected by fluvial factors, such as freshwater and sediment inputs, and marine factors, including tidal influence, waves, and the inflow of salt water. The inherently dynamic nature of estuaries results in extreme changes in hydrographic characteristics, with the distribution of zooplankton being highly influenced. Zooplankton provide a crucial role in the biological communities of big rivers. However, they are extremely sensitive to environmental changes, particularly in estuarine environments. Consequently, this study aimed to examine how changes in physicochemical properties, such as salinity, affect species richness and zooplankton community composition along the Waikato River estuary, fed by New Zealand's largest river. Eight sites were selected to represent the salinity gradient from the river mouth to a point where salinity was undetectable. Environmental variables, including temperature, dissolved oxygen, pH, salinity, and chlorophyll-a, as well as zooplankton communities, were examined at spring high tide (25 January 2023), spring low tide (8 March 2023) and neap high tide (15 March 2023).

A canonical correspondence analysis (CCA) indicated that salinity explained the greatest proportion of variation in zooplankton community composition (26.8%, P=0.002). At the lower extreme of the gradient, zooplankton assemblages of the freshwater sites were dominated by freshwater rotifers such as *Lecane luna*, *Synchaeta oblonga*, *Brachionus calyciflorus* and *Filinia novaezealandiae*. The zooplankton assemblages of the saltwater sites were dominated by marine copepods, such as *Oithona similis*, *Paracalanus parvus*, *Microsetella norvegica*, and marine cladocerans such as *Pleopis polyphemoides*. Dissolved oxygen also significantly influenced zooplankton composition, independent of salinity (9.3%, P=0.004).

At sites characterised by intermediate salinities in the Waikato River estuary, typical estuarine copepods (e.g., *Gladioferens pectinatus*) were absent. Sampling was conducted after high precipitation as well as a severe tropical cyclone which caused heavy rainfall and flooding. The strong influence of the tidal and riverine forces caused an unstable habitat for mid-salinity zooplankton communities to survive. It is predicted that with climate change, extreme weather events will likely become more frequent and severe in the future which pose a threat to less tolerant zooplankton species.

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Chapter 1: Introduction

1.1 Estuaries

Estuaries form a transitional zone between freshwater rivers or streams and the open shore (Thrush et al., 2013) and are among Earth's most productive habitats. Estuaries provide essential ecosystem services such as water filtration, food provision, storm protection and nutrient regulation (Booi et al., 2022). They are global hotspots for organic matter processing, primary production and nutrient cycling (Swales et al., 2020). Estuaries exhibit extremely dynamic hydrographic characteristics, where the interaction of tides, river discharges, and winds determines the spatial pattern of salinity, temperature, turbidity, and other hydrological factors impacting the horizontal distribution of estuarine organisms (Gao et al., 2008; Benfield, 2012). In addition, estuaries are extremely sensitive to human influences, notably pollution. As a result, this environment can be challenging for the biota due to the extreme dynamics in the physical environment and anthropogenic conditions (Rosenberg, 1976; Kennish, 2002; Lucena-Moya & Duggan, 2017).

A heterogeneous environment is formed due to the dynamic physical characteristics of estuaries (Lucena-Moya & Duggan, 2017). This can be divided into three different sectors: (1) one that is more marine and has a more significant influence from the sea; (2) one that has brackish waters and highly resilient species, but is still subject to strong tidal influence; and (3) one that is more freshwater, but still has some saline influence (Fairbridge, 1980; Cardoso, 2021).

1.2 The significance of zooplankton

Zooplankton live in the water column of almost all water bodies such as oceans, ponds, lakes, large rivers and estuaries, and are considered to be non-motile or weak swimmers that drift within water-bodies via water currents (Brierley, 2017). Zooplankton are typically small animals, usually below two millimetres, but are important components of the aquatic biota (Bakhtiyar et al., 2020; Hendrey, 2001). Freshwater zooplankton primarily consists of the three main groups: rotifers, cladocerans, and copepods. Marine zooplankton, in contrast, are exceedingly varied, comprising of many different taxonomic groups and lifestyles, such as holoplankton and transient constituents known as meroplankton (e.g., larval forms of sea urchin, sea stars and crabs; Lalli & Parsons, 1997; Hamilton & Duggan, 2010).

Zooplankton influence the dynamics of aquatic ecosystems (Lomartire et al., 2021). For example, the biomasses of phytoplankton taxa are strongly influenced by zooplankton. Zooplankton may influence the concentrations of algal populations through consumption and of predator populations such as fish through provision of food (Vanni, 2002). Zooplankton function as primary or secondary consumers, and the preservation of zooplankton is crucial to preventing the collapse of food webs since they are essential for the continuation of ecosystem services (Bakhtiyar et al., 2020; Lomartire et al., 2021). In addition to structuring phytoplankton communities, zooplankton also provides them with nitrogen and phosphorus through a continuous cycle of nutrient recycling. Furthermore, zooplankton play a significant role in the effectiveness of the biological carbon pump, which controls the amount of carbon dioxide in the atmosphere (Lomartire et al., 2021). The biological carbon pump is a crucial part of climate regulation. Zooplankton participate in this process by relocating particulate organic carbon via diel vertical migration (Halfter et al., 2020).

Zooplankton are vulnerable to environmental stressors such as temperature, pH, turbidity, phytoplankton composition, nutrient loading, eutrophication, and warming (Cremona et al., 2021). Additionally, the temporal distribution of zooplankton has been demonstrated to be significantly influenced by physical processes such as tidal advection, wind-induced mixing, and eddies. Thus, the short-term community structure may be impacted by the zooplankton's reaction to external driving processes (Wiafe & Frid, 1996). Due to varying environmental conditions along the river, there is expected to be large differences in biomass and composition (Lair, 2006). Understanding zooplankton community change is beneficial for determining how anthropogenic stressors threaten the ecological status of aquatic ecosystems, biological integrity and ecosystem service provisioning (Cremona et al., 2021).

1.3 The importance of salinity on zooplankton dynamics

The salinity gradients found in estuaries are connected to the hydrogeomorphology of the area and influence changes in species richness and community composition (Lucena-Moya & Duggan, 2017). Zooplankton species have different salinity tolerance (Gao et al., 2008; Hall & Lewandowska, 2022). The capacity of an aquatic organism to osmoregulate, a biochemical mechanism that controls solute transport and numerous metabolic processes, is influenced by salinity (Schallenberg et al., 2001).

The response of zooplankton populations to salinity is a hotly debated subject (Gao et al., 2008). Many studies nationally (Roper & Jones, 1983; Schallenberg et al., 2003; Hall & Burns, 2003; Lucena-Moya & Duggan, 2017; Marshall, 2022) and internationally (Grindley, 1981; Gao et al., 2008; Yuan et al., 2020; Hall & Lewandowska, 2022) indicate that salinity is the most significant environmental factor influencing zooplankton diversity and abundance. Zooplankton species richness is predicted to be greater when salinity levels are nearer to that of freshwaters or totally marine waters than in brackish (transitional) waters areas. This is a characteristic of the biodiversity pattern known as "Remane's curve" (Remane, 1934). The curve shows that between 5 and 8 PSU there is minimum species richness, due to the different physiology of marine and freshwater organisms (Paavola et al., 2005).

Various estuaries and systems with significant seawater intrusions exhibit zooplankton assemblage changes from freshwater to euryhaline marine species along the salinity gradient. Hall & Burns (2003) found that salinity was the main factor influencing changes in the assemblage's species composition and abundance in Lake Waihola, New Zealand. Results from this study showed that communities will shift from freshwater Cladocera to euryhaline copepods as a result of decreased freshwater inflows and increased tidal input. This study also illustrates that sea level rise brought on by climate change may also cause a greater tidal scale change in the distribution of zooplankton populations ultimately contributing to the alteration in trophic relations. This is also further supported by Schallenberg et al. (2003), South Island lakes that receive slight salinity increases from tidal intrusions have significant changes in the composition of zooplankton species and a notable decline in freshwater species. The combination of fresh river water with seawater will result in the majority of planktonic species perishing and the zooplankton species composition resembling that of estuaries (Schallenberg et al., 2003).

Ecosystem health and structure are closely related to species diversity, population size, behaviour, and the ecological roles they play. It is vital to understand how various environmental factors influence assemblage variation, the abundance and distribution of species that perform crucial functions, and the health of ecosystems for natural resource management in marine and freshwater ecosystems (Jones et al., 2020). Knowing how zooplankton distribution and salinity interact could prove crucial to understanding how ecosystems operate when there is a global mean sea level rise brought about by climate change (Yuan et al., 2020). Salinity changes may affect the zooplankton community composition, causing the local extinction of some species and the emergence of more tolerant species (Hall & Lewandowska, 2022).

1.4 New Zealand studies of zooplankton in estuaries

There are a limited number of published studies on zooplankton composition in New Zealand's estuarine environments. Roper & Jones (1983) produced the first comprehensive quantitative data on zooplankton from a New Zealand estuary. This research was conducted in Christchurch's Avon-Heathcote Estuary, a bar-built estuary that receives freshwater inputs from Heathcote and Avon rivers. This study found that estuarine and freshwater species were typically more abundant upstream of the estuary. In contrast, marine species were typically more prevalent near the estuary mouth. The degree of mixing of residual estuary water, freshwater, and coastal water significantly impacted zooplankton composition more than other environmental factors. This study also found distinct assemblages of euryhaline, estuarine species at intermediate salinities, including the ostracod *Leptocythere* spp. and copepods such as *Gladioferens pectinatus* (Roper & Jones, 1983).

A more recent study undertaken by Lucena-Moya & Duggan (2017) investigated whether variation in zooplankton assemblages were in accordance with the categories of estuary type described by the Estuary Environment Classification System (EEC) (Hume et al., 2007). Additionally, they investigated if zooplankton assemblages among New Zealand estuaries could be predicted using Remane's curve. This is the only research conducted in New Zealand that has concentrated on zooplankton assemblages on a salinity gradient that is represented among estuarine ecosystems across a wide geographic region. The results from this study showed that rotifers dominated the freshwater category, while estuarine copepods (including *Gladioferans pectinatus*) dominated the intermediate salinity range. Marine copepods (e.g., *Euterpina acutifrons*) and other oceanic species dominated the marine sites. Zooplankton diversity was influenced by the salinity gradient as predicted by Remane's curve (Lucena-Moya & Duggan, 2017).

Overall, Roper & Jones (1983) and Lucena-Moya & Duggan (2017) showed that zooplankton diversity responded to the salinity gradient as predicted by Remane's curve. The findings of these studies corroborate those from overseas that have demonstrated salinity to be the primary driver of the diversity and composition of zooplankton communities.

1.5 Waikato River and Estuary

New Zealand has a vast coastline, stretching approximately 18,000 km. It comprises over 400 estuaries covering a combined area of around 5,300 km^2 (Thrush et al., 2013). The Waikato River, the longest river in New Zealand, travels 425 kilometres from Mount Ruapehu to Port Waikato, where it discharges into the Tasman Sea (Chapman, 1996). Eight hydroelectric dams regulate the river's flow, and the tide can affect water levels upstream as far as at least Mercer, 42 kilometres from the entrance (Collier et al., 2010). This river drains a total catchment area of c. 14,000 km^2 with a mean annual discharge of c. 600 m^3 s^{-1} (Jones & Hamilton, 2014).

The Waikato River delta is situated in the lowest reaches of the Waikato River, approximately 15 km from the sea (Jones & Hamilton, 2014). The river delta divides into several intricate networks of channels interspersed by islands and reed beds created by deposited sediment (Collier et al., 2010; Jones & Hamilton, 2014). Daily tidal cycles and river flows affect the lower part of the Waikato River. This can cause changes in water levels at different points upstream between Mercer (42 km from the sea) and Rangiriri (63 km) (Collier et al., 2010).

The lower Waikato River and estuary meet the standards for international importance (Myers et al., 2013; Ryer et al., 2016). Various freshwater and saltwater fish species inhabit the lower Waikato River, such as whitebait (*Galaxias maculatus*), taking advantage of the abundant resources of the Waikato River delta. Port Waikato is a habitat, nursery, and passageway for migrating freshwater species. For instance, Caspian terns breed in this area, and oystercatchers are commonly found at Port Waikato during winter (Ryer et al., 2016). Furthermore, diverse mudflat, sandflat, salt marsh, and wetland habitats are used by numerous native birds in the delta for feeding and nesting (Collier et al., 2010).

The Estuary Environment Classification (EEC) system identifies several environmental factors that greatly influence estuaries' physical and biological characteristics. These factors include climate, oceans, rivers, and catchments (Hume et al., 2007). Based on the EEC scheme of Hume et al. (2007), the Waikato River estuary is categorised as a tidal river mouth, where river flows dominate hydrodynamic processes. The primary channel of the river is effectively cleared of saltwater. Still, there may be a salt wedge formation creating a hypopycnal flow (Hume et al., 2007). The hypopycnal flow is developed when the density of inflowing water (freshwater) is lower than oceanic density, the river water is therefore buoyant and flows over the denser salt water (Valle-Levinson, 2011).

1.6 Zooplankton composition along the Waikato River

A comprehensive study along the Waikato River in 1999 found that numerically small-sized rotifers made up 85% of the zooplankton community, followed by cladocerans (9%) and copepods (6%) (Burger et al., 2002). Although the zooplankton composition in the Waikato River has been extensively investigated, nothing is known about the zooplankton in the Waikato River estuary and delta (Hamilton & Duggan, 2010). Larger zooplankton species are more apparent along the upper river such as the cladoceran *Ceriodaphnia* and copepod *Boeckella propinqua*, and these decline in abundance downstream. Rotifer species such as *Lepadella accuminata* and *Trichotria tetractis* are also found in high abundances upstream (Hamilton & Duggan, 2010). Within hydroelectric lakes, larger zooplankton species are usually unable to proliferate (Lair, 2006). Hydroelectric lakes are known to be a major source of planktonic crustaceans in the Waikato River, where large cladocerans such as *Daphnia galeata* are abundant in or just below dams (Burger et al. 2002). Along the lower Waikato River, Benfield (1990) examined zooplankton from Lake Karapiro to Huntly. This study showed a notable decline in the abundance of zooplankton, especially crustaceans downstream of Lake Karapiro.

1.7 Aim and hypotheses

The aim of this study was to determine how changes in salinity within the Waikato River estuary, New Zealand, affect zooplankton species richness and community composition. In order to achieve this, eight sites along the Waikato River estuary were selected to represent a gradient in salinity from the river mouth to an upstream site where salinity is undetectable. The results of this study will advance the knowledge of how environmental changes, such as climate change, may influence zooplankton communities. Previous studies in New Zealand estuaries and coastal ponds have shown that salinity is the key factor determining the spatial distribution of zooplankton. According to the Remane curve model, the marine and freshwater sections will each have similar number of species, but within transitional sections, the number of species decreases, occupied by a few brackish species (Remane, 1934). Therefore, my hypotheses are that salinity will have the greatest influence on the zooplankton community composition along the Waikato River estuary and that along the salinity gradient (with freshwater, transitional and marine taxa in different sections of the river), there will be distinct zooplankton assemblages, with higher but equal species diversity in the freshwater and marine sites and the transitional water will comprise of lower diversity of brackish species.

Chapter 2: Methods

2.1 Site Selection

The limit of saltwater intrusion in the Waikato River estuary is considered to be in the mid-islands region of the delta, approximately 10 km from the entrance of the river mouth during neap high tide and about 13 km from the entrance during spring high tide, according to a field study undertaken over a spring-neap tidal cycle in 2013 (Jones & Hamilton, 2014). Utilising this information, Google Maps satellite images were used to identify areas that were easily accessible and had a spread relatively equidistant along the gradient.

On 23 January 2023, an initial search was performed to identify suitable sampling locations along the estuary. However, some sites were ruled out due to concerns over accessibility and health and safety issues. From the scoping assessment, eight sites (*Figure 1; Table 1*) were chosen to represent the gradient of salinity, ranging from high salinity close to the river mouth (salinity over 30 PSU during spring high tide) to undetectable salinity upstream (below 0.1 PSU) in the Waikato River. The survey covered around 25 km of the Waikato River (*Figure 1; Table 1*). On average, sites 1-7 were approximately 850 m apart, covering a total distance of over 5 km. Site 8 was located at Les Batkin Reserve, Tuakau. This area had undetectable salinity; however, variance in river flows and tidal cycles can result in regular changes in water levels at this site, which can reach a distance from the river mouth to Rangiriri (63 km from the river mouth).

Table 1 Location (latitude and longitude) of the eight monitored sites in the lower Waikato River and estuary.

Site	Latitude	Longitude
1	37°22'52.13"S	174°42'38.82"E
2	37°23'02.91"S	174°43'00.34"E
3	37°23'19.86"S	174°43'35.00"E
4	37°23'9.00"S	174°43'55.57"E
5	37°22'56.52"S	174°44'43.51"E
6	37°22'33.33"S	174°44'55.78"E
7	37°22'8.53"S	174°45'16.13"E
8	37°17'45.86"S	174°56'43.97"E

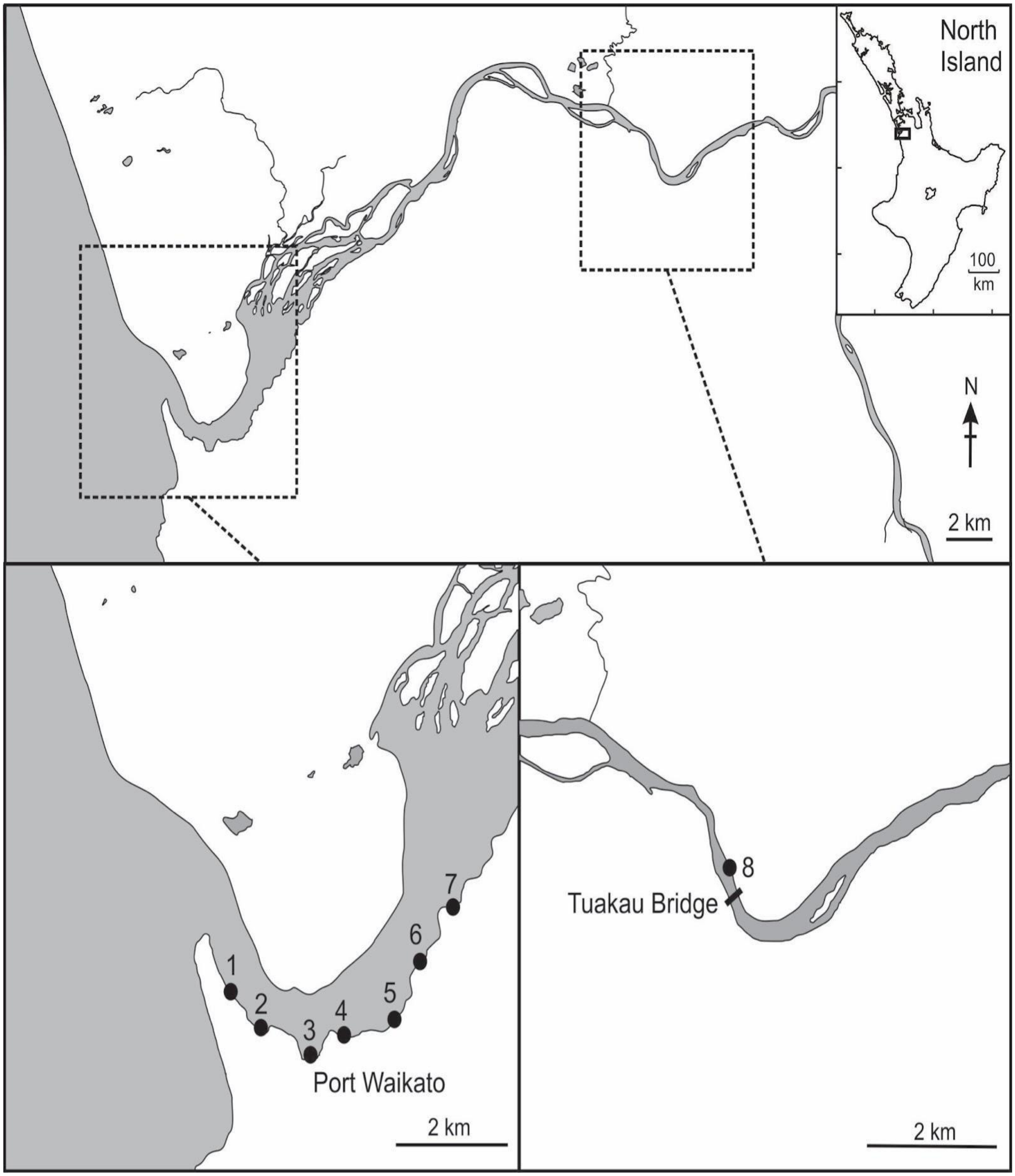

Figure 1. Map of the Waikato River estuary, New Zealand, indicating the positions of the eight-site locations.

2.2 Fieldwork sampling

Sampling was undertaken by wading during spring high tide (25 January 2023), spring low tide (8 March 2023) and neap high tide (15 March 2023) and consisted of two parts; 1) obtaining water quality parameters and 2) collection of zooplankton samples.

2.2.1 Water quality parameters

The Waikato Regional Council frequently (readings every five minutes) monitors water levels in the Waikato River at Mercer. Additionally, tidal waves often propagate as far as at least Mercer (Jones & Hamilton, 2014). Therefore, river flow data at Mercer was obtained from the Waikato Regional Council environment data hub at the time of the high tide during spring high tide, neap high tide and spring low tide on the days of sampling. The closest tidal station to the estuary was Port Waikato-Sunset Beach, which is 4.0 kilometres from the river mouth. Tidal conditions such as the time of high tide and height of tide were obtained from the Metservice website sourced from MetOcean Solutions. Meteorological conditions for Port Waikato (wind speed, air temperature and rainfall) represented daily average values; these meteorological data were also obtained from Metservice website. River flows at Mercer for previous years (2020-2022) were obtained from the Waikato Regional Council environment data hub; the river flow values from previous years were obtained from the same dates as for the 2023 survey.

Salinity, temperature and dissolved oxygen were measured using the YSI Pro2030 probe on each of the three sampling dates. A hand-held waterproof pH probe (pHTestr 30 11 Series) was used to measure the pH at all sites. Prior to measurements, all devices were calibrated. Probes were deployed at a depth approximately 10 cm below the surface in all eight sites (*Table 1*).

Samples for chlorophyll-a analysis were collected using a 50 mL syringe. A volume of 20 mL of water was collected from each site at a depth of around 5 cm. Each sample was promptly filtered using a Whatman GF/C glass microfiber filter with a nominal pore size of 1.2 µm. The filters were folded in half, with the sample side facing inwards, shielded from light with aluminium foil. For each location, this was repeated to yield duplicate samples. The filters were kept cold with ice until returned to the laboratory and stored in a freezer until analysed.

2.2.2 Zooplankton sampling

Zooplankton samples were collected using 20 duplicate fillings of a 2 L measuring jug plunged into the water to a minimum depth of approximately 0.25 m. A plankton net with a mesh size of 40 µm was used to filter zooplankton from the collected water, with a total volume filtered of 40 L for each sample. At each site, two quantitative zooplankton replicates were collected. Each sample was immediately washed into a 250 mL container using distilled water and preserved with ethanol (>50% final concentration).

2.3 Laboratory analyses

2.3.1 Chlorophyll-a analysis

Prior to the extraction of chlorophyll-a, saturated magnesium carbonate was prepared by adding 1000 mL of ultrapure water to 10 g magnesium carbonate and mixing well. This was then allowed to settle for a minimum of 48 hours and was decanted to a separate clean container ensuring there was no visible precipitation in the solution, and this was then utilised to make buffered acetone. Buffered acetone (90%) was made by adding 100 mL of the saturated magnesium carbonate to 900 mL of analytical-grade acetone. In order to prepare 0.1 N hydrochloric acid, 0.85 mL of concentrated hydrochloric acid was pipetted into 50 mL of ultrapure water and was then made up to

100 mL with additional ultrapure water. This was prepared in a fume hood wearing nitrile gloves.

One 50 mL screw cap centrifuge tube was used for each sample taken from each site (a total of 48 samples). Filters were removed from the freezer and homogenised in a fume hood away from direct sunlight. One filter was added to each 50 mL centrifuge tube with 20 mL buffered acetone solution. This was grinded into a slurry using a homogeniser Rotary tool (Ozito, RTG-920VT), and another 20 mL of buffered acetone was then added to the centrifuge tube. To avoid cross-contamination, the homogeniser was rinsed with buffered acetone before being used again with the remaining samples. Following the homogenisation of samples, centrifuge tubes were sealed, covered with aluminium foil, and maintained at 4 °C for approximately 12 hours in a dark room. Following this steeping time, the tubes were violently agitated, and placed in a centrifuge (Universal 320 R Hettich) for 10 minutes at 1,461 G with a high brake. Before starting chlorophyll-a measurements, samples were set aside for thirty minutes in the dark to attain room temperature.

Chlorophyll-a measurement was performed using a Turner Design 10-AU fluorometer. Prior to measurements, the glass cuvettes were thoroughly cleaned and visually inspected for scratches that might affect measurements. The fluorometer was turned on at least 15 minutes before use. Five mL of 90% buffered acetone was pipetted into a clean cuvette to take a blank reading. This initial blank reading was recorded, and the cuvette was removed from the fluorometer. To account for phaeophytin degradation 150 µL of 0.1 N hydrochloric acid was added to the glass cuvettes, and this was then wiped and left to stand for 90 seconds before inserting it into the fluorometer again to obtain acidified readings. This procedure was repeated for all samples ensuring that the tip of the pipette was changed between samples to prevent cross-contamination. All data were then entered into a pre-programmed Microsoft Excel spreadsheet to determine total chlorophyll-a concentration using calibration curves generated from known chlorophyll-a concentrations.

2.3.2 Zooplankton community composition

Ethanol was removed from the zooplankton samples prior to counting by filtering the samples with water using a clean 40 µm mesh. Samples were then transferred to a clean 50 mL measuring cylinder using distilled water, and the volume made up to 50 mL. The sample was counted in 5 mL aliquots using an auto-pipette. The auto-pipette was moved in a figure of eight-like motion and the sample was then placed in a gridded Perspex tray for the zooplankton count and identification. Individual zooplankton were tallied and identified in 5 mL aliquots until at least 300 were counted in total. Individuals were counted under the stereomicroscope (Nikon SMZ645) and compound microscopes (Olympus BH-2) for identification using magnifications as high as 400x.

Copepods were identified by examination of the fifth pair of pleopods, removed from the body using minuten pins. For cyclopoid copepods, females were utilised, and for calanoid copepods the males. These were dissected using a stereo dissecting microscope, covered with a cover slip, and further inspected using a compound microscope.

The ideal way of identifying rotifers is when they are alive because preservation causes contraction and other changes. Preserved rotifers, however, can still be identified to species. To achieve this, rotifer specimens were placed on a slide with a coverslip with a small drop of sodium hypochlorite added to the edge of the coverslip using a microcapillary pipette. The addition of sodium hypochlorite allowed the tissue to be dissolved from the trophi. Filter paper was used to draw out fluid from the edge of the coverslip, and when the trophi was cleared from tissues, it was examined and identified under high magnification (Shiel, 1995). Bdelloid rotifers were also found in the estuary but could not be identified to species level due to taxonomic issues with this group and the loss of diagnostic characteristics once preserved.

Zooplankton were identified to species level, where possible, utilising relevant taxonomic keys (Bradford,1972; Voigt & Koste,1978; Bradford-Grieve, 1994; Shiel, 1995; Rivier, 1998; Bradford-Grieve, 1999; Chapman et al., 2011; Suthers & Rissik, 2019).

2.4 Statistical analyses

2.4.1 Species richness

The compilation of entire species inventories is a goal that is practically impossible to achieve in practical applications and frequently necessitates exceptional efforts in most biodiversity research. Further, almost every taxonomic census or species inventory contains unidentified species. As such, the true species richness (observed plus undiscovered) is underestimated by counts of species (empirical or observed richness) in a sample, with the degree of the negative bias possibly being significant (Chao & Chiu, 2016). The Chao estimator was developed to provide an approximation of species richness when there are many undetectable species in a diverse assemblage. This approach focuses on determining the number of species in an assemblage based on the number of rarer species rather than the number of dominating species (Colwell, R. K. & Coddington, 1994).
Chao 1 & 2 estimators are calculated as:

$$S_{\text{Chao 1}} = S_{\text{obs}} + f_1^2/(2f_2) \qquad \text{If } f_2 > 0 \qquad \text{(Equation 1)}$$

$$S_{\text{Chao 1}} = S_{\text{obs}} + f_1\,(f_1 - 1)/2 \qquad \text{If } f_2 = 0 \qquad \text{(Equation 2)}$$

'$S_{\text{Chao 1}}$' corresponds to estimated species richness, and 'S_{obs}' refers to the overall number of species observed in the sample. The terms 'f_1' and 'f_2' refer to the number of taxa represented by a single individual (number of singletons) and two individuals (number of doubleton species), respectively.

The associated lower bound of species richness (Equation 1 & 2) is known as the Chao1 estimator. All computations employed the conventional Chao-1 estimator (Equation 1), where the results of the lower bound of the interval are not less than S_{obs}. A bias-corrected estimator is used in the special situation of homogeneous cases when all species detection probabilities or rates are equal. When the number of doubletons was zero ($f_2 = 0$) (Equation 2), the bias-corrected version was applied (Chao & Chiu, 2016).

Shannon-Wiener diversity index calculates the effective number of species, whereas Chao-1 estimates the total number of species (Ortiz-Burgos, 2015; Safari et al., 2020). The Shannon-Wiener index presumes that each species is represented in the sample and that individuals are chosen at random from a large independent population (Shannon 1948; Ortiz-Burgos, 2015). The Shannon-Wiener diversity index value typically falls between 1.5 and 3.5 and seldom rises over 4.5 (Ortiz-Burgos, 2015). The Shannon-Wiener diversity index is calculated as:

$$H' = -\sum_{n=1}^{n} [(p_i) \times \log(p_i)] \qquad \text{(Equation 3)}$$

Where 'H'' is the Shannon-Wiener diversity index and 'p_i' is the proportion of all individuals that belong to the ith species in each species and '$\ln p_i$' is the natural logarithm of this proportion.

Juveniles such as copepod nauplii, unidentified calanoid, harpacticoid and cyclopoid copepods were removed from the taxa list in diversity calculations as they could not be appropriately assigned to a taxon and were likely already represented among identified (adult) taxa.

The Shannon-Wiener diversity index and Chao-1 estimated species richness were both compared with underlying gradients of the Remane's curve (Remane, 1934).

2.4.2 Zooplankton community composition

A preliminary Detrended Correspondence Analysis determined that zooplankton communities were responding to underlying environmental gradients in a unimodal manner (ter Braak & Smilauer, 1998). Canonical Correspondence Analysis (CCA) was therefore used to elucidate relationships between zooplankton community composition among sites and environment conditions (ter Braak & Verdonschot, 1995). The analyses was performed using CANOCO version 5.12 (Centre for Biometry, Wageningen, The Netherlands)

Zooplankton taxa were only included in multivariate analyses if they were present in at least two sites to lessen the impact of species sampled by chance. Copepod nauplii and unidentified juvenile copepods were not included in this analysis because they could not be positively identified as belonging to any particular taxa and were likely present in proportionally high abundances across the entire salinity gradient. Species abundance data were log(x+1) transformed to reduce the influence of highly abundant species. Environmental variables were normalised where necessary, and all variables were then standardised to zero mean and unit variance to remove the impact of varying measurement scales.

Chapter 3: Results

3.1 Environmental variables

The first survey was conducted during spring high tide on 25 January 2023. The height of the high tide was 3.9 metres at 13:17, the river flow 350.4 m^3/s, the air temperature 26.0°C, and there was a recorded rainfall of 0.2 mm over the day (*Table 2*). During spring low tide, on 8 March 2023, the height of the tide was 0.5 metres at 17:28. The river flow was 337.1 m^3/s, the air temperature was 20.0°C, and there was no rainfall recorded. On the neap high tide survey, on 15 March 2023, the height of the tide was 3.0 metres at 16:10 and river flow at Mercer of 315.6 m^3/s. There was no rainfall recorded, and the air temperature was 22.0 °C (*Table 2*). Prior and during sampling parts of the North Island experienced the wettest January on record. Additionally, on the 12 and 14 February, between the first and second surveys, the North Island of New Zealand experienced extreme rainfall caused by cyclone Gabrielle, with parts of the country recording rainfall of up to 400 millimetres. Therefore, the high river flow at Mercer experienced during this survey can be attributed to the heavy rainfall. The river flow obtained in this study was between 315.6 m^3/s to 350.4 m^3/s, whereas in previous years (2020-2022), the river flow ranged between 199.0 m^3/s to 245.5 m^3/s (*Table 3*).

There was no substantial variation in water temperature throughout the field survey (*Figure 2. A*). Water temperature during spring high tide, neap high tide and spring low tide ranged between 20.6 and 23.0 °C (*Figure 2. A*), with an average temperature of 22.1 °C during spring high tide and spring low tide, and 21.4 °C during neap high tide.

The saltwater intrusion was at its maximum during spring high tide. This is shown through the high salinity values at site 1 (32.3 PSU) (*Figure 2. B*). Sites 4 and 5 in the spring high tide survey had the highest salinity concentration (32.5 PSU) (Figure 2. B). Neap high tide had a moderate saltwater influence, with salinity being highest at site 1 (26.0 PSU). Spring low tide had the lowest influence of saltwater with salinity concentration at site 1 being only 11.6 PSU. Salinity was highest at site 1 during neap high tide and spring low tide and salinity declined further upstream. The salinity concentration was lowest at site 8 (0.1 PSU) on all days during the spring-neap cycle.

There were no major trends observed for dissolved oxygen concentrations along the estuary. Dissolved oxygen ranged from 6.4 to 9.6 mg/L during the spring-neap cycle. During spring high tide, the average dissolved oxygen was 7.9 mg/L and during neap high tide 8.2 mg/L, whereas during spring low tide the average dissolved oxygen concentration was 8.8 mg/L (*Figure 2. C*)

During the spring-neap cycle, pH ranged from 7.4 to 8.3. There was generally a decline in pH upstream from the river mouth (*Figure 2. D*). This can be clearly seen during spring low tide, where pH was at its highest at site 1 (7.9) and declined to 7.6 at site 8 (*Figure 2. D*). Site 8 had the lowest pH on each sampling date over the spring-neap tidal cycle survey.

Chlorophyll-a concentration ranged from 3.6 µg/L to 30.9 µg/L during the spring-neap cycle. There was no clear spatial trend with the chlorophyll-a concentration. Spring high tide had the highest chlorophyll-a concentration at site 7 (30.9 µg/L) (*Figure 2. E*). During neap high tide, it was highest at site 2 (17.5 µg/L) and during spring low tide, it was highest at site 6 (22.9 µg/L) (*Figure 2. E*). Site 4 had the lowest chlorophyll-a concentration during spring high tide (5.3 µg/L), neap high tide (3.6 µg/L) and spring low tide (8.9 µg/L).

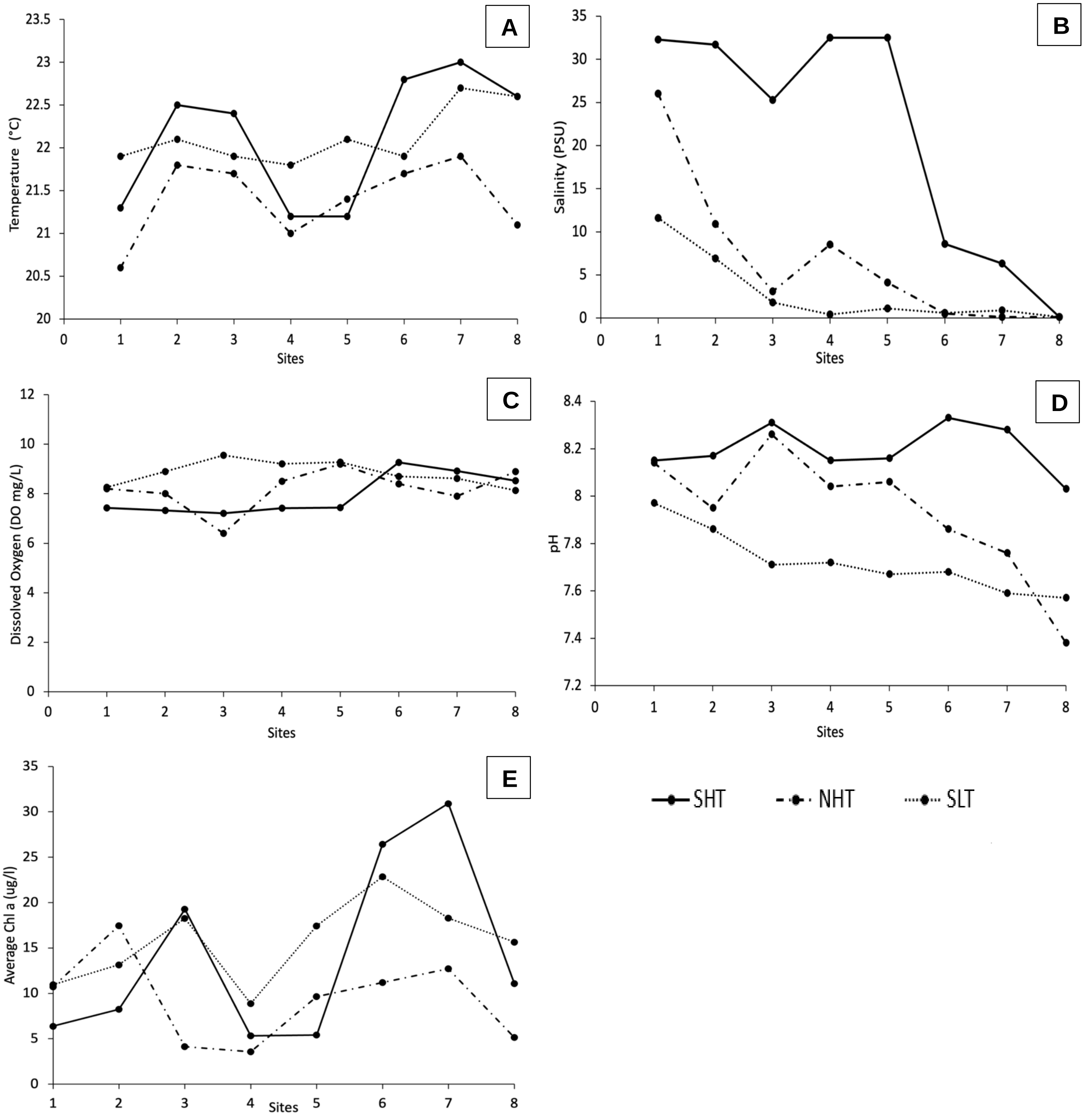

Figure 2. Environmental variables (A: temperature, B: salinity, C: dissolved oxygen, D: pH and E: chlorophyll-a) for eight sites. Environmental variables are shown for spring high tide (SHT), neap high tide (NHT) and spring low tide (SLT).

Table 2 River flow, tidal, and meteorological conditions during Waikato River estuary survey at spring high tide, spring low tide and neap high tide. River flow at Mercer (data obtained from Waikato Regional Council). Tidal conditions (Time of high/low tide and height of tide) (data obtained from Met service website sourced from MetOcean Solutions). Meteorological conditions (wind speed, air temperature and rainfall) represented daily average values (Meteorological data obtained from MetService).

	Spring high tide	Spring low tide	Neap high tide
Survey date	25/01/23	8/03/23	15/03/23
River flow at Mercer (m^3/s)	350.4	337.1	315.6
Time of high tide (hh: mm)	13:17	17:28	16:10
Height of tide (m)	3.94	0.53	2.96
Wind speed (km/h)	28	22	20
Air temperature (°C)	26.1	20.0	22.0
Rainfall (mm day^{-1})	0.2	0.0	0.0

Table 3 River flow (m^3/s) at Mercer for previous years. Spring high tide data was obtained on 25 January, spring low tide on 8 March, and neap high tide data was on the 15 March (data obtained from Waikato Regional Council website). The number in bold indicates the river flow that was taken during this study's field sampling.

Year	Spring high tide	Spring low tide	Neap high tide
2020	245.5	217.8	234.8
2021	199.0	228.2	210.5
2022	204.8	210.0	203.7
2023	**350.4**	**337.1**	**315.6**

3.2 Zooplankton assemblages

A total of 50 zooplankton taxa was recorded in this study, including 24 rotifer species, 12 copepod species, five cladoceran species and nine other taxa (*Table* 4). The dominant taxonomic group was the rotifers (48% of the community diversity), followed by copepods (24%) and cladocerans (10%), with other taxa making up the remaining 18%.

The dominant taxa were *Polyarthra dolichoptera* (21.9%), *Keratella cochlearis* (15.5%), copepod nauplii (13.2%) and *Synchaeta pectinata* (9.2%). *Keratella cochlearis* and copepod nauplii were found in all the sites during spring high tide, neap high tide and spring low tide (*Table* 4).

Species diversity for all eight sites from spring high tide, neap high tide and spring low tide was extrapolated using the Chao-1 species richness estimator (*Figure 3*) and the Shannon-Wiener index of zooplankton species diversity. The Chao-1 estimated species richness for spring high tide ranged between 10.0 and 21.6, for neap high tide 12.6 and 30.3, and for spring low tide 11.3 and 18.9. Overall, species richness was high towards the marine and freshwater sites and was low in the transitional sites (*Figure 3 & Figure 4*). Results from Chao-1 estimated species richness showed that during neap high tide and spring low tide, species richness was high in the marine site (sites 1) and towards the freshwater site (site 6) (*Figure 3*). The Shannon-Wiener index showed the same trends for both neap high tide and spring low tide, where the taxon richness was highest at the most marine site (site 1) (*Figure 4*). However, for spring high tide, the Shannon-Wiener index was highest at the freshwater site (site 8).

Table 4 List of zooplankton species recorded in the Waikato River Estuary during this study. Species and numbers refer to species present in spring high tide (SHT), neap high tide (NHT) and/or spring low tide (SLT). Site location number refers to *Figure 1 & Table 1*. Zooplankton composition (%) of each species is also recorded.

Rotifera	SHT	NHT	SLT	% Composition
Ascomorphella volvocicola	8		8	0.26
Asplanchna priodonta	1,3,5,6,8	2,3,4,5,6,7,8	1,2,3,4,5,8	1.31
Bdelloid spp.	1,3,4,6,7,8	1,2,3,5,6,7,8	1,2,3,4,5,6,7,8	2.22
Brachionidae budapestinensis			3	0.07
Brachionus calyciflorus	6,7,8	4,6,7,8	2,3,4,5,7,8	1.36
Brachionus quadridentatus melhini	1,2,6	1,2,6,7	1,2,3	0.22
Epiphanes clavulata	7,8		3,4,6,7,8	0.34
Filinia novaezealandiae	6,7,8	2,3,4,5,6,7,8	1,2,3,4,5,6,7,8	1.53
Hexarthra intermedia	3,6,7,8	2,4,6,7,8	3,4,5,7,8	0.46
Keratella cochlearis	1,2,3,4,5,6,7,8	1,2,3,4,5,6,7,8	1,2,3,4,5,6,7,8	15.53
Keratella tecta	1,3,5,6,7,8	1,2,3,4,5,6,7,8	1,2,3,4,5,6,7,8	3.36
Keratella tropica	3,4,6,7,8	1,2,3,5,6,8	1,2,3,4,5,8	0.67
Lecane bulla	7,8		8	0.22
Lecane luna	8	1,2,5	3	0.06
Penilia avirostris	1,2,6			0.07
Polyarthra dolichoptera	2,3,6,7,8	1,2,3,4,5,6,7,8	1,2,3,4,5,6,7,8	21.9
Synchaeta grimpei	1,2,4,5	1		0.51
Synchaeta oblonga	2,7,8	6,7,8	3,4,6,7,8	0.64
Synchaeta pectinata	3,5,6,7,8	2,4,5,6,7,8	1,2,3,4,5,6,7,8	9.16

Table 4 continued. List of zooplankton species recorded in the Waikato River Estuary during this study. Species and numbers refer to species present in spring high tide (SHT), neap high tide (NHT) and/or spring low tide (SLT). Site location number refers to *Figure 1 & Table 1*. Zooplankton composition (%) of each species is also recorded.

Rotifera	SHT	NHT	SLT	% Composition
Synchaeta triopthalma		2,8	1,2	0.26
Trichocerca pusilla	4,5,6,7,8	2,4,5,8	1,2,3,4,5,8	0.71
Trichocerca similis	3,4,8		5	0.15
Trichocerca stylata	1,3,4,6,7,8	2,5,6,7,8	1,2,3,4,5,6,7,8	2.11
Trichotria tetractis		3	3,5,8	0.15
Copepods				
Acanthocyclops robustus	1,2,3,5,6	1,2,3,5,7	2,8	3.19
Calamoecia lucasi		2	4	0.02
Clytemnestra rostrata		1,3	3	0.05
Copepod nauplii	1,2,3,4,5,6,7,8	1,2,3,4,5,6,7,8	1,2,3,4,5,6,7,8	13.21
Euterpina acutifrons	1,2,3,5,7	1,5	1	2.86
Indeterminate Calanoids	4,5,6,7	2,3,4,5,6	2,3,5	0.65
Indeterminate Cyclopoids	1,2,3,5,6,7,8	1,2,3,4,57	1,2,5,6	0.52
Indeterminate Harpacticoids		1,2,3		0.04
Mesochra parva		4,5	3	0.09
Microstella norvegica	1,2,4,5,6			0.09
Oithona similis	1,2,4,5,6,7	1,2,3,4,5,6,7,8	1,2,5,7	5.22
Paracalanus parvus	1,2	1	1	1.72

Table 4 continued. List of zooplankton taxa recorded in the Waikato River Estuary during this study. Species and numbers refer to species present in spring high tide (SHT), neap high tide (NHT) and/or spring low tide (SLT). Site location number refers to *Figure 1 & Table 1*. Zooplankton composition (%) of each species is also recorded.

Cladocerana	SHT	NHT	SLT	% Composition
Bosmina meridionalis	1,2,5,7,8	1,2,3,5,6,7,8	1,2,3,4,5,7,8	1.74
Ceriodaphnia dubia	5,8	1,2,3	2	0.08
Evadne nordmanni	1,5	1		0.02
Ilyocryptus sordidus	7	2,3		0.02
Pleopis polyphemoides		1,2,3,4,5		1.32
Other Taxa				
Barnacle cyprid larvae	2	1		0.02
Barnacle nauplii	1,2,4,5	1,2,4,5	1,3,5,6	1.23
Bivalve larvae	1,2,3,4,5,6	1,2,3,4,5	1,2	1.36
Cnidaria		1,2		0.06
Gastropod larvae	2,3,4,6,7	1		0.13
Larvacea	1,2,3,4,5,6	1,2,3	1	1.27
Mysid shrimp	8	4		0.01
Ostracoda	1,2,5,6	1,2,3,6	1,6	1.21
Polychaete larvae	1,2,4,5	1,3,4	1	0.61

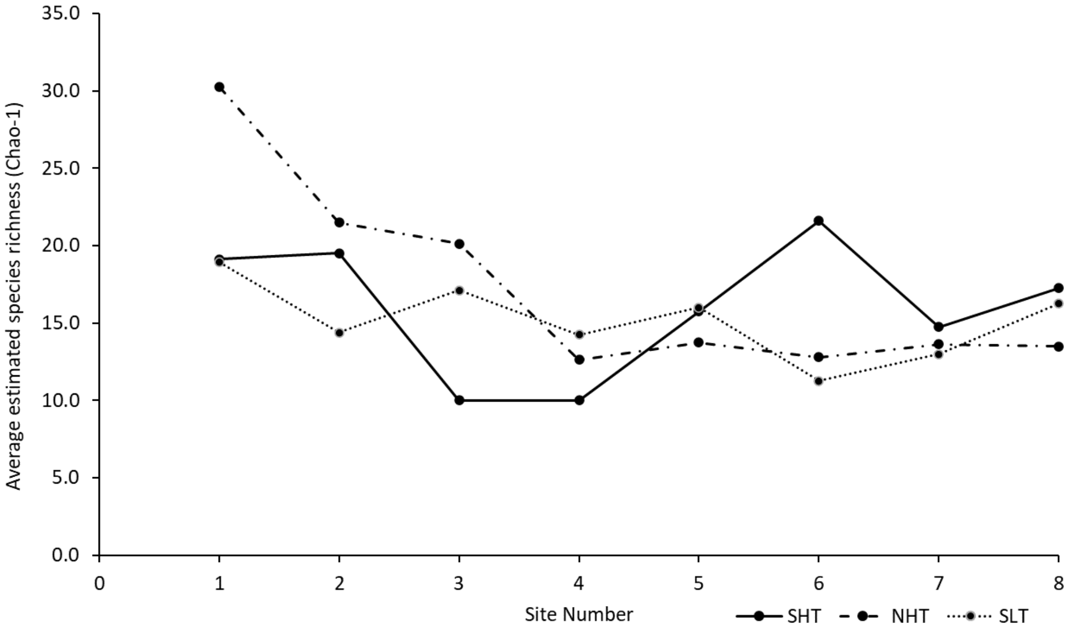

Figure 3. Average Chao-1 estimated zooplankton species richness from eight sites during spring high tide, neap high tide and spring low tide.

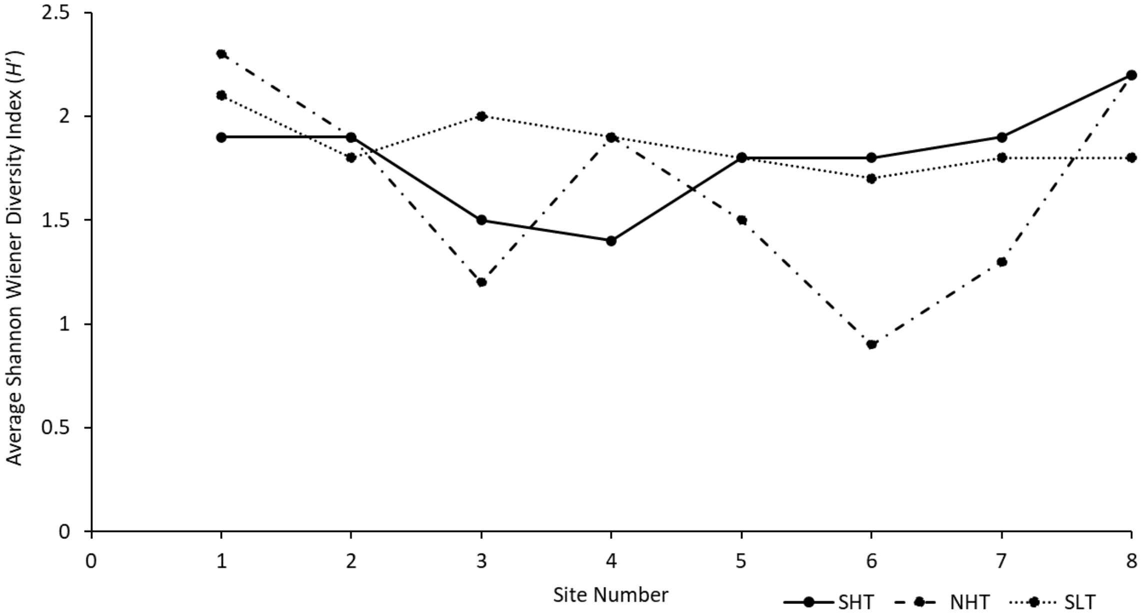

Figure 4. Average Shannon-Wiener (SW) diversity of zooplankton species from eight sites during spring high tide, neap high tide and spring low tide.

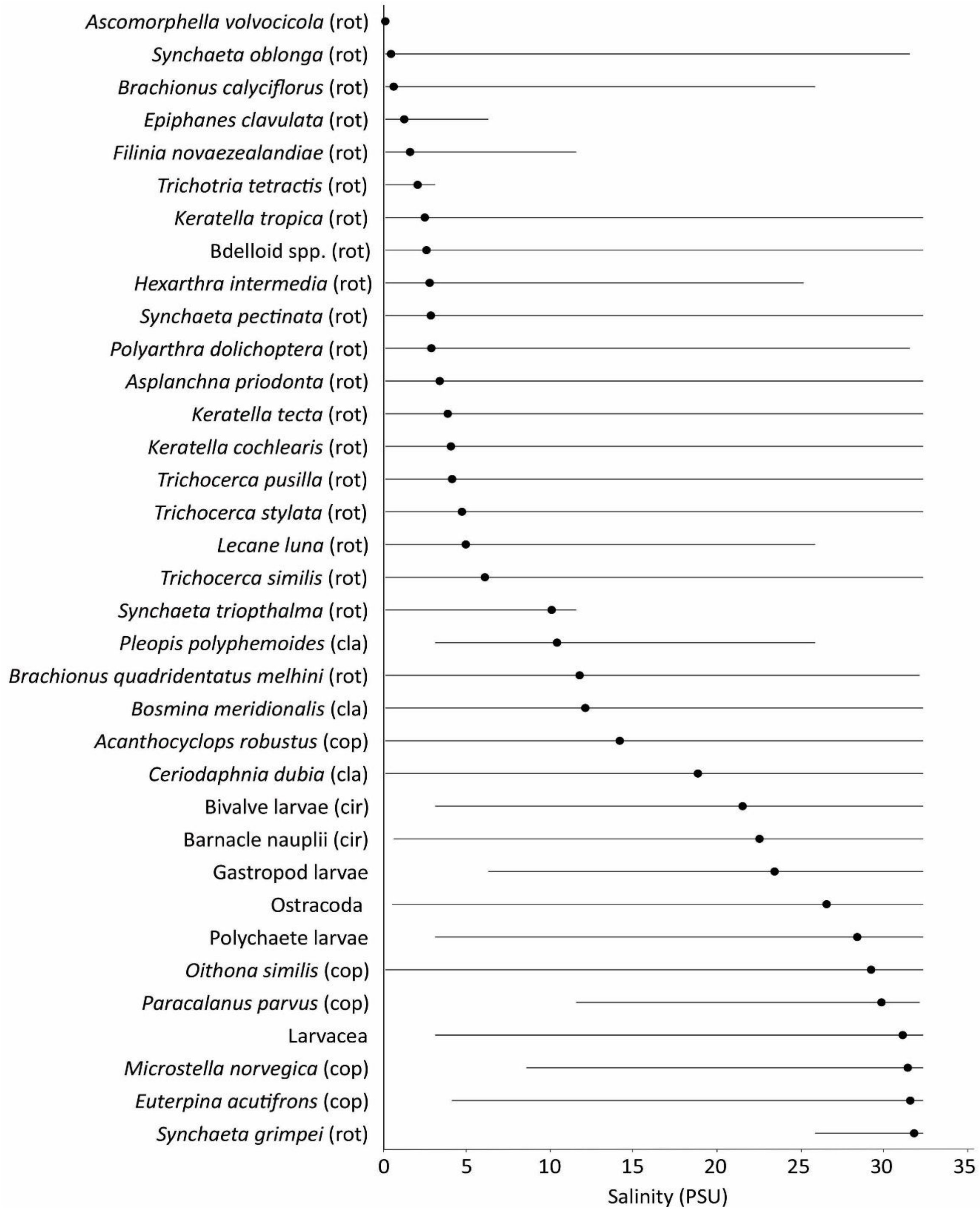

Figure 5. Distribution of zooplankton taxa across a salinity gradient (the black point represents the weighted average, the lower tail represents each taxon's lowest salinity, and the higher tail represents its highest salinity). The y-axis lists the zooplankton taxa and taxonomic groupings in brackets (rot: rotifers, cla: cladocerans, cop: copepods) and the x-axis displays a salinity gradient.

3.3 Variation in zooplankton community composition

The environmental factors that influenced patterns in zooplankton community composition throughout a spring-neap tidal cycle at Port Waikato were investigated using Canonical Correspondence Analysis (CCA), along with accompanying forward selection and Monte Carlo permutation testing. Arrows are used to symbolise environmental factors; the strength of the association is shown by the length of the arrows, which are directional indicators of the associations between the location, species and environmental factors.

The forward selection and accompanying Monte Carlo permutation tests indicated that the measured environmental factors were responsible for 46.9% of the variance in the zooplankton community in the dataset (*Table 5, Figure 6*). Salinity (26.8%, P=0.002; *Table 5*) and dissolved oxygen (9.3%, P=0.004; *Table* 5) had the greatest influence on the variation in zooplankton community composition. No other variables explained any further significant variation in zooplankton composition during the spring-neap tide cycle following the addition of salinity and dissolved oxygen.

The eigenvalue for Axis 1 was 0.6017 and for Axis 2 0.0938. The eigenvalue value for Axis 2 is very low relative to Axis 1, and variability along Axis 2 of the biplot is thus of little importance relative to Axis 1. On the CCA biplot, species such as the freshwater rotifers *Synchaeta oblonga*, *Brachionus calyciflorus*, *Filinia novaezealandiae* and *Lecane luna (Figure 6)* were most strongly negatively associated with Axis 1; this indicates that these taxa were commonly found in sites that have low salinity. The copepods *Oithona similis*, *Paracalanus parvus* and *Microsetella norvegica*, and the cladoceran *Pleopis polyphemoides* (*Figure 6*), were strongly positively associated with Axis 1, indicating that these taxa were associated with high salinity. The sites closest to the river mouth were generally positively associated with Axis 1 (sites 1-5) and generally had high salinity values. The upriver sites (sites 6-8) were negatively associated with Axis 1 and typically had low salinity.

Salinity and pH were positively associated with Axis 1 and temperature, chlorophyll- a and dissolved oxygen negatively associated (*Figure 6*). As such, species and sites associated with low salinity were also associated with lower pH and higher temperature, chlorophyll-a and dissolved oxygen.

Table 5 Forward selection and Monte Carlo permutation test results (Explains %, contribution%, pseudo-F and P) from Canonical Correspondence Analysis (CCA) of zooplankton taxa with respect to environmental variables (Salinity, dissolved oxygen, chlorophyll-a, temperature and pH) sampled at Port Waikato in eight selected sites during spring high tide, neap high tide and spring low tide. Bold values indicate a significant result ($p<0.05$) at the time of their inclusion in the model.

Conditional Term Effects				
Variable	Explains %	Contribution %	pseudo-F	P
Salinity	26.8	57.1	8.0	**0.002**
Dissolved Oxygen	9.3	19.8	3.0	**0.004**
Chlorophyll *a*	5.0	10.7	1.7	0.088
Temperature	3.2	6.8	1.1	0.344
pH	2.6	5.6	0.9	0.522

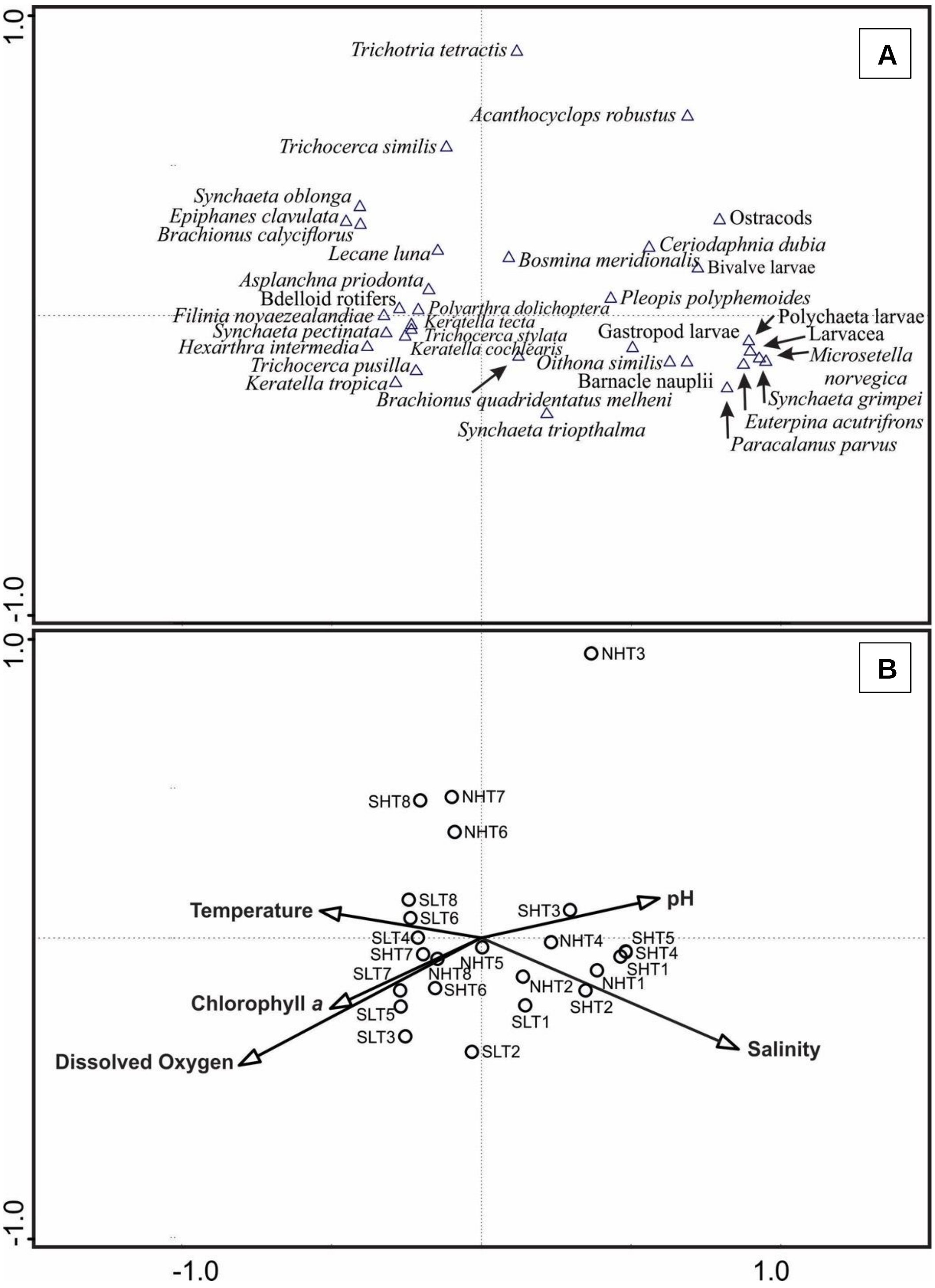

Figure 6. Ordination biplots generated from Canonical Correspondence Analysis (CCA) of zooplankton taxa with respect to environmental variables sampled during spring high tide, neap high tide and spring low tide in 8 selected sites. A= species (close triangle) biplot. B=environmental variables (arrows) with sites (SHT= Spring high tide, NHT= Neap high tide, SLT= Spring low tide). Eigenvalues for axis 1= 0.6017, axis 2= 0.0938.

Chapter 4: Discussion

4.1 Environmental variability of the estuary

During both spring high tide and spring low tide, the average temperatures across sites was 22.1 °C, and during neap high tide 21.4 °C. The maximum and minimum temperatures among sites during the spring high tide was 23.0 °C at site 7 and 21.2 °C at sites 4 and 5, respectively. During neap high tide, the maximum temperature was 21.9 °C at site 7, and the minimum was 20.6 °C at site 1. During spring low tide, the maximum temperature was 22.7 °C at site 7, and the minimum was 21.8 °C at site 4 (*Figure 2. A*). The low temperatures experienced closer to the river mouth of the Waikato River were likely due to the intrusion of colder oceanic water, relative to the warmer summer water from the river. The average water temperature during summer of the Waikato River at The Narrows bridge between 1998-2007 was 21 °C (Vant, 2010). However, temperature downstream can be higher because the lower Waikato River's water temperature is also influenced by tributary inflows, shading, natural heating and cooling in the main channels, and waste heat discharge (Rutherford, 2010). Jones & Hamilton (2014) found variability in temperature along the Waikato River estuary and attributed this to numerous factors such as tidal incursion and diurnal heating. The water temperature can vary diurnally by 0.5-1.0 °C downstream, due to solar heating. As such, the slight temperature differences found among sites are likely due to diurnal heating (Rutherford et al., 1993). The river is shallow in parts of the river downstream below Huntly, and thus experiences greater fluctuations in temperature. Furthermore, the addition of warm water from Waipa River and the Huntly Power Station is also linked to the higher temperature experienced at site 7 (Rutherford, 2010). Site 8 experienced slightly lower water temperature compared to site 7, which is likely because there was more riparian vegetation providing shade, thus lowering water temperature compared to sites sampled downstream.

Salinity concentration at site 1 during the spring high tide survey was 32.3 PSU, followed by neap high tide with 26.0 PSU and spring low tide had the lowest concentration of 11.6 PSU at site 1 (*Figure 2. B*). Salinity concentration declined further upstream. This is seen at site 8, where salinity was always measured as 0.1 PSU. As expected, patterns in salinity concentrations along the estuary varied between spring high tide, spring low tide and neap high tide. The length of the salinity intrusion increases as the tidal range increases (Cho et al., 2022). As such, the spring high tide survey had the highest degree of saltwater intrusion, followed by neap high tide and spring low tide the lowest (*Figure 2.* B). Similarly, in the Sumjin River, South Korea, as saline intrusion strengthens, a large horizontal difference in salinity develops, leading to a salinity gradient (Cho et al. 2022). Conversely, decreasing saline intrusion results in a decrease in the horizontal difference in salinity. Therefore, the high influx of seawater found during spring high tide advances upstream, increasing the salt intrusion length. This is further supported by Jones and Hamilton (2014), who have found that the limit of saltwater intrusion in the Waikato River estuary and delta was in the mid-islands, which is approximately 10 km from the river mouth during neap high tide survey. However, the high influx of seawater during a spring high tide survey revealed that salinity intrusion can extend up to 13 km from the river mouth during low river flow (Jones and Hamilton, 2014).

A study by Liu and Liu (2014) in Taiwan’s Wu River system revealed that during dry seasons, salt intrusion increases. Further, Rice et al. (2012) found that the salt intrusion in Chesapeake Bay, U.S.A., is increased during dry seasons as opposed to other times of the year. Therefore, the salinity intrusion is moved downstream towards the mouth as riverine flow rises. During the current study, there was a high influx of freshwater from the river. As such, the extent of the saltwater intrusion was horizontally decreased. It is important to note that prior to surveying, the river flow reached 350 m^3/s, which is higher than experienced at equivalent times in previous years (*Table 3*). This was due to “unprecedented” rainfall experienced at on January and cyclone Gabrielle, which caused flooding in parts of the North Island (Stillman, 2023). The intensity and increased frequency of precipitation due to climate change is projected to increase erosion and flooding in many coastal areas, especially river mouths (Fitzharris, 2007). It is predicted there would be substantial changes in rainfall patterns and an increase in severe rainfalls of up to 8% more intense rain for every 1 °C of warming (IPCC, 2013.). Heavy rainfall can lead to increased input of freshwater into estuaries, affecting salinity. As previously mentioned, Jones & Hamilton (2014) found that the extent of saltwater intrusion in the Waikato River estuary can range between 10-13 km from the entrance. It is expected that the salinity intrusion during my sampling was significantly impacted by the high river flow. During spring tide, simulations by Jones & Hamilton (2014) predicted that the location where saltwater and freshwater converge can vary between 3 and 4 kilometres depending on the river's flow. For example, when there was a spring tide (1.62 m) and low flow (257 m^3/s), saltwater intrusion occurs in the mid-islands, approximately 10 kilometres east of the river mouth. However, when there is a high flow (837 m^3/s), the saltwater intrusion is limited to the mid-upper estuary, around 6-8 kilometres east of the river mouth. This aligns with my results, as salinity concentrations dramatically declined at site 7, which was approximately 5 kilometres from the river mouth. Overall, this suggests that saltwater intrusion is highly variable in the Waikato River estuary with both tidal and riverine forces having a significant influence.

Dissolved oxygen concentrations ranged between 6.4 to 9.6 mg/L. The average dissolved oxygen concentration was lowest during spring high tide (7.9 mg/L), followed by neap high tide (8.2 mg/L), and spring low tide had the highest average dissolved oxygen concentrations (8.8 mg/L). The concentrations of dissolved oxygen showed little variation across locations. Dissolved oxygen appeared to be higher when there was minimum tidal influence and was typically higher upstream compared to sites downstream. Dissolved oxygen is affected by numerous processes, such as atmospheric diffusion, water aeration, photosynthesis, decomposition and respiration (Abu Shmeis, 2018). During the process of photosynthesis, oxygen is produced as a waste product. This adds to the dissolved oxygen concentration in the water, and this is seen during spring high tide, where sites 6 (26.4 µg/L) and 7 (30.9 µg/L) exhibited high chlorophyll-a concentration, indicating that photosynthesis strongly affects the levels of dissolved oxygen (9.3 mg/L and 8.9 mg/L, respectively) at these locations. It is interesting to note that the river flow was at its highest during the spring high tide of this study due to high precipitation (350.4 m^3/s; *Table 2*). However, dissolved oxygen was low compared to the neap high tide and spring low tide survey. Faster flowing water increases diffusion from the atmosphere, therefore increasing dissolved oxygen concentrations (Dou et al., 2018). It would be expected that the concentration of dissolved oxygen would be higher during spring high tide compared to the spring low tide and neap high tide. However, this was not the case. As previously mentioned, this could be attributed to other processes such as respiration, photosynthesis and decomposition (Abu Shmeis, 2018).

The pH values decreased from Site 1 to Site 8 during the spring-neap tidal cycle. For example, during neap high tide, there was a maximum pH of 8.1 at Site 1 (marine site), which decreased to 7.4 at Site 8 (freshwater site). The typical estuarine pH ranges from 7.0 to 7.5 in the fresher portions to between 8.0 and 8.6 in the more saline regions (Fisher, 1993). This aligns with the pH results from this study. The marine site had slightly high pH due to the naturally occurring buffering from carbonate and bicarbonate dissolved in the water, which gives saltwater a slightly alkaline pH (Ohrel & Register, 2006). Other factors may also have influenced the pH of water in the estuary, including bacterial activity, the rate of photosynthesis, chemical constituents from runoff and water turbulence (Fisher, 1993).

Concentrations of chlorophyll-a did not appear to follow a spatial pattern. The lowest chlorophyll-a concentration was found during neap high tide at site 4 (3.6 µg/l). This suggests that this ecological community is healthy and resilient according to the suggested interim rating limits for phytoplankton concentrations of chlorophyll-a in polyhaline estuaries in New Zealand (Robertson et al., 2016). Sites with very high chlorophyll-a concentrations, such as in Site 2 during neap high tide (*Figure 2. E*), suggest it is in a degraded state with no macrophyte cover (Robertson et al., 2016). Due to the proximity of estuaries to the land, the runoff from the surrounding catchment carries organic matter and nutrients that sustain high rates of primary development (Cloern et al., 2014). The long-standing theory holds that turbidity, independent of nutrient concentrations, is the primary factor regulating light availability and, ultimately, phytoplankton growth in estuaries (Lancelot & Muylaert, 2011).

4.2 Zooplankton composition

In total, 24 rotifer taxa were identified, accounting for 48% of the overall zooplankton diversity. The rotifer community was dominated by *Keratella cochlearis* and *Polyarthra dolichoptera*. There were 12 species of copepod, which made up 24% of the total zooplankton diversity. *Oithona similis* and *Acanthocyclops robustus* were the two most prevalent copepods recorded in this study. Five cladoceran species represented 10% of the total zooplankton diversity. The dominant cladoceran found in this study was the freshwater *Bosmina meridionalis*, followed by the marine cladoceran *Pleopis polyphemoides*. The other 9 taxa made up the remaining 18%; these consist of taxa such as Polychaete larvae, Ostracoda, and Larvacea. The weighted averages of individual species showed that rotifers were typically most abundant at lower salinities, whereas most copepod species were prevalent at higher salinities (*Figure 5*). Zooplankton assemblages from marine sites were typically dominated by marine copepods such as *Oithona similis* and *Paracalanus parvus* (Lucena-Moya & Duggan, 2017; Marshall, 2022).

Zooplankton community composition observed in the freshwater sections of this study was typical of rivers found by others. Burger et al. (2002) found that rotifers were the most diverse and abundant zooplankton group in the Waikato River, at sites above the estuary. Burger et al. (2002) also observed high densities of *Polyarthra dolichoptera* and *Keretella* species in the Waikato River during summer. This aligns with the current study because *Polyarthra dolichoptera* (21.9 %) and *Keratella cochlearis* (15.5 %) were also observed in high densities (*Table 4*). According to Shiel et al. (1982), rotifers typically dominate the diversity of zooplankton assemblages of large rivers, with few cladocerans and copepods represented. This links to the zooplankton taxa found in the freshwater sites of this current study. Large river systems worldwide are commonly dominated by similar rotifer groups. In the River Thames, England, the dominant species found are *Keratella cochlearis*, *Synchaeta oblonga* and *Polyarthra dolichoptera* (May & Bass, 1998). Similarly, the Hudson River, Canada is dominated by *Keratella, Polyarthra* and *Trichocerca* species (Pace et al.,1992). Small rotifer taxa have been observed to dominate the majority of rivers globally over crustaceans; this is due to the shorter development periods, fast turnover, high reproductive rates, and the ability of rotifers to produce parthenogenetically (Gannon & Stemberger, 1978; Lair, 2006; Hamilton & Duggan, 2010). Additionally, larger crustacean species' filter-feeding abilities are presumably hindered in flowing conditions, preventing them from receiving enough energy to survive and reproduce (Hamilton & Duggan, 2010). Rotifers also have adaptations for flowing water, such as anchoring their feet to sediments and adhesive glands, which prevent them from displacement (Lair, 2006). These resilient techniques make it easier for them to propagate in challenging environments (Lair, 2006).

Copepods were the next most abundant following rotifers, which made up 24% of the overall zooplankton diversity. Copepods were prevalent at higher salinity (marine sites) (*Figure 5*). The copepod *Euterpina acutifrons* was present in all sites, indicating their euryhaline character. This is consistent with the Lucena-Moya & Duggan (2017) study as the sites characterised as purely marine and intermediate salinity were dominated by copepods (*Euterpina acutifrons)*. Copepods have longer development times than rotifers, and are, therefore, less dominant in rivers (Lair, 2006).

The dominant cladoceran found in this study was the freshwater species *Bosmina meridionalis*, followed by the marine cladoceran *Pleopis polyphemoides*. The weighted averages showed that cladocerans were typically more abundant in the intermediate salinities. This was similar to observations by Lam & Ronberg (1979) and Burger et al.

(2002), who identified *Bosmina meridionalis* as the most prevalent cladoceran species in the lower Waikato River. Lair (2006) results also showed Bosmina to be a dominant taxon among crustaceans. Crustaceans have neural structures to sense shear forces along body surfaces and are therefore able to escape low water velocities however, microcrustaceans are susceptible to erosion and displacement at modest stream velocity this is why the overall Cladocera diversity is low (Lair, 2006).

The remainder 18% of taxa in this study included Polychaete larvae, Ostracoda, Bivalve larvae, Barnacle nauplii and Larvacea which were found to be more abundant in the marine sites (*Figure 5*). This is consistent with Lucena-Moya & Duggan (2017), who also found larvae of bivalves and barnacle larvae to be present in marine sites. During a two-year period, Duggan & White (2010) conducted research in Waituna Lagoon, New Zealand, where the barrier bar was 'artificially' breached. Salinity increases brought about by the opening of the barrier bar had a significant influence on the zooplankton composition. When the barrier bar was open, the salinity was greater than 30 PSU. Copepods were particularly prevalent during times of high salinity, including *E. acutifrons*, copepod nauplii, and meroplankton (gastropod larvae, polychaete larvae). A study on brackish coastal ponds, Auckland, New Zealand, also showed that copepods (*Oithona similis, Euterpina acutifrons, Paracalanus parvus*) and barnacle nauplii were found at high salinities (Marshall, 2022). These studies align with the copepods and meroplankton found in this current study.

4.3 Taxon diversity

Average estimated taxon richness (Chao1) was highest in the fully marine sites, generally declined towards the brackish sites, and then increased in the freshwater sites (*Figure 3*). The Shannon-Wiener index of zooplankton diversity ranged between 0.9 to 2.3. This also showed a similar pattern, where the average Shannon-Wiener diversity index was at its highest at the freshwater and marine sites and lowest in the brackish sites (*Figure 4*). This pattern of species richness was consistent with what is predicted by Remane's curve (Remane, 1934). Remane’s (1934) model suggests that between salinities of 0.5 and 5.0 PSU, the diversity of freshwater taxa is reduced, with the minimum species richness occurring in the brackish sites (between 5.0 to 7.0 PSU). For aquatic organisms, this is a crucial physicochemical range (Telesh & Khlebovish, 2010). Many freshwater and marine species are unable to endure intermediate salinities. Only a small variety of truly brackish species that are euryhaline are capable of surviving broad fluctuations in salinity, which is reflected in the low estimated average species richness found in brackish water (Remane, 1934; Whitfield et al., 2012; Hall & Lewandowska, 2022).

The site closest to the ocean (Site 1, *Figure 3 & Figure 4*) generally had a higher taxon richness compared to the freshwater site (Site 8; *Figure 3*). This result is different from the Remane curve model, which states that marine and freshwater sections each have a similar number of species (Remane, 1934). This is likely because of the contribution of freshwater species such as *Sychaeta oblonga*, *Keratella cochlearis* and *Polyarthra dolichoptera*, *Ceriodaphnia dubia*, *Acanthocyclops robustus* and *Bosmina meridionalis*, which were found across the gradient contributing to the higher taxon richness found in the most saline site. These freshwater taxa are unlikely to survive or reproduce in these marine sites but because of their small size they are more susceptible to be pushed downstream because of their inability to resist river flow (Lair, 2006). Species diversity of zooplankton in South African estuaries also show a peak in the lower reaches due to the contribution of both estuarine and marine species to the overall population (Grindley, 1981). There were also several types of larval stages dominating the marine sites: Barnacle nauplii, Gastropod larvae and Polychaete larvae. These could not be identified to species and therefore taxa diversity may have been underestimated in the marine sites. Remane’s curve is supported among North Island, New Zealand, estuaries (Lucena-Moya & Duggan, 2017). Lucena-Moya & Duggan (2017) found that the zooplankton assemblages of North Island estuaries matched the diversity patterns predicted by the classic Remane's curve, with freshwater and marine sites having significantly higher average species richness (Chao2) than estuaries with intermediate salinities. However, Remane's model has been misinterpreted and indiscriminately applied to global estuaries and their biological components. Considering the model was based on observations from the Baltic area, where tidal regimes differ from European estuaries and river mouths reach a brackish sea rather than a marine ocean, Remane's curve cannot be quantitatively generalised to any transitional water, including estuaries. Whitfield et al. (2012), therefore, revised Remane's diagram to comprehend the biota distribution patterns in estuaries better. The suggested conceptual model for estuarine species covers the salinity continuum from freshwater to hyperhaline conditions and states that freshwater assemblages in an estuary are not as diverse as the marine taxa in the same estuary.

Overall, my findings support the assumptions of Remane (1934) and other brackish water research, as brackish locations have a comparatively low zooplankton taxon richness due to the limited susceptibility of freshwater and marine species to brackish conditions. However, as stated in Remane (1934), the number of species in both the marine and freshwater components is equal though, the result of the current study shows that the freshwater assemblage is not as diverse as the marine sites (*Figure 3*), The high species richness in the marine sites can be attributed to the passive downstream movement of freshwater zooplankton taxa by strong river flow. Therefore, as emphasised by Whitfield et al. (2012), more caution needs to be applied when applying the Remane model to global estuaries and to all biotic components.

4.4 Variation in zooplankton community composition along the salinity gradient

Salinity had the greatest influence on the composition of zooplankton communities along the Waikato River estuary. Rotifer species such as *Synchaeta oblonga*, *Brachionus calyciflorus*, *Filinia novaezealandiae* and *Lecane luna (Figure 6)* were found in high anbundances at sites that had low salinity. In contrast, saline sites were dominated by copepod species such as *Oithona similis*, *Paracalanus parvus* and *Microsetella norvegica* and the cladoceran *Pleopis polyphemoides* (*Figure 6*). It is interesting to note that there was no estuarine copepod community found in the transitional water of the estuary. Typically, sites characterised by intermediate salinities in New Zealand are dominated by euryhaline copepods such as *Gladioferens pectinatus* and *Sulcanus conflictus,* as well as bdelloid rotifers (Roper et al.,1983; Marshall, 2023); only bdelloid rotifers were present in this study, while these copepods were absent.

Lucena-Moya & Duggan (2017) showed that estuaries on the North Island of New Zealand are defined by three groupings of zooplankton (tidal- freshwater, true estuarine and marine) along a salinity gradient. Rotifers (primarily Bdelloidea), cladocerans and calanoid copepod (*Gladioferens pectinatus*) dominated the tidal- freshwater and brackish groupings of zooplankton. The second group was true estuarine and was dominated by copepods (*Euterpina acutifrons*), and the marine grouping was composed of exclusively marine species, which was also dominated by *E. acutifrons* and other marine taxa (Lucena-Moya & Duggan, 2017). Studies of the Avon-Heathcote Estuary in New Zealand also showed the presence of estuarine species such as the copepods *Gladioferens pectinatus* and *Halicyclops neglectus* in sites characterised by intermediate salinity (Roper et al.,1983*)*. The results of the current study and others (e.g., Roper et al.,1983; Lucena-Moya & Duggan, 2017; Marshall, 2023) thus differ significantly in zooplankton composition in this salinity range.

This current study shows that salinity intrusion during spring high tide is above 5 kilometres from the estuary’s mouth. According to a 1977 assessment of the Waikato River estuary, salinity intrusion is between 3 and 6 kilometres from the estuary's mouth (Heath & Shakespeare, 1977). However, a recent study has shown that under neap tide and spring tide conditions with low river flow the extent of saltwater intrusion into the estuary went up to the upper islands, which was 13 km from the entrance, while the mid-island regions was about 10 km away during the neap tide survey (Jones &

Hamilton, 2014). This distance exceeded what had been previously reported. This indicates that saltwater intrusion over time has been increasing longitudinally, which could be an effect of climate change. The limit of salinity intrusion of the current study was found to be approximately 5 km from the river mouth this is less compared to Jones and Hamilton (2014) this is because of the high river flow, with low river flows it would be expected to have higher limit of salinity intrusion. As such, a possible explanation for the missing estuarine copepod communities in the Waikato River estuary is because of the strong forcing of the river coupled with the influence of the tide (*Table 2*); the result of these two factors may lead to an unstable habitat for the mid-salinity zooplankton, and it is therefore harder for them to develop or survive. Changes in river flow rates and water levels have an impact on the physical characteristics of the flow and the chemical makeup of the waters, which also has an impact on zooplankton. This is evident in a study from the Ob River, Russia, that heavy rainfalls and peaks in water levels during floods resulted in a high zooplankton diversity downstream due to the high river flow pushing zooplankton downstream (Yermolaeva et al., 2021).

The forward selection and accompanying Monte Carlo permutation test results from this study indicated that salinity (26.8%, P=0.002; *Table 5*) had the greatest influence on the variation in zooplankton community composition. Schallenberg et al. (2003) also found that salinity had the greatest impact on the zooplankton ecosystem in Lake Waihola. Hall & Burns (2003) showed similar results in which salinity was shown to be the dominant variable responsible to the species-environment correlation in Lake Waihola. This aligns with this present study as salinity also significantly influenced the composition of zooplankton communities.

The results of my study, therefore, support the hypothesis that salinity has the greatest influence on the composition of the zooplankton community. The other hypothesis is that along the salinity gradient (freshwater, intermediate and marine sites) there will be distinct assemblages of zooplankton with higher but equal species diversity in the freshwater and marine sites and the transitional water will comprise of lower diversity of brackish species. The findings from this research showed a lack of estuarine copepod communities in the transitional waters, such as *Gladioferens pectinatus* and *Sulcanus conflictus* and therefore did not meet this hypothesis.

4.5 Effects of climate change on zooplankton

The Waikato River flow reached 350 m^3/s during this study, which is higher than experienced at equivalent times in previous years (*Table 3*). Prior to the first survey parts of the North Island experienced the wettest January on record. A substantial amount of rain was also produced by a cyclone, named Hale on January 10. In addition, a severe subtropical cyclone, Gabrielle also impacted the North Island which also resulted in heavy rain, destructive flooding and storm surge (Stillman, 2023). Due to these antecedent conditions, the speed with which the river rose was exacerbated during Cyclone Gabrielle (Harrington et al., 2023). The results of this study also showed that salinity concentrations were low at site 7 (approximately 5 km from the river mouth) during neap high tide (0.1 PSU) and spring low tide (0.9 PSU). However, during spring high tide salinity was still relatively high (6.3 PSU). The length of salinity intrusion increases as the tidal range increases, therefore, sea level rise will likely mean marine intrusion extends further upstream. The combination of strong influence in the tidal and riverine forces has caused an unstable habitat for mid-salinity zooplankton communities to survive.

It is widely accepted that climate change has a substantial influence on both individual species and ecosystems at both the macro and micro levels. Estuaries are among the most susceptible and impacted ecosystems (Leal Filho et al., 2022). Along with undeniable climate change and ocean acidification, humans have contributed to an increase in the severity and frequency of extreme weather events (such as droughts, floods, and heat waves) during the past century (Hall & Lewandowska, 2022). Global warming will have significant impact on species' geographic distributions, especially in biogeographic transition zones. Some species with lower temperature limits may go extinct as a result of these worldwide changes, whereas species that were previously absent from a region may colonise it. Sea-level rise and coastal flooding caused by climate change are another issue since they will harm coastal wetlands and increase their vulnerability as well as that of their biotic communities (Cardoso, 2021).

High emissions scenarios show that oceanic sea level rise is anticipated to reach 0.61 to 1.10 metres by 2100 and may potentially surpass 2 metres as a result of instability in the Antarctic and Greenland ice sheets (Khojasteh et al., 2021). Global sea level rise can alter estuaries by modifying hydroperiod and by pushing saline water further upstream making these systems more exposed to saline waters. Global warming can also affect river discharge. Future salinity variations in estuaries can be influenced by the rate of sea level rise, changes in river discharge as well as the estuary's current position on the salinity gradient (Megonigal & Neubauer, 2019). Increases in precipitation are also the result of accelerating global climate change. It is expected that there will be a salinity decrease due to the higher frequency of rainfall and the increase in freshwater runoff from the catchment area (Hall & Lewandowska, 2022). There is medium confidence that the likelihood that river flooding will increase in New Zealand. According to projections, the 1-in-50-year and 1-in-100-year flood maxima for rivers in many regions of New Zealand might rise by 5 to 10% by 2050 and by more than that by 2100 (with significant variance between models and emissions scenarios) (Bodeker et al., 2022). An increase in precipitation will also result in nutrient loading from catchments, therefore, affecting the productivity and composition of the phytoplankton community (Hall & Lewandowska, 2022). Increases in phosphorus in less saline environments and elevated temperatures will result in cyanobacteria blooms (Arafat et al., 2021). Different salinity concentrations have an impact on the ecological processes in estuaries, including zooplankton diversity and abundance. Salinity changes may affect the abundance of zooplankton both directly and indirectly, causing the extinction of certain species and the emergence of new ones. Many organisms will migrate to avoid areas of high or low salinity. Variations in salinity may indirectly contribute to or result in a food scarcity, which would reduce zooplankton abundance (Perumal et al., 2009).

Zooplankton are also susceptible to warming on an individual, population, and community level. A common prediction is that the size of marine ectotherms is expected to generally decrease due to long-term warming (Daufresne et al. 2009; Rice et al., 2015). Daufresne et al. (2009) provided evidence that the three universal biological responses to global warming in aquatic systems are the migration of species towards high latitudes and elevations, seasonal changes in life cycle events, and decreased body sizes with temperature increases. Juvenile zooplankton are expected to grow rapidly and start reproducing at smaller sizes, which might result in a drop in individual mean size at the population level. Two different sorts of changes may take place at the community level: a shift in diversity while smaller warm-water species are introduced, and larger cool-water species are removed, resulting in a change in proportion as native, smaller copepods become more comparatively prevalent (Daufresne et al. 2009; Jansson et al. 2020). This is consistent with Rice et al. (2015), who studied the Long Island Sound estuary, U.S.A., and indicated a warming of 0.03 °C year $^{-1}$ between the 1940s and 2012. This study revealed that there would be a concurrent decrease in the mean body size of dominant copepod species and an increase in the abundance of the small copepod *Oithona* sp. Reduced mean size could change food connectivity and therefore reduce the efficiency of trophic transfer between phytoplankton and endemic larval fish (Rice et al., 2015). Larger cool water calanoid species are also expected to be lost in the system because the warmer water exceeds their thermal tolerance. The elevated temperature has also resulted in cladocerans delaying production of their resting eggs (Chen & Folt, 1996), as well as fish larvae population decreasing due to the quality of food that is zooplankton substantially decreasing (Arula et al., 2014).

4.6 Implications and future research

The negative impacts of climate change on zooplankton are already noticeable and are predicted to exacerbate in the near future. According to the IPCC Special Report on Global Warming of 1.5°C, incremental increases in global warming (+0.5°C) induce statistically significant changes in global and regional extremes (high confidence). This is especially true for temperature extremes (very likely) and the intensification of heavy precipitation (high confidence) (Seneviratne et al., 2021). The increase in global temperature promotes sea level rise, which in turn can change salinity concentrations and, eventually, biotic communities (Perumal et al., 2009). Therefore, there must be policies that would aid in mitigating the factors responsible for climate change, such as for rapid assessment techniques that will help policymakers and managers make thoughtful decisions for the protection and management of the estuarine environment.

My research has examined zooplankton species richness and zooplankton community composition along the Waikato River estuary in a spring-neap tidal cycle. Results from my study show that along the salinity gradient, there was diverse communties in both the freshwater and marine section of the river. However, sites characterised as transitional were missing estuarine copepods, which may be attributed to the effects of climate change. Due to the time constraints of this study, zooplankton composition was only sampled during a spring-neap tidal cycle, and therefore I would recommend examining the long-term zooplankton composition in the Waikato River estuary. Continuous sampling, such as monthly sampling across seasons and multi-year sampling, would allow for more comprehensive data and would better depict the effects of climate change over time on zooplankton composition. The current study showed strong forcing of the river in comparison to previous years due to high freshwater inputs because of high precipitation and flooding upstream. This meant that across the salinity gradient freshwater taxa such as *Polyarthra dolichoptera*, *Ceriodaphnia dubia*, *Acanthocyclops robustus* and *Bosmina meridionalis,* were forced downstream to marine sites, which are not typical of zooplankton assemblages in higher salinity sites. Long-term sampling would allow better understanding of zooplankton communities in estuaries.

This study left significant amounts of variance unexplained. The assessed environmental factors could only account for 46.9% of the variance in zooplankton community composition in the Waikato River estuary, and the remaining 53.1% was not explained. This implies that other stochastic mechanisms and biotic interactions not assessed in the current study may significantly impact the zooplankton populations. For example, biotic interactions such as predation by fish was not examined in this study. Fish species are likely to change along the salinity gradient due to their salinity tolerance range (Kültz, 2015), which could have impacted the zooplankton composition. The Waikato River estuary is of international importance and supports a large recreational whitebait and eel fishery. In estuarine environments, zooplankton is a crucial trophic link and a major source of food for several fish larval species. Thus, a loss in estuarine zooplankton due to climate change implies a decline in pelagic fish (Winder & Jassby, 2011). Therefore, researching this knowledge gap would aid in mitigating stressors in the Waikato River estuary.

4.7 Summary

In summary, the distribution of zooplankton was significantly impacted by the dramatic changes in hydrographic properties that come from estuaries' intrinsic dynamic nature. Zooplankton are critical to the biological communities of large rivers. However, they are susceptible to environmental changes, particularly in estuarine environments. Salinity had the greatest influence on zooplankton community composition along the Waikato River estuary. Overall, findings from this study indicate that the marine section of the river had the highest species richness followed by the freshwater site and the intermediate site having the least species richness. The sites characterised by intermediate salinities showed that estuarine copepods were missing, which may be attributed to the effects of climate change. Further research should be performed to increase our understanding of estuarine systems and ways to manage and conserve them. This study will be beneficial for future research on the composition of zooplankton in estuaries along a salinity gradient. It will provide insights into the susceptibility of these ecosystems to various challenges, including the effects of climate change, such as sea level rise, warmer temperatures and precipitation. Understanding these factors is crucial for the protection and management of estuarine biodiversity.

References

Abu Shmeis, R. M. (2018). Water Chemistry and Microbiology. *Fundamentals of Quorum Sensing, Analytical Methods and Applications in Membrane Bioreactors*, 1–56.

Arafat, M. Y., Bakhtiyar, Y., Mir, Z. A., & Tak, H. I. (2021). Paradigm of Climate Change and its Influence on Zooplankton. *Biosciences Biotechnology Research Asia*, *18*, 423.

Arula, T., Ojaveer, H., & Klais, R. (2014). Impact of extreme climate and bioinvasion on temporal coupling of spring herring (*Clupea harengus* m.) larvae and their prey. *Marine Environmental Research*, 102, 102–109.

Bakhtiyar, Y., Arafat, M. Y., Andrabi, S., & Tak, H. I. (2020). Zooplankton: the significant ecosystem service provider in aquatic environment. *Bioremediation and Biotechnology, Vol 3: Persistent and Recalcitrant Toxic Substances*, 227-244.

Benfield, L. A. (1990). *The composition and distribution of zooplankton in the lower Waikato River, New Zealand* (Doctoral dissertation, University of Waikato).

Benfield, M. C. (2012). Estuarine zooplankton. *Estuarine ecology. Hoboken (NJ): John Wiley & Sons, Inc*, 285-302.

Bodeker, G., Tait, A., Morgenstern, O., Noone, D., Revell, L., McDonald, A., ... & Katurji, M. (2022). Aotearoa New Zealand climate change projections guidance: Interpreting the latest IPCC WG1 report findings.

Booi, S., Mishi, S., & Andersen, O. (2022). Ecosystem services: a systematic review of provisioning and cultural ecosystem services in estuaries. *Sustainability*, *14*(12), 7252.

Bozorg-Haddad, O., Delpasand, M., & Loáiciga, H. A. (2021). Water quality, hygiene, and health. *Economical, Political, and Social Issues in Water Resources*, 217–257.

Bradford, J. (1972). Systematics and ecology of the New Zealand Central East coast plankton sampled at Kaikoura. *Bulletin (New Zealand Department Scientific and Industrial Research), 207*, 1-87.

Bradford-Grieve, J. M. (1994). *The marine fauna of New Zealand: pelagic calanoid Copepoda: Megacalanidae, Calanidae, Paracalanidae, Mecynoceridae, Eucalanidae, Spinocalanidae, Clausocalanidae*. National Institute of Water and Atmospheric Research, Wellington, New Zealand.

Bradford-Grieve, J. M. (1999). *The marine fauna of New Zealand: pelagic calanoid Copepoda: Bathypontiidae, Arietellidae, Augaptilidae, Heterorhabdidae, Lucicutiidae, Metridinidae, Phyllopodidae, Centropagidae, Pseudodiaptomidae, Temoridae, Candaciidae, Pontellidae, Sulcanidae, Acartiidae, Tortanidae.* National Institute of Water and Atmospheric Research, Wellington, New Zealand.

Brierley, A. S. (2017). Plankton. *Current Biology*, *27*(11), R478-R483.

Burger, D. F., Hogg, I. D., & Green, J. D. (2002). Distribution and abundance of zooplankton in the Waikato River, New Zealand. *Hydrobiologia*, *479*, 31-38.

Cardoso, P. G. (2021). Estuaries: Dynamics, Biodiversity, and Impacts. In *Life Below Water* (pp. 1-12). Cham: Springer International Publishing.

Chao, A., & Chiu, C. (2016). Nonparametric estimation and comparison of species richness. *Wiley StatsRef: Statistics Reference Online*, 1–11.

Chapman, M. A., Lewis, M. H., & Winterbourn, M. J. (2011). Guide to the freshwater Crustacea of New Zealand. *Guide to the freshwater crustacea of New Zealand.*

Chapman, M. A. (1996). Human impacts on the Waikato River system, New Zealand. *GeoJournal*, *40*, 85-99.

Chen, C.Y., & Folt, C.L. (1996). Consequences of fall warming for zooplankton over wintering success. *Limnology and Oceanography*, 41, 1077–1086.

Cho, E., Tak, Y., Cho, Y., & Na, H. (2022). Fortnightly variability of horizontal salinity gradient affects exchange flow in the Sumjin River estuary. *Frontiers in Marine Science*, *9*, 1077004.

Clarke, K. R., & Warwick, R. M. (2001). Changes in Marine Communities: An Approach to Statistical Analysis and Interpretation, 2nd edn.

Cloern, J. E., Foster, S. Q., & Kleckner, A. E. (2014). Phytoplankton primary production in the world's estuarine-coastal ecosystems. *Biogeosciences*, *11*(9), 2477–2501.

Collier, K. J., Watene, E. M., McCraw, J. D. (2010). The Waters of the Waikato: Ecology of New Zealand's longest river. *Environment Waikato and the Centre for Biodiversity and Ecology Research, The University of Waikato: Hamilton, New Zealand*.

Colwell, R. K. & Coddington, J. A. (1994). Estimating terrestrial biodiversity through extrapolation*. Philosophical Transactions: Biological Sciences*, 345(1311): 101-118.

Cremona, F., Blank, K., & Haberman, J. (2021). Effects of environmental stressors and their interactions on zooplankton biomass and abundance in a large eutrophic lake. *Hydrobiologia*, *848*(18), 4401–4418.

Daufresne, M., Lengfellner, K., & Sommer, U. (2009). Global warming benefits the small in aquatic ecosystems. *Proceedings of the National Academy of Sciences, 106*(31), 12788-12793.

Dou, B., Hosseini, Y., Lee, C., Rosenberg, C., & Wu, N. (2018). The relationship between stream discharge and dissolved oxygen levels at canyon creek, and implications towards salmon performance. *The Expedition*, *8*

Duggan, I. C., & White, M. A. (2010). Consequences of human-mediated marine intrusions on the zooplankton community of a temperate coastal lagoon. *New Zealand Journal of Marine and Freshwater Research*, *44*(1), 17-28.

Fairbridge, R. W. (1980). The estuary: its definition and geodynamic cycle. *Chemistry and Biochemistry of Estuaries*, 1-35.

Fisher, N. A. (1993). *Volunteer estuary monitoring: A methods manual*. US Environmental Protection Agency, Office of Water, Office of Wetlands, Oceans, and Watersheds, Oceans and Coastal Protection Division.

Fitzharris, B. (2007). How vulnerable is New Zealand to the impacts of climate change? *New Zealand Geographer*, *63*(3), 160-168

Gao, Q., Xu, Z., & Zhuang, P. (2008). The relation between distribution of zooplankton and salinity in the Changjiang Estuary. *Chinese Journal of Oceanology and Limnology*, *26*(2), 178–185.

Grindley, J. R. (1981). Estuarine plankton. *Estuarine Ecology with Particular Reference to South Africa*, 117-146.

Gulyas, P. (1994). Studies on the rotatorian and crustacean plankton in the Hungarian section of the Danube between 1848.4 and 1659.0 RIV. *Km Limnologie Aktuel l2*: 2–14.

Haddout, S., Baimik, I., Maslouhi, A., Igouzal, M., Magrane, B., & Marah, H. (2018). The influence of spring and neap tide on salt intrusion and stratification in Sebou Estuary (Morocco). *International Journal of River Basin Management*, *17*(1), 131–142.

Haddout, S., Maslouhi, A., & Magrane, B. (2018). A 2D fluid motion model of the estuarine water circulation: Physical analysis of the salinity stratification in the Sebou estuary. *The European Physical Journal Plus*, *133*, 1-15.

Halfter, S., Cavan, E. L., Swadling, K. M., Eriksen, R. S., & Boyd, P. W. (2020). The role of zooplankton in establishing carbon export regimes in the Southern Ocean – a comparison of two representative case studies in the Subantarctic region. *Frontiers in Marine Science*, *7*.

Hall, C. A., & Lewandowska, A. M. (2022). Zooplankton dominance shift in response to climate-driven salinity change: A mesocosm study. *Frontiers in Marine Science*, *9*.

Hall, C. J., & Burns, C. W. (2003). Responses of crustacean zooplankton to seasonal and tidal salinity changes in the coastal Lake Waihola, New Zealand. *New Zealand Journal of Marine and Freshwater Research, 37*(1), 31-43.

Hamilton, D. P., Duggan, I. C. (2010). The Waters of the Waikato: Ecology of New Zealand's longest river. *Environment Waikato and the Centre for Biodiversity and Ecology Research, The University of Waikato: Hamilton, New Zealand*.

Harrington, L. J., Dean, S. M., Awatere, S., Rosier, S., Queen, L., Gibson, P. B., ... & Otto, F. E. (2023). *The role of climate change in extreme rainfall associated with Cyclone Gabrielle over Aotearoa New Zealand's East Coast*. Grantham Institute.

Heath, R. A., & Shakespeare, B. S. (1977). Summer temperatures and salinity distribution on three small inlets on the west coast, North Island, New Zealand. *New Zealand Oceanographic Institute, 3*(17), 151-158.

Hendrey, G. R. (2001). Acid rain and deposition. *Encyclopedia of Biodiversity*, 1–15.

Hume, T. M., Snelder, T., Weatherhead, M., & Liefting, R. (2007). A controlling factor approach to estuary classification. *Ocean & coastal management*, *50*(11-12), 905-929.

IPCC, A. (2013). Climate change 2013: the physical science basis. *Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change*, *1535*.

IPCC. (2018). Summary for Policymakers" in Global warming of 1.5° C. An IPCC Special Report on the impacts of global warming of 1.5° C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. *Sustainable Development, and Efforts to Eradicate Poverty. Geneva, Switzerland: World Meteorological Organization*, *32*.

Jansson, A., Klais-Peets, R., Grinienė, E., Rubene, G., Semenova, A., Lewandowska, A., & Engström-Öst, J. (2020). Functional shifts in estuarine zooplankton in response to climate variability. *Ecology and Evolution*, *10*(20), 11591-11606.

Jones, H. F. E., & Hamilton, D. P. (2014). *Assessment of the Waikato River estuary delta for whitebait habitat management: field survey, GIS modelling and hydrodynamic modelling. ERI report 27*. Prepared for Waikato Regional Council. Hamilton, New Zealand: Environmental Research Institute, Faculty of Science and Engineering, The University of Waikato.

Jones, T. R., Henderson, C. J., Olds, A. D., Connolly, R. M., Schlacher, T. A., Hourigan, B. J., Goodridge Gaines, L. A., & Gilby, B. L. (2020). The mouths of estuaries are key transition zones that concentrate the ecological effects of predators. *Estuaries and Coasts*.

Kennish, M. J. (2002). Environmental threats and environmental future of estuaries. *Environmental Conservation*, *29*(1), 78-107.

Khojasteh, D., Glamore, W., Heimhuber, V., & Felder, S. (2021). Sea level rise impacts on Estuarine Dynamics: A Review. *Science of The Total Environment*, *780*, 146470.

Kobayashi, T. (1997). Associations between environmental variables and zooplankton body masses in a regulated Australian river. *Marine and Freshwater Research*, *48*(6), 523-529.

Kültz, D. (2015). Physiological mechanisms used by fish to cope with salinity stress. *The Journal of Experimental Biology*, *218*(12), 1907-1914.

Lair, N. (2006). A review of regulation mechanisms of metazoan plankton in riverine ecosystems: aquatic habitat versus biota. *River research and Applications*, *22*(5), 567-593.

Lalli, C. M., & Parsons, T. R. (1997). Zooplankton. Biological Oceanography: An Introduction, 74–111.

Lam, C. W. Y. & L. A. Ronberg. (1979). The Waikato River: a water resources study. In Strachan, C. J. (ed.), Technical Committee for the Waikato River Water Resources Study. Wellington Water and Soil Division Ministry of Works and Development, New Zealand: 225 pp.

Lancelot, C., & Muylaert, K. (2011). 7.02 Trends in estuarine phytoplankton ecology. In *Treatise on estuarine and coastal science* (pp. 5-15). Academic Press Waltham.

Leal Filho, W., Nagy, G. J., Martinho, F., Saroar, M., Erache, M. G., Primo, A. L., Pardal, M. A., & Li, C. (2022). Influences of climate change and variability on estuarine ecosystems: An impact study in selected European, South American and Asian countries. *International Journal of Environmental Research and Public Health*, *19*(1), 585.

Liu, W. C., & Liu, H. M. (2014). Assessing the impacts of sea level rise on salinity intrusion and transport time scales in a tidal estuary, Taiwan. *Water*, *6*(2), 324-344.

Lomartire, S., Marques, J. C., & Gonçalves, A. M. M. (2021). The key role of Zooplankton in ecosystem services: A perspective of interaction between zooplankton and fish recruitment. *Ecological Indicators*, *129*, 107867.

Lucena-Moya, P., & Duggan, I. C. (2017). Correspondence between zooplankton assemblages and the Estuary Environment Classification System. *Estuarine, Coastal and Shelf Science*, *184*, 1–9.

Marshall, G. M. J. (2023). *Responses of zooplankton assemblages to spatio-temporal variability in environmental conditions of brackish coastal ponds, Auckland, New Zealand* (Doctoral dissertation, The University of Waikato).

May, L., & Bass, J. A. (1998). A study of rotifers in the River Thames, England, April–October, 1996. *In Hydrobiologia*. Springer Netherlands.

Megonigal, J. P., & Neubauer, S. C. (2019). Biogeochemistry of tidal freshwater wetlands. *Coastal Wetlands*, 641–683

Myers, S. C., Clarkson, B. R., Reeves, P. N., & Clarkson, B. D. (2013). Wetland management in New Zealand: are current approaches and policies sustaining wetland ecosystems in agricultural landscapes? *Ecological Engineering*, *56*, 107-120.

Ohrel, R. L., & Register, K. M. (2006). *Volunteer estuary monitoring: a methods manual*. Ocean Conservancy.

Ortiz-Burgos, S. (2015). Shannon-Weaver Diversity index. *Encyclopedia of Estuaries*, 572–573.

Paavola, M., Olenin, S., & Leppäkoski, E. (2005). Are invasive species most successful in habitats of low native species richness across European brackish water seas?. *Estuarine, Coastal and Shelf Science*, *64*(4), 738-750.

Pace, M. L., Findlay, S. E., & Lints, D. (1992). Zooplankton in advective environments: the Hudson River community and a comparative analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, *49*(5), 1060-1069.

Perumal, N. V., Rajkumar, M., Perumal, P., & Rajasekar, K. T. (2009). Seasonal variations of plankton diversity in the Kaduviyar estuary, Nagapattinam, southeast coast of India. *Journal of Environmental Biology*, *30*(6), 1035-1046.

Remane, A. (1934). Die Brackwasserfauna: Mit besonderer Berücksichtigung der Ostsee. *Zoologischer Anzeiger*.

Rice, E., Dam, H. G., & Stewart, G. (2015). Impact of climate change on estuarine zooplankton: surface water warming in Long Island Sound is associated with changes in copepod size and community structure. *Estuaries and Coasts*, *38*, 13-23.

Rice, K. C., Hong, B., & Shen, J. (2012). Assessment of salinity intrusion in the James and Chickahominy Rivers as a result of simulated sea-level rise in Chesapeake Bay, East Coast, USA. *Journal of Environmental Management*, *111*, 61-69.

Rivier, I. K. (1998). The predatory Cladocera (Onychopoda: Podonidae, Polyphemidae, Cercopagidae) and Leptodorida of the World. *Guides to the identification of the micro-invertebrates of the continental waters of the world.*

Robertson, B.M, Stevens, L., Robertson, B., Zeldis, J., Green, M., Madarasz-Smith, A., Plew, D., Storey, R., Oliver, M. (2016). NZ Estuary Trophic Index Screening Tool 2. Determining Monitoring Indicators and Assessing Estuary Trophic State. *Prepared for Envirolink Tools Project: Estuarine Trophic Index, MBIE/NIWA Contract,* C01X1420. 68.

Roper, D. S., Simons, M. J., & Jones, M. B. (1983). Distribution of zooplankton in the Avon-Heathcote Estuary, Christchurch. *New Zealand Journal of Marine and Freshwater Research*, *17*(3), 267-278.

Rosenberg, R. (1976). Benthic faunal dynamics during succession following pollution abatement in a Swedish estuary. *Oikos*, *27*(3), 414.

Rutherford, J. C., Macaskill, J. B., & Williams, B. L. (1993). Natural water temperature variations in the lower Waikato River, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, *27*(1), 71–85.

Rutherford, J.C. (2010). The Waters of the Waikato: Ecology of New Zealand’s longest river. *Environment Waikato and the Centre for Biodiversity and Ecology Research, The University of Waikato: Hamilton, New Zealand*.

Ryer, R., Bentley, J., Saunders, L., & De Luca, S. (2016). Natural Character Study of the Waikato Coastal Environment.

Safari, Z., Bruneau, A., Monnoye, M., Mariadassou, M., Philippe, C., Zatloukal, K., & Gérard, P. (2020). Murine genetic background overcomes gut microbiota changes to explain metabolic response to high-fat diet. *Nutrients*, *12*(2), 287.

Schallenberg, M., Hall, C. J., & Burns, C. W. (2001). Climate change alters zooplankton community structure and biodiversity in coastal wetlands. *Report of Freshwater Ecology Group, University of Otago, Hamilton*.

Schallenberg, M., Hall, C. J., and Burns, C. W. (2003). Consequences of climate-induced salinity increases on zooplankton abundance and diversity in coastal lakes. *Marine Ecology Progress Series, 251*, 181-189.

Seneviratne, S. I., Zhang, X., Adnan, M., Badi, W., Dereczynski, C., Di Luca, A., ... & Zhou, B. (2021). Weather and Climate Extreme Events in a Changing Climate; Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change.

Shannon, C. E. (1948). A mathematical theory of communication. *The Bell System Technical Journal*, *27*(3), 379-423.

Shiel, R. J., Walker, K. F., & Williams, W. D. (1982). Plankton of the lower River Murray, South Australia. *Marine and Freshwater Research*, *33*(2), 301-327.

Shiel, R. J. (1995). A Guide to identification of rotifers, cladocerans and copepods from Australian inland waters. Identification Guide No. 3. *Albury: CRCFE-MDBC*.

Stillman, D. (2023). Severe storm, flooding prompts state of emergency in New Zealand. *The Washington Post*, NA-NA.

Suthers, I., Rissik, D., & Richardson, A. (2019). *Plankton: A guide to their ecology and monitoring for water quality*. CSIRO publishing.

Swales, A., Bell, R. G., & Lohrer, A. (2020). Estuaries and lowland brackish habitats. *Cop right*, 55.

Telesh, I. V., & Khlebovich, V. V. (2010). Principal processes within the estuarine salinity gradient: a review. *Marine Pollution Bulletin*, *61*(4-6), 149-155.

ter Braak, C. J. F., & Smilauer, P. (1998). *Canoco for Windows, Version 4.02.* Centre for Biometry, Wageningen, Netherlands.

ter Braak, C. J., & Verdonschot, P. F. (1995). Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquatic Sciences*, *57*(3), 255–289.

Thrush, S. F., Townsend, M., Hewitt, J. E., Davies, K., Lohrer, A. M., Lundquist, C., & Dymond, J. (2013). The many uses and values of estuarine ecosystems. *Ecosystem Services in New Zealand–Conditions and Trends. Manaaki Whenua Press, Lincoln, New Zealand*, 226-237.

Valle-Levinson, A. (2011). 1.05 Classification of estuarine circulation. *Treatise on Estuarine and Coastal Science*, 75-86.

Vanni, M. J. (2002). Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics*, *33*(1), 341–370.

Vant, W.N. (2010). The Waters of the Waikato: Ecology of New Zealand's longest river. *Environment Waikato and the Centre for Biodiversity and Ecology Research, The University of Waikato: Hamilton, New Zealand*

Voigt, M., & Koste, W. (1978). Rotatoria.

Whitfield, A. K., Elliott, M., Basset, A., Blaber, S. J. M., & West, R. J. (2012). Paradigms in estuarine ecology–a review of the Remane diagram with a suggested revised model for estuaries. *Estuarine, Coastal and Shelf Science, 97*, 78-90.

Wiafe, G., & Frid, C. L. (1996). Short-term temporal variation in coastal zooplankton communities: The relative importance of physical and biological mechanisms. *Journal of Plankton Research*, *18*(8), 1485–1501.

Winder, M., & Jassby, A. D. (2011). Shifts in zooplankton community structure: implications for food web processes in the upper San Francisco Estuary. *Estuaries and Coasts*, *34*, 675-690.

Wooldridge, T. H., & Deyzel, S. H. P. (2009). Temperature and salinity as abiotic drivers of zooplankton community dynamics in the Great Berg Estuary. *Transactions of the Royal Society of South Africa*, *64*(2), 219-237

Xia, Y. (2020). Correlation and association analyses in microbiome study integrating multiomics in health and disease. *Progress in Molecular Biology and Translational Science*, 309–491.

Yermolaeva, N., Dvurechenskaya, S., Kirillov, V., & Puzanov, A. (2021). Dependence of long-term dynamics of zooplankton in the Ob River on interannual changes in hydrological and hydrochemical parameters. *Water*, *13*(14), 1910.

Yuan, D., Chen, L., Luan, L., Wang, Q., & Yang, Y. (2020). Effect of salinity on the zooplankton community in the Pearl River Estuary. *Journal of Ocean University of China*, *19*(6), 1389–1398.